TOXIC METALS, CANCER RISK AND GENOTOXICITY BIOMARKERS IN HUMANS EXPOSED TO ELECTRONIC WASTE IN SOUTHWESTERN NIGERIA

By

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Matric No. 173047

A thesis in the Department of Chemical Pathology

Submitted to the

Faculty of Basic Medical Sciences in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

***May*, 2018**

ABSTRACT

The scourge of electronic waste (e-waste) is currently a major global concern, particularly in developing countries like Nigeria where its reprocessing is substantial but unregulated. The possible contribution of e-waste toxic metals to the risk of cancer development in Nigeria has received little attention. This study was aimed at assessing the exposure of Nigerians to toxic metals in e-waste, using biomarkers of exposure and genotoxicity to evaluate the risk of cancer development.

In this cross-sectional study, 632 consenting participants were enrolled from Benin, Lagos and Ibadan, Southwestern Nigeria; consisting of 381 e-waste workers (EW) and 120 environmental e-waste exposed participants (EEEP) age-matched with 131 unexposed participants (control). Blood (10 mL) was obtained from each participant. Levels of toxic metals (cadmium, lead, mercury, arsenic, chromium, nickel, molybdenum, aluminium, vanadium, thallium, antimony and tin) in blood and essential metals (zinc, selenium, copper and cobalt) in serum were determined using inductively coupled plasma-mass spectrometry. Vitamins A, C and E, oxidative stress biomarkers: malondialdehyde and uric acid (UA), activities of enzymatic antioxidants [catalase, superoxide dismutase (SOD), ɣ-glutamyltransferase (GGT) and glutathione peroxidase (GPx)] were determined in serum using standard methods like spectrophotometry. Genotoxicity biomarkers; [wild-type tumour suppressor protein (wt-p53), 8-oxoguanine-DNA glycosylase (OGG_1), and 8-hydroxy-2′-deoxyguanosine (8-OHdG)]; glutathione (GSH) and tumour markers [prostate-specific antigen (PSA) and alpha-fetoprotein] in serum were determined using ELISA. Micronucleus assay was carried out using microscopy. Data were analysed using ANOVA and Pearson's correlation coefficient at $\alpha_{0.05}$.

Elevated levels of toxic metals (cadmium, lead, mercury, arsenic, chromium, nickel, molybdenum, aluminium, vanadium, and thallium) and decreased levels of zinc, selenium, copper and cobalt were observed in both EW and EEEP compared with control. Vitamins A and C in EEEP (2.20±0.04 µmol/L; 116±2.03 µmol/L) were higher than EW (2.08±0.04 µmol/L; 88.75±3.33 µmol/L) and control (1.97±0.09 µmol/L; 92.19±1.68 µmol/L), respectively while vitamin E in EW (8.91±0.19 µmol/L) and EEEP (8.05±0.18 µmol/L) were lower than control (12.36±0.34 µmol/L). Malondialdehyde and UA levels were raised in EW and EEEP compared with control, while GSH was higher in control (5.41±0.09 µmol/L) than EEEP (4.44±0.09 µmol/L) and EW (3.96±0.91 µmol/L). Catalase, SOD and GPx activities were decreased in EW (122.83±3.54; 140.00±6.32; 31.41±0.84

µmol/min/mL) and EEEP (123.84±2.77; 188.31±8.79; 33.46±0.46 µmol/min/mL) compared with control (215.74±4.55; 328.48±20.44; 39.90±0.26 µmol/min/mL) respectively, while GGT activity was higher in EW and EEEP than control. The levels of wt-p53 in EW (0.45±0.05 ng/mL) and EEEP (0.41±0.04 ng/mL) were lower than control (0.73±0.12 ng/mL), while OGG_1 activity in EEEP (0.35±0.04 ng/mL) was higher than EW (0.24±0.03 ng/mL) and control (0.23±0.05 ng/mL). The levels of 8-OHdG in EW (127.82±18.29 pg/mL), EEEP (126.53±14.01 pg/mL) and control (95.95±5.60 pg/mL) were similar. The PSA and alpha-fetoprotein in EW (7.61±1.93 µg/L; 5.62±0.32 µg/L) were more elevated than EEEP (3.51±0.27 µg/L; 3.34±0.22 µg/L) and control (2.80±0.24 µg/L; 3.56±0.20 µg/L), respectively. The MnPCE/1000PCE in EW (22.7±0.15) was higher compared with EEEP (4.17±0.28) and control (0.99±0.76).

Nigerians exposed to toxic metals in e-waste experienced decreased levels of micronutrients, downregulation of wt-p53 and increased DNA damage which may lead to increased cancer risk.

Keywords: Cancer risk, E-waste, Genotoxicity, Metals, Tumour markers.

Word count: 496

ACKNOWLEDGEMENTS

I wish to express my heart-felt gratitude to my supervisor, Prof. John I. Anetor, whose outstanding mentorship and devotion made this work a success up to this stage. I am also very indebted to Prof. Oladele O. Osibanjo, my co-supeervisor, for his expertise and fatherly attitude, which has positively influenced the level of success achieved so far.

I acknowledge with profound gratitude the Head of Department, Prof. O. G. Arinola for his fatherly advice and encouragement and for providing a conducive environment for learning. I am grateful to Prof. E. O. Agbedana for his exceptional fatherly support. I am grateful to all my lecturers in the Department of Chemical Pathology: Dr. O. M. Akinosun, Dr. K. S. Akinlade, Dr. Mrs Mabel Charles-Davies, Dr. F. M. Abbiyesuku, Dr. K. S. Adedapo, Dr. I. O. Omotosho, Dr. M. A. Kuti and Dr. A. A Onifade for their advice, support, encouragement and constructive criticisms. My warm appreciation also goes to Dr. Elizabeth B. Bolajoko, Dr. Bose E. Orimadegun and Mrs. Olufunke O. Ademola-Aremu for their encouragement.

I am also very grateful to all laboratory staff of the Department of Chemical Pathology, University of Ibadan and School of Basic Medical Sciences, University of Benin, Benin City for their support in the course of laboratory analysis.

Very importantly, I am grateful to all electronic waste workers and artisans for their understanding and consent to be involved in this study.

My special thanks goes to all post-graduate students and friends in the postgraduate programme, non-academic staff of the department of Chemical Pathology, Uche Chukwumelie Zedech, Funmi Mapayi, Popoola Omolara and Mrs Linda Nwobi. Mr Edem Fabian, Funmilola Oladele, Ollope Ajayi, Mr. Stephen Okoli, Mrs Bola Ogiogwa and others.

I also specially thank Prof. Anthony Ekata Ogbeibun of the University of Benin, Miss Otakponwmen Ivie and Isaac Adebanjo for their untiring support and guidance in data management and statistical analysis. The encouragement and support of Dr. Segun Adeola, Terry Ayebor, my dear roommate, Dr. S. U. Ezenkwa and pastor Ikeke, David Micheal Onipe, Igiewe Osaze and David Aiyanyor are highly appreciated.

My profound gratitude also goes to my pastors, Rev. Isaac and Mrs Augusta Ehigiamusoe, and to all ministers and members of Practical Faith Ministry nationwide. I am also grateful to Dr. H.B. Osadolor, Dr. M.A Emokpae, Dr. M. A. Okungbowa and all members of staff of the Department of Medical Laboratory Science, University of Benin, Benin City,

Nigeria. I also specially appreciate the kind support of Prof. E. Igene, and the assistance given me by Dr. Mrs. Olufunke Ola-Davies in the micronuclei assay component of this work.

All members of the Igharo and Okhionkpaimwonyi families are specially appreciated. In addition, I wish to express my profound gratitude to the Royal Society Chemistry for the Analytical Chemistry Trust Fund DW Scholarship, the Trust Secretary, Dr. David Ferguson, and my knowledgeable and inspiring host, Dr. Chris F. Harrington for the scholarship award to carry out aspects of this study at the Supra-regional Assay Service Trace Element Centre (part of the Royal Surrey NHS County Hospital), located in the, Surrey Research Park, Guildford, United Kingdom. I also warmly appreciate the kindness and contribution of all members of staff the SAS Trace Element Lab, very prominently is the Director of the Centre, Dr. Andrew Taylor, who freely gave me a soft copy of his invaluable book on trace elements. Martin Davies, Geoff Carpenter, Jonathan Dart, Shal Kamal, Tom Roe and Sheena Nayee were friendly, helpful and always ready to assist at every stage of the laboratory analysis and training.

I also acknowledge the helpful support of the Tertiary Education Trust Fund. My deep and lovely appreciation goes to my lovely wife, Mrs Edouwaye L. Igharo, who lovingly and understandingly kept the home front whenever I was away; and my loving children Nomase, Oghosa and Etinosa for their love and understanding. To my siblings, Ekundayo, Bose, Babatunde, and Minister-Ogualigho (of blessed memory) who died under suspicious circumstances in Spain in 2014 while this study was at a very critical stage, I love you all deeply.

My greatest and most profound appreciation goes to the almighty God who grants me continuous protection on the highways in all my journeys to and from Ibadan in the course of this program. His unfailing mercies and provisions have brought me this far.

CERTIFICATION

I certify that this work was carried out by Mr Osaretin G. Igharo, in the Department of Chemical Pathology, University of Ibadan, Nigeria.

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DEDICATION

To God Almighty, my family and mentors

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CHAPTER 1

INTRODUCTION

1.1 Background to the Study

From long-standing to emerging hazards, environmental factors are a root cause of a significant burden of death, disease and disability – particularly in the developing countries (Smith *et al*., 2006). The resulting impacts are estimated to cause about 25% of death and disease globally, reaching nearly 35% in regions such as sub-Saharan Africa (Smith *et al*., 2006). This includes environmental hazards in the workplace, home and broader community/living environment (Hull, 2002; Nnorom and Osibanjo, 2008; Nnoron *et al*., 2009).

Environmental problems in Africa especially issues of chemicals and wastes are on the increase with cross-cutting impacts, of which magnitude is yet to be fully assessed and recognized, bearing in mind the connection between waste, climate change and human health (Blacksmith Institute, 2011; Basel Convention, 2012).

What appears to be the biggest problem confronting mankind currently is electronic waste (e-waste) (BAN, 2012). With the usage of electrical and electronic equipment (EEE) on the rise, the amount of electrical and electronic waste produced each day is equally growing enormously around the globe. Recycling of valuable elements contained in e-waste such as copper and gold has become a source of income mostly in the informal sector of developing or emerging industrialized countries (Nnorom and Osibanjo, 2008; Nnoron *et al*., 2009; BAN, 2012). However, primitive recycling techniques such as burning cables for retaining the inherent copper expose both adult and child workers (indicating child labour) as well as their families to a range of hazardous substances. E-waste-connected health risks may result from direct contact with harmful materials such as lead, cadmium, chromium, brominated flame retardants or polychlorinated biphenyls (PCBs), from inhalation of toxic fumes, as well as from accumulation of chemicals in soil, water and food. In addition to its hazardous components, being processed, e-waste can give rise to a number of toxic by-products likely to affect human health. Furthermore, recycling activities such as dismantling of electrical equipment may potentially bear an increased risk of injury (Blacksmith Institute, 2011), and pose adverse public health impacts.

Bearing the potential e-waste-related health risks in mind, it is burdensome that Africa has become the destination for uncontrolled quantities of electronic wastes from developed

countries (Pucket *et al*., 2000, BAN, 2012). Crude and environmentally unsound methods are employed by the informal sector in Africa to recycle e-waste without environmental safeguards, and in the process creating adverse human health impacts ((Pucket *et al*., 2000; Nnorom and Osibanjo, 2008; Nnoron *et al*., 2009; BAN, 2012).

Electronic waste, e-waste, e-scrap, or waste electrical and electronic equipment (WEEE) describes discarded electrical or electronic devices. The term may broadly be defined as discarded computers, office electronic equipment, entertainment device electronics, mobile phones, television sets and refrigerators. This definition includes used electronics which are destined for reuse, resale, salvage, recycling, or disposal (Sthiannopkao and Wong, 2012). Additionally, Puckett *et al.,* 2000 submitted that the term e-waste is the term used to describe old, end-of-life or discarded appliances which contain electrically powered components.

Unregulated recycling of electronic waste has led to environmental degradation and human rights violations- most often in developing countries in Asia and Africa where exporting is easy, labor laws are lax, and communities are poor. While the global market for new high-technology electronic products continues to grow, so does the amount of discarded products. The increasing availability of new electronics, along with the higher number of products built with shorter life spans, has resulted in the current explosion of e-waste. The majority of e-waste currently ends up in domestic landfills or incinerators, although efforts to divert e-waste from landfills, via recycling, have led to a largely unregulated, and oftentimes illegal, e-waste trade that dumps toxic materials from the affluent onto poorer countries in such regions as Asia and Africa, (Puckett *et al.*, 2000)

Informal processing of electronic waste in developing countries may cause serious health and pollution problems, though these countries are also most likely to reuse and repair electronics. E-waste disposal is especially problematic when humans and the environment are exposed to hazardous chemicals during the process of dismantling electronic products. E-waste contains approximately 1,000 chemicals, including mercury, lead oxide, cadmium, and polyvinyl chloride, which are especially hazardous to human health, (Puckett *et al.*, 2000).

Greenpeace, a global Environmental Protection Agency, in a publication highlighted that e-wastes contain health-threatening substances such as Mercury, Lead, Arsenic and Cadmium. Others are Berylium, Hexavant Chromium, Bromated flame retardants (BFRs)

and Polyvinyl chloride (PVC); as well as Phthalates (phthalate esters) and Organotins. Some of these substances are carcinogenic, and listed as restricted hazardous substances in WEEE (EU, 2002a; EU, 2002b).

There is disparity in e-waste recycling cost and regulations between developed and developing countries. Considering the risks to human and environmental health, as well as the higher costs of safer recycling processors, developed countries have found it easier to ship their e-waste to developing countries where regulations are poor and labour is cheap. As a result of this disparity in costs and regulations, much of the world's e-waste is currently exported from more developed, affluent countries to less developed countries, (Terada, 2012). Hull, (2002) reported that the e-waste issue is all about poisoning the poor for profit. Based on a report by the Basel Action Network and Silicon Valley Toxics Coalition, two leading organizations working against the toxic trade, an estimated 50 - 80 percent of the e-waste collected for recycling in the western United States is not recycled domestically, but is sent to countries in Asia for recycling. China has been listed as a leading recipient of e-waste and also repeatedly singled out in the media as posing dangerous risks to the environment and the health of dismantling workers and surrounding communities (Terada, 2012).

E-waste in Nigeria is an issue crying for serious consideration. Terada 2012, in an article titled "Recycling Electronic Wastes in Nigeria: Putting Environmental and Human Rights at Risks", took Nigeria as a Case Study being a major dumping ground for e-waste in Africa. It was reported that the case of Nigeria was significant because Nigeria is a party to the Basel Convention and has signed the Bamako Convention in February 2008, but is yet to ratify the Bamako Convention. The Bamako Convention made the trade of hazardous e-waste illegal in sub-Saharan African countries, yet the e-waste trade continues to thrive in Nigeria. Although Nigeria ratified the Basel Convention on May 24, 2004, it still has not ratified the Bamako Convention, and the country remains a dumping ground for e-waste from European and Asian markets, (Aginam, 2010; Basel, 2004). It is estimated that five hundred (500) containers of second-hand electronics are imported to Nigeria every month from Europe (Aragba-Akore, 2005). About three-quarters of these imported products are reported to be junk that cannot be reused and are dumped in landfills (Terada, 2012).

In 2009, Greenpeace (an international non-governmental environmental organization) completed a three-year undercover investigation, tracking the two-month journey of a broken, irreparable TV from the UK to Nigeria (Greenpeace International, 2009). Acting

on a tip-off, Greenpeace, along with the help of Sky Television (TV), fitted a TV with a satellite tracking device and brought it to the UK's Hampshire County Council for recycling. The organization used a device that is a combination of a mobile phone, Global Positioning System (GPS) receiver and radio frequency transmitter. From the Hampshire County Council waste recycling site, the TV was transported by van to BJ Electronics; the recycling company. The next day, the TV was loaded onto a container and sent to West Africa. The container sat at Tin Can Island Port in Lagos, Nigeria for about two weeks until it was sent to Alaba Market. Alaba Market is a second-hand electronics market, with approximately ten to fifteen containers arriving daily from Europe and Asia. The investigative crew watched as the container with the TV was unloaded and all the TV's were put on the street for resale. The investigative team members, who had traveled to Nigeria, recognized the TV by checking the serial numbers, and (re)purchased it from the owner/importer of the container under the condition that it had not been tested. The investigation conducted by Greenpeace demonstrates the lack of enforcement on both ends of the TV's 4,500-mile journey (Terada, 2012).

In Africa, Nigeria is reported to be the largest e-waste dump yard (Terada, 2012). This is evident in the number of e-waste imported and received in the major e-waste recycling sites such as Westminster Electronic Market and Alaba International market, Lagos State.

It is important to mention that though these Lagos based sites are mainly used to receive e-wastes, a great proportion of the WEEE find their ways to smaller markets, households and small scale electronic/electrical repair outlets nationwide. Therefore, the reception, use, recycling and disposal of WEEE indeed constitute a nationwide issue.

Some studies (Nnorom and Osibanjo, 2008; Nnorom *et al*., 2009; Terada, 2012) on the burden of primitive recycling of e-waste in Nigeria have been carried out. Report by the Nigerian Ministry of Environment showed that basic components such as lead are being recovered and then smelted in people's backyards, which constitutes a huge risk of lead poisoning (Terada, 2012). Excess heavy metals have been reported in the soil and plants, and pollution from e-waste dismantling reportedly led to illness in grazing animals, tainted vegetables, and contaminated drinking water, (Osibanjo and Nnorom, 2007; Nnorom and Osibanjo, 2007; Nnorom and Osibanjo, 2008; Nnorom *et al*., 2009; Terada, 2012).

Humans can become exposed to heavy metals in dust through several routes which include ingestion, inhalation, and dermal absorption (Leung *et al.*, 2006; Leung *et al*, 2008). In

dusty environments, it has been estimated that adults may ingest up to 100 mg dust/day (Hawley, 1985; Calabrese, 1987; Leung *et al*., 2008). Children are usually exposed to greater amounts of dust than adults as a result of pica (a psychological disorder characterized by an appetite for non-nutritive substances such as soil) and play behaviour (Calabrese, 1987; Leung *et al*., 2008). Exposure to high levels of heavy metals can result in acute and chronic toxicity, such as damage to central and peripheral nervous systems, blood composition, lungs, kidneys, liver, and even death. Lead levels in dust have been significantly associated with Pb levels in children's blood (Leung *et al*, 2008) and a blood lead level (BLL) greater than an intervention level of 10 µg Pb/dL has been associated with a decrease in intelligence quotient (Leung *et al*., 2008). The Centre for Disease Control and Prevention now uses 5µg Pb/dL as safe limit (CDC, 2012).

Two studies demonstrated elevated body burden of heavy metals (Huo *et al*., 2007) and persistent toxic substances (Bi *et al*., 2007) in children and e-waste workers, respectively, at Guiyu, China (Leung *et al*., 2008).

Although there is paucity of data on post impact e-waste environmental studies in the African region, a recent study in Nigeria has demonstrated high concentrations of copper, nickel, zinc and lead in soils far in excess of European Union limits at e-waste dump sites in Lagos, Benin, and Aba cities in Nigeria (Nnorom *et al*., 2009). Contamination of plants and nearby surface waters in e-waste disposal sites by heavy metals was also reported in these sites (Nnorom *et al*., 2009). Another study showed that the mean concentration (mg/kg) of Cu and Pb in printed circuit boards (PCB) of central processing unit (CPU) and monitor of computers were found to be over 50 folds higher than the Toxicity Threshold Limit Concentration (TTLC) for the metals in electrical and electronic equipment in developed countries. The excessively high concentrations of Cu and Pb in the components of the computers analyzed suggest that these used computers are hazardous wastes. Consequently, improper disposal of the PCBs of the CPU and monitors in the environment may pose serious risk to humans and the environment (Nnorom and Osibanjo, 2008; Nnorom *et al*., 2009).

There is a consequential association between heavy metal and chemical toxicants, and high cancer risks. China is known to be the largest e-waste dump yard in the world, and the emergence of China Cancer villages is well-documented (Hong, 2005; Liu and Chen, 2007; Yingying, 2011). In brief, a cancer village is a community where rates of cancer are considerably in excess (at least twice that) of China's normal death rate of 6 per 1,000/year

(Liu and Chen, 2007; Yingying, 2011). Presumably, this phenomenom may not be unconnected with the reported heavy chemical pollution, including toxicants in e-wastes. Lack of effective operation system of environmental laws in China has been reported as a contributory factor to the emergence of China Cancer Villages (Liu and Chen, 2007).

With the unregulated heavy inflow of e-waste into Nigeria, coupled with other widespread health-threatening pollutions, there may be the possibility of the emergence of "Nigerian Cancer Villages", should this trend continue.

In the theory of cancer development, the involvement of chemical toxicants and oxidative stress, as well as oxidative DNA damage are well reported (Anetor, *et al.*, 2013; Terada, 2012; Elst *et al*., 2009; Valavanidis, *et al.*, 2009). Biomarkers such as 8-hydroxy2-deoxyguanosine has been used to estimate oxidative stress, oxidative DNA damage and carcinogenesis in humans after exposure to cancer-causing agents like heavy metal, asbestos fibers and tobacco smoke (Valavanidis, *et al*.,2009) .

Cancer, the generic term for carcinoma is the malignant form of uncontrolled proliferation of cells and tissues. It is the most dreaded non-communicable disease in developing countries where it is invariably fatal, due to lack of adequate preventive and curative services (Kolawole, 2011), unlike in the developed countries which have policy, strategies and programs for cancer prevention and management (WHO, 2002; Thun, 2010; Nnodu, 2010). Consequently, though the incidence of cancer is rising globally, the developing countries account for 52% of this increase (Parkin, 2003) and for 70% of cancer deaths (USEPA, 2005) with only possessing 5% of global funds for cancer control and very few human and material resources (Jones, 1999). Available statistics reveal that cancer is the second most common cause of death; constituting 12% of all deaths after cardiovascular disease. It kills more people than Tuberculosis, HIV/AIDS and Malaria combined (WHO 2006a, 2006b). In 2007, there were 11 million cancer cases, 7 million cancer deaths and 25 million people living with cancer. This is projected to increase to 27 million cases, 17 million deaths and 75 million people living with cancer in 2050 (WHO 2006a, 2006b).

The aetiology of many cancers are still unknown, however there are risk factors which are either modifiable or non-modifiable. The modifiable factors include tobacco use, physical inactivity, unhealthy diet, obesity, ultraviolet radiation and infectious agents like Human Papilloma Virus (HPV), Hepatitis Viruses (HBV, HCV) and Helicobacter pylori. The non-modifiable factors include heredity, sex, ethnicity, immunosuppression and ageing (WHO

2006a, 2006b). Moreover, due to the epidemiological shift, increase in ageing population, high rate of infections and entrenchment of the modifiable risk factors (Thun, 2010), cancers will yet pose significant challenge to Nigeria and other developing countries which currently lack cancer control programs directed at reducing cancer incidence and mortality and to improve the quality of life (WHO,2006b). There are very few human and material resources for cancer control in developing countries where cancers occur at younger ages, 70% of cancer deaths occur and only 5% of global funds for cancer control is present (WHO, 2002).

Africa carries an increasing cancer burden; 75% of the 650,000 annual cases present late, at younger ages and about 510,000 deaths occur (Ngoma, 2006). The incidence ranges from 70/100,000 to- 100/100,000 people. Infectious agents like Hepatitis B and C, Human Papilloma Virus (HPV) and *Helicobacter pylori* contribute significantly to cancers in developing countries, in addition to possible contributions by inflammations due to varying infections (Mackay *et. al.*, 2006).

The prevalence of cancer in Nigeria has grown over the years. Reports from the National Cancer Prevention Agency, courtesy of Ibadan Cancer Registry and Abuja Cancer Registry, showed that incidence rate of cancer in Nigeria in 2012 stood at 66.4 per 100 000 men and 130.9 per 100 000 women (Jeddy-Agba *et al*., 2012).

WHO (2008) estimated that the incidence of cancer in Nigerian men and women by 2020 will be 90.7/100,000 and 100.9/100,000 and the deaths rates will be 72.7/100,000 and 76,000/100,000, respectively.

Prostate cancer is a leading cause of cancer-related death in men globally (Thorne, *et al*., 2012; Velonas *et al*., 2013). Prostate Cancer is the most common cancer in Nigerian males; having overtaken liver cancer (Jeddy-Agba *et al*., 2012). Although, prostate cancer is the leading cause of cancer deaths globally and the most common cancer in Nigerian men, liver cancer has been reported as the most common cause of cancer death in Nigeria. (Jeddy-Agba *et al*., 2012; Ukah and Nwofor, 2017). The most common liver malignancy in Nigeria has been reported as hepatocellular carcinoma (Jeddy-Agba *et al*., 2012). Liver disease is listed as a risk factor for cancer .The liver plays an important role in sex hormone metabolism by making binding proteins that carry the hormones in the blood. These binding proteins affect the hormones' activity. Men with severe liver disease such as cirrhosis have relatively low levels of androgens and higher estrogen levels. They have a

higher rate of benign male breast growth (gynecomastia) and also have an increased risk of developing breast cancer (ACS, 2012).

The role of the liver in xenobiotics metabolism is well known. Enzymes located in the endoplasmic reticulum of liver cells protect against accumulation of lipid-soluble exogenous and endogenous compounds by converting them to water-soluble metabolites which can be easily excreted by the kidney. But only few drugs possess suitable groups which are conjugated with glucuronic or sulfuric acid. Most compounds have to be hydroxylated first. For this purpose the endoplasmic reticulum has at its disposal an enzymatic system, completely unspecific, which activates molecular oxygen for the oxidation of lipid-soluble compounds. This takes place in the cytochrome, P_{450}, which is available in the endoplasmic membranes abundantly. The oxidation rate, however, is extremely slow and dependent on the chemical configuration of the compound and on genetically determined differences of the protein moiety of the enzyme. Since more specific enzymes located in the liver cells metabolize most of the endogenous compounds, such as steroids, at a much higher rate, the slow hydroxylation by the non-specific endoplasmic enzyme does not play an important role in their conversion to inactive compounds (Remmer, 1970; Palatini and De Martin, 2016). Owing to the lack of specificity of this enzyme, drugs compete for the binding sites if high concentrations of several drugs are present in the liver cells. A slower metabolism of these drugs with less affinity is the result. Metabolism of drugs by this enzyme system leads sometimes to more active and toxic compounds which produce liver injury, for example, in the case of carbon tetrachloride. Drug metabolism is inhibited only in severe hepatitis, and exceptionally in liver cirrhosis (Remmer, 1970; Palatini and De Martin, 2016)

1.2 Rationale for the Study

The need to carry out studies involving human populations occupationally or environmentally exposed to e-waste with emphasis on the risks of cancer, particularly prostate and liver cancers, cannot be overemphasized. Such studies may provide evidence of the extent of damage to human health as a result of primitive and unregulated e-waste recycling. From this kind of study, it is also possible to generate data and findings that could stimulate government interest to fully subscribe to and implement relevant trans-boundary and international regulations to reduce or totally stop e-waste toxic trade as well as establish formal technology-based recycling of locally generated e-wastes.

In china where there is heavy e-waste burden (similar to, although higher than the Nigerian situation), limited investigations have been carried out on the health effects of its Guiyu's poisoned environment. Two studies have demonstrated elevated body loadings of heavy metals (Huo *et al*., 2007) and persistent toxic substances (Bi *et al*., 2007) in children and e-waste workers, respectively, at Guiyu, China (Leung *et al*., 2008). From available literature, no similar work has been documented in Nigeria.

Although there is paucity of data on post impact e-waste environmental studies in the African region, a study in Nigeria has demonstrated high concentrations of copper, nickel, zinc and lead in soils far in excess of European Union limits at e-waste dump sites in Lagos, Benin, and Aba cities in Nigeria (Nnorom *et al*., 2009). Contamination of plants and nearby surface waters in e-waste disposal sites by heavy metals was also reported in these sites (Nnorom *et al*., 2009). Another study observed that the mean concentration (mg/kg) of Cu and Pb in Printed Circuit Boards (PCB) of CPU and monitor of computers were found to be over 50 folds higher than the Toxicity Threshold Limit Concentration for the metals in electrical and electronic equipment in developed countries (Olubanjo *et al*., 2015). The excessively high concentrations of Cu and Pb in the components of the computers analyzed suggest that these used computers are hazardous wastes. Consequently, improper disposal of the PCBs of the CPU and monitors in the environment may pose serious risk to humans and the environment (Osibanjo and Nnorom, 2007; Nnorom and Osibanjo, 2007; Nnorom and Osibanjo, 2008; Nnorom *et al*., 2009). Report by the Nigerian Ministry of Environment showed that basic components such as Pb are being recovered and then smelted in people's backyards, which poses a huge risk of lead poisoning (Terada, 2012). Excess heavy metals have been found in the soil and plants, and pollution from e-waste dismantling is reported to have led to illness in grazing animals, tainted vegetables, and contaminated drinking water (Terada, 2012).

Copper alone, for instance is seriously linked with carcinogenesis. Metal ions play an important role in biological systems, and without their catalytic presence in trace or ultra-trace amounts, many essential co-factors for many biochemical reactions would not take place. However, they become toxic to cells when their concentrations surpass certain optimal (natural) levels. Copper is an essential metal. Catalytic copper, because of its mobilization and redox activity, is believed to play a central role in the formation of reactive oxygen species (ROS), such as O^{2-}* and *OH radicals, that bind very fast to DNA, and produce damage by breaking the DNA strands or modifying the bases and / or

deoxyribose leading to carcinogenesis. The chemistry and biochemistry of copper is briefly accounted together with its involvement in cancer and other diseases (Theophanides and Anastassopoulou, 2002).

There is need for human-population based studies on the Nigerian e-waste burden. Humans can become exposed to heavy metals in dust through several routes which include ingestion, inhalation, and dermal absorption (Leung *et al*., 2006; Leung *et al*, 2008). In dusty environments, it has been estimated that adults could ingest up to 100 mg dust / day (Leung *et al*., 2008; Hawley, 1985; Calabrese, 1987). Most e-waste recycling environments (crude waste sites) are dusty owing largely to the accumulation of dust particles in and on the internal parts of WEEE.

It is expected that data generated from this study will not only be useful in demonstrating the toxicological and biochemical disorders and risks associated with e-waste occupational exposure, but will provide more scientific basis for existing actions towards prompting the Nigerian Government to see the need to enact new policies and enforce or ratify existing regulations on e-waste local and transboundary operations. Specifically, agencies such as Federal Ministries of Health, Environment, Science and Technology, and Commerce and Industry would find data generated from this study valuable with respect to bringing unauthorized trans-boundary inflow of WEEE to a lowest ebb as well as prompting the nation towards the establishment of formal technologically driven e-waste recycling facility nationwide to take care of locally generated e-waste.

Likewise, appropriate actions and regulations sparked by research of this nature may help prevent the emergence of the hypothetical "*Nigerian Cancer Villages*", expand the scope and essence of public health and environmental education in Nigeria with respect to occupational and environmental exposure to e-waste-borne toxicants.

1.3 Aim of the Study

The aim of this study is to evaluate the exposure of Nigerian e-waste workers to toxic metals, using biomarkers of exposure in combination with alterations in micronutrient status, and the contributions of these to the risk of cancer development.

1.4 Specific Objectives of the Study

The specific objectives of this study include:

1. To identify the occupational exposure pattern and assess workers' risk awareness levels regarding e-waste hazards.

2. To determine the levels of toxic metals in hand wash water and blood samples of Nigerian e-waste workers.

3. To determine serum levels of some essential trace elements and antioxidant vitamins (A, C and E) useful in establishing the connection between metal toxicity, inorganic micronutrients and antioxidant vitamin metabolism, and by extension their specific and collective effects on systemic antioxidant and pro-oxidant capacities in the study groups.

4. To determine Serum Calcium and Vitamin D levels as a window into the role of vitamin D and Calcium Signaling in carcinogenesis.

5. To examine antioxidant status, oxidative stress level and oxidative DNA damage/repair in Nigerian e- waste workers using genotoxicity and cancer risk predictive biomarkers such as tumour suppressor protein (p53), DNA repair protein; 8-oxoguanine-DNA glycosylase (OGG_1), and oxidative DNA damage biomarker; 8-hydroxy-2'-deoxyguanosine (8-OHdG).

6. Additionally, to determine serum levels of simple biomarkers for prostate cancer - prostate specific antigen (PSA), and for liver cancer- total alpha-fetoprotein (AFP) in combination with liver function assessment indices; prostate and liver cancers being the two commonest cancers in men. Total AFP is reported to have the specificity of 90% and sensitivity of 60% for hepatocellular carcinoma (HCC) (Yuen and Lai, 2005).

7. Lastly, to find out the existence or extent of chromosomal aberration in the studied e-waste exposed human populations using frequency of micronuclei (MN) expression in peripheral blood as guide.

1.5 Research Hypothesis

Chronic exposure to toxic metals and chemicals in e-waste could provoke biochemical changes, including genotoxicity and oxidative responses leading to systemic damage and increased risk of cancer development in Nigerian e-waste workers.

CHAPTER 2

LITERATURE REVIEW

2.1 Metals and health: an overview

Several metals are toxic to humans exposed through occupational sources, environmental contamination, water and food. Upon chronic exposure, most of these metals accumulate in the body throughout human life and their biological effect contributes to shorten the natural lifespan of organs such as the kidney and ultimately determine their early failure (Barregard *et al.*, 1999). However, the mechanisms through which individual metals target specific organs and cause a distinctive pattern of pathological signs is understood in limited detail (Rubino, 2015). Metals occur naturally in the earth's crust, and their contents in the environment can vary between different regions resulting in spatial variations of background concentrations. The distribution of metals in the environment is governed by the properties of the metal and influences of environmental factors (Khlifi and Hamza-Chaffai, 2010). Of the ninety two naturally occurring elements, approximately thirty metals and metalloids are potentially toxic to humans, and these include but not limited to Be, B, Li, Al, Ti, V, Cr, Mn and Co; as well as Ni, Cu, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, W, Pt, Au, Hg, Pb, and Bi [Barregard *et al.*, 1999; Agency for Toxic Substance and Disease Registry (ATSDR), 2003a, 2003b, 2007, 2008; Castro-González and Méndez-Armenta, 2008]

These metals enter the environment by natural and anthropogenic means. Such sources include: natural weathering of the earth’s crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout, and a number of others (Ming-Ho, 2005). Although some individuals are primarily exposed to these contaminants in the workplace, for most people the main route of exposure to these toxic elements is through the diet (food and water). The contamination chain of toxic metals almost always follows a cyclic order: industry, atmosphere, soil, water, foods and human. Although toxicity and the resulting threat to human health of any contaminant are, of course, a function of concentration, it is well-known that chronic exposure to heavy metals and metalloids at relatively low levels can cause adverse effects (ATSDR, 2003a, 2003b, 2007, 2008; Castro-González and Méndez-Armenta, 2008). Therefore, there has been increasing concern, mainly in the developed world, about exposures, intakes and absorption of heavy metals by humans. Populations are increasingly demanding a cleaner environment in general, and reductions in the amounts of

contaminants reaching people as a result of increasing human activities. A practical implication of this trend, in the developed countries, has been the imposition of new and more restrictive regulations [European Commission (EC), 2006; Figueroa, 2008].

2.2 Metals

Metals are often characterized and distinguished from non-metals by their properties. Such properties are the ability to conduct heat, an electrical resistance that is directly proportional to temperature, malleability, ductility and even lustre (Müller, 2007). Generally, most metals are solid while a few of them are liquid at room temperature, e.g. Hg. There are still a few others, which lie in between metallic and non-metallic, and have properties of both, e.g Arsenic. A toxicologically important characteristic of metals is that they may react in biological systems by losing one or more electrons to form cations (Dinis and Fiuza, 2011).

2.2.1 Properties of metals

Some of the basic properties of Metals include, but not limited to the following; ability to conduct electricity and heat, high reflectivity (Lustre), mechanical ductility and magnetic properties (Dinis and Fiuza, 2011).

Metallic properties increase from top to bottom in the column and decreases from left to right in the row in the periodic table. A wide variety of metal compounds and their salts impart beneficial properties to products like plastics in terms of colour, brightness, heat resistance and resistance to degradation. Metals emits electrons when exposed to radiation e.g. light) of a short wavelength or when heated in sufficiently high temperature. These phenomena are exploited in the design of television screen using rare earth metal oxides and in a variety of electronic devices and instruments. The circuitry systems in household appliances, television sets, computers and general electronic equipment relies on the electrical conductivity of metals to function (Dinis and Fiuza, 2011).

2.2.2 Definition of Heavy Metals

In medical usage, heavy metals include all metals that have high relative density greater than 5g/cm^3 and are toxic at low concentrations (Dinis and Fiuza, 2011). The term, ‘heavy metals’ refers to the group of metals and metalloids with atomic density greater than 4 g/cm^3 or 5 times or more, greater than water (Nriagu and Pacyna, 1988; Nriagu, 1989; Hawkes, 1997). They include but not limited to the following, Hg, Pd, Cd, and As, as well as Cr, Ag, Ni, Cu and Fe. They are natural components of the earth crust. Heavy metals are

also called trace element due to their presence in trace (10mg/kg) or in ultratrace (1µg/kg) quantities in the environmental matrices (Hawkes, 1997).

Conventionally, heavy metals is the generic term for metallic elements having an atomic weight higher than 40.04 (the atomic mass of Ca) (Ming-Ho, 2005). However, the use of the term "*heavy metal*" has been reviewed owing to the fact that some of the metals classified, based on atomic densities as heavy metals are essential for life, amongst other considerations (IUPAC, 2002). The use of the terms: *toxic metals* (based on confirmed toxicity) and *essential metals* (based on essentiality to life) may be more useful regardless of atomic densities.

2.2.3 Classification of Metals

Metals can be classified into two major groups on the basis of their health importance. These are essential trace elements and non-essential trace elements. Essential trace elements are metals that are essential to human health and are therefore known as micronutrients being required at trace amount for their biological roles. However, at higher concentrations they can become toxic and cause serious adverse health effects. Zinc, for example is a known micronutrient, but the toxicity of zinc may lead to suppression of the immune system and reduced high density liporoteins. Copper, Cobalt, Chromium (iii), Manganese, iron and Selenium, are other examples of essential trace elements (Blaylock and Huang, 2000).

Non-essential trace elements include Mercury, Cadmium, Arsenic and lead etc. (Raikwar *et al*., 2008). Toxicity with these elements in low concentrations has been largely reported in literature (Prasad, 1976; Fairweather-Tait, 1988; Blaylock and Huang, 2000). The high intake of cadmium caused *itai itai* disease, renal dysfunction and mercury intake lead to Minamata disease, first reported in Japan in 1950s (Fairweather-Tait, 1988; Blaylock and Huang, 2000)

2.3 Sources of Exposure to Heavy Metals

Metals are ubiquitous in the environment as a result of both natural and anthropogenic activities. Human exposure occurs through various means which include but not limited to those listed below.

1. Human Exposure through the usage of Industrial Products

   Industrial products are among the lead sources of heavy metal exposure. For example most products used in homes have one or more heavy metals as one of its

major constituents, which eventually become a source of human exposure to such metals. Mercury (in form of mercurochrome) is present in most disinfectants, antifungal agents, toiletries, mercury lambs, creams and organo-metallics (McCluggage, 1991); cadmium exposure may be through nickel/cadmium batteries and artist paints; lead exposure could be through wine bottle wraps, mirror coatings, batteries, old paints and tiles and linolein amongst others (Duruibe, *et al*., 2007)

2. Human Exposure through Occupational means

   Occupational exposure to heavy metals is a significant phenomenon in toxicology. Occupational lead exposure mostly occurs in lead smelting plants and battery factories, as well as in renovation of old houses, when workers inhale or ingest fumes and dust contaminated with lead. Workers engaged in mining and production of cadmium, chromium, lead, mercury, gold and silver have been reported to be consequently exposed, these also includes workers in electrical industries. Occupational risk exists where lead and its compounds are present in the form of vapour or aerosol (lead fume, lead dust) in the working environment, especially in lead ore smelting (Duruibe, *et al*., 2007).

3. Human Exposure through Contamination of the Environment

Emission from the exhaust of vehicles running on leaded gasoline can contaminate and pollute the environment by the release of lead (Pb). This process can lead to the contamination of open food items in market places along road sides. Consumption of such contaminated food items exposes the individual to the risk of heavy metal exposure. Environmental pollution by heavy metals is also very common in areas of mining and old mine sites and pollution reduces with increasing distance away from mining sites (Peplow, 1999). These metals are leached out and in sloppy areas, are carried by acid water downstream or run-off to the sea. Water bodies are most emphatically polluted (Garbarino *et al*., 1995; INECAR, 2000). Through rivers and streams, the metals are transported as either dissolved species in water or as an integral part of suspended sediments. They may then be stored in river bed sediments or seep into the underground water thereby contaminating water from underground sources, particularly wells; and the extent of contamination will depend on the nearness of the well to the mining site. Wells located near mining sites have been reported to contain heavy metals at levels that exceed drinking water criteria (Garbarino *et al*., 1995). Also inhabitants around industrial sites of heavy metal mining and processing, are exposed through air by suspended particulate matters

(Garbarino *et al*., 1995; Ogwuegbu and Muhanga, 2005; Duruibe, *et al*., 2007).

2.3.1 Routes of exposure to metals

Exposure to metals may take place by inhalation, ingestion, or skin penetration. For organometallic compounds, dermal uptake can cause substantial, sometimes lethal, doses. Inhalation is usually the most important occupational exposure route. Ambient air, except in the vicinity of an emission source, does not usually contribute significantly to the total exposure. Contaminated air may pollute soil and water secondarily, resulting in contaminated crops and vegetables. Drinking water is sometimes a significant route of exposure (Garbarino *et al*., 1995; Ogwuegbu and Muhanga, 2005; Duruibe, *et al*., 2007).

2.4 Heavy metals and biological system: biochemical reactions of heavy metals

Heavy metals interfere with the normal body physiological and biochemical processes. Subsequent to exposure beyond the toxic limit of the metal, in the acid medium of the stomach, they are converted to their stable oxidation states (Zn^{2+}, $Pb^{2+,}$ $Cd^{2+,}$ $As^{2+,}$ As^{3+}, Hg^{2+} and Ag^{+}) and combine with biomolecules such as proteins and enzymes to form strong and stable chemical bonds. As shown in the equations below (Fig. 2.1), heavy metals react with the sulphydryl groups (SH) of cysteine and sulphur atoms of methionine (SCH_3) to form a complex with a stable bond (Ogwuegbu and Muhanga, 2005).

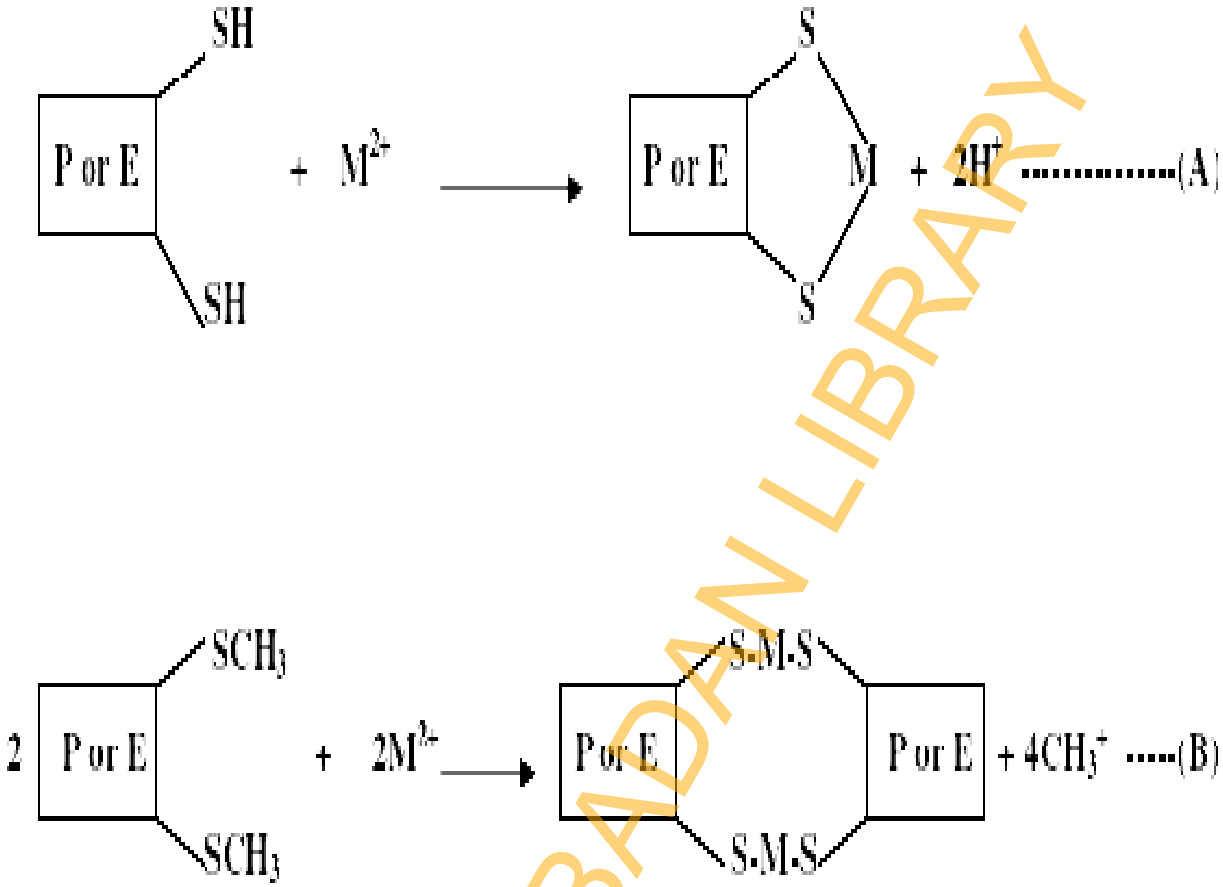


Fig. 2.1 Reaction of heavy metals with sulfhydryl groups of protein (Duruibe *et al.*, 2007)

As seen above sketch, equation (A) forms Intra-molecular bond; equation (B) forms Inter-molecular bond; P represents Protein molecule; E represent an Enzyme system; M represents the toxic Metal. The toxic metal brings about inhibition of the enzyme by replacing the hydrogen atoms or the essential metal groups. The protein–metal complex thus formed acts as a substrate and reacts with a metabolic enzyme.

In a schematic representation shown below: (equation C), enzyme (E) reacts with substrate (S) in either the lock-and-key pattern or the induced-fit pattern. In both cases, a substrate fits into an enzyme in a highly specific fashion to form an enzyme–substrate complex (E-S*) as follows:

$E + S \rightarrow E\text{-}S \rightarrow E - S^* \rightarrow E - P \rightarrow E + P$ ----- (C)

(E = Enzyme; S = Substrate; P = Product; * = Activated Complex)

While at the E-S, E–S* and E-P states, an enzyme cannot accommodate any other substrate until it is freed. Sometimes, the enzymes for an entire sequence coexist together in one multi-enzyme complex consisting of three or four enzymes. The product from one enzyme reacts with a second enzyme in a chain process, with the last enzyme yielding the final product as follows:

$A \rightarrow^{E1} B \rightarrow^{E2} C \rightarrow^{E3} D \rightarrow^{E4} F$ (E1 to E4 are enzymes) ----- (D)

The final product (F) goes back to react with the first enzyme thereby inhibiting further reaction since it is not the starting material for the process. Hence, the enzyme E1 becomes incapable of accommodating any other substrate until F leaves and F can only leave if the body utilizes it. If the body cannot utilize the product formed from the heavy metal-protein substrate complex, there will be a permanent blockage of the enzyme E1, which then cannot initiate any other reaction. Therefore, the metal remains embedded in the tissue, and will result in bio-dysfunctions of various gravities (Prasad, 1995; Stohs and Bagchi, 1995). Furthermore, a metal ion in the body's metallo-enzyme can be replaced by another metal ion of similar size, but due to the specificity of essential metals for an enzyme catalyzed reactions, the enzyme cannot function. Thus Cd^{2+} being an antagonist of Zn^{2+}, can replace Zn^{2+} in enzymes that requires Zn as a Co-factor, leading to cadmium toxicity. In the process of inhibition, the tertiary structure of a protein molecule can be affected rendering the protein molecule inactive (Ogwuegbu and Ijioma, 2003; Duruibe *et al.*, 2007).

2.4.1 Metal induced oxidative stress

2.4.1.1 Metal-induced reactive oxygen species (ROS) production

Heavy metal ions, such as iron, copper, cadmium, mercury, nickel, lead, arsenic and most of the transition metal ions, can induce generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA (Stohs and Bagchi, 1995). One of the most important mechanisms of metal mediated free radical generation is via a Fenton-type reaction. Superoxide ion and hydrogen peroxide can interact with transition metals, such as iron and copper, via the metal catalyzed Haber-Weiss/Fenton reaction to form OH radicals.

$$Metal^{3+} + {}^{\cdot}O_2 \longrightarrow Metal^{2+} + O_2 \qquad \text{Haber-Weiss}$$

$$Metal^{2+} + H_2O_2 \longrightarrow Metal^{3+} + OH^- + {}^{\cdot}OH \qquad \text{Fenton reaction}$$

Besides the Fenton-type and Haber-Weiss-type mechanisms, certain metal ions can react directly with cellular molecules to generate free radicals, such as thiol radicals, or induce cell signaling pathways. These radicals may also react with other thiol molecules to generate $O_2^{-\cdot}$. Further, O_2^- is converted to H_2O_2, which causes additional oxygen radical generation. Some metals, such as arsenite, induce ROS formation indirectly by activation of radical producing systems in cells (Stohs and Bagchi, 1995). Cellular components may also generate ROS due to metabolic activities going on in the cells.

2.4.1.2 Production of ROS in the Mitochondria

The mitochondrion is considered the power house of the biological cells due to its role in energy generation. Mitochondria are unique organelles, as they are the main site of oxygen metabolism, accounting for approximately 85-90% of the oxygen consumed by the cell (Chance *et al.*, 1979; Shigenaga *et al.*, 1994). Incomplete processing of oxygen and/or release of free electrons results in the production of oxygen radicals. Mitochondria constantly metabolize oxygen thereby producing reactive oxygen species (ROS) as a byproduct. These organelles have their own ROS scavenging mechanisms that are required for cell survival (Melov *et al.*, 1998). However, mitochondria produce ROS at a rate higher than their scavenging capacity, resulting in the incomplete metabolism of approximately 1–3% of the consumed oxygen (Boveris and Chance, 1973; Nohl and Hegner, 1978) The byproducts of incomplete oxygen metabolism are superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). The formation of superoxide occurs via the transfer of a free electron to molecular oxygen. This reaction occurs at specific sites of the electron

transport chain, which resides in the inner mitochondrial membrane. The electron transport chain complexes I (NADH dehydrogenase) and III (ubisemiquinone) produce most of the superoxide, which is then scavenged by the mitochondrial enzyme manganese superoxide dismutase (MnSOD) to produce H_2O_2. Since mitochondria do not contain catalase, their only defense against the potentially toxic properties of H_2O_2 is the enzyme glutathione peroxidase (GPx). Glutathione peroxidase requires reduced glutathione (GSH) as a coenzyme and converts H_2O_2 to water, thus completely detoxifying ROS. However, in the presence of reduced transition metals, H_2O_2 can produce the highly reactive $OH^{\bullet}$, which can cause extensive damage to DNA, proteins, and lipids (Goldstein *et al.*, 1993).

Two other important radical species are nitric oxide (NO) and Peroxynitrite ($ONOO^-$) which have been reported to be produced by the mitochondria because they posses their own nitric oxide synthase (mtNOS) and can produce endogenous NO and $ONOO^-$ (Goldstein *et al.*, 1993). Mitochondrial nitric oxide decays mainly via $ONOO^-$ formation, ubiquinol oxidation, and reversible binding to cytochrome *C* oxidase (Poderoso *et al.*, 1999).

Metal induced toxicity has been well reported in literature (Lanphear and Roghmann, 1997; Leung *et al*, 2008; Anetor, *et al.*, 2013). One of the mechanisms of heavy metal toxicity has been attributed to oxidative stress. A large body of data provides evidence that heavy metals are capable of interacting with nucleoproteins and DNA causing oxidative deterioration of biological macromolecules. In-depth studies in the past few decades have shown that metals like Fe, Cu, Cd, Hg, Pd, Ni, As etc., possess the ability to generate free reactive radicals, resulting in cellular damage, depletion of antioxidant enzymes, damage to lipid bilayer and DNA. Common mechanism involving the Fenton reaction, generating superoxide and hydroxyl radicals appear to be involved for Iron, copper, Chromium, etc. primarily associated with mitochondria, microsome and peroxisomes. These reactive radical species includes a wide variety of Oxygen-, carbon-, Sulful- and Nitrogen- radicals originating not only from superoxide radicals, hydrogen peroxides, lipid peroxidations but also in chelates of amino acids, peptides, and proteins complexed with toxic metals. These metals generate reactive species which in turn causes neurotoxicity, hepatotoxicity, nephrotoxicity and genotoxicity in humans (Valko *et al.*, 2005; Ercal, *et al.*, 2001). A scheme of metal-induced oxidative stress in biological systems is shown in fig.2.2.

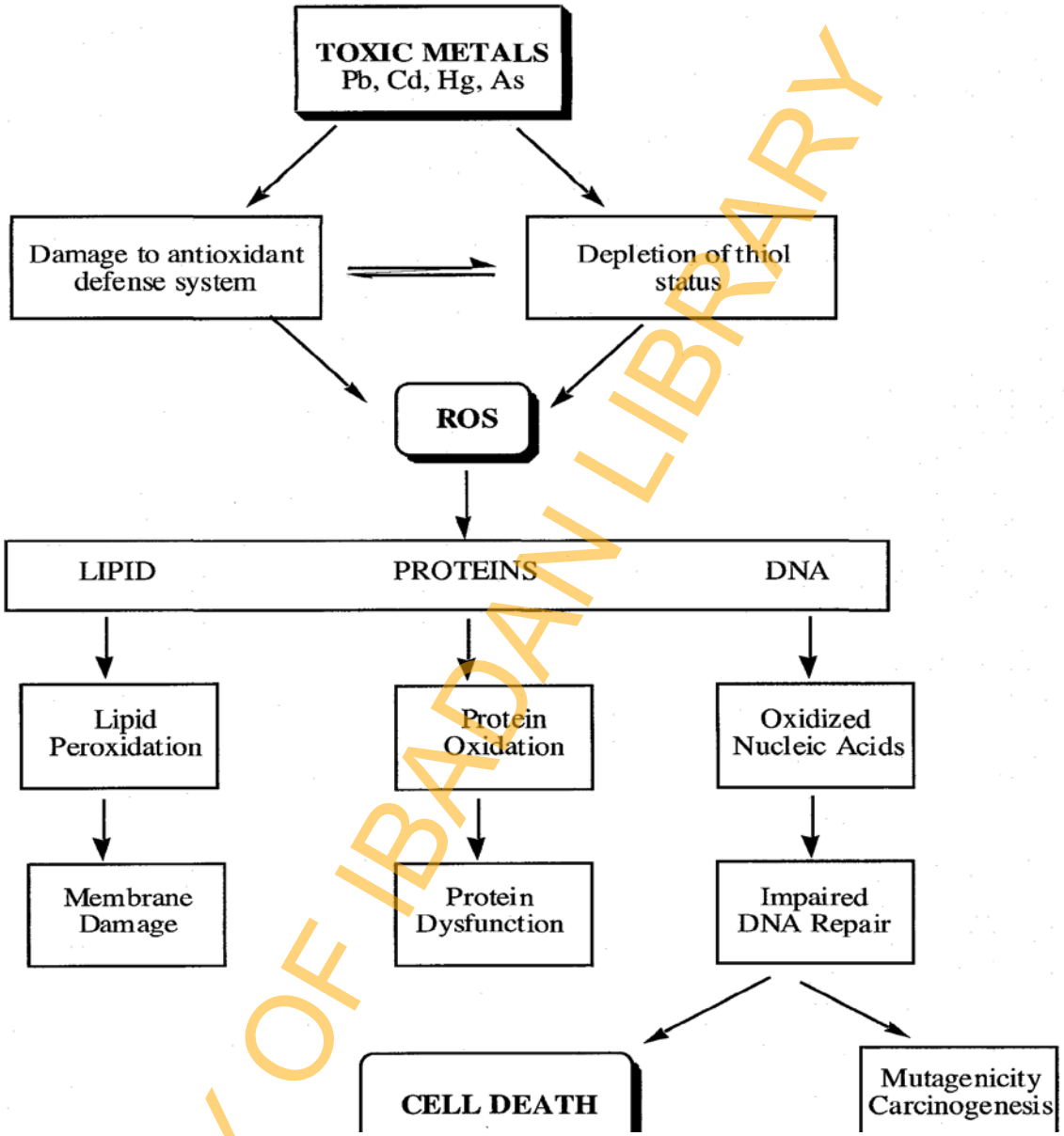


Fig 2.2 Metal induced Oxidative Stress (Ercal, *et al*., 2001)

2.4.3 Metal-metal interaction (MMI)

Metals are involved in a wide range of biochemical reactions within the body and are critical to the functioning and stability of most enzymes involved in these pathways. Copper and zinc in superoxide dismutase, zinc in aminolevulinate dehydratase, Fe in ferrochelatase, are important examples. They stabilize the structure of enzymes and facilitate substrate binding. More often, toxic metals exert their toxicity by interacting with essential metals in biological systems leading to the displacement of these metals from the active site of the enzyme structure. Non-essential toxic metals can mimic essential elements and disrupt homeostasis, as with cadmium which will potentially displace zinc to bind to zinc-dependent transcription factors and enzymes (Waalkes, 2003). Exposure to multiple metals may precipitate metal-metal interactions at uptake sites, binding sites on transport proteins or at cellular target and storage sites.

The metallothioneins are a very important class of metal binding proteins that function in essential metal homeostasis and metal detoxification (Klaassen *et al*., 1999). They are small (6000 Da), soluble, and rich in thiol ligands. These thiol ligands provide the basis for high-affinity binding of several essential and toxic metals including zinc, cadmium, copper, and mercury. The metallothioneins have higher affinity for copper, Cadmium and mercury than for Zinc and they are highly inducible by a variety of metals. Cadmium binding to metallothionein displaces Zinc from the protein and the displaced Zinc will induce more synthesis of the protein which in turn binds more of the toxic metal. These interactions are thought to result from physical and chemical similarities among these metals, occurring via the mechanism of ionic and molecular mimicry (Bridges and Zalups, 2005) and can influence metals accumulation and toxicity (Klaassen *et al*., 2009). Due to the fact that Cd, Cu and Zn belong to the borderline group of metals and have comparable ionic radii and affinity for similar binding sites (sulphur-, oxygen- and nitrogen-containing ligands) in organic macromolecules, it is logical to infer that their interactions are consistent with the isomorphous competitive binding theory (Bridges and Zalups, 2005; Klaassen *et al*., 2009).

2.5 Waste Electrical and Electronic Equipment (e-waste)

E-waste broadly covers waste from all electronic and electrical appliances and comprises items such as computers, mobile phones, digital music recorders/players, refrigerators, washing machines, televisions (TVs) and many other household consumer items (Sinha, 2007; Pinto, 2008). E-waste products contain intricate blends of plastics and chemicals, which when not properly handled can be harmful to humans and pose environment hazard

(Leung, *et al*., 2006). The composition of e-waste is very diverse and differs in products across different categories. It contains more than 1000 different substances, which fall under 'hazardous' and 'non-hazardous' categories. The presence of elements like lead, mercury, arsenic, cadmium, selenium and hexavalent chromium and flame retardants beyond threshold quantities in e-waste classifies them as hazardous waste. The fraction including Iron, Copper, Aluminium, Gold and other metals in e-waste is over 60%, while plastics account for about 30% and the hazardous pollutants comprise only about 2.70% (Leung, *et al*., 2006). Electronic waste or e-waste is one of the rapidly growing problems of the world. It is estimated that 20-50 million tons of electric and electronic waste is generated per year of which 75-80% is shipped to countries in Asia and Africa for recycling and disposal (Adaramodu, *et al*., 2012).

2.5.1 E-Waste in Developing Nations

The electronic industry is the world's largest and fastest growing manufacturing industry (Robinson, 2009). During the last decade, it has assumed the role of providing a forceful leverage to the socio - economic and technological growth of a developing society. The consequence of its consumer oriented growth combined with rapid product obsolescence and technological advances are a new environmental challenge. It is an emerging problem in developing nations as well as a business opportunity of increasing significance, given the volumes of e-waste being generated and the content of both toxic and valuable materials in them. E-waste from developed countries find an easy way into developing countries and Africa is seen as a dumping site for e-waste (Terada, 2012).

In global e-waste trade perspectives, Hull, (2002) opined that the e-waste trade was simply an act "Poisoning the poor for profit".

2.5.2 Management of Waste Electrical and Electronic Equipment in Nigeria

Nigeria has ratified the Basel Convention, the BAN Amendment and the Bamako Convention (Wanjiku, 2008). The National Environmental (Electrical Electronics Sector) Regulations and the Harmful Waste (Special Criminal Provisions) Act entered into force in 2011. The law restricts the import of hazardous wastes for final disposal in the country (Lagos State Environmental Protection Agency, 2011). In addition, a multi-stakeholder Consultative Committee on e-waste has been established to prepare national policy guidelines on e-waste management and an action plan. The National Environmental Standards and Regulations Enforcement Agency (NESREA) was established in 2007 to

enforce all environmental laws, regulations and guidelines, including monitoring and control of e-waste (Benebo, 2009). The National Toxic Dump Watch Programme (NTCWP) has recently been reactivated. It requires importers of e-waste to be registered with NESREA (Lagos State Environmental Protection Agency, 2011). Overall, control of e-waste in Nigeria is inadequate. There has been insufficient enforcement of environmental laws and difficulties in implementing extended producer responsibility (EPR) and producer take-back, together with a general lack of awareness and funds. With no material recovery facility for e-waste and/or appropriate solid waste management infrastructure in place, waste materials often end up in open dumps and unlined landfills (Wanjiku, 2008).

In Nigeria, Alaba International Market in Lagos is becoming as popular as the electronic goods district in Tokyo's Akihabara, reportedly filled with electronic items imported mainly from Japan and South Korea ((Lagos State Environmental Protection Agency, 2011, Terada, 2012). The market reverberates to the sound of car horns and is overloaded with second hand appliances such as television sets, DVD players, and stereo sets etc. that arrive daily in truckloads from around the world. Over 80 per cent of the world's high-tech e-wastes lands up in land-fills in Asia and Africa, Nigeria is emerging as one of the top dumping grounds for toxic chemicals and electronic waste from the developed countries. According to the Basel Action Network (BAN) (2012), a Seattle-based environmental group, an estimated 500 shipping containers with a load equal in volume to 400,000 computer monitors or 175,000 large TV sets enter Lagos, Nigeria each month. As much as 75 percent of such shipments are classified as e-waste. The recycling, parts salvaging, distribution and the disposal of these discarded electronic devices are now creating a new set of environmental and public health challenges in Nigeria (Adaramodu, *et al.*, 2012).

Major e-waste recycling sites in Nigeria include Ikeja Computer Village, Westminster Electronics and Alaba International Markets in Lagos state, Olodo and Ogunpa markets in Ibadan, New Benin Electronics market in Benin, Onitsha Main Market, Anambra state as well as Ariaria Market, Aba (Aragba-Akore, 2005, Nnoron, 2009; Osibanjo, 2009)

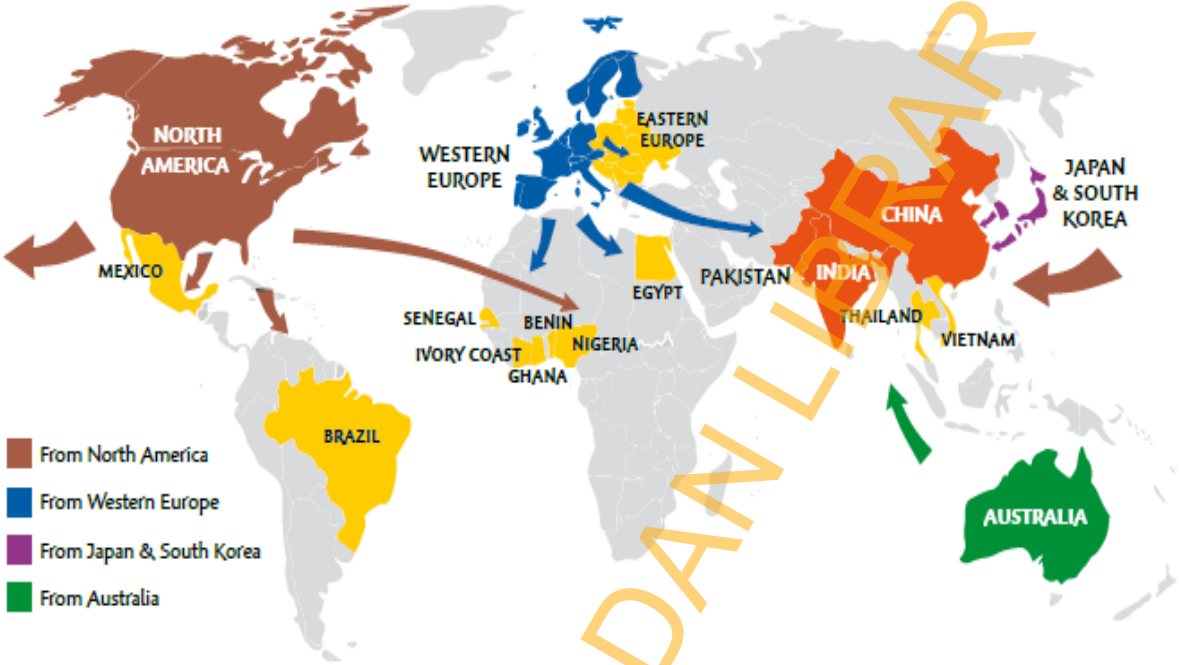


Fig. 2.3 International Flow of E-waste (Lewis, 2011).

Fig. 2.4 Image of e-waste dealers in Nigeria (Adaramodu, *et al.*, 2012)

Informal electronic waste recycling includes the dismantling of end-of-life electronics to retrieve valuable elements with primitive techniques, with or without very little technology to minimise exposure or protective equipment, allowing the emission of dangerous chemicals (Wong *et al*., 2007).

Formal electronic waste recycling facilities use specifically designed equipment to safely remove salvageable materials from obsolete electronics while protecting workers from adverse health effects. However, these centres are very expensive to build and run and are rare in less developed countries. Varying national safety standards can mean that workers at formal or semiformal recycling centres still risk exposure at low doses (Schluep *et al*., 2007).

The cost of recycling and the laws guiding WEEE recycling has led to improper disposal of WEEE. For example California has the strictest e-waste laws in the nation, but an investigation discovered the strict laws have led to dumping of electronic junk in someone else's back yard, causing serious damage. A mountain in the Arizona desert that's not on any map was discovered in 2009. A closer look reveals the mountain is made of glass from old TVs and monitors, full of lead and other toxic heavy metals. Most of the e-waste, about 41 million pounds comes from California (BAN, 2012). E-waste management practices in Nigeria are summarized in table 2.1.

Table 2.1 Summary of e-waste management practices in Nigeria

MANUAL DISMANTLING	OPEN BURNING	OPEN DUMPING OF SCRAPS	REPAIR	DIRECT REUSE
*Obtains copper wires	*Recover copper wires	*Open burning	*Dismantling	* < 25% reusable EEE(end-of-life)
-Recover Printed Circuits Boards	*Toxicants released into soil, air, water, plants *PHI	*Water bodies (scraps or burnt remnants)	-Associated exposures	-Becomes WEEE sooner or later
*Dermal absorption of toxicants	*WEEW dermal absorption	*WEEW systemic accumulation	*Component recovery	
*Ingestion :dust and hands *Inhalation of fumes during soldering	*Direct ingestion *Inhalation(smoke)	-Surface contact *People and environment *PHI	*Trouble shooting; soldering	

*emphasis

WEEW = Waste Electronic and Electrical Workers

PHI= Activities with Public Health Implication

Over 1000 toxic substances including toxic metals (Hg, Pb, As, Cd, Ag, etc.), Bromated flame retardants, polyvinyl chlorides, Phthalates and Organotins, amongst others, have been reported to be contained in e-waste. (Puckett *et al*., 2002). Some of these substances are known carcinogens, neurotoxicants, nephrotoxicants and immunotoxicants. Also, some are recognised as genotoxicants, endocrine disruptors and toxicants, as well as prohibited and listed as Restricted Hazardous Substances. Others are substances whose health effects have not been fully established (EU, 2000a; EU, 2002b; Terada, 2012).

Toxic substances in e-waste and their suspected hazardous effects are summarized in tables 2.2 and 2.3.

Table 2.2 Toxic Metals in Waste Electrical and Electronic Equipment, their Sources in Electronic Products and their Health Concerns

WEEE toxic metals	Source in EEE	Suspected Health Concerns
Antimony	CRTs, printed circuit boards, etc.	Very hazardous in event of ingestion, hazardous in event of skin and eye contact, and inhalation. Causes damage to the blood, kidneys, lungs, nervous system, liver and mucous membranes.
Arsenic	Used to make Transistors	Soluble inorganic arsenic is acutely toxic and intake of inorganic arsenic over a long period can lead to chronic arsenic poisoning. Effects, which can take years to develop, include skin lesions, peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease and cancer.
Barium	Front panel of CRTs	Short-term exposure causes muscle weakness and damage to heart, liver and spleen. It also produces brain swelling after short exposure.
Beryllium	Motherboards of Computers	Carcinogenic (causing lung cancer), and inhalation of fumes and dust can cause chronic beryllium disease or beryllicosis and skin diseases such as warts.
Cadmium	Chip resistors and Semiconductors	Has toxic, irreversible effects on human health and accumulates in kidney and liver. Has toxic effects on the kidney, the skeletal system and the respiratory system, and is classified as a human carcinogen.
Cobalt	Rechargeable batteries and coatings for hard disk drives	Hazardous in case of inhalation and ingestion, and is an irritant of the skin. Has carcinogenic effects and is toxic to lungs. Repeated or prolonged exposure can produce target organs damage.
Copper	Used as a conductor	Very hazardous in case of ingestion, in contact with the eyes and when inhaled. An irritant of the skin and toxic to lungs and mucous membranes. Repeated or prolonged exposure can produce target organ damage
Gallium	Integrated circuits, optical electronics, etc.	Hazardous in case of skin (may produce burns) and eye contact, ingestion and inhalation. Severe over-exposure can result in death. Toxic to lungs and mucous membranes. Repeated or prolonged exposure can produce target organ damage
Indium	LCD screens	Can be absorbed into the body by inhalation or ingestion. Is irritating to the eyes and respiratory tract and may have long-term effects on the kidneys. Environmental effects have not been investigated and information on its effects on human health is lacking; therefore utmost care must be taken.

Modified from Chemicals of Primary Concern in E-waste, (Lungren, 2012).

Table 2.1b Toxic Metals in Waste Electrical and Electronic Equipment, their Sources in Electronic Products and their Health Concerns.

WEEE toxic metals	Source in EEE	Suspected Health concerns
Lead	Solder of printed circuit boards, glass panels and gaskets in computer monitors	Causes damage to central and peripheral nervous systems, blood systems and kidneys, and affects the brain development of children. A cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular and renal systems.
Lithium	Rechargeable Batteries	Extremely hazardous in case of ingestion as it passes through the placenta. It is hazardous and an irritant of the skin and eye, and when inhaled. Lithium can be excreted in maternal milk.
Mercury	Relays, switches and printed circuit boards	Elemental and methyl-mercury are toxic to the central and peripheral nervous system. Inhalation of mercury vapour can produce harmful effects on the nervous, digestive and immune systems, lungs and kidneys, and may be fatal. The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract, and may induce kidney toxicity if ingested.
Nickel	Rechargeable Batteries	Slightly hazardous in case of skin contact, ingestion and inhalation. May be toxic to kidneys, lungs, liver and upper respiratory tract. Also has carcinogenic effects.
Silver	Wiring circuit boards, etc.	Very hazardous in case of eye contact, ingestion and inhalation. Severe over-exposure can result in death. Repeated exposure may produce general deterioration of health by an accumulation in one or many human organs.
Thallium	Batteries, semiconductors, etc.	Very hazardous in case of ingestion and inhalation. Also hazardous in case of skin and eye contact. May be toxic to kidneys, the nervous system, liver and heart, and may cause birth defects. Severe over-exposure can result in death.
Tin	Lead-free solder	Causes irritation in case of skin and eye contact, ingestion and inhalation. Can cause gastrointestinal tract disturbances.
Zinc (chromates)	Plating material.	Contact with eyes can cause irritation; powdered zinc is highly flammable, if inhaled, causes a cough, and if ingested, abdominal pain, diarrhoea and vomiting is common.

Modified from Chemicals of Primary Concern in E-waste, (Lungren, 2012).

Table 2.3 Summary of other toxic substances in e-waste and their adverse health effects

Substance	Occurrence in e-waste	Toxicity/Effect
Halogenated compounds		
PCB(polychlorinated biphenyls)	Condensers, Transformers	Hepatoxicity, Neurotoxicity, Endocrine disruption
-TBBA (tetrabromo-bisphenol-A) -PBB (polybrominated biphenyls) PBDE (polybrominated diphenyl ethers)	Fire retardants for plastics (thermoplastic components, cable insulation) TBBA is presently the most widely used flame retardant in printed wiring boards and casings.	Neurotoxicity, Endocrine disruption, Carcinogenicity
-Chlorofluorocarbon (CFC)	Cooling unit, Insulation foam	Global warming potential
PVC (polyvinyl chloride)	Cable insulation	Carcinogenicity

Culled from Puckett, *et al.*, 2002

E-waste chemicals constitute public health risks due to crude management methods in that teenagers may pick electrical panels for re-sale; and are then exposed to toxic metal, the indiscriminate disposal may cause raised levels of toxicants in soil, and subsequent leaching of toxic chemicals into underground water. There is the transfer of toxic chemicals in e-wastes through e-waste workers to family members and household environment. Ultimately, the e-waste chemicals enter the food chain (Man- Animal-Plant Cycle).

It is therefore agreeable that there are serious public health risks and consequences associated with both occupational and non-occupational exposure to e-waste toxicants. Most e-waste borne chemicals may predispose to serious disease conditions, for example, cancer, which is one of the most feared diseases presently; and this is the focus of this study.

2.6 Heavy Metals, Chemical Toxicants and High Cancer Risks revise here

In the theory of cancer development, the involvement of chemical toxicants and oxidative stress, as well as oxidative DNA damage are well reported (Elst, 2007; Valavanidis, *et al*., 2009; Terada, 2012; Anetor, *et al*., 2013). In China (largest e-waste dump yard in the world): the emergence of China Cancer villages is well-documented (Hong, 2005; Liu and Chen, 2007 and Yingying, 2011).With the unregulated heavy inflow of e-waste into Nigeria, coupled with other widespread health-threatening pollution, the emergence of "Nigerian Cancer villages" is not impossible (Anetor *et al*., 2016).

2.7 Cancer

Cancer is a disease of cellular mutation, proliferation, and aberrant cell growth. It ranks as one of the leading causes of death in the world. In the United States, cancer ranks as the second leading cause of death, with over one million new cases of cancer diagnosed and more than one half million Americans die from cancer annually. Cancer is group of diseases characterised by unregulated cell growth and invasion and spread of cells from the site of origin or primary site, to other sites in the body (metastasis) (Pecorino, 2012)

Molecular pathways- (cellular and molecular routes) differ; the accumulation of mutations occurs only after the cell's defence mechanisms such as DNA repairs have been compromised or evaded. Multiple causes of cancer have been either firmly established or suggested, including infectious agents, radiation, and chemicals. Estimates suggest that 70–90% of all human cancers have a linkage to environmental, dietary, and behavioural

factors. While the understanding of the biology of the progression from a normal cell to a malignant one has advanced considerably in the past several decades, many aspects of the causes, prevention, and treatment of human cancers remain unresolved (James *et al*., 2013)

2.7.1 Properties of Cancer Cells

Cancer cells are characterised by three important properties:

1. Diminished or unrestricted control of growth.
2. Capability of invasion of local tissues, and
3. Capable of spreading to distant parts of body by metastasis (Chatterjea and shinde, 2012).

Cells are constantly faced with the decision of whether to proliferate (through cell division), differentiate (by expressing specialized properties that distinguish one tissue or organ from the others), or die. Involved in these decisions are a small number of genes, about 100 of the tens of thousands of genes that make up the human genome. Genes are encoded in the DNA molecules of the chromosomes, which are found in the cell nucleus. A gene can be thought of as a recipe that the cell follows to make a protein, each gene providing directions for a different protein. The genes that regulate the growth of cells can be divided into two categories: proto-oncogenes, which encourage cell growth, and tumour suppressor genes, which inhibit it. Many of the agents known to cause cancer (chemicals, viruses, and radiation) exert their effects by inducing changes in these genes or by interfering with the function of the proteins encoded by these genes. Mutations in proto-oncogenes tend to over stimulate cell growth, keeping the cell active when it should be at rest, whereas mutations in tumour suppressor genes eliminate necessary brakes on cell growth, also keeping the cell constantly active.

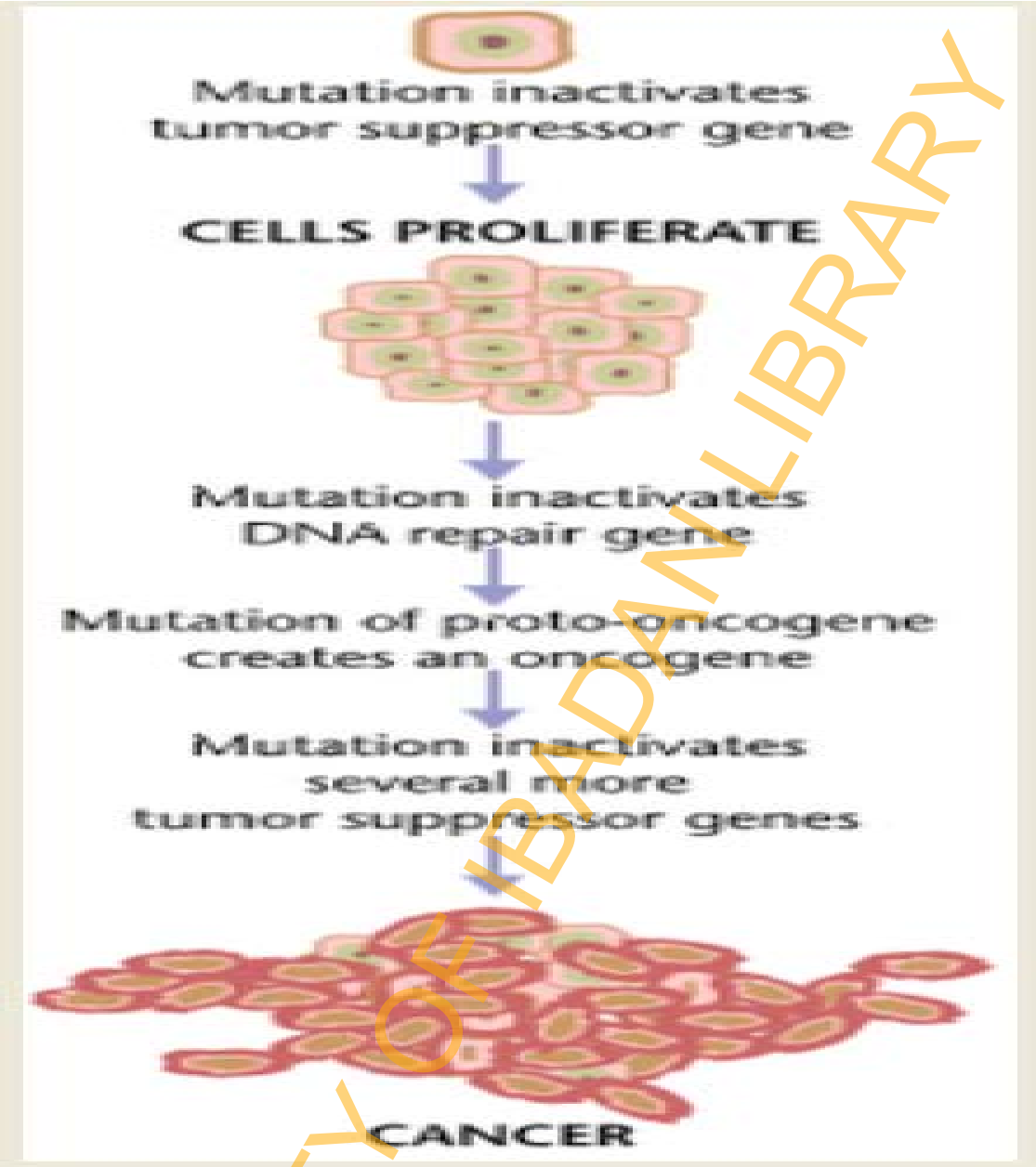


Fig. 2.5 Molecular basis of cancer (Pecorino, 2012).

2.7.2 Causes of Cancer

The billions of cells that make up a tumour are from a single cell that has found a way to escape the normal controls of its growth. This loss of control is caused by damage of the genetic material in the cell, specifically the long, coiled chains of deoxyribonucleic acid (DNA) found in the chromosomes. Such damage can arise during cell division, be induced by environmental agents, or be inherited. Regardless of how the damage is caused, genetic changes and the abnormal growth pattern that they promote are passed on to a cell's progeny (its daughter cells) as the cell divides. Still, a single damaging genetic event is not enough to convert a healthy cell to a cancer cell. Evidence shows that several damage must occur to the DNA of one cell for it to become cancerous, however in many cases this is a slow process that takes years (Anand *et al*., 2011; Kushi *et al*., 2012)

Information on cancer causation has come from investigation of the patterns of cancer in human populations and the induction of tumours in experimental animals following treatment with cancer-causing agents. The most important human carcinogens include tobacco, asbestos, aflatoxins and ultraviolet light. Almost 20% of cancers are associated with chronic infections, the most significant ones being hepatitis viruses (HBV, HCV), papillomaviruses (HPV) and Helicobacter pylori. There is increasing recognition of the causative role of lifestyle factors, including diet, physical activity, and alcohol consumption. Genetic susceptibility may significantly alter the risk from environmental exposures (Anguiano *et al*., 2012; Parkin *et al.*, 2011).

The majority of cancers, some 90–95% of cases, are due to environmental factors. The remaining 5–10% are due to inherited genetics (Parkin *et al.*, 2011). Environmental, as used by cancer researchers, means any cause that is not inherited genetically, such as lifestyle, economic and behavioral factors and not merely pollution (Anand *et al*., 2011; Kushi *et al*., 2012). Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and environmental pollutants (Anand *et al*., 2011; Kushi *et al*., 2012).

2.7.3 Prevalence of Cancer in Developing Countries

In 2010, it was projected that cancer would overtake ischemic heart disease as the leading cause of death in the world (Anand *et al*., 2011; Kushi *et al*., 2012). Between 2005 – when some 7.6 million people died from cancer, accounting for 13% of global deaths – and

2015, it was speculated that 84 million people will die of cancer (WHO, 2011). In 2008, approximately 72% of cancer deaths occurred in low and middle income countries where, although cancer has a lower incidence, survival rates are also much lower, largely because of delays in diagnosis leading to presentation with advanced disease. Many poor patients receive affordable (by their standards) or available treatment rather than optimal treatment, and those with little chance from benefitting from cancer treatment, or without financial support, are not infrequently sent home to die – without even the comfort of palliative care. Many patients – who remain uncounted – never reach a centre capable of providing appropriate treatment. This catastrophe will soon become a crisis, since the global cancer burden is increasing rapidly in developing countries where populations continue to expand and communicable diseases are better controlled, resulting in longer life spans. Even so, approximately 50% of cancer in developing countries occurs in individuals less than 65 years of age.

The increased risk of cancer with age is, to a large degree, a function of prolonged exposure to risk factors. Unfortunately, any reduction in cancer incidence resulting from better control of chronic infections will almost certainly be overwhelmed by the consequences of failure to pre-empt the effects of smoking and dietary factors. In less developed countries, the fact that a higher fraction of patients die from cancer demonstrates that their attempts to control cancer are much less effective, which is hardly surprising, given the remarkable disparities in resources between the lowest and highest income countries (WHO,2011). The poorest populations, particularly those in Africa find themselves trapped in a vicious cycle from which escape is difficult, but essential if their socioeconomic status and health care is to improve (WHO, 2011).

2.8 Multistage Carcinogenesis

Through extensive experimental studies with animal models and evaluation of human cancers, it has been shown that the carcinogenesis process involves a series of definable and reproducible stages.

Operationally, these stages have been defined as initiation, promotion, and progression. These steps follow a temporal sequence of events that have been observed in a wide variety of target tissues (Klaunig and Kamendulis, 2004; Klaunig *et al*., 2009).

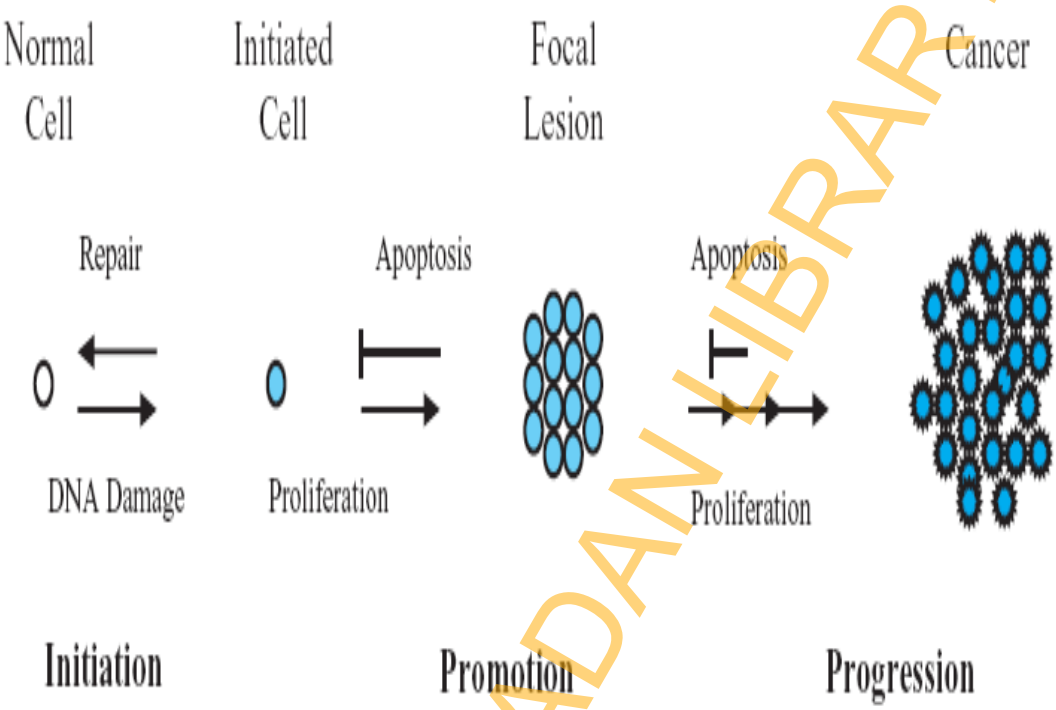


Figure 2.6 Multistage model of carcinogenesis (Klaunig and Kamendulis, 2004)

2.8.1 Initiation

The first stage of the cancer process involves initiation, a process that is defined as a stable, heritable change. This stage is a rapid, irreversible process that results in a carcinogen-induced mutational event. Chemical and physical agents that function at this stage are referred to as initiators or initiating agents. Initiating agents lead to genetic changes including mutations and deletions Initiation by itself does not appear to be sufficient for neoplastic formation. Once initiated cells are formed, their fate has multiple potential outcomes (Klaunig and Kamendulis, 2004; Klaunig *et al.*, 2009):

(1) The initiated cell can remain in a static non-dividing state through influences by growth control via either normal surrounding cells or through endocrine influence;

(2) The initiated cell may possess mutations incompatible with viability or normal function and be deleted through apoptotic mechanisms; or

(3) The cell, through stimuli such as intrinsic factors or from chemical exposure, may undergo cell division resulting in the growth in the proliferation of the initiated cell. In some instances, typically following relatively high doses and/or repeated exposure to the genotoxic carcinogen, a chemical carcinogen may function as a complete carcinogen, i.e., it is capable of progressing through all stages of the cancer process.

2.8.2 Promotion

Derived from either endogenous or exogenous stimuli of cell growth, the second stage of the carcinogenesis process involves the selective clonal expansion of initiated cells to produce a preneoplastic lesion. This is referred to as the promotion stage of the carcinogenesis process. Both exogenous and endogenous agents that function at this stage are referred to as tumor promoters. Tumor promoters are not mutagenic and generally are not able to induce tumors by themselves; rather they act through several mechanisms involving gene expression changes that result in sustained cell proliferation, either through increases in cell proliferation and/or the inhibition of apoptosis.

2.8.3 Progression

The final stage of the carcinogenesis process, progression, involves the conversion of benign preneoplastic lesions into neoplastic cancer. In this stage, due to increase in DNA synthesis cell proliferation in the preneoplastic lesions, additional genotoxic events may occur resulting in additional DNA damage including chromosomal aberrations and

translocations. These events result in the transfer from preneoplastic, clonally derived cell populations into neoplastic cell populations. Chemicals that impact on the progression stage are usually genotoxic agents. By definition, the progression stage is an irreversible stage in that neoplasm formation, whether benign or malignant, occurs (Klaunig and Kamendulis, 2004; Klaunig *et al*., 2009).

2.8.4 Failure of DNA repair mechanisms

The normal cell is able to repair such genetic damage through its DNA repair mechanisms, such as the so-called mismatch repair genes, whose normal function is to identify and repair defective DNA segments that arise in the normal course of a cell's life. However, if the cell's repair mechanisms are faulty, mutations will accumulate, and genetic damage that has not been repaired will be reproduced and passed to all daughter cells whenever the cell divides. In this way malfunctioning DNA repair machinery contributes to the genesis of some cancers. When a normal cell senses that its DNA has been damaged, it will stop dividing until the damage has been repaired. But when the damage is massive, the cell may abandon any attempt at repair and instead activate a suicide program called, apoptosis or programmed cell death. The life of a cell can be prolonged for a number of reasons; for example, an excess of molecules that prevent the suicide program from occurring may be present, or the molecules that trigger the apoptotic process may be defective. Significant prolongation of a cell's life increases the chances that it will accumulate mutations in its DNA that transform the cell. Thus, the failure of a cell to die when it should is another factor that can contribute to carcinogenesis (the development of cancer) (Klaunig and Kamendulis, 2004; Klaunig et al., 2009).

2.9 Occupational exposure and cancer risks

The association of occupational exposure with cancer risk has been long recognized. High incidence of cancer has been associated with some forms of occupational exposure to carcinogens. In 1775, Pott described a linkage between the increased occurrence of scrotal and nasal cancer among chimney sweepers and their profession. Pott (1775) concluded that chimney soot was the causative agent for cancer induction in these individuals (Androutsos, 2006; Herr, 2011). Other investigators also recognized an association between exposure to chemicals and the induction of human cancer. A linkage between an increased incidence of lung cancer and uranium mining was noted by Harting and Hesse (1879). Butlin, in a follow-up of Potts' observations, noted that scrotal cancer in chimney sweepers in the European continent was relatively rare compared to that seen in England

Androutsos, 2006; Herr, 2011). He attributed this difference to better hygiene practices by the European sweepers as well as to the use of younger boys as sweeps in England, suggesting that the age of exposure and the duration of exposure influence the formation of the cancer.

In 1997, occupational exposure to carcinogens and risk of lung cancer was investigated in a Netherland cohort study by van Loon and co-workers (van Loon *et al.*, 1997). Their study showed that occupational exposure to asbestos or paint dust is associated with higher risk for lung cancer.

A recent review on occupational exposure and risk of breast cancer by Fenga (2016) documented that epidemiological and experimental studies have demonstrated that ionizing and non-ionizing radiation exposure, night-shift work, pesticides, polycyclic aromatic hydrocarbons and metals are defined environmental factors for breast cancer, particularly at young ages.

Based on epidemiological and experimental studies, there exists a clear relationship between the induction of cancer in humans by prolong unprotected exposure to a specific chemical or or a mixture of chemicals.

2.10 Toxic metals

2.10.1 Aluminium

Aluminium (Al) is a silvery-white, soft, nonmagnetic, ductile metal it is a chemical element in the boron group with atomic number 13. It is recognised as the third most abundant element in the Earth's crust (after oxygen and silicon) and its most abundant metal. It is a corrosion resistance metal because a thin surface layer of aluminium oxide forms when the bare metal is exposed to air, effectively preventing further oxidation, in a process termed passivation (Savory and Wills, 1989). Aluminium is a non-essential element of ubiquitous distribution. Aluminium in the diet comes from foods, food additives, storage, cooking materials and drinking water. Pharmaceutical agents such as aluminium-containing antacids and buffered analgesics potentially contribute substantial amounts of aluminium to the total body burden. Aluminium is also found in toothpastes, cosmetics and antiperspirants. Consequently, exposure to aluminium is unavoidable with a daily dietary intake in the population probably being in the range of 3 to 5 mg. However, only a small fraction of this aluminium is absorbed (approximately 15 µg) with the majority excreted through the kidneys (Savory and Wills, 1989).

From a toxicological point of view, greatly elevated concentrations of plasma aluminium were found to be associated with dialysis encephalopathy (speech disorder, dementia, convulsions, myoclonus, depression, anxiety, malaise and memory loss) after 3- 7 years on dialysis. Other conditions associated with aluminium toxicity include contact dermatitis, digestive disorders, vomiting and other symptoms especially in people, who are allergic to aluminium. Aluminium has also been controversially implicated as a factor in Alzheimer's disease (Ferreira *et al*., 2008).

2.10.2 Vanadium

Vanadium is a chemical element with symbol **V** and atomic number 23. It is a hard, silvery grey, ductile and malleable transition metal. The elemental metal is rarely found in nature, but once isolated artificially, the formation of an oxide layer (passivation) stabilizes the free metal somewhat against further oxidation. Some sources describe vanadium as "soft", perhaps because it is ductile, malleable and not brittle (Francois, 2008).

The toxicity of vanadium compounds is low. The toxicity of vanadium compounds usually increases with increase valence and pentavalent compounds are the most toxic. Most of the toxic effects of vanadium compounds result from local irritation of the eyes and upper respiratory tract rather than systemic toxicity. The only clearly documented effect of exposure to vanadium dust is upper respiratory tract irritation characterised by rhinitis, wheezing, nasal haemorrhage, conjunctivitis, cough, sore throat, and chest pain. Ingestion of the dust may result in diarrhoea and vomiting. Long-term exposure may cause chronic bronchitis. Vanadium is absorbed from the digestive and respiratory tract (Taylor, 2006).

2.10.3 Chromium

Chromium is a chemical element with symbol **Cr** and atomic number 24. It is the first element in Group 6. It is a steely-grey, lustrous, hard and brittle metal (Brandes *et al*., 1956). Chromium was named from the Greek word "chroma" meaning colour, because of the many colorful compounds made from it (Liu *et al*., 2008). Chromium occurs ubiquitously in nature, the geological forms being almost entirely trivalent; hexavalent chromium in the environment is the result of human industrial activity within the last 200 years (Taylor, 2006).

In mammals, it is considered to have an essential role as a component of a postulated glucose tolerance factor required to potentiate insulin action. Identification of this factor

has been difficult, but it has been suggested that it is an oligopeptide of ~ 1500 Da which has been isolated from liver (mouse, rat, rabbit, cow & dog), kidney (pig) and colostrum (dog). This molecule, named chromodulin, is capable of binding four chromic ions (Vincent, 2004). In humans, normal blood and plasma concentrations are at analytically challenging levels and specimen collection requires scrupulous avoidance of contamination.

More recently, it has been demonstrated that serum insulin and glucose concentrations in Type 2 diabetics can be lowered by chromium supplementation, but only at pharmacological intakes (> 200 µg/day) (Morns *et al*., 1999).

Cr (VI) is very toxic even at low concentration and it is known human carcinogen. It is also corrosive and can readily cause allergic skin reactions, following skin contact independent of the dose. Damage to kidney and liver has also been reported. Occupational exposure to chromium may be a cause of asthma (O'Brien and Patierno, 2003; Bright *et al*., 1997). Hexavalent chromium compounds are genotoxic. Occupational exposure to hexavalent chromium compounds, particularly in the chrome production and pigment industries, is associated with increased risk of lung cancer, and hexavalent chromium-containing compounds are considered to be human Carcinogens (Costa *et al*., 2006). Once hexavalent chromium enters the cell, it is reduced by various intracellular reductants to give reactive trivalent chromium species. During the reduction process, various genetic lesions can be generated, including chromium DNA-adducts, DNA protein cross-links, DNA-chromium interstrands cross-links, DNA strand breaks, and oxidized DNA bases. Hexavalent chromium can also cause disturbances of the p53 signaling pathway, cell cycle arrest, apoptosis, interference of DNA damage repair, and neoplastic transformation (Valko *et al*., 2005). All these effects could well play an integrated role in chromium carcinogenesis (Costa *et al*., 2006).

2.10.4 Nickel

Nickel is a chemical element with symbol **Ni** and atomic number 28. It is a silvery-white lustrous metal with a slight golden tinge. Nickel belongs to the transition metals and is hard and ductile. It exists as oxidation states of 0, +1, +2, and +3. Predominate compounds of interest include nickel oxide, nickel hydroxide, nickel subsulfide, nickel sulphate, nickel chloride, and nickel carbonyl (Catherine and Max, 2007). Nickel is an essential trace element for several animal species. The average human daily intake is around 100-300 µg, of which only 1% is absorbed, the rest being eliminated in the faeces. Foods relatively rich

in nickel include cocoa, soya beans, dried legumes, nuts and certain grains. Drinking water also contains nickel and absorption from this source may be up to 25% (Taylor, 2006).

The exact role of nickel in human metabolism has not been identified but there is some evidence that it acts in synergy with Vitamin B12 in stimulating haematopoiesis. There are no known clinical conditions associated with nickel deficiency and no established daily requirement or allowance. The element may be essential at levels as low as <1µg/Kg/day (Taylor, 2006).

The average daily exposure does not pose a threat to human health. Most of the nickel absorbed every day by humans is removed by the kidneys and passed out of the body through urine or is eliminated through the gastrointestinal tract without being absorbed. Nickel is not a cumulative poison, but larger doses or chronic exposure may be toxic, even carcinogenic, and constitute an occupational hazard (Buttice, 2015). The e-waste workers are at the risk of nickel toxicity as nickel is one of the elemental constituents of e-waste.

2.10.5 Arsenic

Arsenic (Symbol; As and an Atomic Number; 33) occurs in many minerals, usually in combination with sulfur and other metals, but also as a pure elemental crystal. Arsenic has a similar electronegativity and ionisation energies to its lighter congener phosphorus and as such readily forms covalent molecules with most of the non-metals. Though stable in dry air, arsenic forms a golden-bronze tarnish upon exposure to humidity which eventually becomes a black surface layer. Arsenic (and some arsenic compounds) sublimes upon heating at atmospheric pressure, converting directly to a gaseous form without an intervening liquid state at 887 K (614 °C) (Gokcen, 1989). The triple point is 3.63 MPa and 1,090 K (820 °C) (Holleman *et al*., 1985; Gokcen, 1989).

Arsenic has many applications in electronics, it is used in the design of varieties of semiconductor devices, solar cells, light-emiting diodes, lasers, and integrated circuits in electronics appliances (Uryu *et al*., 2003).

Arsenic exposure produces characteristic transverse white bands across fingernails (Mees' line), which appear about 6 weeks after the onset of symptoms of arsenic toxicity. Arsenic interferes with mitochondrial ATP production (Young-Seoub *et al.*, 2014). Arsenic has

been reported to be associated with hypertension and have serious impacts on the cardiovascular system, and even hepatic damage at high dose (Lee *et al.*, 2002).

The trivalent compounds of arsenic are thiol-reactive, and thereby inhibit enzymes or alter proteins by reacting with proteinaceous thiol groups (Rossman, 2003). Pentavalent arsenate is an uncoupler of mitochondrial oxidative phosphorylation, by a mechanism likely related to competitive substitution (mimicry) of arsenate for inorganic phosphate in the formation of adenosine triphosphate. Arsenic and its metabolites have been shown to produce oxidants and oxidative DNA damage, alteration in DNA methylation status and genomic instability, impaired DNA damage repair, and enhanced cell proliferation (Rossman, 2003). Arsenic can also act as a co-mutagen and/or co-carcinogen. These mechanisms are not mutually exclusive and multiple mechanisms likely account for arsenic toxicity and carcinogenesis.

The association of internal tumours in humans with arsenic exposure is well recognized (Wei *et al.*, 2002). This includes arsenic induced tumours of the urinary bladder, lung, skin, the liver, kidney, and prostate (Young-Seoub *et al.*, 2014). In rats, the methylated arsenic species, $DMA5^{+}$, is a urinary bladder tumour initiator and promoter (Wei *et al.*, 2002) and produces urothelial cytotoxicity and proliferative regeneration with continuous exposure (Cohen *et al.*, 2001).

2.10.6 Molybdenum

Molybdenum is a chemical element with symbol Mo and atomic number 42, its ores were initially confused with lead ores. In its pure form, molybdenum is a silvery-grey metal with a Mohs hardness of 5.5. It has a melting point of 2,623 °C (4,753 °F); of the naturally occurring elements, only tantalum, osmium, rhenium, tungsten, and carbon have higher melting points (Lide, 1994).

Molybdenum is an essential element for humans, and dietary recommendations have been established. Dietary intakes in humans are usually within the range of the recommendations. Soluble molybdenum compounds are readily absorbed when ingested. The highest molybdenum concentrations are found in the kidneys, liver, and bone. Excretion, primarily through the urine, is rapid. The biological half-life ranges from a few hours to a few days. Turnover is much more rapid when intake is high than when intake is low (Turnlund and Friberg, 2007).

The average daily intake of molybdenum varies between 0.12 and 0.24 mg, depending on the molybdenum content of the food (Coughlan, 1983). Significant dietary sources include green beans, eggs, sunflower seeds, wheat flour, lentils, cucumbers and cereal grain (Emsley, 2001)

2.10.7 Cadmium

Cadmium (Cd) is a toxic transition metal that was discovered in 1817 as an impurity of "calamine" (zinc carbonate) for which it is named from the Latin cadmia (Liu *et al*., 2008). Cadmium and its compounds are used in a number of applications within electrical and electronic products. An industrial use of Cd ranges from acting as a stabilizer in plastic components to being added to components as a colour pigment. Cadmium sulphide is used as a phosphorescent coating on the inside of fluorescent monitor screens. Colour in a monitor screen is achieved using three electron guns, a shadow mask and the Cd phosphorescent coating. Cd is also added to PVC plastic insulation of wires and cables as a plastic stabilizer and flame-retardant (Liu *et al*., 2008).

Cadmium is readily absorbed in the gastrointestinal tract, due to chemical similarity with calcium, its transports into cells is mediated through calcium channels and through molecular mimicry (Zalpus and Ahmad, 2003). Gastrointestinal absorption of cadmium is limited to 5–10% of a given dose. Absorption can be increased by dietary deficiencies of calcium or iron and by diets low in protein. Absorption of cadmium after inhalation is generally greater, ranging from 5 to 35%, depending on the specific compound, site of deposition, and particle size. After absorption, cadmium is transported in blood initially bound to albumin and other large molecular weight proteins. Cadmium bound to albumin is preferentially taken up by the liver hepatocytes. In the liver, kidney, and other tissues; cadmium induces the synthesis of metallothionein (MT), a low-molecular-weight, high affinity metal-binding protein (Klaassen *et al.*, 1999).

Cadmium is stored in the liver primarily as cadmium-Metallothionein complex. Cadmium-MT complex may be released from the liver and transported via blood to the kidney, where it is reabsorbed and degraded in the lysosomes of the renal tubules. The free cadmium induces the synthesis of more metallothionein forming more cadmium-MT complex again or cause renal toxicity. Due to its low molecular weight, cadmium-MT complex is efficiently filtered through the glomeruli and thereafter taken up by the tubules. Cadmium accumulates in the body and it is stored in the bone with a half life of about 17 – 30 years

(Klaassen *et al.*, 1999). Once absorbed, cadmium is very poorly excreted and only about 0.001% of the body burden is excreted per day. Both urinary and faecal excretory routes are operative However, the body lacks an effective mechanism of cadmium excretion (Zalpus and Ahmad, 2003).

Cadmium exposure occurs occupationally through inhalation of fume or dust containing Cd and its compounds. It is a cumulative toxicant and long term exposure can result in damage to kidney, bone structure and hepatocytes. Cadmium damages a specific structure of the functional unit of the kidney (the proximal tubules of the nephron) in a way that is first manifested by leakage of low molecular weight proteins and essential ions, such as calcium, into urine, which progresses over time to complete kidney failure (Elinder and Jarup, 1996). The loss of calcium caused by Cd effect on the kidney can be severe enough to lead to weakening of the bones. Itai-itai disease, an epidemic of bone fractures in Japan from gross cadmium contamination of rice stocks grown on contaminated soil with Cd is prominent in the history of toxicology. In addition, carcinogenesis, development of hypertension and cardiovascular diseases are also mediated by Cd toxicity (ATSDR, 2008).

2.10.8 Tin

Tin is a chemical element with the symbol Sn (for Latin: *stannum*) and atomic number 50. It is a main group metal in group 14 of the periodic table. Tin shows a chemical similarity to two neighbouring group-14 elements, germanium and lead, and has two possible oxidation states, +2 and the slightly more stable +4 (Holleman *et al*., 1985).

In the electrical and electronics industries, tin has long been used as a solder in the form of an alloy with lead, tin accounting for 5 to 70% w/w. Tin forms a eutectic mixture with lead, containing 63% tin and 37% lead. Such solders are primarily used for joining pipes or electric circuits. Since the European Union Waste Electrical and Electronic Equipment Directive (WEEE Directive) and the Restriction of Hazardous Substances Directive came into effect on 1 July 2006 (ATSDR, 2008), the use of lead in such alloys has decreased.

Ingestion of food items contaminated with high levels of inorganic tins may cause acute gastroenteritis, while chronic inhalation of inorganic tins (e.g. stannic oxide dust or fumes) may lead to benign non-fibrotic pneumoconiosis called *stannosis* (ATSDR, 2005; Ostrakhovitch and Cherian, 2007). Some organic tin compounds are highly neurotoxic,

particularly triethyltin and trimethyltin, and cause encephalopathy and cerebral oedema (Ostrakhovitch and Cherian, 2007). There is inadequate information to assess carcinogenic potential of organotin compounds in animals, and they are considered not classifiable as to human carcinogenicity (ATSDR, 2005).

2.10.9 Antimony

Antimony is a chemical element with symbol Sb (from Latin: *stibium*) and an atomic number of 51. A lustrous gray metalloid, it is found in nature mainly as the sulphide mineral stibnite (Sb_2S_3). Antimony compounds have been known since ancient times and were used for cosmetics; metallic antimony was also known, but it was erroneously identified as lead upon its discovery. In the West, it was first isolated by Vannoccio Biringuccio and described in 1540. In primitive cultures, its powder was used to cure eye ailments, as well as also for eye shadow and it was often referred to by its Arabic name, kohl (Wang, 1919).

Most antimony compounds are of trivalent and pentavalent states. Antimony has many uses including in alloys, and in production of fire proofing chemicals, ceramics, glassware, and pigments. It is used medically in the treatment of the parasitic diseases, schistosomiasis and leishmaniasis (De Boeck *et al.*, 2003; Sundar and Chakravarty, 2010). Occupational antinomy exposure comes from industrial emissions. Food is the major route for environmental exposure, but the exposure levels are generally low. The average daily intake from food and water is estimated at about 5µg (ATSDR, 1992; Sundar and Chakravarty, 2010).

Occupational exposures are usually by inhalation of dust containing antimony compounds, such as the pentachloride, trichloride, trioxide, and trisulphide (Sundar and Chakravarty, 2010). Acute toxicity from the pentachloride and trichloride exposures includes rhinitis and, in severe exposures, even acute pulmonary oedema. Chronic exposure by inhalation of other antimony compounds results in rhinitis, pharyngitis, trachitis, and, over the longer term, bronchitis and eventually pneumoconiosis with obstructive lung disease and emphysema. Transient skin eruptions (antimony spots) may occur in workers with chronic exposure (Sundar and Chakravarty, 2010). Trivalent forms of antimony appear more toxic and may produce cardiotoxicity involving arrhythmias and myocardial damage, although the evidence of heart disease from industrial exposure to antimony is not strong (Sundar

and Chakravarty, 2010). In rodent sub-chronic/chronic studies with antimony potassium tartrate, relatively low toxicity was reported (Lynch *et al*., 1999).

Antimony compounds are generally negative in non-mammalian genotoxicity tests, whereas mammalian tests usually give positive results for trivalent but negative results for pentavalent antimony compounds. The in vivo potential of antimony to induce chromosome aberrations appears inconsistent (De Boeck *et al*., 2003; Sundar and Chakravarty, 2010).

2.10.10 Mercury

Mercury (Hg) was named after the Greco-Roman god known for swift flight. Also called quicksilver, metallic mercury is in liquid state at room temperature. The symbol Hg was derived from the Latinized Greek hydrargyrum, meaning "water" and "silver." it has an atomic weight of 80. It is a Silvery-white metal (Liu *et al*., 2008). Mercury is used in lighting devices that illuminates flat screens. Backlight bulbs and lamp bulbs that illuminate laptop and other flat screen panel displays. Relay and Hg switches (Devi *et al*., 2004).

Mercury vapour is readily absorbed (about 80%) in the lungs, rapidly diffuses across alveolar membranes into the blood, and distributes to all tissues in the body due to its high lipid solubility. Once the vapour has entered the cell, it is oxidized to divalent inorganic mercury (Hg^{2+}) by tissue and erythrocyte catalase (Neustadt and Pieczenik, 2007). A significant portion of mercury vapour crosses the blood–brain barrier and placenta before it is oxidized by erythrocytes, and thus shows more neurotoxicity and developmental toxicity compared to administration of inorganic mercury salts which cross membranes less rapidly (Neustadt and Pieczenik, 2007).

Inorganic mercury is poorly absorbed from the gastrointestinal tract. Absorption ranges 7% to 15% of ingested dose, depending on the inorganic compound. Renal uptake of mercury salts occurs through two routes: from luminal membranes in renal proximal tubule in the form of the cysteine S-conjugates (Cys-SHg-S-Cys) or from the basolateral membrane through organic anion transporters (Bridges and Zalpus, 2005). Inorganic mercury salts do not readily pass blood–brain barrier or placenta and are mainly excreted in urine and faeces, with a half-life of about 2 months. In contrast to inorganic mercury, 90% of the methylmercury is eliminated from the body in the faeces, and less than 10% is in the urine, with a half-life of 45–70 days (Bridges and Zalpus, 2005).

Chronic exposure to mercury vapour has the major effect on the central nervous system. Early signs are nonspecific, and this condition is termed the asthenic-vegetative syndrome or micromercurialism (Neustadt and Pieczenik, 2007). The triad of tremors, gingivitis, and erethism (memory loss, increased excitability, insomnia, depression, and shyness) has been recognized historically as the major manifestation of mercury poisoning from inhalation of mercury vapour. The major human health effect from exposure to methylmercury is neurotoxicity (Neustadt and Pieczenik, 2007). Clinical manifestations of neurotoxicity include paresthesia (a numbness and tingling sensation around the mouth, lips) and ataxia, Insomnia, Blindness, depression, memory loss, mental retardation, keratosis, melanosis, Fatigue, ulcer manifested as a clumsy, stumbling gait, and difficulty in swallowing and articulating words (Neustadt and Pieczenik, 2007).

2.10.11 Thallium

Thallium (Tl) atomic, with number 81, is a soft gray post-transition metal which is not found free in nature. When isolated, it resembles tin, but discolours when exposed to air. Thallium is extremely soft, malleable and sectile enough to be cut with a knife at room temperature. It has a metallic lustre that, when exposed to air, quickly tarnishes to a bluish-gray tinge, resembling lead. It may be preserved by immersion in oil. A heavy layer of oxide builds up on thallium if left in air. In the presence of water, thallium hydroxide is formed. Sulphuric and nitric aciddissolve thallium rapidly to make the sulphate and nitrate salts, while hydrochloric acid forms an insoluble thallium (I) chloride layer (Holleman *et al*., 1985).

In the electrical and electronics industry Thallium selenide has been used in a bolometer for infrared detection due to its photoresistivity (Jain and Zaret, 2005). Doping selenium semiconductors with thallium improves their performance, and therefore it is used in trace amounts in selenium rectifiers. Thallium is also applied in scintillation generators and in some of the dissolved oxygen analysers' electrode. Before the widespread application of technetium-99m in nuclear medicine, the radioactive isotope thallium-201, with a half-life of 73 hours, was the main substance for nuclear cardiography. The nuclide is still used for stress tests for risk stratification in patients with coronary artery disease (Jain and Zaret, 2005).

The triad of gastroenteritis, polyneuropathy, and alopecia is regarded as the classic syndrome of thallium poisoning (WHO, 1996). Other signs and symptoms also occur

depending on the dose and duration of exposure. The estimated acute lethal dose in humans is 10 to 15 mg/kg. Death is due mainly to renal, central nervous system, and cardiac failure within a few days to two weeks (WHO, 1996; Peter and Viraraghavan, 2005). Alopecia is the best known effect of thallium poisoning (Peter and Viraraghavan, 2005)

2.10.12 Lead

Lead is a chemical element in the carbon group with symbol Pb (from Latin: plumbum) and atomic number 82. Lead is a soft and malleable metal, which is regarded as a heavy metal and a post-transition metal (Liu *et al.*, 2008).

Lead has many uses in the electronic products, including design of capacitors, cabling, Lead-acid batteries, printed circuit boards and fluorescent tubes. Metallic Pd is used in electrical tin-solder primarily on printed circuit boards. Lead Oxide is used in cathode ray tube (CRT) displays and monitors. Pd in CRTs acts as radiation shield and lowers the melting temperature of the glass. Pd compounds have been used as a stabilizer in PVC formulations. The second largest use of lead in electronics is found in tin-lead solder for connecting many component parts together (Mahaffey, 1985; Liu *et al.*, 2008).

The absorption of Pb from the intestine is mediated by both passive and facilitated diffusion, though passive diffusion plays a minor role in total absorption. Pb absorption can be enhanced by low dietary iron (Fe) and calcium (Ca), especially in children (Mahaffey, 1985). Some evidence supports the hypothesis that divalent metal transporter1 (DMT1) is responsible for transporting Pb. It is known that DMT1 is a metal ion transporter that can transport metals such as Pb, Cd, and Zn in addition to its physiological substrate, iron. The Ca binding protein, calbindin, which is responsible for basolateral Ca^{2+} transfer in enterocytes and has been shown to bind both Pb and Ca with similar affinity (5µM) (Liu *et al.*, 2008). Absorbed Pb is carried to the bone. Pb in blood is primarily (99%) in erythrocytes bound to haemoglobin, only 1% of circulating Pb in serum is available for tissue distribution. Metabolic balance studies indicate that Pb is predominately excreted through faeces, with urinary excretion playing a secondary role. Trace amounts of Pb can also be excreted through hair, sweat, breast milk, and nails (Liu *et al.*, 2008).

Lead has a wide range of effects including damage to the nervous system, kidney, blood components especially red cells, and on reproductive system (Ercal *et al.*, 2001). Footdrop (a gait abnormality in which the dropping of the forefoot happens due to weakness,

irritation or damage to the common fibular nerve) and wrist drop (condition in which the wrist and the fingers cannot extend at the metacarpophalangeal joints) characterized the house painter and other workers with excessive occupational exposure to lead more than half century ago (Mahaffey, 1985; Liu *et al.*, 2008). Adults with high blood Pb levels (>40µg/dL) may have impaired haem synthesis and chronic kidney disease (blood Pd levels above 60 µg/dL), and sustained blood Pb levels above 80µg/dL can cause lethargy and impairment of cognitive function. Epidemiological studies suggest a small dose-effect on blood pressure for blood levels up to 30 to 40µg/dL (Mahaffey, 1985; Liu *et al*., 2008).

2.10.12.1 Lead Haemoglobin (Hb) Interactions

Lead and some heavy metals including Ag^{+}, $Hg^{2+,}$ Cu^{2+} cause haemolysis and lipid peroxidation (Ribarov and Benov,1981). It has been suggested that lead causes superoxide radical formation in erythrocytes by its interaction with oxyhaemoglobin. Ribarov *et al*. found that Pb^{2+} substantially increased the auto-oxidation of haemoglobin in an in-vitro liposome model. Haemoglobin auto-oxidation by lead was inhibited by antioxidant enzymes, suggesting that $O_2^{\cdot -}$ and H_2O_2 were involved in the process. Therefore, it was speculated that lead might induce oxidative stress by interacting with oxyhaemoglobin, leading to peroxidative haemolysis in erythrocyte membranes (Ribarov and Bochev, 1982).

2.10.12.2 Delta-Aminolevulinic Acid (δ-ALA) - Induced Generation of Reactive Oxygen Species in Lead Poisoning

Anaemia is a well-known symptom of lead poisoning, as it is seen in plumbism (an old term for lead poisoning), because lead inhibits haemoglobin synthesis and changes erythrocyte morphorlogy and survival (Gurer and Ercal, 2000). In haem synthesis, the activities of δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase, which are involved in haem synthesis, are inhibited by lead. It is known that ALAD is a sulphydryl-containing enzyme. Lead binds to the sulphydryl group of this enzyme, making it inactive. Condensation of two molecules of ALA, which is catalysed by ALAD, is then blocked and levels of ALA become very high in blood. Studies showed that ALA undergoes enolisation at physiological pH. The enolised ALA then autoxidises and generates superoxide anion, as evidenced by the parallel reduction of ferricytochrome C and also by Electron Spin Resonance spin-trapping experiments. It has been suggested that ALA/oxyHb coupled oxidation also results in reactive oxygen species ROS. As shown in the figure below, HO. Radicals, which are the most reactive free radicals, are generated as a result of both ALA

and ALA/oxyHb coupled autoxidation. Inhibition of ALA/oxyHb coupled oxidation by antioxidants supports the hypothesis that free radicals are involved in the process (Gurer and Ercal, 2000).

It was demonstrated that the final oxidation product of ALA, 4, 5-dioxovaleric acid, is an effective alkylating agent (of the guanine moieties within both nucleosides and isolated DNA) (Ercal *et al*., 2001). Rats chronically treated with ALA, have high levels of DNA oxidation products such as 8-oxo-7, 8-dhydro-2'-deoxyguanosine and 5-hydroxyl-2'-deoxycytidine. These studies suggest that ALA accumulation in lead toxicity might be partially responsible for ROS generation (Gurer and Ercal, 2000; Ercal *et al*., 2001).

Ferrochelatase catalyzes the insertion of Iron into the protoporphyrin ring to form haem. Inhibition of ferrochelatase results in accumulation of protophorphyrin IX, which takes the place of haem in the haemoglobin molecule and, as the erythrocytes containing protoporphyrin IX circulate, zinc is chelated at the site usually occupied by iron. Erythrocytes containing zinc protoporphyrin are intensely fluorescent and may be used to diagnose lead exposure (Ercal *et al*., 2001).

The association of lead exposure with increased human cancer risk has been reported. A study carried out by Anttila *et al.* (1995) on workers exposed to lead and engine exhaust found a 1.4 fold increase in the overall cancer incidence and a 1.8 fold increase in lung cancer among those who ever had elevated blood lead levels. Another epidemiological study conducted by Cocco, (1998) provides evidence for a potential link between occupational exposure to lead and brain cancer. Silbergeld (2003) in his study concluded that Pd did not appear to be directly genotoxic in vivo or in vitro, and that lead may interact with other toxicants to facilitate chemical carcinogenesis. Several mechanisms have been proposed for lead-induced carcinogenesis, including regenerative repair, inhibition of DNA synthesis or repair, generation of reactive oxygen species with oxidative damage to DNA, substitution of lead for zinc in transcriptional regulators, interaction with DNA-binding proteins, and aberrant gene expression (Qu *et al*., 2002).

2.11 Essential metals

2.11.1 Magnesium

Magnesium is an alkali earth metal with atomic number 12 and symbol Mg; it is an essential mineral in man. It is required for the activities of enzymes, for neuromuscular function and cell signalling, and as a structural component e.g. within mineralised bone

and for the conformation of macromolecules such as DNA and proteins. There is current interest in the pharmacological use of magnesium in conditions such as acute myocardial infarction and eclampsia (Ryan, 1991). Magnesium is the fourth most abundant and important cation in humans. It is extremely essential for life and is present as intracellular ion in all living cells and tissues. It is widely distributed in vegetables, found in porphyrin group of chlorophyll of vegetable cells and also found in almost all animal tissues. Other important sources are cereals, beans, green vegetables, potatoes, almonds and dairy products, e.g. cheese (Chatterjea and Shinde, 2012).

Deficiency of magnesium may occur as a complication of various disease states such as malabsorption syndromes, renal dysfunction, and endocrine disorders. Magnesium deficiency in humans causes neuromuscular irritability, frank tetany and even convulsions. Magnesium deficiency induces an inflammatory syndrome (Mazur *et al*., 2007), and is a risk factor for diabetes mellitus, hypertension, hyperlipidemia, and ischemic heart diseases (Ueshima, 2005).

In industrial exposures, no ill effects are produced with a twofold increase in serum magnesium, although concurrent increases occur in serum calcium. Inhaled freshly generated magnesium oxide can cause metal fume fever, similar to that caused by zinc oxide. In non-occupationally exposed individuals, toxicity can occur when magnesium-containing drugs, usually antacids, are ingested chronically by persons with serious renal failure. The toxic effects may progress from nausea and vomiting to hypotension, electrocardiograph abnormalities, central nervous system effects, coma, and systolic cardiac arrest (Herroeder *et al*., 2011).

2.11.2 Manganese

Manganese (Mn) has an atomic weight of 54.94, an atomic number of 25 and a density of 7.21–7.44, depending on the allotropic form. Its melting and boiling points are is 1245°C and 2097°C respectively; it is a whitish grey metal, harder than iron, and very brittle. It belongs to group VIIB in the system of element and occurs in 11 oxidation states of which 2, 4, and 7 are the most important ones. Most manganese is mined in open pit or shallow underground mines. More than 80% of the high-grade manganese ore (>35% manganese) is mined in South Africa, and also Australia, Brazil, Gabon, and, India, and, more recently, China, and the Commonwealth of Independent States (CIS) (Marko and Roberto, 2007).

Manganese is an essential trace element that acts as a catalyst for several enzymes involved in carbohydrate, glycoaminoglycan, and cholesterol metabolism (TEHD, 1985). Manganese superoxide dismutase, is the principal antioxidant found in mitochondria and is responsible for the inactivation of superoxide radicals (Macmillan and Cruthirds, 2001).

The classes of enzymes that have manganese cofactors is large and includes oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins, and integrins. The reverse transcriptases of many retroviruses contain manganese. The best-known manganese-containing polypeptides may be arginase, the diphtheria toxin, and Mn-containing superoxide dismutase (Mn-SOD) (Law *et al.*, 1998). The manganese dietary reference intake for a 44 year old human male is 2.3 mg per day from food, with 11 mg estimated as the tolerable upper limit for daily intake to avoid toxicity. Estimates for females and children are generally less (DRI, 2004). The essential minimum intake is unknown since manganese deficiency is so rare. The human body contains about 12 mg of manganese, mostly in the bones. The soft tissue remainder is concentrated in the liver and kidneys (Emsley, 2001). In the human brain, the manganese is bound to manganese metalloproteins, most notably glutamine synthetase in astrocytes (Takeda, 2003).

Dietary manganese deficiency in experimental animals results in numerous biochemical and structural abnormalities. Manganese deficient animals have demonstrated impaired insulin production, lipoprotein metabolism, anti-oxidant defence mechanisms and perturbations in growth factor metabolism; deficiency also produced skeletal abnormalities and irreversible ataxia during early animal development (Keen *et al.*, 1999). In humans, experimental studies using manganese deficient diets have demonstrated mild glucose intolerance in young women (Johnson and Lykken, 1991) and a fleeting dermatitis with reduced cholesterol levels in male subjects (Friedman *et al.*, 1987). Epidemiology studies (Hall *et al.*, 1989) have linked low blood manganese levels with Perthes' disease, a vascular necrosis of the head of femur, in children in the Liverpool area of the United Kingdom. Low levels of whole blood manganese have also been associated with epilepsy although patients whose epilepsy was caused by trauma have shown significantly higher blood manganese levels (Carl *et al.*, 1986).

The body is protected against manganese toxicity primarily by low absorption and/or rapid pre-systemic elimination of manganese by the liver. However, manganese intoxication resulting in a neurotoxic syndrome known as Manganism may occur from industrial

exposure to the metal or as a complication of Total Parenteral Nutrition (TPN) in patients with hepatobiliary disease. Higher levels of exposure to manganese in drinking water are associated with increased intellectual impairment and reduced intelligence quotients in school-age children. It is hypothesized that long-term exposure to the naturally occurring manganese in shower water puts up to 8.7 million Americans at risk (Finley and Davies 1999; Elsner and Spangler, 2005).

2.11.3 Zinc

Zinc is a metallic chemical element with atomic number 30. It is the first element of group 12 of the periodic table. Its ion is of similar size and its only common oxidation state is +2. Zinc is the 24th most abundant element in the Earth's crust and has five stable isotopes. The most common zinc ore is sphalerite (zinc blende), a zinc sulphide mineral. The largest mineable amounts are found in Australia, Asia and the United States. Zinc production includes froth flotation of the ore, roasting, and final extraction using electricity (Debjit *et al.*, 2010)

2.11.3.1 Functions of zinc

The biological function of zinc are said to be numerous. On the cellular level, the function of zinc can be divided into three categories: Catalytic, Structural and Regulatory.

1. Catalytic Functions of Zinc

   Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc dependent enzymes can be found in all known classes of enzymes. (Debjit *et al.*, 2010)
2. Structural Functions of Zinc

   Zinc plays an important role in the structure of proteins and cell membrane. The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their functions. (Debjit *et al.*, 2010)
3. Regulatory Functions of Zinc

   Zinc finger proteins have been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signalling and has been found to influence hormone release and nerves impulse transmission. (Debjit *et al.*, 2010).

Others functions are:

4. Zinc affects multiple aspects of the immune system. It is needed for DNA synthesis and RNA transcription, cell division and cell activation. (Overberk *et al*., 2008, Prasad 2008)
5. It also functions as antioxidant and stabilizes membrane.
6. Zinc is critical to tissue growth, wound healing, taste, prostaglandin production, bone mineralization, proper thyroid function, blood clotting foetal growth and sperm distribution.

After iron, zinc is the most common natural trace element in the body. Its requirement is obligatory by virtue of the fact that the activity of over 200 enzymes involved in vital cellular processes (e.g. Alkaline Phosphatase, DNA polymerase, Alcohol Dehydrogenase, and Carbonic Anhydrase) need Zn as a cofactor/stabilizing ion. Zinc is also an important structural component of biomembranes and of certain eukaryotic DNA-binding proteins (e.g. zinc finger motifs (Moore and Ullman, 2003; Rhodes and Klug, 1993) which bind DNA transcription factors) and is thus important in protein-nucleic acid and protein-protein interactions. Zinc has also been found to play an important role in cell signalling and in nerve impulse transmission. The necessity for zinc is demonstrated by the fact that nutritional deficiency in this element is associated with growth and development failure, delayed sexual maturation, loss of taste and smell, night blindness, characteristic skin rashes, behaviour disturbances, poor wound healing and impaired immune function (Prasad, 1995).

The body contains about 2-3 g of zinc, with the bulk (over 80%) found in the bone and skeletal muscle. As with other elements, homeostatic mechanisms operate to maintain tissue/body zinc concentrations, despite fluctuations in dietary intake. The Recommended daily allowance (RDA) for zinc is age dependent and varies from ~ 2mg in neonates to 10-15mg in adults. Food sources include beans, nuts, certain types of seafood (such as crab and lobster), whole grains, fortified breakfast cereals, and dairy product. Normally only about 20-40% of dietary zinc is absorbed by the small intestine, via both saturable and non-saturable components. Uptake is believed to be confined to the mature enterocytes (Steel and Cousins, 1985). Members of the recently identified zinc/iron-regulated (ZIP) family of metal ion transporters are believed to mediate intestinal zinc influx: the expression of these zinc transporters is governed by dietary zinc levels (Eide, 2004).

Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhoea, delayed

sexual maturation, impotence, hypogonadism in males, and eye and skin lesions (Prasad, 2004; Wang and Busbey, 2005; Maret and Sandstead, 2006). Weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur (Krasovec and Fenk, 1996; Nishi, 1996; King and Cousins, 2005; Maret and Sandstead, 2006).

Zinc nutritional status is difficult to measure adequately using laboratory tests due to its distribution throughout the body as a component of various proteins and nucleic acids. Plasma or serum zinc levels are the most commonly used indices for evaluating zinc deficiency, but these levels do not necessarily reflect cellular zinc status due to tight homeostatic control mechanisms (Maret and Sandstead, 2006).

Zinc toxicity is rare and can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea, and headaches (Hopper *et al.*, 1980; Johnson *et al.*, 2007).

2.11.4 Copper

Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome C oxidase. Copper is present in every tissue of the body, but is stored primarily in the liver, with fewer amounts found in the brain, heart, kidney, and muscles. The main areas where copper is found in humans are liver, muscle and bone. (Johnson *et al.*, 2007). Copper in humans, is essential for the proper functioning of organs and metabolic process. Although copper is the third most abundant trace metal in the body (after iron and zinc), the total amount of copper in the body is only 75-100 milligrams (Willis *et al.*, 2005). Copper is absorbed in the gut and transported to the liver bound to albumin. It enters the bloodstream via the plasma protein called ceruloplasmin, where its metabolism is controlled, and is excreted in bile (Adelstein, 1961).

Approximately 90% of the copper in the blood is incorporated into ceruloplasmin, which is responsible for carrying copper to tissues that need the mineral (Adelstein, 1961). Since excretion of copper is so slow (10%in 72 hours) an excessive dose of Cu is a lingering problem.

In addition to the role of ceruloplasmin as a transport protein, it also acts as an enzyme; catalyzing the oxidation of minerals, most notably iron. The oxidation of iron by ceruloplasmin is necessary for iron to be bound to its transport protein- transferrin, so iron deficiency anaemia may be a symptom of copper deficiency (Araya, 2006). Nutritionally,

rich sources of copper include oysters, beef and lamb liver, Brazil nuts, blackstrap molasses, cocoa, and black pepper. Other sources include lobster, nuts and sunflower seeds, green olives, avocados, and wheat bran. Copper homeostasis is controlled mainly at the level of excretion (Linder, 2002). The average safe daily intake of dietary copper in adults is 1.5 –3.0 mg (24 - 47 μmol) (NRC, 2007).

Copper is known to play important role in body metabolism, largely because it allows many critical enzymes to function properly (Araya, 2006). Copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Also, it plays a role in the production of haemoglobin, myelin, melanin and it also keeps thyroid gland functioning normally (Araya, 2006). In addition, Copper can act as both an antioxidant and a pro-oxidant. As an antioxidant, Copper scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause (Araya, 2006).

When copper acts as a pro-oxidant at times, it promotes free radical damage and may contribute to the development of Alzheimer's disease. Maintaining the proper dietary balance of Copper, along with other minerals such as zinc and manganese, is important (Araya, 2006). A significant portion of the toxicity of copper comes from its ability to accept and donate single electrons as it changes oxidation, this catalyses the production of very reactive radical ions, such as hydroxyl radical. This catalytic activity of copper is used by the enzymes with which it is associated, thus is only toxic when unsequestered and unmediated. This increase in unmediated reactive radicals is generally termed oxidative stress and is said to be an active area of research in a variety of diseases where copper may play an important but more subtle role than in acute toxicity. Some of the effects of aging was also said to be associated with excess copper (Araya, 2006).

2.11.5 Selenium

Selenium with symbol Se and atomic number 34 belongs to the oxygen group in the periodic table. Although technically a non-metal, certain forms have metal-like properties. Selenium is an essential element found in selenoproteins and deficiency is recognized in humans and animals (Högberg and Alexander, 2007). It is also toxic and high doses cause overt selenium poisoning (*selenosis*). The availability and the toxic potential of selenium compounds are related to their chemical forms and, most importantly, to solubility. Selenium occurs in nature and biological systems as selenate (Se^{6+}), selenite (Se^{4+}), selenide (Se^{2+}), and elemental selenium (Se^{0}) (Fairweather-Tait *et al*., 2010).

As an essential metal, foods are a good source of selenium. Seafood (especially shrimp), meat, milk products, and grains provide the largest amounts in the diet. Levels of selenium in river water vary depending on environmental and geologic factors. Combustion of coal and other fossil fuels are the primary sources of airborne selenium compounds. Occupational exposure comes from selenium refining operations, metal smelting, and milling operations, incineration of rubber tires, and municipal waste. Rocks and soil, plants, and tobacco are other sources of selenium exposure (Högberg and Alexander, 2007; Fairweather-Tait *et al.*, 2010).

Metabolic balance studies in adults indicate about 50 to 70 µg per day is required to maintain selenium balance and presumably to satisfy selenium requirement (Högberg and Alexander, 2007).

Selenium is notable for its actions in antioxidant systems through involvement in over 20 selenoproteins (Högberg and Alexander, 2007; Fairweather-Tait *et al.*, 2010). For instance, glutathione peroxidase is the selenium-dependent enzyme that reduces peroxides using glutathione, and thereby protects membrane lipids, proteins, and nucleic acids from damage by oxidants or free radicals.

The most extensively documented deficiency of selenium in humans is Keshan disease. This is an endemic cardiomyopathy first discovered in Keshan County in China where there are very low concentrations of selenium in the soil and food. Keshan disease patients show very low plasma selenium levels. This deficiency occurs most frequently in children under 15 years of age and in women of childbearing age and is characterized by various degrees of cardiomegaly and cardiac decompensation. Deficiency of selenium also occurs in domestic animals and rodents. Selenium supplementation reduces these adverse effects (Fairweather-Tait *et al.*, 2010).

Kashin–Beck disease is an osteoarthropathy found in areas where combined deficiency of selenium and iodine occurs with elevated exposure to mycotoxin and fulvic acids (Fairweather-Tait *et al.*, 2010). Selenium deficiency is a major contributing factor in this disease. Other potential effects of selenium deficiency include immune dysfunction, and susceptibility to cancer or infectious/inflammatory diseases (Högberg and Alexander, 2007; Fairweather-Tait *et al.*, 2010).

Established tolerable upper intake limits of selenium in adults are 200 to 300 µg per day (Duffield-Lillico *et al.*, 2002; Högberg and Alexander, 2007). Symptoms of acute selenium

toxicity include nausea and vomiting, followed by pulmonary oedema and rapid cardiovascular collapse (Fairweather-Tait *et al.*, 2010). Chronic selenium toxicity (*selenosis*) can occur with environmental exposure when the intake exceeds the excretory capacity (Fairweather-Tait *et al.*, 2010). Some epidemiological data have linked low blood selenium levels and increased cancer risk in various populations (ATSDR, 2003a, b). Selenium supplementations appear to decrease human cancer rates, especially for prostate cancer (Duffield-Lillico *et al.*, 2002; Fairweather-Tait *et al.*, 2010). On the other hand, selenium sulphide is considered reasonably anticipated to be a human carcinogen based on multiple positive rodent studies where tumours of the lung or liver were produced after exposure via stomach tube (NTP, 2011).

2.11.6 Chromium

Chromium is a transition metal with the symbol Cr and atomic number 24; most naturally occurring chromium is found in the trivalent state in chromite ores, which are generally refined to ferrochromium or metallic chromium for use in industrial processes. Trivalent chromium (Cr^{3+}) is an essential trace nutrient important for glucose metabolism. Hexavalent chromium (Cr^{6+}) is rarely found in nature and is formed as a by-product of various industrial processes. Most chromite ores are processed to sodium dichromate, a hexavalent chromium compound, which is used as an oxidizing agent in stainless steel production and welding, chromium plating, ferrochrome alloys and chrome pigment production, and tanning industries (Ashley *et al.*, 2003).

Chromium is absorbed poorly in the diet. It is absorbed mainly in the small intestine by a pathway it appears to share with zinc. It is transported to tissues, bound to 'transferrin' and appears in liver mitochondria, microsomes and the cytosol. Serum level of chromium in normal healthy adult is about 6 to 20 µg/100 ml. Recommended daily requirement for an adult is approximately 50–200 µg/day. Chromium has being shown to play important role in the metabolism of carbohydrate, lipid and protein (Chatterjea and Shinde, 2012).

Hexavalent chromium is a human carcinogen and produces a variety of toxic effects (ATSDR, 2008). Chromium in ambient air originates primarily from industrial sources, particularly ferrochrome production, ore refining, and chemical processing. Chromium fallout is deposited on land and water, and, eventually, in sediments. Widespread industrial uses have increased chromium levels in the environment. The hexavalent chromium compounds are also toxic to ecosystems and microbial and plant variants occur that adapt

to high chromium levels in eco-environment (ATSDR, 2008). Toxic effects have been attributed primarily to airborne hexavalent chromium compounds in industrial settings. Hexavalent chromium is corrosive and may cause chronic ulceration and perforation of the nasal septum, as well as chronic ulceration of other skin surfaces (ATSDR, 2008). It elicits allergic contact dermatitis among previously sensitized individuals, which is a type IV allergic reaction inducing skin erythema, pruritus, oedema, papule, and scars. The prevalence of chromium sensitivity is less than 1% among the general population (Proctor *et al*., 1998). Occupational exposure to chromium may be a cause of asthma (Bright *et al*., 1997). Hexavalent chromium compounds are classified as known to be human carcinogens by the National Toxicology Program (NTP, 2011). Occupational exposure to hexavalent chromium compounds, particularly in the chrome production and pigment industries, is associated with increased risk of lung cancer and hexavalent chromium–containing compounds are considered to be human carcinogens (IARC, 1990).

2.11.7 Cobalt

Cobalt is a transition metal with the symbol Co and atomic number 27. Cobalt forms an integral part of vitamin B_{12} and is required as a constituent of this vitamin as such is an essential element. The metal is principally used to make high temperature, high strength alloys and for the preparation of tough carbide-tipped cutting and drilling tools. Cobalt is also used in the manufacture of semi-conductors, magnetic alloys and catalysts. Cobalt salts are highly coloured and have been used from the earliest times for pigment production. Dicobalt edetate is used in the treatment of cyanide poisoning. Vitamin B_{12} deficiency causes pernicious anaemia and is due to absence of intrinsic factor which is required for its absorption, or general malabsorption. There is little value in measuring cobalt for nutritional purposes (Taylor, 2007).

Occupational inhalation of cobalt-containing dust in industrial settings may cause respiratory irritation at air concentrations between 0.002 and 0.01 mg/m^3. Higher concentrations may be a cause of “hard metal” pneumoconiosis, a progressive form of pulmonary interstitial fibrosis. This disease is observed in workers exposed to cobalt–tungsten carbide but not observed with exposure to cobalt alone (ATSDR, 2004; NTP, 2011). Occupational dermal exposure is sometimes associated with an allergic dermatitis. The goitrogenic effect has been elicited by the oral administration of 3 to 4 mg/kg to children in the course of treatment of sickle cell anaemia. Intravenous exposure to cobalt can cause increased blood pressure, slowed respiration, tinnitus, and deafness due to nerve

damage. Cardiomyopathy with signs of congestive heart failure has been associated with excessive cobalt intake (>10 mg per day), particularly from drinking beer to which cobalt was added as a foaming agent (Lison, 2007). Based on experimental studies in animals, cobalt sulphate has been classified as reasonably anticipated to be carcinogenic in humans (NTP, 2011). In rodents, inhalation of cobalt sulphate induces lung tumours, including carcinoma, in rats and mice (Bucher *et al*., 1999). Repository injections or implantation of various cobalt compounds can produce local sarcomas in rodents (IARC, 1991).

Incidence of adrenal gland tumours was also increased in female rats expose to cobalt sulphate (NTP, 2011). Cobalt–tungsten carbide powders and hard metals are reasonably anticipated to be human carcinogens based on limited evidence of carcinogenicity from human studies and supporting evidence from studies on mechanisms of carcinogenesis (NTP, 2011).

2.12 Enzymatic antioxidant biomarkers

Generally, enzymes are biological catalysts of protein origin that speeds up the rate of biochemical reactions. Enzymatic system directly/indirectly contributes to defence against the reactive oxygen species (ROS). Enzymatic antioxidants catalyse the degradation of toxic products of oxidation. Important enzymes in this regard include; Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin reductase, haem oxygenase and biliverdin reductase (Noori, 2012).

2.12.1 Superoxide Dismutase

Superoxide dismutases (SOD, EC 1.15.1.1) are enzymes that catalyze the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide. Thus they are an important antioxidant defence in nearly all cells exposed to oxygen, they are proteins co-factored with copper and zinc, or manganese, iron, or nickel (Noori, 2012). Generally, the SOD-catalysed dismutation of superoxide may be written with the following half-reactions:

$$M^{(n+1)+}\text{-SOD} + O_2^- \longrightarrow M^{n+}\text{-SOD} + O_2$$

$$M^{n+}\text{-SOD} + O_2^- + 2H^+ \longrightarrow M^{(n+1)+}\text{-SOD} + H_2O_2.$$

Where M = Cu (n=1); Mn (n=2); Fe (n=2); Ni (n=2).

In this reaction the oxidation state of the metal cation oscillates between n and n+1 (Vanaporn *et al*., 2011). In biological systems, the main reactions of superoxides are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (approximately 10^5 $M^{-1}s^{-1}$ at pH 7). SOD is necessary because superoxide reacts with sensitive and critical cellular targets to cause pathological condition. Superoxide dismutase has the largest k_{cat}/K_M (an approximation of catalytic efficiency) of any known enzyme (approximately 7×10^9 $M^{-1}s^{-1}$), this reaction being limited only by the frequency of collision between itself and superoxide. That is, the reaction rate is "diffusion-limited" (Heinrich *et al*., 2006).

Depending on the cofactor of the SOD enzyme, there are three major families of SOD: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and the Ni type, which binds nickel. The Cu/Zn-SOD is used by the eukaryotes (Richardson *et al*., 1975). Copper and zinc is most commonly used by eukaryotes. The cytosol of virtually all eukaryotic cells contains an SOD enzyme with copper and zinc (Cu-Zn-SOD). For example, Cu-Zn-SOD available commercially is normally purified from the bovine erythrocytes- the Cu-Zn enzyme is a homodimer of molecular weight 32,500. The bovine Cu-Zn protein was the first SOD structure to be solved in 1975 (Richardson *et al*., 1975). It is an 8-stranded beta-barrel, with the active site held between the barrel and two surface loops. The two subunits are tightly joined back-to-back, mostly by hydrophobic and some electrostatic interactions. The ligands of the copper and zinc are six histidine and one aspartate side-chains; one Histidine is bound between the two metals (Tainer *et al*., 1983).

2.12.2 Catalase (CAT)

Catalase is a common enzyme (with an EC number of 1.11.1.6) found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al*., 2004). It is a very important enzyme in protecting the cell from oxidative damage by ROS. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2004). Catalase was first noticed in 1818 when Louis Jacques Thénard, who discovered H_2O_2 (hydrogen peroxide), suggested its breakdown is caused by an unknown substance

(Davies *et al.*, 1979). In 1900, Oscar Loew was the first to give it the name catalase, and found it in many plants and animals (Loew, 1900).

It is mainly localized in the peroxisomes but is also found in the cytoplasm and mitochondria in minor amounts (Davies *et al.*, 1979). It has been reported that catalase has a higher Km (Michealis Costant for enzyme kinetics) than GPx, which suggests a major role for CAT at higher levels of H_2O_2 but a minor role at physiological levels of H_2O_2 (Halliwell and Gutteridge, 1985). The optimum pH for human catalase is approximately 7, and has a fairly broad maximum (the rate of reaction does not change appreciably at pH between 6.8 and 7.5). The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species (Aebi, 1984).

The reaction of catalase with H_2O_2 occurs in two stages and these involve the haem group of the enzyme. In the first step, one hydrogen peroxide molecule oxidizes the haem to an oxy-ferryl species, in the second step, a second hydrogen peroxide molecule is used as a reductant to regenerate the enzyme, producing water and oxygen (Halliwell and Gutteridge, 1985).

$$H_2O_2 + \text{Fe- (III) -E} \longrightarrow H_2O + \text{O - Fe-(IV) - E (+)}$$

$$H_2O_2 + \text{O-Fe- (IV) -E (+)} \longrightarrow H_2O + \text{Fe (III) - E} + O_2$$

Fe (III)-E represents the iron center of the haem group attached to the enzyme. Fe (IV)-E (+) is a mesomeric form of Fe (V) -E, meaning the iron is not completely oxidized to +V, but receives some "supporting electrons" from the haem ligand. This haem has to be drawn then as a radical cation (+) (Halliwell and Gutteridge, 1985).

2.12.3 Glutathione Peroxide (GPx)

Glutathione peroxidase (GPx) (EC 1.11.1.9) is the general name of selenium-dependent enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. It was discovered in 1957 by Gordon C. Mills (Mills, 1957).

The main reaction that glutathione peroxidase catalyzes is the reduction of oxidised glutathione (GS-SG) to reduced glutathione (GSH). Glutathione is oxidised when it reacts with H_2O_2 to form H_2O.

$$2GSH + H_2O_2 \longrightarrow GS–SG + 2H_2O$$

The mechanism involves the oxidation of the selenol of a selenocysteine residue by hydrogen peroxide. This process gives the derivative with a seleninic acid (RSeOH) group. The selenenic acid is then converted back to the selenol (RSeH) by a two step process that begins with reaction with GSH to form the GS-SeR and water. A second GSH molecule reduces the GS-SeR intermediate back to the selenol, releasing GS-SG as the by-product (Muller *et al*., 2007). A simplified representation is shown below

$$RSeH + H_2O_2 \longrightarrow RSeOH + H_2O$$
$$RSeOH + GSH \longrightarrow GS\text{-}SeR + H_2O$$
$$GS\text{-}SeR + GSH \longrightarrow GS\text{-}SG + RSeH$$

Glutathione reductase then reduces the oxidized glutathione to complete the cycle:

$GS\text{-}SG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$ (Muller *et al*., 2007).

Several isozymes of glutathione peroxidase are encoded by different genes, which vary in cellular location and substrate specificity. Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. Glutathione peroxidase 4 (GPx4) has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels. Glutathione peroxidase 2 is an intestinal and extracellular enzyme, while glutathione peroxidase 3 is extracellular, especially abundant in plasma (Muller *et al*., 2007). So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans (Muller *et al*., 2007).

2.12.4 Glutathione Reductase

Glutathione reductase (GR), also known as glutathione-disulfide reductase (GSR) is an enzyme that in humans is encoded by the GSR gene. Glutathione reductase (EC 1.8.1.7) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell (Meister, 1988). Glutathione reductase functions as dimeric disulfide oxido-reductase and utilizes an FAD prosthetic group and NADPH to reduce one mole of GSSG to two moles of GSH. Glutathione plays a key role in maintaining proper function and preventing oxidative stress in human cells. It can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles (Muller *et al*., 2007). Reduced glutathione reduces the oxidized form of the enzyme glutathione

peroxidase, which in turn reduces hydrogen peroxide (H_2O_2), a dangerously reactive species within the cell. In addition, it plays a key role in the metabolism and clearance of xenobiotics, acts as a cofactor in ceratin detoxifying enzymes, participates in transport, and regenerates antioxidants such and Vitamins E and C to their reactive forms. The ratio of GSSH/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell, that is, it is critical that the cell maintains high levels of the reduced glutathione and a low level of the oxidized Glutathione disulfide. This narrow balance is maintained by glutathione reductase, which catalyzes the reduction of GSSG to GSH (Meister, 1988).

The reaction catalysed by glutathione reductase is the reduction of oxidised form of glutathione to the reduced form, this is very important to ensure a normal pool of the antioxidant glutathione. The figure below shows the mechanism of reaction of glutathione reductase. Glutathione reductase and glutathione peroxidase work in synergism to protect cells from oxidative stress in the cycle called glutathione redox cycle. Glutathione reductase was first purified in 1955 at Yale by E. Racker (Racker, 1955).

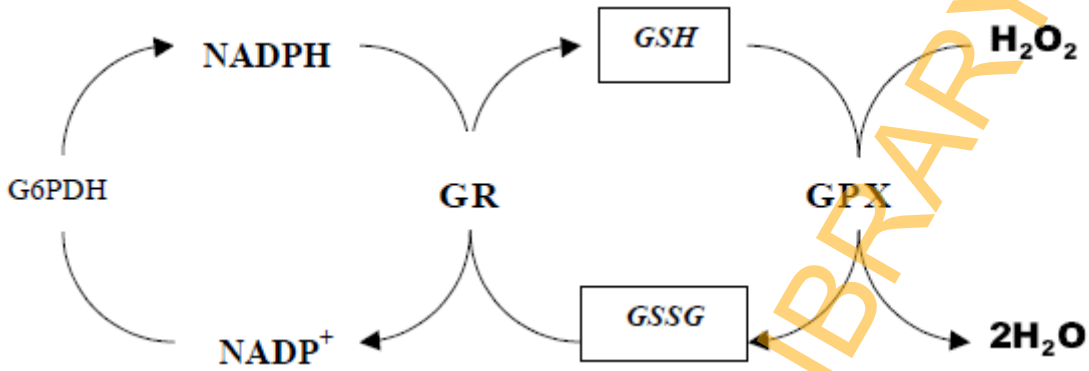


Fig. 2.7 The glutathione redox cycle. G6PDH = glucose-6-phosphate dehydrogenase, GR = glutathione reductase, GSSG = oxidized glutathione, GPx = glutathione peroxidase and GSH = glutathione (Jomova and Valko, 2010).

2.12.5 Gamma-glutamyltransferase

Gamma-glutamyltransferase (GGT), also called transpeptidase, is an enzyme found in the liver, kidney, and pancreas with the highest value in the kidney than the liver. It has an enzyme code number EC 2.3.2.2. It is not found in the bone and muscle (Sheehan and Haythom, 1979).

Gamma glutamyl transferase plays an important role in amino acid transport in the course of glutathione metabolism. It catalyzes the transfer of γ glutamyl group of amino acid and short peptide. It is more useful in liver diagnosis than ALP as it is more specific but ALP is more sensitive. Normal range is between 0-51U/L. It remains normal in bone disease even as ALP rises, hence it used to rule out bone disease (Lum and Gambino, 1972), but it rises along with ALP in hepatobiliary injury. Hence, GGT is a specific biomarker of hepatobiliary injury, especially cholestasis and biliary effects (Sheehan and Haythom, 1979).

2.13 Non-enzymatic biomarkers of oxidative stress

Non-enzymatic antioxidants scavange ROS and reactive nitrogen species (RNS) and some of them are glutathione, vitamins E, A and C. Also, uric acid is the scavenger of peroxynitrite in plasma. Essential metals such as zinc and selenium; polyphenols such as flavonoids, phenolic acid, gingerol, and curcumin as well as proteins (albumin, bilirubin and caeruloplasmin) have been shown to have antioxidant potentials (Esterbauer *et al*., 1991; Mazza *et al*., 2001; Weir *et al*., 2003).

2.13.1 Malondialdehyde (MDA)

Reactive intermediates produced under conditions of oxidative stress cause the oxidation of polyunsaturated fatty acids (PUFAs) in membrane lipid bilayer, leading eventually to the formation of aldehydes. Among these, the most abundant aldehydes are 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA), while acrolein is the most reactive one (Esterbauer *et al*., 1991). Even though MDA shows little reactivity under physiological conditions, at low pH its reactivity increases, when beta-hydroxyacrolein becomes the predominant species and, analogously to acrolein and HNE, MDA can form 1, 4-Michael type adducts with nucleophiles (Esterbauer *et al*., 1991). The accepted markers for oxidative stress are aldehydic secondary products MDA and 4-HNE (Mattill, 1947). Michael's reaction is the 1, 4-addition of a doubly stabilized carbon nucleophiles to α, β-unsaturated carbonyl compound. The reactants are called Michael acceptor and Michael

donor respectively. Phosphatidylcholine γ-hydroxyalkenals (PC-HAs) are the most abundant and biologically relevant compounds in the class of γ-hydroxyalkenal phospholipids, deriving from the peroxidation of polyunsaturated fatty acids (PUFAs) esterified to phosphoglycerides at the *sn*-2 position of phosphatidylcholine (Mattill, 1947).

2.13.2 Uric Acid (UA)

Uric acid is a Nitrogenous compound with the chemical formular $C_5H_4N_4O_3$ and name 2, 6, 8-tridroxylpurine. In human, uric acid is the major product of catabolism of the purine nucleosides; adenosine and guanosine. The bulk of purine excreted as uric acid arises from catabolism of endogenous nucleic acids. The daily synthesis of uric acid is approximately 400mg while dietary sources contribute 300mg (Kellogg and Fridovich, 1977). Uric acid has been said to play important antioxidant role in the body, studies has also proven this. Kellogg and Fridovich originally described the ability of urate to scavenge oxygen radicals and protect the erythrocyte membrane from lipid oxidation (Kellogg and Fridovich, 1977). Ames and co-worker also shows the protective ability of urate against peroxidation of erythrocyte membrane ghosts induced by t-butylhydroperoxide. (Ames *et al*., 1985). One of the major sites where the anti-oxidant effects of uric acid have been proposed is in the central nervous system, particularly in conditions such as multiple sclerosis, Parkinson's disease, and acute stroke (Amaro *et al*., 2007; Duan *et al*., 2002). While chronic elevations in uric acid are associated with increased stroke risk, acute elevations in uric acid may provide some anti-oxidant protection. For example, uric acid protects cultured rat hippocampal neuronal cells from oxidative stress and administration of uric acid 24 hours prior to middle artery occlusion also attenuated brain injury induced by acute ischemia in rats (Weir *et al*., 2003; Mazza *et al*., 2001). Uric acid administration was also beneficial in the mouse model of experimental allergic encephalomyelitis (EAE) that has been proposed to have similarities to multiple sclerosis. In EAE uric acid was found to block peroxynitrite ($ONOO^-$) mediated nitrosylation of neuronal proteins and to block the increase in the blood brain barrier resulting in less infiltration of leukocytes (Hopper *et al*., 1998). Although uric acid can act as an antioxidant, excess serum accumulation is often associated with cardiovascular disease. It is not known whether this is causative or a protective reaction taking advantage of urate's antioxidant properties. The same may account for the putative role of uric acid in the aetiology of stroke (Toncev, 2006). However, even in the plasma, urate can prevent lipid peroxidation only as long as ascorbic acid is present (Frei *et al*., 1988).

2.13.3 Total Glutathione

Glutathione (GSH) is an important antioxidant in plants, animals, fungi, and some bacteria and archaea. Glutathione is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals (Pompella *et al.*, 2003). Decreased levels of GSH after exposure to metals such as arsenic have been reported. It was reported that one hour after exposure to arsenic (15.86 mg/kg body wt), the GSH concentration was significantly decreased in the liver of male Wistar rats (Maiti and Chatterjee, 2001). After 6 months exposure to arsenic (3.2 mg/l), hepatic GSH and the enzymes glucose-6-phosphate dehydrogenase and GPx were significantly lowered in mice. GSH plays an important role in maintaining cellular redox status and its GSH level is considered a significant marker of oxidative stress.

Glutathione levels are highly correlated with the cellular redox status induced by metals. Generally, there are three pathways by which metals can decrease cellular levels of GSH. In the first pathway GSH possibly acts as an electron donor for the reduction of pentavalent to trivalent metallic ions such as arsenicals. Secondly, metals with high affinity for GSH can directly bind to it and inhibit it. The third pathway involves oxidation of GSH by metal-induced generation of free radicals. Taken together, exposure to toxic levels of metals is likely to cause depletion of GSH level (Varfolomeev and Ashkenazi, 2004).

2.13.4 Albumin

Albumin is a plasma protein containing 585 amino acids and has a molecular weight of 66 kDa. This highly soluble protein is present in human plasma at normal concentrations between 35 and 50 g/L (El-Benna *et al.*, 2005). Albumin is synthesized by the liver and has several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, bile pigments, and drugs. It is a key factor in the regulation of osmotic pressure and distribution of fluid between different compartments. In normal conditions, its half-life is about 20 days, and its plasma concentration represents equilibrium not only between its synthesis in the liver and its catabolism, but also its transcapillary escape. In general, albumin represents the major and predominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress. A large proportion of total serum antioxidant properties can be attributed to albumin. Studies have shown that more than 70% of the free radical-trapping activity of serum was due to human serum

albumin (HSA) as assayed using the free radical-induced haemolysis test (Kerr *et al*., 1999).

Free Cu (II) and Fe (II) ions can interact with hydrogen peroxide (H_2O_2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. Bound to proteins, copper and iron are generally less susceptible to participate in the Fenton reaction. Concerning metals bound to albumin, hydroxyl radicals released from Fenton reaction are mostly directed to the protein sparing more important targets. In plasma, most of the copper is bound to caeruloplasmin, but a high percentage of the metal ion may exist bounded to albumin (Vorbach *et al*., 2003). HSA contains one high affinity site for copper, the N-terminal tripeptide Asp-Ala-His (Gomez-Cabrera *et al*., 2003). Protein sequestration of Cu (II) ions has been shown to prevent ROS-generating reactions. The first four amino acids of the N-terminus of human albumin, Asp-Ala-His-Lys (DAHK), form a tight-binding site for Cu (II) ions. Despite iron being present at higher physiological concentrations, copper can react with H_2O_2 to form hydroxyl radicals 60 times faster than iron (Judge and Dodd, 2004). HSA contains one reduced cysteine residue (Cys34) which, due to the large amount of albumin in plasma, constitutes the largest pool of thiols in the circulation. In healthy adults, about 70–80% of the Cys34 in albumin contains a free sulphydryl group; the rest forms a disulfide with several compounds like cysteine, homocysteine, or glutathione (Peterhans, 1997).

2.13.5 Total Bilirubin and Conjugated Bilirubin

Bilirubin, the end product of haem catabolism in mammals, is generally regarded as a potentially cytotoxic, lipid-soluble waste product that needs to be excreted. Bilirubin, at micromolar concentrations in vitro, efficiently scavenges peroxyl radicals generated chemically in either homogeneous solution or multi-lamellar liposomes. The antioxidant activity of bilirubin was found to increase as the experimental concentration of oxygen decreased from 20% (that of normal air) to 2% (physiologically relevant concentration) (Stoker *et al*., 1987). Furthermore, below 2% oxygen in liposomes bilirubin suppresses the oxidation more than alpha-tocopherol, which is regarded as the best antioxidant of lipid peroxidation (Stoker *et al*., 1987). This work of Stocker *et al* supports the idea of a beneficial role for bilirubin as a physiological, chain-breaking antioxidant. It has also been shown in studies (Stocker *et al*., 1987; Stocker *et al* 2005) that bilirubin, when bound to human albumin and at concentrations present in normal human plasma, protects albumin-bound linoleic acid from peroxyl radical induced oxidation in vitro. Initially, albumin-

bound bilirubin (Alb-BR) is oxidized at the same rate as peroxyl radicals are formed and biliverdin is produced stoichiometrically as the oxidation product. On an equimolar basis, Alb-BR successfully competes with uric acid for peroxyl radicals but is less efficient in scavenging these radicals than vitamin C. These observations of Stocker and is co-workers showed that 1 mole of Alb-BR can scavenge 2 moles of peroxyl radicals and that small amounts of plasma bilirubin are sufficient to prevent oxidation of albumin-bound fatty acids as well as of the protein itself (Stocker *et al*., 1987; Stocker *et al* 2005). The observation indicates a role for Alb-BR as a physiological antioxidant in plasma and the extravascular space.

2.14 Antioxidant vitamins

2.14.1 Vitamin A

Vitamin A is the nutritional term for the group of compounds with a 20-carbon structure containing a methyl-substituted cyclohexenyl ring (β-ionone ring) and an isoprenoid side chain. The group includes such nutritional organic compounds as retinol, retinal, retinoic acid, and several pro-vitamin A carotenoids (most notably, beta-carotene) (Fennema, 2008). It is important for growth and development, for the maintenance of the immune system and good vision (Tanumihardjo, 2011). Vitamin A is needed by the retina of the eye in the form of retinal, which combines with protein opsin to form rhodopsin, the light-absorbing molecule (Wolf, 2001), necessary for both low-light (scotopic vision) and colour vision. Vitamin A also functions in a very different role as retinoic acid (an irreversibly oxidized form of retinol), which is an important hormone-like growth factor for epithelial and other cells (Tanumihardjo, 2011).

2.14.2 Vitamin C

Vitamin C (L-ascorbate) is a hydrophilic molecule, and, therefore, it is found mostly in bodily fluids. Vitamin C is abundant in fruits and vegetables and they serve as the main source for dietary vitamin C intake. However, modern food processing methods lead to the loss of vitamin C, as well as many other vitamins and nutrients (Deruelle and Baron, 2008). Isolated in 1928, vitamin C was recognized as the bioactive molecule that was missing in the diet of sailors, causing scurvy (Baron, 2009).

Vitamin C is known to take part in many physiological processes, and has been proposed to have a beneficial or therapeutic role in immune responses, cardiovascular disease and cancer (Deruelle and Baron, 2008). The unique structure of L-Ascorbate that includes two

adjacent hydroxyl groups and a carbonyl makes this molecule an excellent hydrogen or electron donor. Therefore, it takes part as a co-factor in many enzymatic reactions, and also acts as a plasma localized anti-oxidant. Once oxidized, ascorbate is turned into ascorbate free radical (AFR), a molecule that is relatively stable due to electron delocalization. Although AFR can donate another electron, it does not undergo further oxidation. Rather, it is reduced back to ascorbate via NADH-dependent and independent mechanisms. The accumulation of AFR, resulting from increased oxidative conditions, leads to a reaction between two AFR molecules that form one molecule of ascorbate and one molecule of dehydroascorbate (DHA). The DHA itself can either be reduced back to ascorbate, or hydrolyzed to gulonic acid (Aguirre and May, 2008). L-Ascorbate fulfils the requirements of an antioxidant, since it can react with radicals and terminate their reaction. Indeed, in the cellular environment where its concentrations are high and recycling mechanisms are abundant, L-ascorbate protects the cell from oxidative stress (Carr and Frei, 1999).

However, L-ascorbate radical can also serve as an electron donor, and actually accelerate redox reactions in the presence of transition metals such as iron or copper. Thus, in the atherosclerotic plaque where ferric iron is present, vitamin C might serve as a pro-oxidant rather than as an anti-oxidant (Stocker and Kaeney, 2005).

2.14.3 Vitamin E

Vitamin E is a group of eight antioxidant lipophilic molecules, four of which are tocopherols and four are tocotrienols. It is mostly found in green vegetables, grains, nuts and various vegetable oils, as well as in eggs and milk (Dan *et al*., 2010). Although it is commonly known today for its antioxidant properties, the first biological role attributed to vitamin E was its necessity for foetal survival. Today vitamin E is known to possess many biological properties, including antioxidant activity and the ability to modulate protein function and gene expression (Dan *et al*., 2010). All vitamin E compounds are lipophilic. The lipophilicity of the compounds is attributed to their hydrophobic tail being a lipophilic molecule; vitamin E is most abundant in lipid phase compartments such as the plasma membrane and lipoproteins. It is also found in the membranes of cellular organelles and most notably in the lysosome and the golgi membrane, where its concentration is more than ten times higher than in other membranes (Zhang e*t al*., 1996).

Vitamin E is classified as an antioxidant due to its ability to scavenge lipid radicals and terminate oxidative chain reactions. It can terminate radical chain reactions by interacting with the lipid peroxyl radical, preventing it from generating a new radical and perpetuating

the chain reaction by oxidizing other lipids. Following its oxidation, vitamin E can be recycled back to its native un-oxidized form by various soluble antioxidants such as vitamin C and ubiquinol. This process prevents the accumulation of vitamin E radicals and their subsequent peroxidation of lipids (Brigelius-Flohe, 2009), and is considered by some to be critical for the antioxidant activity of vitamin E (Carr *et al*., 2000). It has been suggested that all of the other biological functions of vitamin E are actually a result of its antioxidant activity (Traber and Atkinson, 2007).

2.15 Calcium and 25(OH) Vitamin D

2.15.1 Calcium

Calcium is a very important macro element, total calcium is absorbed and released by the intestines, bones, and kidneys and is biologically regulated by hormones and vitamins. The calcium ion is an essential structural component of the skeleton and plays a key role in muscle contraction, blood coagulation, enzyme activity, neural excitability, secondary messengers, hormone release, and membrane permeability. Precise control of calcium ion in extracellular fluids is vital to health. Three major hormones (parathyroid hormone, vitamin D, and calcitonin) interact to maintain a constant concentration of calcium, despite variations in intake and excretion. Other hormones, such as adrenal corticosteroids, estrogens, thyroxine, somatotropin, and glucagon, may also contribute to the maintenance of calcium homeostasis. Plasma calcium level depends on the exogenous intake (feed), resorption, excretion and the functional state of the kidneys (Rodica *et al*., 2013).

Calcium in plasma or serum exists in three forms or fractions: ionized or free calcium, calcium bound to proteins (primarily albumin), and complexed or chelated calcium, bound to a variety of anions with small molecular weight (phosphate, bicarbonate, sulfate, citrate, and lactate) (Burnett *et al*., 2000). Together, the ionized and complexed calcium constitute the diffusible fraction of calcium. This portion may also be called the ultrafilterable calcium, since it passes through biologic membranes. Ionized calcium is the physiologically active fraction of serum calcium, with important biological roles in bone homeostasis, facilitates the transmission of the nerve impulse, couples the excitation with the muscle contraction, interferes in the blood clotting and control of hormone secretions such as vitamin D_3 and parathyroid hormone, reduces the membrane permeability, activates metabolic and digestive enzymes, influences the utilization of iron (Fujita, 2006). About 90% of the protein-bound calcium is linked to albumin with the remaining 10% bound to a variety of globulins.

2.15.2 25(OH) Vitamin D

Cholecalciferol also known as vitamin D with a molecular weight of 384.62 Dalton, is a fat soluble sterol compound that occurs naturally, chiefly in animals, plants and yeasts. Cholecalciferol is generated from the provitamins: ergosterol and 7-dehydrocholesterol which are found in plants and animals respectively. Ultraviolet irradiations of a variety of animal and plant sterols result in the conversion of provitamins to compounds with cholecalciferol activities (Silver *et al*, 1986; Wolf, 2004). The hormonally active form of cholecalciferol: 1, 25-dihydroxycholecalciferol is formed by hydroxylation using 25-hydroxylase in the liver and finally 1- α -hydroxylase in the kidney. Its physiological functions include calcium homeostasis and bone metabolism. Synthesis in the skin involves ultraviolet beta-radiation which effectively penetrates only the epidermic layers of the skin, where 7-dehydrocholesterol absorbs ultraviolet light at wavelength between 270-300nm, optimal synthesis occur in a narrow band of ultraviolet beta spectrum between 295-297nm. The two most important factors that govern the generation of pre-vitamin D are the quantity (intensity) and quality (appropriate wavelength) of ultraviolet beta irradiation reaching the 7-dehydrocholesterol deep in stratum basale and stratum spinosum. The 7-dehydrocholesterol, a derivative of cholesterol is photolysed by ultraviolet light in the 6-electron conrotatory (in conrotatory mode, of electrocyclic reaction both atomic orbitals of the end groups turn in the same direction such as both atomic orbitals rotating clockwise or counter-clockwise)) electrocyclic reaction. The product is pre-vitamin D3, then spontaneously isomerises to vitamin D_3 in an antarafacial hydride (Silver *et al.*, 1986; Wolf, 2004) sigmatropic shift. At room temperature, the transformation of pre-vitamin D_3 to vitamin D_3 takes about 12 days to complete. Adequate amount of cholecalciferol can be made in the skin after only 10-15 minutes of sun exposure at least 2 times per week to the face, hands, back and other parts without sunscreen (Wolf, 2004).

A critical determinant of vitamin D_3 production in the skin is the presence and concentration of melanin. Melanin functions as a light filter in the skin and therefore the concentration in the skin is related to the ability of ultraviolet-B light to penetrate the epidermal strata and reach 7-dehydrocholesterol containing stratum basale and stratum spinosum. Under normal circumstances, about 25-50 micrograms of 7-dehydrocholesterol per centimetre of skin are available in the stratum basale and stratum spinosum to meet the body's vitamin D requirement (Matsuoka *et al.*, 1991; Zittermann, 2003; Holick *et al.*, 2007).

The individual with higher skin melanin content will simply require more time in sunlight to produce the same amount of vitamin D as an individual with lower melanin content. Exposure to sunlight for extended period of time does not normally cause vitamin D toxicity. This is because within 20 minutes of ultraviolet exposure in light-skinned individual (3-6 times longer for pigmented skin) the concentration of vitamin D precursor produced in the skin reaches an equilibrium and any further vitamin D produced is degraded (Holick, 2009). Production is 250 µg (10,000 International Units)/day (Vieth, 1999). The recommended dietary allowance for vitamin D for infants and children is 400 units, or 10µg per day. On the basis that adults require less vitamin D than infants, the adults dose was set arbitrarily at 200 International Units per day.

2.16 Biomarkers of genotoxicity

2.16.1 Tumour Suppressor Protein

Tumour Suppressor Protein (p53), also known as **TP53** or **tumour protein** is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumour suppressor. It is very important for cells in multicellular organisms to suppress cancer. Tumour suppressor protein has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation (Strachan and Read, 1999). The name is due to its molecular mass, as it is in the 53 kilodalton fraction of cell proteins. It plays an important role in cell cycle control and apoptosis. Defective p53 could allow abnormal cells to proliferate, resulting in cancer; as many as 50% of all human tumours contain p53 mutants (Blagosklonny, 2002). In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the last resort to avoid proliferation of cells containing abnormal DNA. The cellular concentration of p53 must be tightly regulated. While it can suppress tumours, high level of p53 may accelerate the aging process by excessive apoptosis. The major regulator of p53 is the mouse double minute 2 (MDM-2) homolog, also known as E3 ubiquitin-protein ligase. In brief, MDM-2 is a protein that in humans is encoded by the MDM2 gene. It can trigger the degradation of p53 by the ubiquitin system MDM-2 and it is an important negative regulator of p53 (Blagosklonny, 2002).

If the $_{P}$53 gene is damaged, tumour suppression is severely reduced. People who inherit only one functional copy of $_{P}$53 will most likely develop tumours in early adulthood, a disease known as Li-Fraumeni syndrome (Blagosklonny, 2002; Samantha *et al*., 2013). It is known that $_{P}$53 can also be damaged in cells by mutagens (chemicals, radiation or viruses), increasing the likelihood that the cell will begin uncontrolled division. More than 50 percent of human tumours contain a mutation or deletion of the p53 gene. In health, p53 is continually produced and degraded in the cell. The degradation of p53 is, as mentioned, associated with MDM-2 binding. In a negative feedback loop MDM-2 is itself induced by p53. However mutant p53 type often does not induce MDM-2, and are thus able to accumulate at very high concentrations. Worse, mutant p53 protein itself can inhibit normal p53 (Blagosklonny, 2002).

Samantha *et al*., (2013), experimented the effect of metal on the expression of p53, they reported a very strong upregulation of the transcriptional factor p53 by a 24-hour treatment with 400µM Co (II) prompted us to investigate the presence of apoptotic responses.

Metals have also been described to interactions with zinc finger proteins, notably DNA repair proteins, transcription factors, and tumour suppressors, which are thought to be more relevant for metal-mediated carcinogenesis rather than direct binding to DNA (Beyersmann and Hartwig, 2008). Possible mechanisms for zinc finger-interference by carcinogenic metals include isostructural substitution, replacement with altered geometry, mixed complex formation, and catalysis of thiol oxidation (Hartwig, 2001). These modes related to metal carcinogenesis are dedicated to the altered gene expression (Sunderman and Barber, 1988). Definite zinc finger proteins might be regarded as predictive direct biomarkers for the initiation of cancer.

2.16.2 8-Hydroxy-2-Deoxyguanosine

Generally, metals toxicity have been linked to diseases and their effects on the major biomolecules (carbohydrate, protein, lipids and nucleic acids) have been explained (Kaur *et al*., 2011; Valko *et al*., 2005; Steinberg, 1995; Stadtman and Oliver, 1991). Nucleic acids (especially DNA) play crucial role as the cells genetic make-up and the mean of transferring parental character to the offspring (Chatterjea and Shinde, 2012). Therefore, any damage to the DNA may cause great abnormality.

The molecular damage formation after exposure to metals is still not well understood. Direct oxidation of DNA, generation of reactive oxygen species and consequential

production of lipid peroxides which also attack DNA are proposed pathological mechanisms by which metal toxicity damages DNA (Takane *et al.*, 2002; Swaran and Vidhu, 2010).

Igharo *et al*., (2016), reported elevated uric acid in participants occupationally exposed to toxic metals in e-waste, the study also suggested that the elevated uric acid may be a consequence of DNA damage. Uric acid, being an end product of purine (Adenosine and Guanosine) metabolism; adenosine and guanosine are released from DNA in their triphosphate forms (Chatterjea and Shinde, 2012).

Another effect of excess ROS on DNA is the activation of specific metabolic systems, e.g. calcium-dependent endonucleases, which split the DNA chain. Damaged DNA becomes more immunogenic, causing autoantibody production. Studies on hypoxanthine/xanthine oxidase-dependent damage to DNA structure in the presence of calcium ions identified products which may be used as oxidative DNA damage markers. These are 5, 6-dihydrocytozine, 4, 6-diamino-5-formamidopirymidine, 2, 6-diamino-4-hydoxy-5-form-amidopirymidine, 8-hydroxyadenine, 8-hydroxyguanine, and 8-hydroxy-2´-deoxyguanosine (8-OHdG) (Takane *et al*., 2002).

It is known that 8-OHdG is a modified nucleoside base, which is the most commonly studied and detected product of DNA damage that is excreted in urine upon DNA repair (Wu *et al*., 2004). Also, 8-OHdG is one of the most abundant base modifications and has attracted special attention because it is premutagenic, causing G-to-T transversions; thus, the presence of 8-OHdG may lead to mutagenesis. Moreover, the repair process for 8-OHdG–inflicted damage results in excised 8-OHdG adduct being excreted into the urine (Takane *et al*., 2002; Shigenaga *et al*., 1989). Partly due to easy collection, urinary 8-OHdG is thus regarded as a suitable biomarker of oxidative stress (Toraason 1999; Wong *et al*., 2003).

2.16.3 8-Oxoguanine DNA Glycosylase (OGG_1)

The pathophysiology of metals depends primarily on the generation of oxidative stress, which is characterized by increased ROS and Reactive Nitrogen Species (RNS) production; depletion of intracellular antioxidant stores and free-radical scavengers and inhibition or reduction of the activity of enzymes that contribute significantly to the metabolism and detoxification of reactive oxygen species (Arif *et al*., 2015).

One of such radicals is hydroxyl (·OH) radical, the most powerful oxidising radical, is responsible for causing damage to most biomolecules. In nucleic acids, both nitrogenous bases and sugars are highly susceptible to attack by electrophiles, particularly hydroxyl (·OH) radical. Upon interaction with the sugar moiety of DNA, ·OH radical cause abstraction of H from sugar, thereby resulting in strand breaks. This leads to purine and pyrimidine adducts formation, this adduct has a tendency to depurinate. Besides that, it has also been found to inhibit DNA repair enzymes, such as Formamido Pyrmidine (FAPY) DNA glycosylase, associated with the removal of 7, 8-dihydro-8-oxoguanine (abbreviated as 8-oxo-G or 8-OH-G). Together, it points towards existence of synergism between generation of peroxynitrite and ability to inhibit the repair of this damage (Jaiswal *et al*., 2001; Laval *et al*., 1997). 8-hydroxyguanine (8-OHG) and 8 hydroxydeoxyguanosine (8-OHdG), the most frequent base lesions, are used as a marker of oxidative DNA damage. Due to its premutagenic ability, 8-oxo-guanine is well known for its contribution to human diseases (Jaiswal *et al*., 2001; Haghdoost *et al*., 2005).

To prevent 8-oxoG accumulation and its mutagenic effects, mammalian cells express DNA repair proteins that are analogous to the *Escherichia coli* proteins MutM (formamidopyrimidine DNA glycosylase, also known as Fpg), Nei (endonuclease VIII-like DNA glycosylase), and MutT (8-oxo-7,8-dihydrodeoxyguanosine triphosphate GTPase) (Izumi *et al*., 2003). It is known that OGG_1 is a eukaryotic functional homolog for MutM that excises 8-oxoG and its open-ringed form 2, 6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) from the 8-oxoG/FapyG: C mis-pairing (Dizdaroglu *et al*., 2008). OGG_1 has targeting signals for both mitochondrial import (amino acid residues 9–26) and nuclear localization (amino acids 335–342) (Nishioka *et al*., 1999). The 8-oxoG DNA glycosylase (OGG_1) specifically repairs oxidative DNA damage, 8-oxoG being a critical mutagenic lesion (Kasprzak, 2002). OGG_1 is a bifunctional DNA glycosylase with an associated apurinic/apyrimidinic (AP) lyase activity that cleaves DNA at abasic sites via a β-elimination mechanism (Izumi *et al*., 2003; Mitra *et al*., 1997). The OGG_1-initiated DNA base excision repair pathway (OGG_1–BER) is a multistep process, which includes lesion recognition, changes in DNA structure, insertion of the 8-oxoG-containing DNA double helix into the base-binding pocket (active site) of OGG_1, and base excision and strand cleavage (Izumi *et al*., 2003; Bruner *et al*., 2000; Mitra *et al*., 1997). It has been shown that heavy metal such as cadmium toxicity causes decrease of OGG_1 activity in both mouse and human models (Youn *et al*., 2005; Zharkov *et al*., 2002).

2.17 Liver Damage Risk Assessment Indices

2.17.1 Total protein

Primarily, a molecule of protein is made up of amino acids join to each other by the peptide bonds and sequentially arranged to form a polypeptide chain. The sequence of amino acids in the chain determines the biological properties of the protein. Specific proteins are formed by joining of amino acids by hydrogen bonds, covalent bonds, or electrostatic bonds, resulting in the winding of the polypeptide chain. Proteins are found in almost all the cells and fluids of the body. They are synthesised within the cells of the body, released in the tissue fluid and then into the plasma (Thapa and Walia, 2007).

The liver performs the function of the synthesis of proteins in the body such as albumin, globulins and other important proteins. Its estimation is helpful in differentiating a functioning and a damaged liver as the liver produces the serum albumin and globulins (Thapa and Walia, 2007). Hence it is used to estimate the productive capacity of the liver. Increase in total protein is due to increase in globulin fraction as seen in chronic liver disease, dehydration, multiple myeloma, autoimmune disease, massive burn and nephrotic syndrome, while decreased value is due to low albumin caused by waste overload, sever haemorrhage, increased protein breakdown, insufficient diet and decreased liver formation or chronic liver disease. Total protein may reduce slightly but albumin/globulin ratio shows a sharp decline in hepatocellular injury (Ochei and Kokhartar, 2008).

2.17.2 Globulin

Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. They are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins are produced in the liver, while others are made by the immune system. Globulins, albumin, and fibrinogen are the major blood proteins. The normal concentration of globulins in human blood is about 2.6-4.6 g/dL. The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. Globulins are divided into four groups by electrophoresis. The four fractions are α_1, α_2, β and γ, depending on their migratory pattern between the anode and the cathode. Malnutrition and congenital immune deficiency

can cause a decrease in total globulins due to decreased synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney (Thapa and Walia, 2007).

2.17.2 Alanine Aminotransferase

Alanine Aminotransferase (ALT) is widely distributed throughout the body's tissues, with the greatest amounts in liver and the kidneys. The systematic name is L-alanine:2-oxoglutarate aminotransferase (EC 2.6.1.2) and it is also called pyruvate aminotransferase and glutamate pyruvate aminotransferase. It is found in the cytoplasm and mitochondria. ALT is responsible for the metabolism (transamination) of alanine and is found at much higher concentrations in the liver compared to other organs. When hepatocellular injury occurs, the liver-abundant enzyme ALT will leak into the extracellular space and enter the blood, wherein it shows a slow clearance rate with a half-life of approximately 42 hours (Ozer *et al*., 2008). It leaks from damaged tissues in hepatocellular necrosis. It catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. This reaction requires the coenzyme pyridoxal phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid. Normal levels are in the range of 5-50 U/L. Elevated level of this enzyme is released during liver damage. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver (Amacher, 2002).

An increase in ALT is an indicator of (hepatic) tissue damage. The highest increases (often >20-fold) are observed with acute hepatocellular injuries, such as xenobiotic-induced necrosis or acute viral hepatitis (Clement and Chalmers, 1967). Cholestasis or chronic liver disease rarely cause increases, and serum activities are generally within the reference interval or only slightly increased in alcoholic liver disease (Matloff *et al*., 1980).

In an established liver disease, its falling value usually reflects decreasing cell damage or recovery. If measured alongside aspartate aminotransferase (AST), the ratio AST/ALT is used to identify alcohol induced hepatotoxicity with value >2; if the value is less than this value, it isconsidered non-alcohol induced (Matloff *et al*., 1980; Ozer *et al*., 2008).

2.17.3 Aspartate Aminotransferase

Aspartate Aminotransferase (AST), also called serum glutamic oxaloacetate transaminase (SGOT), is another liver enzyme with a biochemical enzyme code EC 2.6.1.1. Two

isoenzymes are present in a wide variety of eukaryotes. In humans, GOT1/cAST, the cytosolic isoenzyme is derived mainly from red cells and heart, while GOT2/mAST, the mitochondrial isoenzyme is present predominantly in liver. These isoenzymes are thought to have evolved from a common ancestral AST via gene duplication, and they share a sequence homology of approximately 45% (Hayashi *et al*., 1990).

It is located in the cytoplasm and mitochondria. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Besides liver, it is also found in other organs like heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level (Nathwani *et al*., 2005). Normal levels are in the range of 7-40 U/L. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury (Ozer *et al*., 2008).

2.17.4 AST/ALT (De Ritis ratio)

The AST to ALT ratio can also occasionally be elevated in a liver disease pattern in patients with non-alcoholic steatohepatitis, and it is frequently elevated in an alcoholic liver disease pattern in patients with hepatitis C who have developed cirrhosis. In addition, patients with Wilson's disease or cirrhosis due to viral hepatitis may have an AST that is greater than the ALT, though the ratio typically is not greater than two. Most causes of liver cell injury are associated with an AST that is lower than the ALT. However, an AST to ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease, particularly in the setting of an elevated gamma-glutamyl transferase (De Ritis *et al*., 2006).

2.17.5 Alkaline Phosphatase

Alkaline phosphatase (ALP) (EC 3.1.3.1; orthophosphoric-monoester phosphohdrolase) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates (Panteghini and Bais, 2012). Alkaline phosphatase is a zinc-containing dimeric enzyme with the molecular weight: 86,000 kDa. It is heat stable and its function is to remove phosphate groups from phosphorylated compounds facilitating transport across membranes and providing the cell with a source of inorganic phosphate. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. Four separate genes encode this family of isoenzymes, found primarily the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta, and the kidney (in the proximal convoluted tubules)

(Milan, 1988).Hence, sources may include the liver, bone, leukocytes, kidneys, and first-trimester placenta. Serum values ordinarily range from 20U/L-120U/L, but are affected by several physiologic variables. Alkaline phosphatase activities are markedly increased in children and adolescents, as well as the third trimester of pregnancy. In young to middle-aged adults serum activities are usually higher in men; in elderly individuals the activities are often higher in women (Gordon, 1993).

2.18 Cancer Risk Biomarkers

Serum levels of simple biomarkers for prostate cancer - prostate specific antigen (PSA) (Grunkemeier and Vollmer, 2006; Bjartell, 2011); and for liver cancer- total alpha-fetoprotein (AFP) (Lee *et al*., 2004; Baig *et al*, 2009) have also been used as predictive indicators for risk of cancer development in the prostate and liver respectively. Prostate and liver cancers reportedly the two commonest cancers in men. Total AFP is reported to have the specificity of 90% and sensitivity of 60% for hepatocellular carcinoma (HCC) (Yuen and Lai, 2005).

2.18.1 Total and free prostate specific antigen

Human biomarkers in body fluids or tissue-bound are important diagnostic tools in disease diagnosis and clinical decision making. Prostate-specific antigen, also known as gamma-seminoprotein or kallikrein-3 (KLK3), is one of these biomarkers. It is a glycoprotein enzyme encoded in humans by the KLK3 gene. Prostate-specific antigen is a member of the kallikrein-related peptidase family and is secreted by the epithelial cells of the prostate gland. It is produced for the ejaculate, where it liquefies semen in the seminal coagulum and allows sperm to swim freely (Balk *et al*., 2003). It is also believed to be instrumental in dissolving cervical mucus, allowing the entry of sperm into the uterus (Carter and Partin, 2002). Also, PSA is an organ-specific and not a carcinoma-specific marker, serum PSA may be elevated due to a number of factors such as hyperplastic growth of prostatic tissues, inflammation, prostatic manipulation, urinary retention, sexual activity, and, hypothetically, the presence of undetectable clinically insignificant foci of cancer (Carter and Partin, 2002). In addition, more leaky prostates with regard to PSA have been postulated for older men (Hans-Joachim *et al*., 2007). Although, some authors are objecting the use of PSA as marker for prostate cancer diagnosis with the claim that it is insensitive and have poor specificity for carcinogenesis (Babian *et al*., 1992; Schröder and Bangma, 1997; Luboldt *et al*., 2001).

However, PSA is a biomarker of choice for preliminary prostate cancer diagnosis and prostate biopsy recommendation. Hans-Joachim *et al.* also stated that the standard reference range of (0.0 to 4.0 ng/ml) for PSA does not compensate for age-related volume changes in the prostate primarily due to hyperplastic growth of prostatic tissue (Hans-Joachim *et al*., 2007). Elevated PSA level has been reported in people exposed to heavy metals which is predictive of prostate cancer (Igharo *et al*., 2015; Mudgal *et al*., 2010; Kampa *et al*., 2008; Zaichick *et al*., 1997).

In some clinical settings, free PSA (fPSA) rather than total PSA (tPSA) is used in the diagnosis of prostate cancer. According to Catalona *et al*., (1995), about 20% of the false positive results caused by benign prostatic hyperplasia (BPH) can be eliminated by determination of the proportion of free PSA, often called %fPSA. The fact that %fPSA can reduce the number of false positive results is well known, but in spite of this clinical decisions are still mainly based on tPSA. However, there are compelling reasons to base the decision more on %fPSA than on tPSA (Ulf-Hakan, 2005).

On the basis of results from the Finnish Randomized Screening Study as reported by Finne *et al*., (2002), the average probability of finding a prostate cancer on biopsy is 17% and 32% when tPSA is 4 and 10µg/l, respectively. However, in this tPSA range the probability of prostate cancer is only 2% to 5% when %fPSA is 35% while the probability is 39% to 60% when %fPSA is 7%. Thus, at a certain value of tPSA, changes in %fPSA may cause a more than 10-fold difference in prostate cancer risk. Even when tPSA is 3µg/l, i.e. 'normal', the prostate cancer probability is over 17% when %fPSA is below 14% (Finne *et al*., 2002)

2.18.2 Alpha fetoprotein

Alpha-fetoprotein (AFP) is a glycoprotein, mainly from the yolk sac and embryo liver during foetal development. It is thought to be the foetal form of serum albumin. It is encoded by the AFP gene (Harper and Dugaiczyk, 1983). The AFP gene is located at chromosome 4. Serum AFP levels are very low in adults. Since the 1970s, AFP has been used as a tumour marker for the diagnosis of hepatocellular carcinoma (HCC). Serum AFP levels in nearly 75% of cases of HCC are higher than 10ug/L (Johnson, 1999). In patients with cirrhosis or chronic hepatitis B or hepatitis C infections, AFP is the most important serum marker to predict liver cancer occurrence (Lee *et al*., 2004; Baig *et al*, 2009). The

serum AFP level does not only has diagnostic value but also has predictive value for the prognosis of hepatocellular carcinoma.

It is synthesised in the liver, yolk sac and gastrointestinal tract in foetal life and is released into the serum of foetus. It is a normal component of serum protein in human foetus. The concentration is highest during embryonic and foetal life. It has a molecular weight of 61,000 -70,000, and has similar chemical and physical properties with albumin. In adult males and non-pregnant females, normal value is less than 15ng/ml. A value of AFP above 300 ng/ml is often associated with cancer, (Elias *et al*., 2013). Although levels in this range may be seen in non-malignant liver diseases, levels above 1000ng/ml are almost always associated with cancer (except in pregnancy). The gene for AFP is located in chromosome number 4, the fucosylated fraction of AFP (AFP-L3) has been reported to be a more specific marker for hepatocellular carcinoma (Elias *et al*., 2013).

2.19 Biomarker of Chromosomal Damage

2.19.1 Micronuclei

Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division. MN can be induced by defects in the cell repair machinery and accumulation of DNA damages and chromosomal aberrations. A variety of genotoxic agents may induce MN formation leading to cell death, genomic instability, or cancer development (Luzhna *et al*., 2013)

Micronuclei, also known as Howell–Jolly bodies, were first identified at the end of nineteenth century in red cell precursors by William Howell, an American, and Justin Jolly, a Frenchman (Sears and Udden, 2011). At that time, Howell–Jolly bodies were described as remnants of nuclei of red blood cells circulating in organs with pathological features (Sears and Udden, 2011). The significance of MN was evaluated in the mid-twentieth century. Dawson and Bury (1961) found MN in red cells within the bone marrow during different pathological states. The authors also mentioned that the formation of Howell–Jolly bodies was paralleled with folic acid and vitamin B deficiency (Dawson and Bury, 1961).

Micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis (cell division), shown in fig 2.8. They are small, extranuclear bodies that are formed during mitosis from lagging chromosomes. In anaphase, the microtubules are not attached

properly to the chromosomes, which can cause pulling in a different direction. This results in parts of the chromatids or chromosomes being broken off and enveloped as an extra nucleus in one of the daughter cells. This is the main way that micronuclei are formed. Micronuclei can also be spontaneously formed as a by-product of cellular defence. If the cell senses extra chromosomes, the cell can attempt to remove the extra chromosome in another cell membrane, separate from the other normal chromosome. Another mechanism to micronuclei formation is by a double-strand break in the DNA, creating a separate linear fragment. Furthermore, the breaking of an anaphase bridge could also lead to formation of a micronucleus. It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei. Due to their rapid formation and easy detection, MN have become the most prevalent biomarker of chromosomal defects induced by genotoxic agents (Luzhna *et al*., 2013)

The summarized roles of both genetic and epigenetic factors in MN formation are outlined in Fig. 2.9.

2.19.2 The Fate of Micronuclei in Cells

Analysis of the fate of MN in the cells has regained a lot of interest. The recent study by Utani *et al*. (2007; 2010) suggested that MN formed after mitosis were stably maintained in the cells for up to one cell cycle. Furthermore, it was observed that mitotic division of cells with MN led to formation of daughter cells either with or without MN. The ability of MN DNA to replicate itself remains obscure, but some suggestions have been made that MN replication depends on MN nature, and if it happens, usually it occurs at the same time as main nucleus replication (Obe *et al*., 1975). Similarly, MN transcription events depend mainly on MN structure. Micronuclei containing whole chromosomes showed active transcription (Labidi *et al*., 1987), whereas acentric fragments were not able to synthesize RNA (Hoffelder *et al*., 2004), unless they represented transcriptionally competent double minutes (DMs) (Utani *et al*., 2007). The DM chromosomes are selectively located at the periphery of the nucleus and are eliminated from the nucleus by nuclear budding during S phase (Shimizu *et al*., 1998). Any possible transcriptional activity in MN depends on nuclear envelope integrity and the presence of nuclear pore complexes (NPCs) (Geraud *et al*., 1989; Sukegawa and Blobel, 1993).

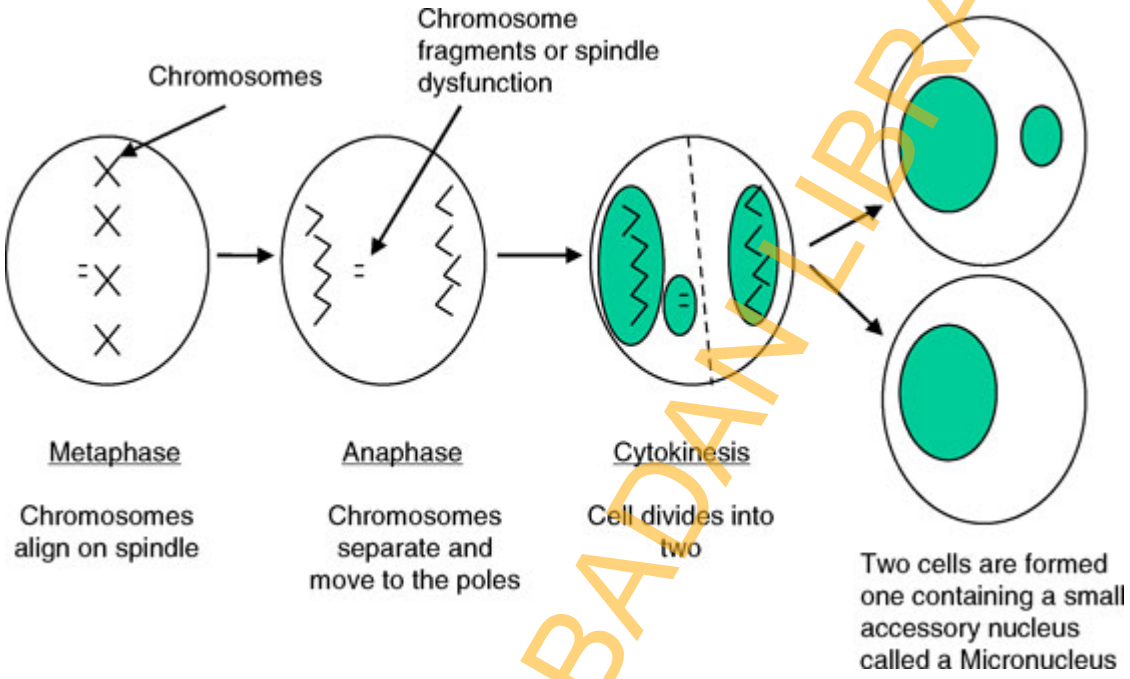


Fig. 2.8 Illustration of Micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis (cell division) (Shimizu *et al.*, 1998)

The DDR in MN involves the formation of ionizing radiation-induced foci (IRIF), similar to ones in the main nucleus. The phosphorylated histone, γ-H2AX foci were co-localized with ataxia telangiectasia mutated (ATM) and MDC1 proteins (Medvedeva *et al.*, 2007). The observation of HR protein Rad51 and ss-DNA-binding protein RPA in radiation-induced MN leads to the suggestion that DDR proteins are either randomly entrapped in MN together with damaged DNA or are recruited to MN that contain damages (Terradas *et al.*, 2010). Similarly, TP53 accumulation in MN was observed to trigger DNA damage response (Granetto *et al.*, 1996). Cells treated with colchicine, vinblastine, bleomycin, and arsenic showed a significant induction of MN and p53 (Salazar *et al.*, 2009).

Micronuclei formation in humans is associated with various medical conditions. MN in spermatids may lead to infertility, while a high number of MN in lymphocytes is associated with pregnancy complications and miscarriages (Fenech, 2011). MN are one of the four main endpoints, together with chromosomal aberrations, aneuploidy, and sister chromatid exchange (SCE) in the identification of cancer initiation (Tucker and Preston, 1996; Hagmar *et al.*, 2001). Some studies have described the correlation between MN and cancer development (Guler *et al.*, 2005). A significant increase in MN in lymphocytes was shown in untreated cancer patients (Iarmarcovai *et al.*, 2008b). Furthermore, healthy women with breast cancer genes (BRCA1 and BRCA2) mutations showed a higher increase in MN frequency and a higher radiation sensitivity than women without family history of breast cancer (Rothfuss *et al.*, 2000; Trenz *et al.*, 2003). Similar outcomes were shown in lung cancer patients with a high frequency of spontaneous MN (Guler *et al.*, 2005), as well as in patients with pleural malignant mesothelioma (Bolognesi *et al.*, 2002), and adenocarcinoma patients (Karaman *et al.*, 2008). Cancer-prone patients with Bloom syndrome and ataxia telangiectasia also possess a high frequency of MN in lymphocytes (Rosin and German, 1985). Analysis of European cohorts indicates that individuals with increased MN are more likely to get cancer 12–15 years after the test was performed (Bonassi *et al.*, 2007).

The involvement of selected genotoxic agents in micronuclei formation are tabulated in table 2.4 and photomicrographs showing micronuclei and micronucleated polychromatic erythrocytes (MnPCE) aberrations are shown in fig. 2.10.

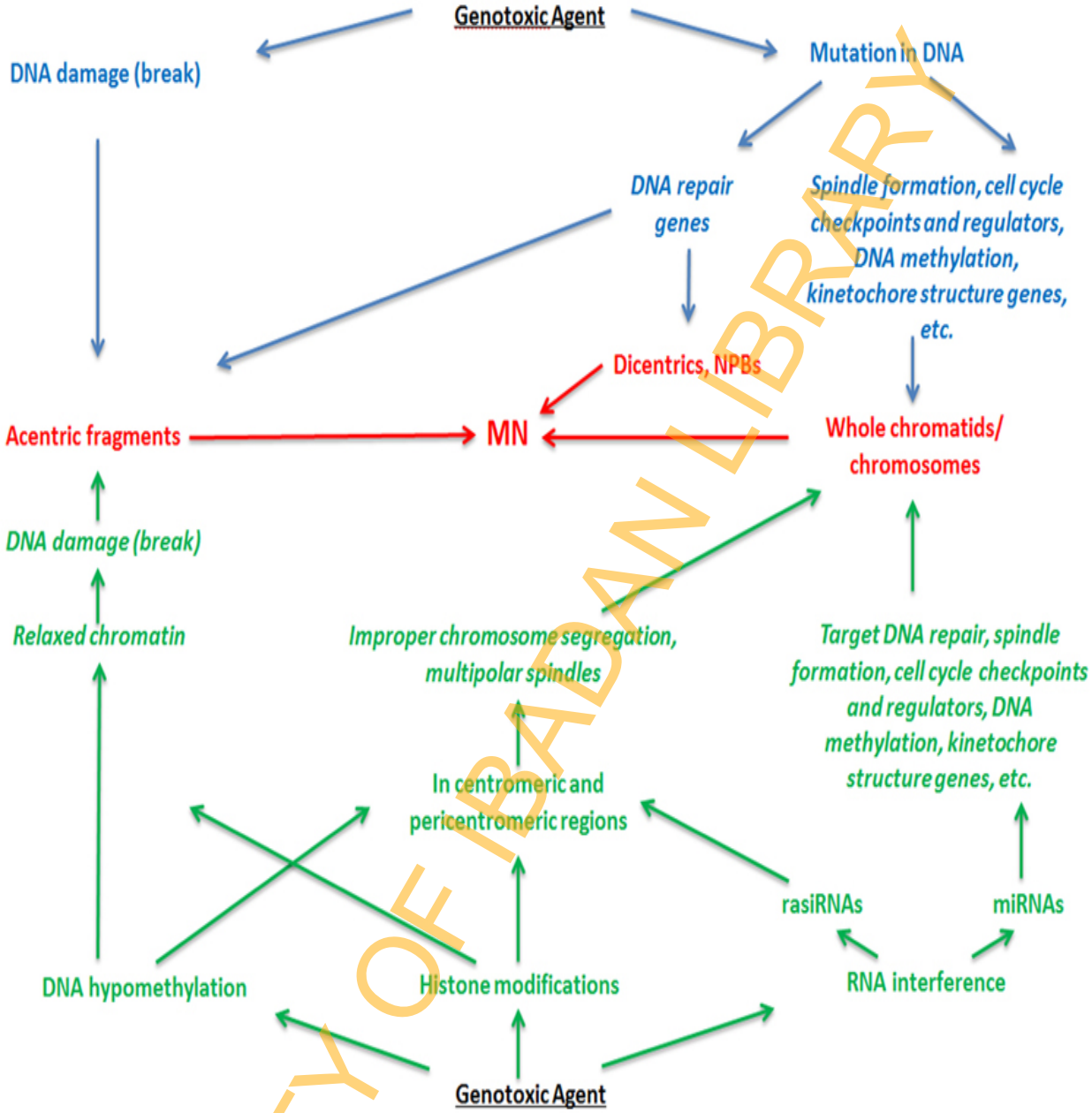


Fig. 2.9 Role of the genetic and epigenetic factors in the formation of micronuclei. MN and its components are reflected in red, genetic factors are reflected in blue, and epigenetic factors are reflected in green (Terradas *et al*., 2010)

Table 2.4 Role of selected genotoxic agents in micronuclei formation

Ionizing radiation	Anti-mitotic agents (vinca alkaloids)	Metals	DNA methylating agents	Anthracycline drugs
DNA and protein adducts, DNA strand breaks, crosslinks, gene mutations	Mitotic spindle disruption, chromosome malsegregation	By binding to DNA and proteins cause damage to DNA, altered gene expression, mutations, altered cell cycle, chromosome non-disjunction, cytoskeleton dysfunction	Loss of methylation in centromeric and paracentromeric regions, sister chromatid decondensation, improper chromosome segregation, chromosome breaks	DNA damages, disruption of DNA replication and DNA repair, DNA breaks
Mainly clastogenic effect, but mutations in certain cell cycle and repair genes may cause aneugenic MN	Mainly aneugenic	Both clastogenic and aneugenic effects, depending on the metal	Aneugenic, if hypomethylation in centromeric regions; clastogenic if global hypomethylation leading to DNA strand breaks	Mainly clastogenic

Source: Luzhna *et al*., 2013; Terradas *et al*., 2010.

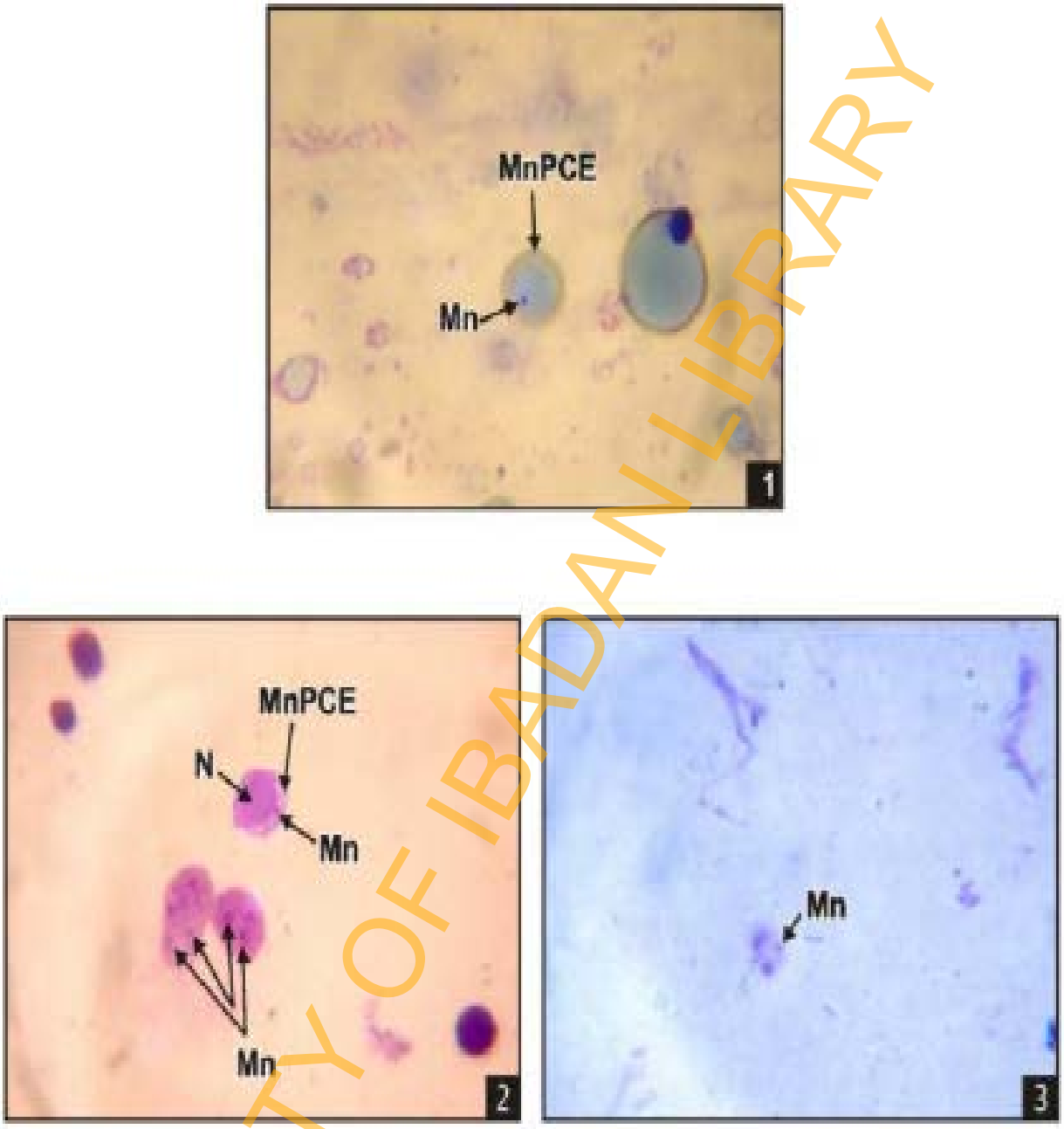


Fig. 2.10 Photomicrographs showing micronuclei (Mn) and micronucleated polychromatic erythrocytes (MnPCE) aberrations in lindane treated animals (1000 X) 1. Polychromatic erythrocyte (PCE) with micronuclei (MN). 2. A micronucleated polychromatic erythrocyte with the main nucleus (N). 3. A binucleated cell with micronuclei (Mn)

Source: (Holland *et al.*, 2008).

Overall, by binding to DNA and proteins, metals have the potential to damage DNA, alter gene expression and cause mutation. Also, altered cell circle, chromosome non disjunction cytoskeleton dysfunction, both clastogenic and aneugenic effects, depending on the type of metals, have been documented with metal exposure (Luzhna *et al*., 2013; Terradas *et al*., 2010).

A micronucleus test is a test used in toxicological screening for potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens, i.e., carcinogens that act by causing genetic damage and is the Organisation for Economic Co-operation and Development (OECD) guideline for the testing of chemicals (Luzhna *et al*., 2013).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study Design

The study was designed as a cross-sectional study with purposive approach in the recruitment of participants into the study groups. The groups comprised e-waste occupationally exposed participants (e-waste workers), participants trading or working around the e-waste repair/recycling sites/locations considered for the study (environmentally exposed), and non-occupationally and minimally environmentally exposed; apparently healthy participants (unexposed or control participants).

3.2 Study Area

The study was carried out in three (3) urban cities (Lagos, Ibadan and Benin) that have been identified and reported (Aragba-Akore, 2005; Nnorom 2009; Osibanjo, 2009; Terada, 2012) as high impact locations for e-waste activities in South –Western Nigeria.

3.3 Study participants

A total of six hundred and thirty two (632) participants were enrolled into the study, viz, three hundred and eighty one (381) e-waste workers; one hundred and twenty (120) environmental e-waste exposed participants and one hundred and thirty one (131) age-matched unexposed individuals, serving as controls.

3.3.1 E-waste workers

Male Waste Electric and Electronic Equipment (WEEE) Workers who were daily involved with four main work tasks, namely, purchasing / reception [i.e., buying and /or receiving second-hand electrical and electronic equipment (EEE)]; dismantling (i.e., all work tasks involving manual dismantling of the goods); repair (i.e., all work tasks involving repair, like circuit board cleaning, soldering, rewiring, etc.) and resale (reselling repaired or inadvertently functional WEEE/EEE, or components thereof), formed the group of e-waste workers enrolled into the study.

Of this group, one hundred and seventy eight (178) e-waste workers from Alaba International Market and Ikeja Computer village, Lagos state; one hundred and twenty (120) e-waste workers from Ogunpa Market and environs, Ibadan, Oyo state; and eighty three (83) e-waste workers from Lawani Close/Upper Mission Road/ New Benin Market Area, Benin City, Edo state met the inclusion criteria.

Consenting participants with a minimum of five (5) years of occupational exposure to toxic substances in WEEE were enrolled into the study.

3.3.2 Environmentally exposed participants

The environmentally exposed group comprised traders and non-e-waste workers involved in work and business activities around the e-waste high impact areas in the aforementioned three study locations selected for this study. Of this group, fifty two (52) participants from Lagos; thirty eight (38) participants form Ibadan; and thirty (30) participants from Benin met the inclusion criteria.

3.3.3 Unexposed participants

Non-occupationally and minimally environmentally exposed, and apparently healthy age- and sex -matched participants formed the unexposed or control group. These included forty (40) participants from Lagos State University community, Lagos Badagry Expressway, Ojo, Lagos state; fifty (50) participants from the University College Hospital / University of Ibadan communities, Ibadan, Oyo State; and forty one (41) participants recruited from the Ugbowo Campus Community of the University of Benin, Benin City, Edo State.

3.3.4 Inclusion Criteria

(a) E-waste workers: comprised electronics technicians carrying out informal (crude) e-waste recycling, repair, dismantling, purchasing and resale of EEE. Workers who were occupationally exposed to e-waste for a period of five years and above at the time of sample collection were enrolled into the study. The five-year duration of exposure used in this study is based on E-waste Risk Assessment Report of Adaramodu *et al*. (2012); suggesting that a five-year of duration is sufficient for the health risks and effects of e-waste crude reprocessing to become apparent in exposed human populations.

(b) Environmentally exposed participants: comprised of apparently healthy age- and sex-matched traders and non-e-waste workers who had been involved in work and business activities around the e-waste high impact areas for a minimum of five years, and as such were environmentally exposed to e-waste-borne toxicants (including metals), due to unregulated and environmentally unfriendly WEEE disposal practices previously identified (Aragba-Akore, 2005; Nnorom 2009; Osibanjo, 2009; Terada, 2012).

(b) Control subjects were healthy male individuals with minimal or no occupational exposure and with no hobby involving e-waste exposure or other toxic substances.

3.3.5 Exclusion Criteria

E-waste workers who were not exposed to e-waste for a period up to five years at the time of sample collection were excluded from the study. Participants with demographic or medical history of any form of cancer, frequent/ habitual tobacco smoking and alcohol consumption were excluded from the study. Demographic and medical history of incidence of cancer, frequent tobacco smoking and alcohol consumption also served as a basis of exclusion for recruiting the apparently healthy control participants.

3.4 Ethical Approval

The protocol for this study was approved by the Health Research Ethics Committee of University of Ibadan/University College Hospital, Ibadan, Nigeria, with a reference number UI/UCH EC: NHREC/05/01/2008a (appendix I) and the Ethical Clearance Committee, Edo State Ministry of Health, with reference number HA.577/Vol.11/164 (appendix II)

3.5 Informed Consent

Participants for this study were adults who were adequately educated on the benefits of the study, sufficiently briefed of the research protocol, and informed consent was obtained from them prior to sample collection. The informed consent form (appendix III) used for this study was explicitly explained to each participant in English and Pidgin English languages.

3.6 Collection of data by administration of questionnaires

A twenty-item questionnaire was validated and administered to consenting participants to obtain information on:

a) Age and state of origin
b) the use of personal protective equipment among e-waste workers
c) frequency and duration of exposure
d) methods of disposal of scraps,
e) Occupational hazards/risk awareness level among participants
f) Basic health information
g) Tobacco and alcohol use
h) Dietary habits

3.7 Generation of anthropometric index data

Standing height and weight readings were obtained using a standard 160kg mechanical height weight scale (Model ZZJKH-01, Focus Technology Company limited, Zhejiang, China). The instrument was calibrated using standardized weights and height rods.

3.7.1 Normal height measurement procedure

Participants were asked to remove their shoes, heavy outer garments, and caps where applicable. The participant were asked to stand with their back to the height rule, with the back of the head, back, buttocks, calves and heels touching the upright, and feet together. The top of the external auditory meatus (ear canal) was level with the inferior margin of the bony orbit (cheek bone). The participant were then asked to look straight. The head piece of the stadiometer or the sliding part of the measuring rod was lowered so that the hair (if present) was pressed flat. Height was recorded in metre to the resolution of the height rule (i.e. nearest millimetre/half a centimetre).

3.7.2 Normal weighing procedure

Participants were asked to remove their heavy outer garments (jacket, coat, trousers, skirts, etc.) and shoes as well as empty their pockets of heavy items. The weight scale was stably and flatly positioned. Then, the participant were made to stand on the centre of the platform, weight distributed evenly to both feet. The scale beam was allowed to balance and the weight was recorded to the resolution of the scale (the nearest 0.1 kg or 0.2 kg).

3.7.3 Body mass index calculation

Body Mass Index (BMI) was calculated from height and weight values using the formula,

BMI= Weight (kg)/ Height^2 (m^2) as previously reported (Romero-Corral *et al*., 2008)

Blood pressure values were obtained using a clinically validated semi-automated Blood Pressure Device (HANA, United States). The systolic and diastolic readings, as well as pulse rates were expressed in mmHg and beats/minute respectively.

3.8 Sample collection and preservation

3.8.1 Preparation of participants, blood collection and preservation

Prior to blood collection, participants were urged to abstain from using herbal medications, drugs and vitamin/mineral supplements for 12 - 24hours. Some medications may contain metals, vitamins and minerals that may influence biochemical processes in the body.

About ten (10) millilitres (mL) of venous blood was collected from each participant using standard phlebotomy technique as summarized below.

All necessary materials needed for the procedure were assembled (10 mL capacity syringes, blood collection tubes, tourniquets, antiseptic – 70% isopropyl alcohol wipes, cotton balls, sharps disposal containers and strip plasters, in addition to personal protective equipment (PPE),viz, laboratory coats and hand gloves.

The participants were identified and given numbers each to match with that on questionnaires. Each of them was positioned with the arm extended to form a straight-line from shoulder to wrist. Appropriate vein was selected and tourniquet applied 3-4 inches above the collection site. Puncture site was cleaned by making a smooth circular pass over the site with the 70% alcohol pad and the skin allowed to dry. A sheathed needle was placed on the syringe and the cap removed, with the bevel of the needle turned up. The skin was pulled tightly with the thumb just below the puncture site. Holding the needle in line with the vein, a quick small thrust was used to penetrate the skin and vein in one motion. The desired amount of blood was drawn by pulling back slowly on the syringe stopper. Tourniquet was released and a cotton ball placed over the puncture site while removing the needle quickly. Subjects were asked to apply pressure to the cotton ball for at least 2 minutes. Plaster strip was applied when bleeding stopped.

Blood sample obtained was dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulant specimen bottles (5 mL). Another 5 millilitres was dispensed into anticoagulant-free specimen bottles to obtain serum. To achieve this, the blood samples were allowed to clot, and were centrifuged at 3000 revolution per minute for 3 minutes and the serum from each sample was collected and stored in another anticoagulant-free bottle. Samples were immediately analyzed for the research parameters and where this was not possible, the EDTA blood specimen were stored for four weeks in the refrigerator (2 to 8 ^{o}C), while the serum samples were kept frozen (0 to -4^{o}C) for less than three weeks until analyzed. Frozen samples were thawed and brought to room temperature before analysis was carried out on them. All samples for the different groups where properly labelled to avoid sample mix-up. All samples were handled using safety gloves and protective lab clothing. Sodium hypochlorite (10%) solution was used to disinfect working areas after analysis. It was ensured that all handling and analysis of samples took place in approved working areas only.

3.8.2 Hand wash collection and preservation

Adequate quantity of distilled and deionized water (DD H_2O) was stored in plastic jerrican that was washed with detergent and rinsed with 1% nitric acid (HNO_3) solution, and this was used for hand washing by all participants. It was ensured that detergents and cosmetics were not used by participants prior to hand washing. The participants were adequately briefed to avoid the use of cosmetics and detergents after their early morning baths (i.e between the hours of 8:00am to 1:00pm. Thirty (30) centilitre (0.3 Litre) of DDH_20 was dispensed into a bow for use by each participant for hand washing. The hand wash water was thereafter well stirred for even particle distribution and ten (10) mL was aliquoted into a clean labelled universal bottle previously rinsed with 1% HNO_3 solution. For the e-waste workers, hand washing took place after involving in the usual daily work task (e.g. dismantling, repair, displaying of goods/wares for sale etc.), while for the environmentally exposed and unexposed participants, hand washing took place while being involved in daily business tasks. The aliquoted hand wash samples were stored in frozen state in refrigerator at 0 to -4^{o}C until metal analysis was carried out.

3.9 Laboratory methods

3.9.1 Metal determination using Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

Toxic and essential metals in whole blood and serum respectively were determined at the Supra-regional Assay Service Trace Elements Laboratory, Surrey Research Park, Guildford, Surrey, United Kingdom, using ICP-MS (Thermo Scientific X series 2, Darmstadt, Germany) based on standard methods described by Fong *et al.*, (2007).

3.9.1.1 Principle

In ICP-MS, elemental abundances or isotopic ratios are determined by the mass spectrometry (MS) of ions generated in an inductively coupled plasma (ICP). By using a stream of Argon carrier gas, sample material is introduced into ICP which serves to provide a source of positively charged analyte ions. The ions are extracted from the atmospheric pressure environment of the plasma into a high vacuum enclosure via an interface region containing two sequential apertures (cones). Focused by an ion lens systems, analyte isotopes are separated according to their mass/charge ratio by a quadrupole mass spectrometer, and detected and measured by an electron multiplier detector. A schematic overview of ICP-MS principle is given in fig. 3.1

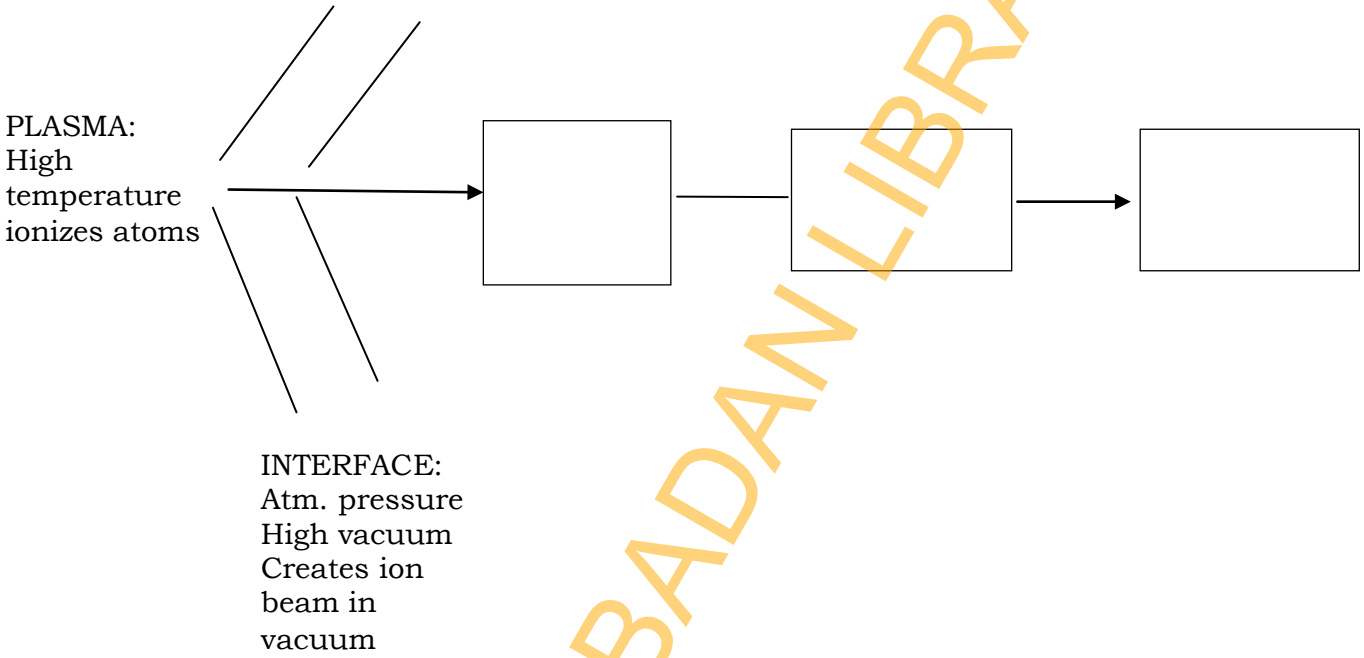


Fig. 3.1 Schematic Overview of ICP-MS principle

3.10 Analysis of toxic and essential metals

3.10.1 Sample preparation

Whole blood and serum were diluted using 1 in 50 dilution while hand wash water was diluted using 1 in 25 dilution with the diluent. The internal standard diluent was prepared by diluting 10mL of concentrated nitric acid to 1 litre with reverse osmosis/deionized (RODI) water with a resistivity of >18 M Ω cm. To this, 1mL of internal standard solution containing the internal standards element: Ge, Ir, Rh, Re (1500µg/L) was added, in addition to 1 mL 5% Triton X – 10; with final volume remaining as 1 Litre.

Trace element scan analysis in the diluted whole blood, serum and hand wash water samples were quantitatively determined by ICP – MS in the collision cell mode (CCT). The samples were analysed against a calibration standard with inclusion of blood internal quality controls (IQCs), the method is validated and data generated are reliable in quantitative sense.

Details of equipment and reagents used are attached in appendix

In whole blood, toxic metals determined included aluminium (Al), antimony (Sb), arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), thallium(Tl), vanadium (V) and Tin (Sn).

In serum, the essential metals determined included chromium (Cr), cobalt (Co), copper (Cu), Magnesium (Mg), Manganese (Mn), Selenium (Se) and zinc (Zn)

In hand wash water the elements measured included toxic metal: (Al), Antimony (Sb), Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Thallium (Tl), Vanadium (V) and Tin (Sn), and essential metals: Chromium (Cr), Cobalt (Co), Copper (Cu), Magnesium (Mg), Manganese (Mn), Selenium (Se) and Zinc (Zn).

3.10.1.1 Procedure

Sample Dilution

The autodilutor was used to dispense working diluents in 5.0, 4.9 and 4.8 µL volume into 10mL Teklab tubes, as shown in the table below, table 3.1.

The Teklab tubes were given identifiers corresponding to the laboratory numbers on the IQCs, whole blood, serum and hand wash water, exemplified using fig. 3.2.

Dilution protocol for internal standard, whole blood, serum and hand wash water for ICP-MS analysis.

Sample type	**Volume (µL)**	**Internal Standard Diluent**
Blank	-	50mL (5000µL)
Standard	100	4.9mL (4900µL)
Blood/serum	100	49mL (4900µL)
Hand wash water	200	4.8mL (4800µL)

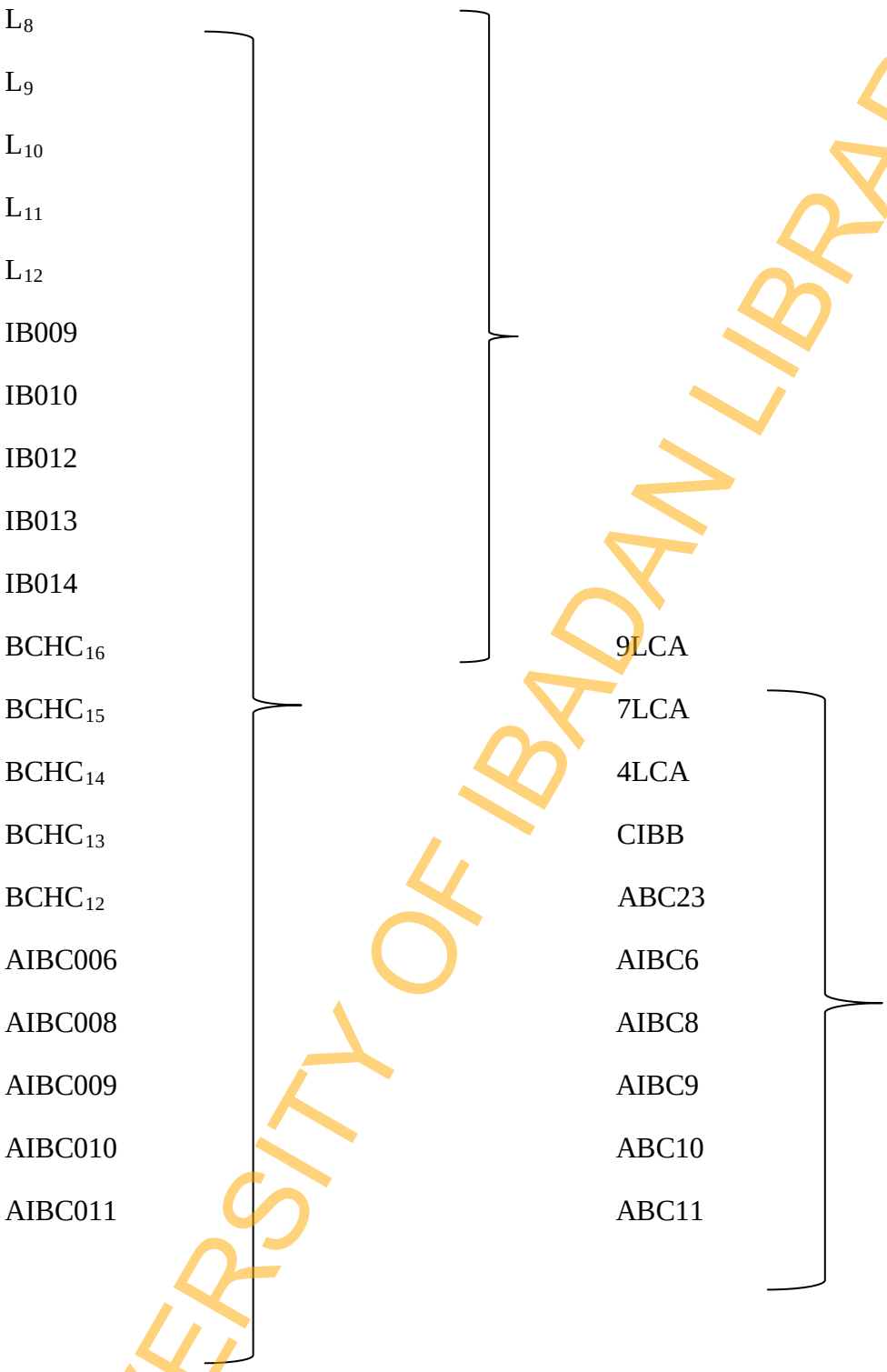


Fig 3.2 A typical structure used for sample arrangement in an ICP-MS run

Note: L=label identity for IQCs; AIBC=absolute Ibadan control (unexposed); ABC=absolute Benin control; IB=Ibadan; BCHC=Benin controls health centre; LCA= Lagos control absolute

After dilutions, the whole blood and hand wash water samples were centrifuged for 10mins at 3000rpm before being transferred to analyser and running in CCT mode. The centrifugation step is required to enhance the viscosity of whole blood and form a subnatant of retained particles in samples before introduction to the analyser. This step is not routinely applicable to serum sample analysis.

Calibrations were generated for selected elements across the mass range. The sensitivity of these elements (i.e. calibration slope) were recorded. The sensitivity was corrected for isotope abundance and ionisation efficiency. The corrected sensitivity (in part per million/parts per billion) was plotted for each calibrated element. A curve (called the mass response curve) was fitted through these points. Sensitivities can be estimated for all analytes across the mass range from the curve. Fresh standard solutions for the external calibrations (CPI International, Amsterdam, The Netherlands; Ultra Scientific Analytical Solutions, North Kingstown, RI, US) and internal standards (High-Purity Standards; Charleston, SC, USA) in 20% (v/v) nitric acid were prepared before every run. The limit of detection (LOD) was set to 3 times the standard deviation (SD) of the blank values.

3.10.1.2 Introduction of sample to the analyser

The teklab tubes containing the samples were loaded unto the autosampler sample tube rack (ASTR).The ICP-MS X series II used had four ASTR technically identified as racks I, II (from left to right on first row) and III, IV (from left to right on second row), and the tubes were loaded in this order from position 1 -12 row by row.

To set up the analyser, the computer with the analyser's output software (Plasmalab) was switched on along with the chiller (water coolant unit). On the desktop, the "plasmalab icon" was checked, and the level of the wash solution was monitored to check for adequacy. The nebulizer and wash bottles were emptied. The peristaltic pump tubing was tensioned over the peristaltic pump and switched on. The analyser software was turned on to ignite the plasma. Thereafter, the system was purged with the CCT gas by manually increasing the amount of gas running through for 15-20 minutes. The system was thereafter tuned with Tune A solution (indicated in appendix), and tuning for standard and CCT modes was done in turns. Further turning was done to achieve target settings by checking the internal standards (Indium and Uranium) counts per seconds as well as ion lens, plasma formation and nebularization efficiency. When performance check was passed, the setting was saved for use in the next analysis.

The analyser was made to begin new analysis by clicking 'begin new analysis), and each sample was serially aspirated from the ASTR. The aspirated sample was converted from liquid form to fine aerosol in the nebulizer, injected into the ~5000K plasma torch, making the MS of the ions generated in the ICP possible.

The analyser was set to run each sample in triplicate and the mean of the results were viewed on the Plasmalab software display, transferred / saved on the system drive for further processing and use as research results.

3.11 Determination of enzymatic antioxidant biomarkers

3.11.1 Superoxide Dismutase (SOD) activity: by the kinetic method of Misra and Fridovich, (1972)

Assay Principle:

Adrenaline auto-oxidises rapidly in aqueous solution to adrenochrome, whose concentration can be determined at 420nm. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme, SOD inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anions. The degree of inhibition is thus a reflection of the activity of SOD.

Assay procedure and protocol:

Assay procedure and protocol for assay of Superoxide Dismutase

	Blank	Reference	Test
Distilled water	3.0ml	0.2ml	
Sample			0.2ml
Phosphate buffer		2.5ml	2.5ml
Adrenaline		0.3ml	0.3ml

Solutions were mixed properly and the absorbances were read at 420nm.

Calculation

$$\% \text{ Inhibition} = \frac{ABS_{ref} - ABS_{test}}{ABS_{ref}} \times 100$$

$$\text{Thus, SOD (Unit/ml)} = \frac{\% \text{ Inhibition}}{50 \times S}$$

Where: ABS_{ref} = Absorbance of reference

ABS_{test} = Absorbance of Test

S = Total protein (g/dl) for each sample

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL

Quality control measures

The spectrophotometer was put on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

3.11.2 Determination of Catalase enzyme activity by the kinetic method of Cohen *et al*., 1970.

Principle

Catalase reacts with and catalyses the break-down of reagent hydrogen peroxide to water and oxygen. The absorbance of hydrogen peroxide at 480nm is measured directly to calculate the reaction rate since water and oxygen does not absorb at this wavelength. In the presence of Catalase, the reaction rate is proportionally (linearly) enhanced.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Test procedure and protocol for catalase assay

	Blank	Test
0.34M H_2O_2	5.0ml	5.0ml
Distilled water	0.5ml	
6M H_2SO_4	1.0ml	1.0ml
0.01M $KMnO_4$	1.0ml	1.0ml
Sample		0.5ml

Absorbances of test samples were read at 480nm, and at 0sec, 20sec, 40sec, 60sec and 80sec for each sample.

Calculation:

The activity of serum Catalase was calculated using the formula:

$$\text{Catalase (Unit/ml serum)} = \frac{\Delta\text{ABS of test} \times V \times 1000}{m \times v \times L \times y}$$

Where; ΔOD = Mean of the differences in the absorbance for each test

V = Total volume of the reaction mixture

m = molar extinction coefficient for H_2O_2 = 40 $M^{-1}cm^{-1}$

L = Light path = 1cm

v = Volume of sample used

y = Total protein (g/dl) for the respective sample

The activity; Unit/g = mole of H_2O_2 consumed per minute.

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL

Quality control:

The assay was done in a cold water bath using ice block to prevent the undesirable deterioration of H_2O_2.

The spectrophotometer was on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

3.11.3 Determination of Glutathione Peroxidase activity (GPx) by kinetic method (Flohe and Gunzler, 1984).

Principle

GPx catalyses the reaction of pyrogallol with hydrogen peroxide to form purpurogallin (purple to black coloured) whose absorbance is read at 420nm.

2pyrogallol + $3H_2O_2$ + purpurogallen + $5H_2O$ + CO_2

Procedure and protocol for assay of Glutathione Peroxidase

	Blank	Sample
Sample		200µl
Phosphate buffer	2.5ml	2.5ml
H_2O_2	2.5ml	2.5ml
Distilled water	1.7ml	1.5ml
Pyrogallol solution	2.5ml	2.5ml

Absorbance of each sample was read at 420nm and at 0sec, 20sec, 40sec, 60sec, 80sec and 100sec.

Calculation:

Concentration of GPx was calculated using the formula below

$$\text{GPx (Unit/ml)} = \frac{\Delta ABS \times V \times Df}{A \times v \times L \times Y}$$

Where; ΔABS = mean of the differences in the absorbance

V = Total volume of the reaction mixture

Df = Dilution factor

A = Molar extinction coefficient of purpurogallin = 12.0 $M^{-1}cm^{-1}$

v = Volume of serum sample used

L = Light path = 1cm

Y = Total protein (g/dl) for each sample.

Expression of result: The result was expressed in Units/ml of serum, where 1 unit = mole of pyrogallol oxidized per minute.

Conversion: Units/mL (nmol/min/mL) x 1000 = 1 µmol/min/mL

Quality control measures

The pyrogallol solution was kept in brown bottle to avoid degeneration from reaction with light

The spectrophotometer was on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

Detrmination of Glutathione Reductase activity (Ellman, 1959).

Principle

Dithionitrobenzoic acid; 5, 51-Dithiobis (2-nitrobenzoic acid) (DTNB) reacts with the GSH generated from the reduction of GSSG by the GR in a sample to form a yellow product 3-thio-6-nitrobenzoate (TNB^{2-}). The rate of change in the optical density, measured at 412 nm, is directly proportional to GR activity in the sample.

Assay procedure and protocol for Assay of glutathione reductase

	Blank (µl)	Standard (µl)	Sample (µl)
Sample			500
TCA			2000

Was mixed properly and allowed to stand for 5minutes at room temperature and was spun for 10minutes at 4000rpm

Supernatant			500
Ellman's reagent	500	500	500
Distilled water	500		
Standard		500	
Buffer	3000	3000	3000

The absorbance of was read at 412nm.

Calculation:

$$\text{Glutathione Reductase (U/g/dl)} = \frac{ABS_{sample}}{ABS_{std}} \times \text{Standard Conc. (g/dl).}$$

Quality control

The spectrophotometer was put on and allowed to run for 15 minutes before use as a quality control measure according to the manufacturer.

The spectrophotometer was inspected to ensure proper functioning of the light source and light path

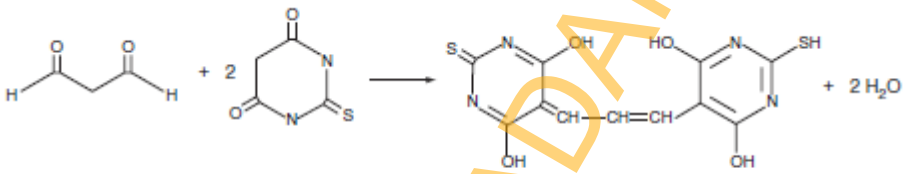
3. 12 Determination of selected biomarkers of oxidative Stress

3.12.1 Malondialdehyde (MDA) determination by the method of Varshney and Kale (1990)

Principle

MDA which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient marker for the determination of the extent of lipid peroxidation. Assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming a MDA-2TBA adduct (Pink-red coloured complex) that absorbs light at 535nm.

Reaction equation;



MDA TBA MDA-TBA Adduct

Procedure

1. 50µl of serum sample was dispensed into a clean test tube
2. 100µl of TCA/TBA working solution was added
3. 1.85ml of distilled water was added
4. The mixture was placed in a boiling water bath for 15 minutes
5. This was centrifuged after cooling
6. The absorbance of the supernatant fluid was read at 535nm using reagent blank.

	Sample	Blank
Serum	50µl	
TBA/TCA Solution	100µl	100µl
Distilled water	1850µl	1900 µl

Calculation:

TBARS activity (mmol/ml) = Absorbance of test x V x 1000

$$/ \frac{A \times v \times L \times Y}{}$$

Where;

V = Total volume to which test was diluted

A = Molar extinction coefficient of product = $1.56 \times 10^{5} M^{-1} cm^{-1}$

v = Volume of serum sample used

Y = Total protein estimated for a particular sample

L = Ligth path = 1cm.

3.12.2 Determination of uric acid by the method of Gjrup *et al.*, (1955).

Principle of assay

Uric Acid is oxidised by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro- 2 hydroxy-benzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound.

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantion} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{Indicator} \xrightarrow{\text{Peroxidase}} \text{Colour complex} + H_2O$$

Test procedure for assay of uric acid as described by Gjrup *et al.*, (1955).

	Reagent blank (ul)	sample (ul)	standard(ul)
Sample	--	20	--
Standard	--	--	20
Reagent	1000	1000	1000

It was mixed and incubated for 5minutes at 37^{o}C and absorbance was read at 520nm against a reagent blank within 30minutes.

Calculation

$$\text{Uric acid concentration} = \frac{\text{Abs sample}}{\text{Abs standard}} \times \text{Standard Conc (mg/dL)}$$

Conversion: mg/dL x 0.059 = mmol/L

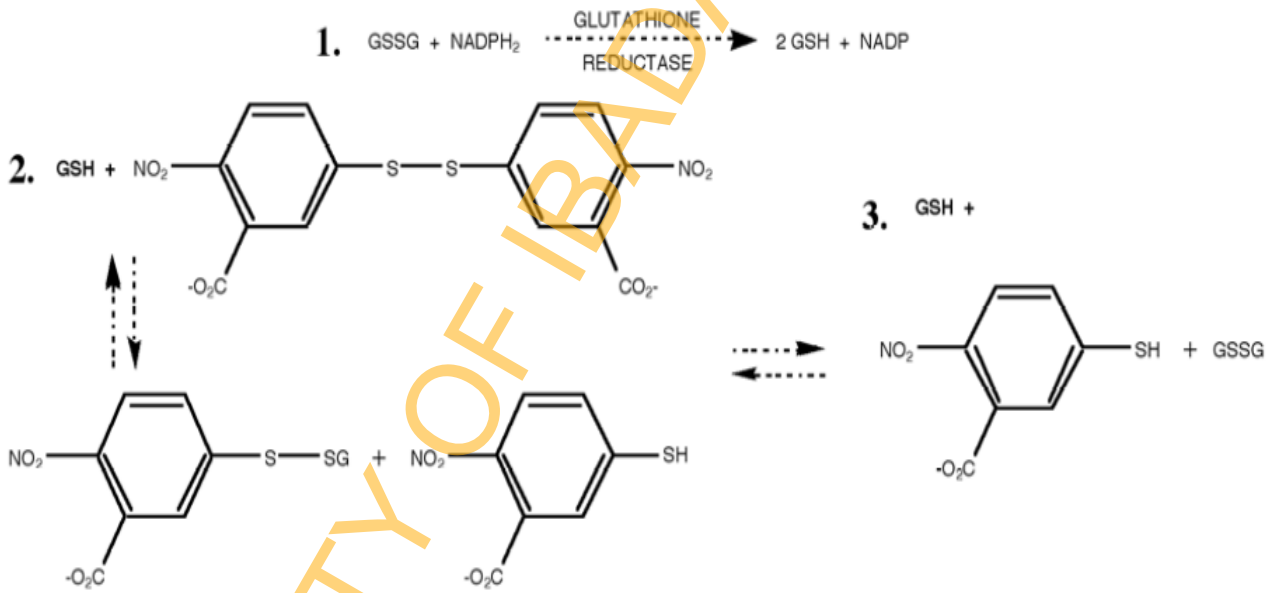
Quality control

Randox Bovine Precision Multi Sera Control Level 1, 2 and 3 were ran. Result of quality control is attached appendix

3.12.3 Determination of total glutathione (tGSH) (Teitze, 1969)

Principle

The reaction is based oxidation of GSH by 5,5'-dithiobis (2-nitrobenzoic acid) [DTNB] to measure the total glutathione (tGSH) content of biological samples. The quantitative determination of the total amount of glutathione [GSH (reduced glutathione) + GSSG (oxidized glutathione)] employs the enzymatic method first reported by Tietze. Briefly, the reaction of GSH with Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) gives rise to a product that can be quantified spectrophotometrically at 412 nm. This reaction is used to measure the reduction of GSSG to GSH. The rate of the reaction is proportional to the GSH and GSSG concentration as shown below



Procedure

1. 50 µL of each GSH standard or sample were added to each well.
2. 50 µL each of DTNB and Oxido-reductase solutions were added to each well.
3. The plate was incubated for 10 minutes at room temperature.
4. 50 µL of β-NADPH2 solution was added to each well to start the reaction.
5. The plates were incubated for 10 minutes and then read at 412 nm

Calculation

The standard curve was plotted with OD versus GSH concentrations. The total GSH concentrations of the samples were calculated from the standard curve.

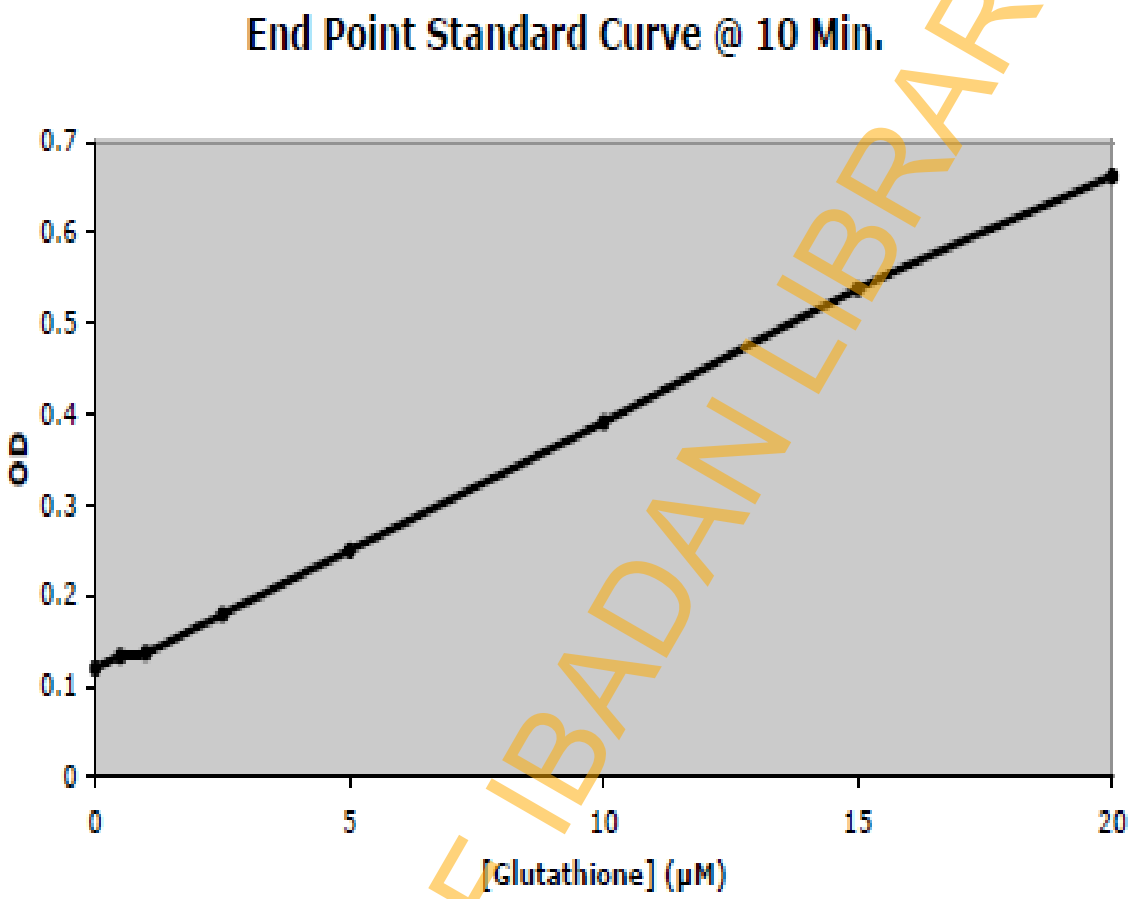


Fig. 3.3 Glutathione assay validation curve

3.13 Determination of antioxidant vitamins using spectrophotometry

3.13.1 Determination of Vitamin A

Vitamin A was determined by the method of Rutkowski *et al*., (2006).

Principle

The test sample was deproteinated and hydrolyzed with KOH solution in ethanol, which allows gentler and shorter heating (60°C, 20 minutes) without the necessity to use the nitrogen atmosphere. The xylene mixture in which KOH does not dissolve was used for extraction. The extract was measured at an absorbance of 335 nm against xylene. Measurement I was the sum absorbance of vitamin A and the interfering substances; while measurement II was of the interfering substances absorbance. Calculation of the determined vitamin A absorbance was the difference between the results of measurement I and II.

Procedure

100µl of the plasma was pipetted into test-tube I. 1ml of KOH solution was added. The tube was plugged with a tight stopper and shaken vigorously for 1minute. The tube was heated in a water bath at 60°C for 20 minutes, and cooled in cold water. 1ml of xylene was added and the tube shaken vigorously again for 1 minute. The tube was centrifuged at 1500xg for 10 minutes. The whole of the separated extract (upper layer) was transferred into test-tube II made of soft (sodium) glass. The absorbance A_1 of the obtained extract was measured at 335nm against xylene using Genesys 10S UV-VIS Spectrophotometer, (Thermo Scientific, Fischer). The extract of test tube II was irradiated with UV light for 30 minutes, then the absorbance of A_2 was measured at 335nm.

The concentration C_x of vitamin A (µM) in the plasma was calculated using the formula:

$$C_x = (A_1 - A_2). 22.23$$

Where: 22.23- multiplier was obtained on the basis of the absorbance coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335nm in a measuring cuvette about 1cm thickness.

3.13.2 Determination of Vitamin C

Vitamin C was determined by the method of Rutkowski and Grzegorczyk, (2007) using phosphotungstate reagent.

Principle

This is a selective and fast method for determination of the reduced form of vitamin C, using a periodically prepared phosphotungstate reagent (PR). This reagent becomes reduced by the L-ascorbic acid which is contained in the sample and produces the tungsten blue, absorbance of which is measured at 700nm using the Genesys 10S UV-VIS Spectrophotometer, (Thermo Scientific, Fischer). The PR also denatures proteins contained in the sample, thus eliminating the necessity of protein removal as a separate step.

Procedure

0.1ml of the plasma was pipette into the centrifugal test-tube, 1 ml of the PR was added and, mixed thoroughly at room temperature for 30minutes. The tube was centrifuged at 7000xg for10minutes and the whole of the separated supernatant was collected with a pipette. The supernatant is a test samples for spectrophotometric measurements. 56.8µM vitamin C (L-ascorbic acid) standard solution was prepared with 50 mmol/L solution of oxalic acid as solvent. 0.1ml of the standard solution was added to 1ml of PR, without centrifugation. The absorbance of the test sample A_x and of the standard solution A_s were measured at 700nm against the mixture PR: 50 mmol/L solution of oxalic acid=1:1 (v/v) as a reference sample.

Concentration C_x of vitamin C (µM) in the plasma was calculated using the formula:

$$C_x = A_x / A_s . C_s$$

Where: C_s = concentration of the standard solution

3.13.3 Determination of Vitamin E

Vitamin E was determined according to the method of Rutkowski *et al*., (2005).

Principle

Vitamin E which is a mixture of four tocopherols and four tocotrienols was extracted with xylene from the test samples and the extracts were exposed to batophenanthroline (and H_3PO_4 to increase stability of the colour). The resulting colour allows for absorbance measurement spectrophotometrically at 539nm using the Genesys 10S UV-VIS Spectrophotometer, (Thermo Scientific, Fischer). To ensure stability of the produced colour reaction, all the applied reagents were in anhydrous form including ethanol.

Procedure

0.1ml of the plasma was pipette into test tube I with a tight stopper, 0.5ml of anhydrous ethanol was added and shaken vigorously for 1 minute. Three ml of xylene was added and shaken vigorously for another 1 minute. The tube was centrifuged to separate the extract at 1500xg for10 minutes; 0.25ml of bathophenanthroline was measured into test tube II. 1.5ml of the extract (upper layer), was transferred to test tube II and mixed. 0.25ml of $FeCl_3$ solution was added to test tube II and mixed, 0.25ml of H_3PO_4 solution was added and mixed again for spectrophotometric measurements. Prepare the standand sample (0.5ml of the standard solution instead of the analysed liquid); using α-tocopherol and prepared as the test. The absorbance of the test sample A_x and of the standard sample A_s at 539nm were measured against the blank test (preparation- as the test sample but using water instead of plasma).

The concentration C_x of vitamin E (µmol) in the plasma was calculated using the formula:

$$C_x = A_x/A_s . C_s$$

Vitamins A, C and E assays were validated and values compared with High Performance Liquid Chromatography assays.

3.14 Vitamins assay using high performance liquid chromatography (HPLC) (Snyder, *et al.*, 1997)

The plasma vitamins A, C and E were assayed using the Waters 616 HPLC machine manufactured by the Waters Corporation USA. Serum samples previously stored at -20°C were used. The Chromosystem HPLC kit (manufactured by chromosystems Instruments & Chemicals, Munchen, Germany) assay procedures are as stated below:

3.14.1 Vitamin A (retinol) analysis

Sample extraction for vitamin A (retinol determination)

Step 1: 0.125ul of the blood sample(s) was measured into a set of clean test tubes and made to 500ul volume with ultra-pure water.

Step 2: 10g/l of ascorbic acid as an antioxidant was added and shaken for 15minutes followed by 5 minutes of sonication.

Step 3: 0.5g/l of Triton X100 was added (this acts as detergent).

Step 4: 400ul acetonitrile was added and mixed properly. (This acts as an internal standard)

Step 5: 400ul n-hexane was added. (This contains 5g/L BHT).

Step 6: The mixture was vigorously shaken for 4 minutes.

Step 7: The mixture was centrifuged for 2 minutes at 3000 revolutions per minute (RPM).

Step 8: The supernatant was collected for Vit A (Retinol) determination on HPLC

Step 9: The sample extracts was injected in to the HPLC through the sample injection valve via the vial septum.

- The flow rate was fixed at 1.5ml/ minute at a temperature of 30°C.
- The wavelength was set at 325nm

3.14.2 Method of vitamin C analysis

Procedure:

- 0.25mL of the sample(s) were pipetted into 250ml volumetric flasks
- 50mL of ultra-pure water was added and whirled gently.
- 20.0mL of 20% metaphosphoric acid was added and shaken gently.
- 2.5mL of 0.5% oxalic acid was added and carefully shaken.
- 2.5.0 mL of acetone was also added and made to 250ml volume with ultra-pure water.
- The whole set up were shaken on a mechanical shaker for 30 minutes
- The sample solution was centrifuged for 20 minutes at the revolution of 5000 rpm.
- The supernatants were sampled to a set of vials ready for determination on HPLC.

Preparation of Standards (Stock Standard)

100 ppm stock standard of ascorbic acid was prepared from 1000 ppm reference standard as follows:

$C_1V_1 = C_2 V_2$

$\therefore V_1 = \frac{100 \times 250}{1000} = 25ml$

i.e. 25ml of 1000 ppm reference standard was pipetted into 250ml volumetric flask, and made to volume with the mixture of (Ultrapure water and oxalic and + acetone) (2:1:1).

Preparation of working standard

Working standards – (0.0, 0.25, 0.50, 0.75, 1.00ppm) were prepared from 100 ppm stock standard, as follows:

$C_1V_1 = C_2 V_2$

1000 x 3 No x 250 (i.e. size of the volumetric flask)

$\therefore V_1 = 100 \times 25/1000 = 0.25ml$

i.e. 0.25ml of 100 ppm stock standard was pipetted into 100ml volumetric flask and made to volume with the same mixture of (ultra-pure water + oxalic acid + Acetone), to get 0.25 ppm etc.

3.14.3 Method of vitamin E analysis

Procedure

- 0.25ml of the sample(s) were pipetted into 100ml volumetric flask(s) each
- 20 ml of ultrapure water was added.
- 25ml of Benzene solution was added and shaken properly
- A solution of Dehydrated ethanol: Diethyl ether: HCL in the ration of (4: 3:1) was made
- 25ml of the extraction mixture was added into the sample solution and mixed properly.
- The whole set up was transferred to a mechanical shaker and shaken for 20mintes.
- It was then centrifuged for 25 minutes at 5000 rpm.
- The supernatant was transferred to a set of vials.
- Then analyzed on HPLC as stated below:

Preparation of standards from 1000 ppm reference standard

- The range of the working standards were : (0.0, 1.0, 2.0, 3.0, 4.0 PPM) prepared as follows:

Preparation of stock standard (100 ppm)

$C_1V_1 = C_2 V_2$

$1000 \times V_1 = 100 \times 100ml$ volumetric flask

i.e. V_1 = 100 x 100/100 = 10ml

i. e. pipetted 10ml of 1000ppm vitamin E reference standard into a 100 ml volumetric flaks and made to volume to get 100 ppm vitamin E stock standards of 0.0, 1.0, 2.0, 3.0, 4.0 ppm were prepared from 100 ppm stock standard as follows

$C_1V_1 = C_2 V_2$

NO x V_1 = 1.0 x 100ml of volumetric flask

i.e. V1 = 1.0 x 100/100 = 1.0ml

i. e. pipetted 1.0 ml of the prepared 100 ppm stock standard into a 100ml volumetric flask and made to volume with the extraction mixture to get 1.0 ppm etc.

3.15 Determination of total calcium, ionized calcium and 25(OH) vitamin D

3.15.1 Determination of total calcium by colorimetric method as described by Ray-Sarkar and Chauhan (1967).

Principle

Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium which maximally absorbs light at 578nm.

Procedure

	Reagent Blank	Standard	Sample
Sample	------	------	25µl
Distilled water	25µl	------	------
Standard	------	25µl	------
Working reagent	1.0ml	1.0ml	1.0ml

These were mixed and absorbance read at 578nm after 10 minutes at room temperature range of 20-25^{o}C.

Calculation

$$\text{Concentration (mmol/L)} = \frac{A \text{ sample}}{A \text{ standard}} \times [\text{Standard}] \text{ (mmol/L)}$$

3.15.2 Determination of ionized calcium

Ionized calcium was calculated (in mg/dL) with the formula (Toffaletti, 2011):

iCa = [0.9 + (0.55 x tCa – 0.3 x albumin)]

iCa = (6 x tCa – TP/3)/(6 + TP); where tCa is in mg/dL, albumin and TP are in g/dL.

3.15.3 Determination of 25 (OH) Vitamin D by enzyme linked immunosorbent assay (ELISA) (Holick, 2009)

Principle

The solid phase enzyme-linked immunoassay (ELISA) was based on the principle of competitive binding. Anti-Vitamin D antibody coated wells are incubated with Vitamin D standards, controls, samples, and Vitamin D-Biotin conjugate at room temperature for 90 minutes. During the incubation, a fixed amount of biotin-labelled vitamin D competes with the endogenous Vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti-vitamin D antibody. Following a wash step, bound Vitamin D-Biotin is detected with Streptavidin-HRP (SA-HRP). SA-HRP conjugate immunologically bound to the well progressively decreases as the concentration of Vitamin D in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 30 minutes, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The colour intensity is inversely proportional to the amount of 25-OH Vitamin D in the sample.

Procedure

1. 10µl of 25-OH Vitamin D Standards, controls and samples were dispensed into each well, as required.
2. 200µl working solution of biotinylated 25 (OH) D reagents was dispensed into each well.
3. Carefully the contents in the wells were mixed for 20 seconds using a plate shaker at 400 RPM.
4. The plate was incubated for 90 minutes at room temperature.
5. The contents of the wells were briskly shaken out into a waste reservoir.

6. 300µl of 1X Wash Buffer was dispensed into each well, and then briskly shaken out into a waste reservoir. The wells were sharply stroke on absorbent paper to remove residual droplets. This was repeated 2 more times for a total of 3 washes.
7. 200µl of enzyme conjugate (Streptavidin-HRP) was dispensed into each well.
8. Incubation was done for 30 minutes, at room temperature.
9. The contents of the wells were briskly shaken out into a waste reservoir.
10. 300µl of 1X Wash Buffer was dispensed into each well, and then briskly shaken out into a waste reservoir. The wells were sharply stroke on absorbent paper to remove residual droplets. This was repeated 2 more times for a total of 3 washes.
11. Using a multi-channel pipette, 200 µl of TMB Substrate was dispensed into each well.
12. Incubation was done for 30 minutes at room temperature in the dark.
13. 50 µl of Stop Solution was dispensed into each well to stop the enzymatic reaction. Plate contents carefully mixed for 20 seconds.
14. Absorbance and concentration were read on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution.

Calculation

Concentration of each sample can also be read from a standard graph, this can be done by plotting O.D of each standard against the corresponding concentrations on a log-log graph.

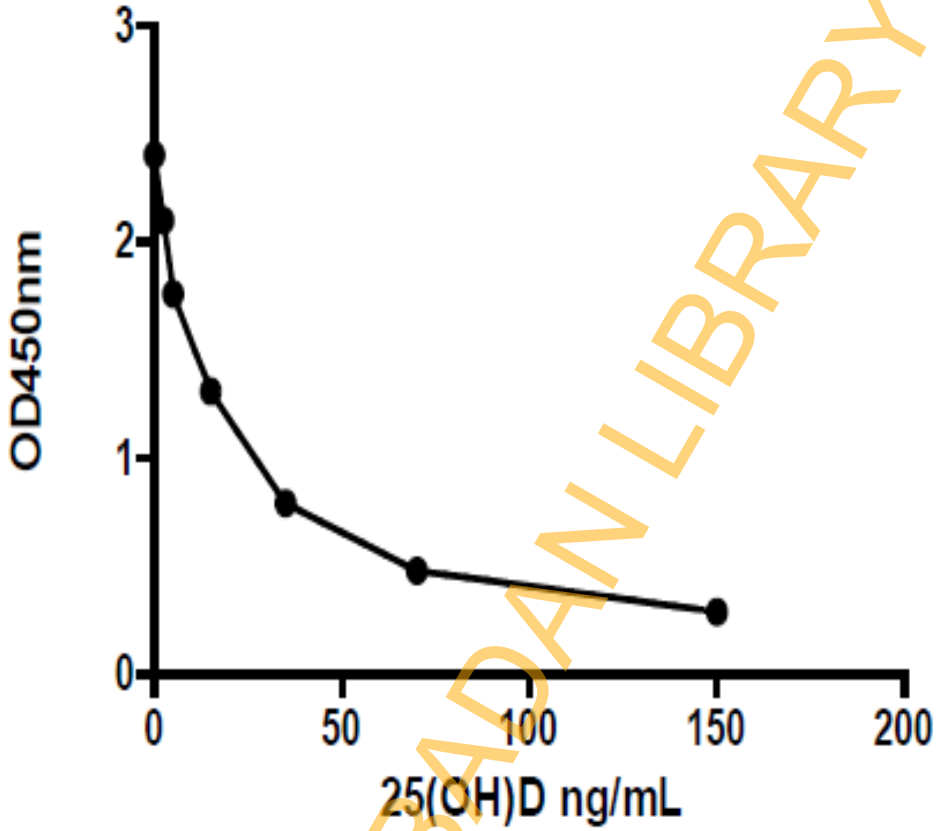


Fig. 3.4 Standard curve for 25(OH) vitamin D determination

3.16 Determination of levels of wild-type p53 and selected biomarkers of Genotoxicity

3.16.1 Determination of wild-type p53 level by ELISA method (RayBio Human p53 assay, USA) (Flaman *et al*., 1995).

Principle

This assay employs an antibody specific for human p53 coated on a 96-well plate. Standards and samples are pipetted into the wells and p53 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human p53 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a 3,3,5,5-tetramethylbenzidine (TMB) substrate solution is added to the wells and colour develops in proportion to the amount of p53 bound. The Stop Solution changes the colour from blue to yellow, and the intensity of the colour is measured at 450 nm.

Procedure

1. All reagents and samples were brought to room temperature (18 - 25°C) before use.
2. 100 µl of each standard and sample were added into appropriate wells. The well was covered and incubated for 2.5 hours at room temperature
3. The solution was discarded and washed 4 times with 1X Wash solution using autowasher.
4. After the last wash, any remaining Wash Buffer was removed by decanting. The plate was inverted and blotted against clean paper towels.
5. 100 µl of 1X prepared biotinylated antibody was added to each well. It was incubated for 1 hour at room temperature with gentle shaking.
6. The solution discarded and the wash step was repeated as in 3.
7. 100µl of prepared Streptavidin solution was added to each well and was incubated for 45 minutes at room temperature with gentle shaking.
8. The solution was discarded and washing was repeated as in step 3.
9. 100µl of TMB One-Step Substrate Reagent (Item H) was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking.
10. 50 µl of Stop Solution (Item I) was added to each well and was read at 450nm immediately.

Calculation

The mean absorbance for each set of duplicate standards, controls and samples, calculated and the average zero standard optical density was subtracted. The standard curve was plotted on log-log graph paper or using sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points was drawn

3.16.2 Determination of 8-hydroxy-2´- deoxyguanosine (8-OHdG) by enzyme linked immunosorbent assay method (Cloud-Clone Corp. USA) (Kasai, 1988)

Principle

This assay employs the competitive inhibitor enzyme immunoassay technique. A monoclonal antibody specific to 8-OHdG has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labelled 8-OHdG and unlabelled 8-OHdG (Standards or samples) with the pre-coated antibody specific for 8-OHdG After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplates well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of 8-OHdG in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of 8-OHdG in the sample.

Procedure

1. 50µl each of standard, blank and samples were added into the appropriate wells, respectively. And then 50µL of Detection Reagent A was added to each well immediately. The plate was shaken gently (using a microplates shaker). Incubation was done for 1 hour at 37°C.
2. The solution was aspirated and washing was done with 350µl of 1X Wash Solution using autowasher, and was allowed to sit for 1-2 minutes. The remaining liquid was removed from ail wells completely by snapping the plate onto absorbent paper. This was repeated 3 times. After the last wash, any remaining Wash Buffer was removed by inverting the plate and blotted it against absorbent paper.
3. 100µL of Detection Reagent B working solution was added to each well. Incubation was done for 30 minutes at 37°C after covering it with the Plate sealer
4. Wash process was repeated has was performed in step 2.

5. 50µL of Substrate Solution was added to each well and incubated for 20 minutes at 37°C.
6. 50µL of Stop Solution was added to each well. Proper mixing of the liquid was done by gently tapping the side of the plate.
7. Remaining water was removed, the microplate reader was ran and measurement conducted at 450nm immediately.

Calculation of result

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between 8-OHdG concentration in the sample and the assay signal intensity.

Average of the duplicate readings for each standard, control, and samples were calculated. A standard curve was created on log-log graph paper, with the log of 8-OHdG concentration on the y-axis and absorbance on the x-axis. The best fit straight line was drawn through the standard points and it can be determined by regression analysis. Concentrations were read from the graph.

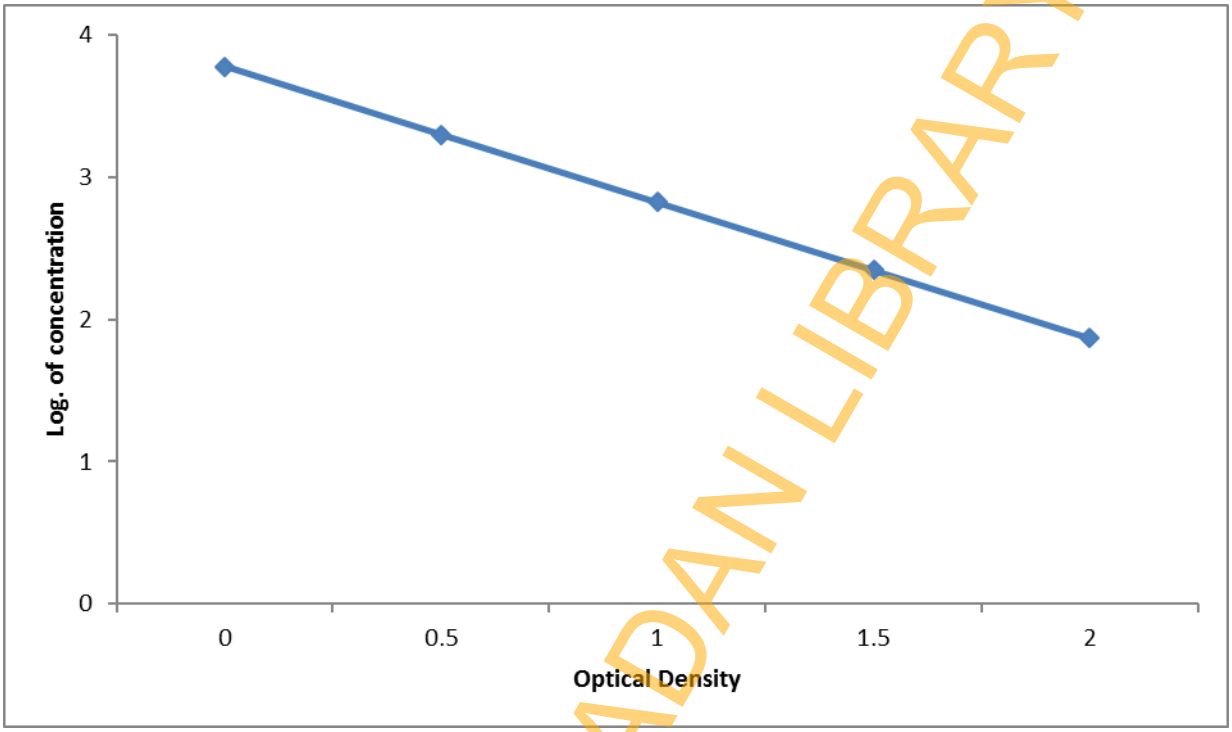


Fig. 3.5 Standard Validation Curve for 8 –OHdG ELISA

3.16.3 Determination of 8-Oxoguanine DNA Gycoxylase activity (OGG_1) by sandwich enzyme immunoassay method (Cloud-Clone Corp. USA)

Principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to OGG_1. Standards or samples are then added to the appropriate microtiter plate wells with biotin-conjugated antibody specie to OGG_1. Next Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain OGG_1, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentrator of OGG_1 in the samples is then determined by comparing the O.D. at the samples to the standard curve.

Procedure

1. Wells were determined for diluted standard, blank and sample. 7 wells were prepared for standard, 1 well for blank.
2. l00µL each of dilutions of standard, blank and samples were added into the appropriate wells and incubation was done for 2 hours at 37^0C.
3. The liquid removed from each well but was not washed.
4. 100µL of Detection Reagent A working solution was added to each well. This was incubated for 1 hour at 37°C after covering it with the Plate sealer.
5. The solution was aspirated and washing was done by adding 350µL of 1X Wash Solution to each well using autowasher. The remaining liquid was removed from all wells completely by snapping the plate onto absorbent paper. It was totally washed 3 times, after the last wash, any remaining Wash Buffer removed by decanting. The plate was inverted and blotted against absorbent paper.
6. l00 µl of Detection Reagent B working solution was added to each well. Incubation was done for 30 minutes at 37°C after covering it with the Plate sealer.
7. The aspiration/wash process was repeated for total 5 times as conducted in step 5.
8. 90µl of Substrate Solution was added to each well and incubated for 20 minutes at 37°C.
9. 50µL of Stop Solution added to each well. The liquid was mixed by tapping the side of the plate.
10. The microplate reader was run and measurement conducted at 450nm immediately.

Calculation

Average the duplicate readings for each standard, control, and samples were done and the average zero standard optical density was subtracted. A standard curve was constructed by plotting the mean O.D and concentration for each standard and drawing a best fit curve through the points on the graph, the concentration read from the standard curve was multiplied by the dilution factor.

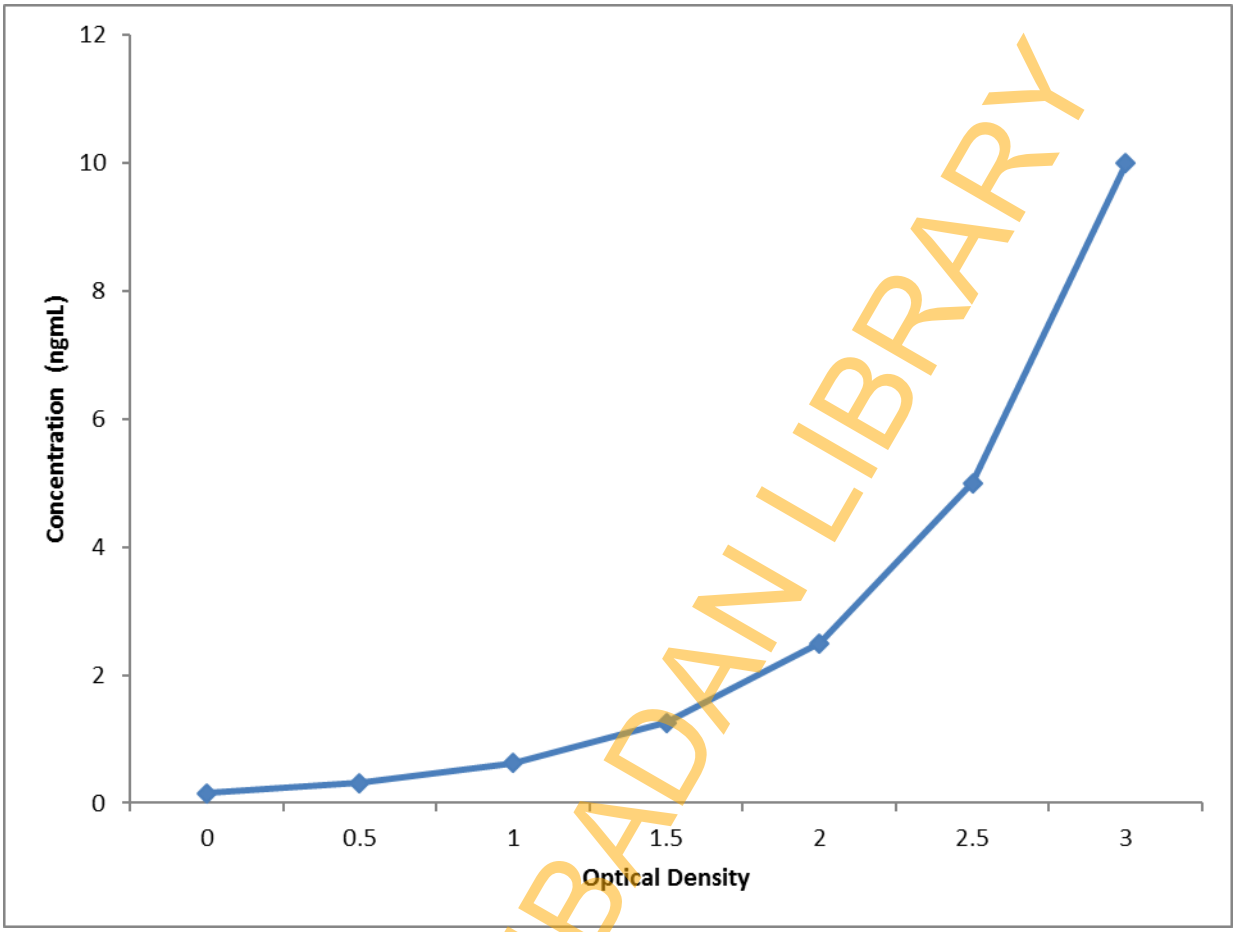


Fig. 3.6 Standard validation curve for 8-Oxoguanine DNA Glycosylase (OGG_1) assay

3.16.4 Micronuclei assay

Principle

The Micronucleus test is a comprehensive, quick and sensitive method for measuring DNA damage – micronuclei (MN). Micronuclei are chromatin-containing structures in cytoplasm surrounded by a membrane without any detectable link to the cell nucleus. They are formed by exclusion of whole chromosomes or chromatin fragments during cell division. Micronuclei are scored specifically in once divided binucleated cells.

Micronuclei as biomarkers of chromosome breakage and / or whole chromosome loss were evaluated using the micronucleus test described by Fenech and Morley, (1985) and modified by Holland *et al.*, (2008).

Procedure:

1) A small drop of Peripheral blood was collected over a clean coded slide, thin smear made and air-dried.
2) Slides were fixed after 24hrs in absolute methanol for 5mins. The slides were then stained with May-Gruenwald and Giemsa.
3) The dry slides were stained with 5% Giemsa solution for 3 – 4 minutes, washed properly with water and let dried at room temperature.

The 5% Giemsa solution was prepared by adding Giemsa (5 ml) to Sörens buffer (15 ml) making up to 100 ml with 80 milliliters of distilled water.

4) Slides were mounted using DPX mountant, dried (20-30°C) and cleaned.
5) The frequencies of micronuclei (MN) in polychromatic erythrocytes (PCE) were estimated by scoring 1000 PCE per human (Zaizuhana *et al.*, 2006).

Criteria for scoring micrunuclei (MNi):

MNi appeared morphologically identical to but smaller than nuclei.

The diameter of MNi usually varied between 1/16th and 1/3rd of the mean diameter, which corresponds to 1/256th and 1/9th of the area of one of the main nuclei in a binucleated cell.

MNi usually had the same staining intensity as the main nuclei but occasionally staining appeared more intense.

MNi occasionally touched but did not overlap the main nuclei and the micronuclear boundary was distinguishable from the nuclear boundary.

Microscopical slides evaluation

The micronuclei were scored in at least 2,000 binucleated cells using a microscope Olympus BX41 at 1000-fold magnification. Then the number of micronuclei in 1,000 binucleated cells was calculated (MN/1000 binucleated cell = micronuclei / 1000 binucleated cells).

Microscopical analysis:

Objects scored during manual analysis shown in fig. 3.9 below:

Binucleated cells without micronuclei – scored as intact

Binucleated cells with 1 micronucleus

Binucleated cells with 2 micronuclei

In the manual microscopic analysis, the following were also scored; nucleoplasmic bridges (NPB – a biomarker of DNA misrepair and/or telomere end-fusion) and nuclear buds (NBUD – a biomarker of elimination of amplified DNA and/or DNA repair complexes).

Overall, selection of the cells followed the criteria summarized below:

The cells should be binucleated with an intact cytoplasm and normal nucleus morphology. The two nuclei in a binucleated cell should be approximately equal in size, staining pattern and staining intensity. The two main nuclei in a binucleated cell should not overlap. The two main nuclei may be attached by a nucleoplasmic bridge, which is no wider than 1/4th of the nuclear diameter.

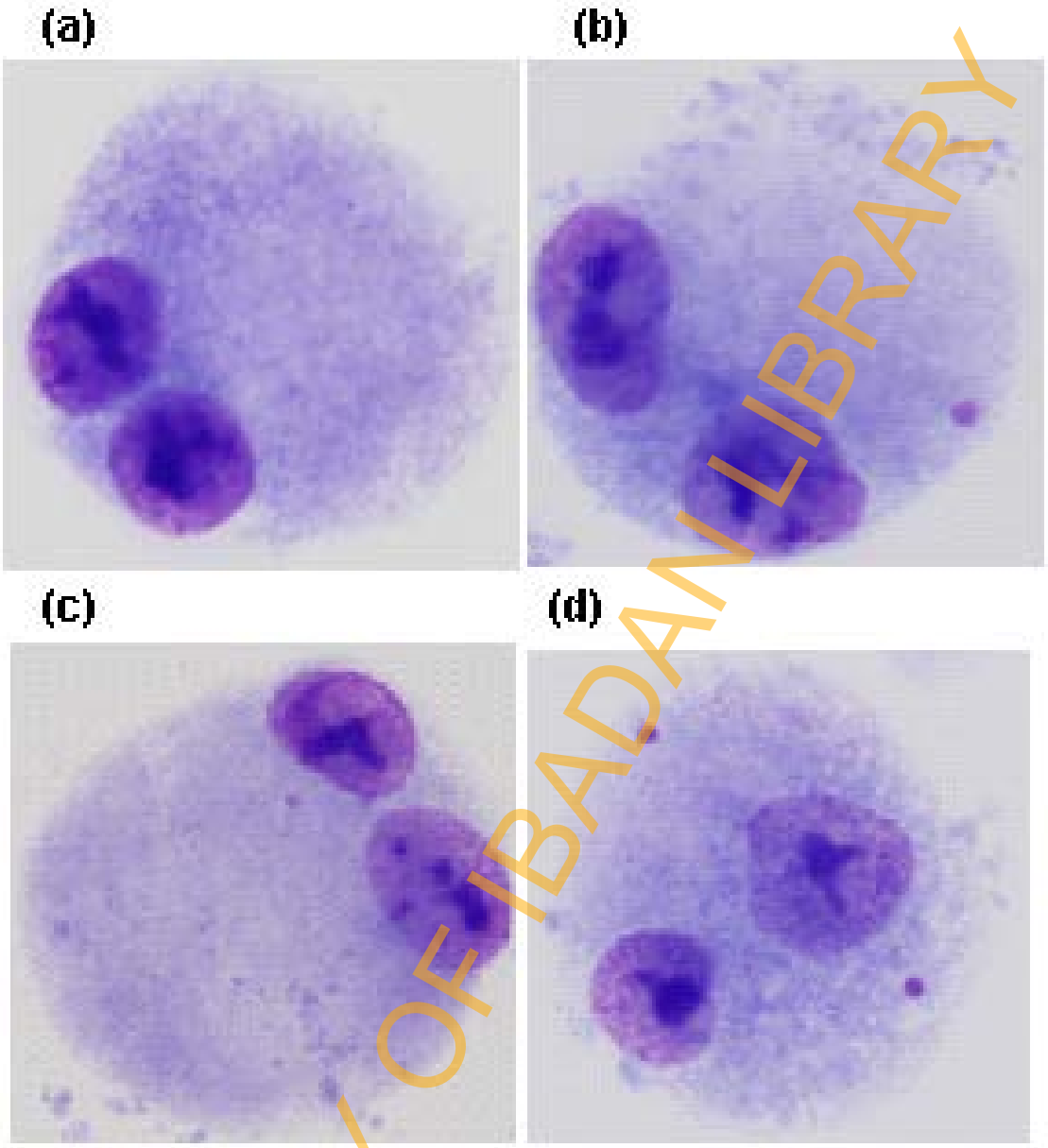


Fig. 3.7 Scoring of binucleated cell – Manual analysis: (a) binucleated cell without the micronucleus; (b,c) binucleated cells with the micronucleus of different size; (d) binucleated cell with two micronuclei.

3.17 Determination of selected liver function tests

3.17.1 Determination of total protein by colorimetric method (Biuret reaction) (Henry *et al.*, 1974)

Principle

Cu^{2+} in alkaline solution reacts with peptide bonds in proteins, producing a violet colour whose intensity is proportional to the amount of protein present.

Protocol

	Reagent blank	Standard	Sample
Distilled water	0.02ml	--	--
Standard(CAL)	--	0.02ml	--
Serum	--	--	0.02ml
R1	1.0ml	1.0ml	1.0ml

- The tubes were mixed and incubated for 30 minutes at room temperature (20-25^{0}C).
- The solutions were mixed and dispensed into 1cm light-path cuvette and placed in the sample cell in the spectrophotometer.
- Absorbance was read using VIS 7220G spectrophotometer (Biotech Engineering Ltd, United Kingdom) at a wavelength of 540nm against a reagent blank.

Calculation

$$\text{Total protein conc. (g/L)} = \frac{\text{Abs sample} \times \text{Conc. of standard (g/L)}}{\text{Abs Standard}}$$

Calculation of globulin concentration

Globulin (g/l) = Total protein (g/l) – Albumin (g/l)

3.17.2 Determination of Alanine Aminotransferase (ALT) activity

Alanine Aminotransferase activity was determined using Reflotron Chemical Auto-analyzer by Awareness Tecnology, Inc. Palm City, United States, Model: 4700, S/N: 4700-1333

Principle of reaction

$$\alpha\text{-ketoglutarate} + \text{alanine} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{pyruvate}$$

$$\text{pyruvate} + PO_4^{3-} + O_2 + H_2O \xrightarrow{\text{Pyruvate oxidase}} \text{acetyl phosphate} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{indicator (red)} \xrightarrow{\text{Peroxidase}} \text{indicator (oxd)} + H_2O$$

This was read at 567nm in the Reflotron Chemistry analyzer.

Procedure for the Test

- The test strip specific for ALT estimation was placed carefully on the working bench.
- The aluminum foil covering the test area was removed. The Reflotron micro-pipette was used to dispense 30µl of serum sample unto the test area on the test strip.
- The flap of the Reflotron insertion chamber was raised and the test strip placed in it carefully and horizontally.
- The flap was closed and the start function key was tapped.
- The result was displayed digitally on the screen after 3 minutes and also printed.

Quality Control

The precinorm and precipath tests were preformed to check the sensitivity of the equipment for reading of values within in health and disease respectively.

3.17.3 Determination of Aspartate Aminotransferase (AST) activity

Aspartate Aminotransferase activity was determined using Reflotron Chemical Auto-analyzer by Awareness Tecnology, Inc. Palm City, United States, Model: 4700, S/N: 4700-1333

Principle of the Assay

After the sample has been applied on the AST specific test strip, the sample flows to the reaction zone. In the presence of AST α-ketoglutarate and alanine sulfinate are converted to pyruvate and glutamate. In a second reaction, catalyzed by pyruvate oxidase, pyruvate is cleaved into acetyl phosphate, carbon dioxide and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide is converted to water and oxygen released is accepted by a

chromogen. The dye formed was measured kinetically at 567nm as a measure of the activity of AST at 37^0C.

α-ketoglutarate + alanine sulfinate $\xrightarrow{\text{AST}}$ glutamate + pyruvate + SO_3^{2-}

pyruvate + $PO4_3^{2-}$ + O_2 + H_2O $\xrightarrow{\text{Py-Oxd}}$ acetyl phosphate + H_2O + CO_2

H_2O_2 + indicator (reduced) $\xrightarrow{\text{Peroxidase}}$ Indicator (oxidized) + H_2O

Procedure

- The test strip with magnetic code specific for AST estimation was placed carefully on the working bench.
- The aluminum foil covering the test area was peeled off carefully.
- The reflotron micro-pipette was used to dispense 30µl of serum sample unto the test area on the test strip.
- The flap of the reflotron insertion chamber was raised and the test strip placed in it carefully and horizontally.
- The flap was closed and the start function key was tapped.
- The result was displayed digitally on the screen after 3 minutes and also printed.

Quality Control

The precinorm and precipath tests were preformed to check the sensitivity of the equipment for reading of values within in health and disease respectively.

Determination of AST/ALT ratio (reference value is

AST/ALT ratio was calculated by dividing AST values with the corresponding ALT (AST to ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease).

3.17.4 Determination of alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was determined using Reflotron Chemical Auto-analyzer by Awareness Tecnology, Inc. Palm City, United States, Model: 4700, S/N: 4700-1333

Reaction principle

O-Cresolphthalein phosphate + methylglucamine

$\Downarrow$ ALP

O-Cresolphthalein + methylglucamine phosphate

The absorbance was read in the Reflotron autoanalyzer at 567nm.

Procedure

- The test strip with magnetic code specific for ALP estimation was placed carefully on the working bench.
- The aluminum foil covering the test area was removed.
- The Reflotron micro-pipette was used to dispense 30µl of serum sample unto the test area on the test strip.
- The flap of the Reflotron insertion chamber was raised and the test strip placed in it carefully and horizontally.
- The flap was closed and the start function key was tapped.
- The result was displayed digitally on the screen after 3 minutes and also printed.

Quality control

The precinorm and precipath tests were preformed to check the sensitivity of the equipment for reading of values within in health and disease respectively.

3.17.5 Determination of Gamma-glutamyltransferase by colorimetric method (Szasz, 1969)

Principle

The substrate L-γ-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by γ-GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405nm.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine

L-γ-glutamylglycylglycine + 5-amino-2-nitrobenzoate

Procedure

100µl of each sample and standard were pipetted into their respective tubes

1ml of reagent was added to each of the tubes

These were mixed and initial absorbance was read

Timer was started simultaneously and reading was made at 1, 2 and 3 minutes.

Calculation

The following formula was used to calculate the GGT activity

U/L = 1158 x ΔA 405 nm/min

3.17.6 Determination of serum albumin by Bromocresol green method (Gustafson, 1976)

Principle

Albumin binds to bromocresol green (3, 3', 5, 5'-tetrabromo-cresol sulphonephthalein) to form a colour complex. The albumin-BCG complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Assay procedure and protocol for Randox albumin assay kit (Randox Inc., USA)

	Blank (µL)	Standard (µL)	Sample (µL)
Sample	----	----	10
Standard	----	10	----
Reagent	3000	3000	3000

Absorbance was read at 578nm after an incubation period of 10 minutes at room temperature.

Calculation

$$\text{Albumin concentration (g/dl)} = \frac{ABS_{sample}}{ABS_{std}} \times \text{Standard conc (g/dl).}$$

Conversion: g/dL x 10 = g/L

Quality control measures

Randox Bovine Precision Multi Sera Control Level 1, 2 and 3 were included for quality control.

3.17.7 Determination of total and conjugated bilirubin based on the colorimetric method described by Jendrassik and Grof (1938).

Principle

Direct (conjugated) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid.

Procedure/protocol table: total bilirubin (TB)

	Sample blank (µl)	Sample (µl)
Reagent 1	200	200
Reagent 2		50
Reagent 3	1000	1000
Sample	200	200

The tubes were mixed, and were incubated for 10 min at room temperature

Reagent 4	1000	1000

The solutions were mixed and were further incubated for 5min at room temperature, after which the absorbance were read spetrophotometrically at 578nm.

Total bilirubin reagent: Sulfanilic acid 16 mM, hydrochloric acid 164mM, dimethyl sulfoxide 4.4M, surfactant. Sodium nitrite reagent: Sodium nitrite 0.6M.

Protocol: conjugated bilirubin (CB)

	Sample blank (µl)	Sample (µl)
Reagent 1	200	200
Reagent 2		50
0.9% NaCl solution	2000	2000
Sample	200	200

The reaction tubes were properly mixed, and were incubated for 10 min at room temperature absorbance was read at 546nm.

Calculation

Total bilirubin (µmol/L) = 185 x Absorbance of test at 578nm

Conjugated bilirubin (µmol/L) = 246 x Absorbance of test at 546nm

Quality control

Quality control was performed as directed by the reagent producer.

Randox Bovine Precision Multi Sera Control Level 1, 2 and 3 were included. Result of quality control is attached in appendix

3.18 Determination of selected cancer risk biomarkers

3.18.1 Determination of total prostate-specific antigen (tPSA) by microplate immunoenzymometric assay (Espana *et al*., 1996).

Principle

The essential reagents required for an Immuno-enzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotin-labeled monoclonal anti-PSA antibody. Upon mixing monoclonal biotin-labeled antibody, the enzyme-labelled antibody and a serum containing the native antigen, reaction result between the native e antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

$Enz_{Ab(p)} + Ag_{PSA}{}^{+\,Btn}Ab_{(m)} \qquad Enz_{AB(p)} - Ag_{PSA} - {}^{Btn}Ab_{(m)}$

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below

$Enz_{Ab(p)} - Ag_{PSA} - {}^{Btn}Ab_{(m)} + spretavidin_{\,c.w}$ immobilised complex

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

Procedure

1. The micro plates' wells were formatted in duplicate for each standard, serum reference, control and patient specimen to be assayed.
2. 25µl of the appropriate serum reference, control or specimen was pipette into the assigned well.
3. 100µl of tPSA enzyme reagent was added to each well.
4. The micro plate was swirled gently for 20-30 seconds to mix and then covered.
5. It was incubated for 30 minutes at room temperature.

6. The content of the micro plate was discarded by decantation and the plate was tapped and blotted dry with absorbent paper.
7. 350µl of wash buffer was added, decant (tap and blot) or aspirate. This was repeated three times.
8. 100µl of the working substrate was added to all the wells.
9. It was incubated at room temperature for 15 minutes.
10. 50µl of stop solution was added to each well and mixed gently for 15-20 seconds.
11. The absorbance of each well was read at 450nm in a micro plate reader (using a reference wavelength of 620-630nm to minimize well imperfection).

Calculation

A dose response curve was plotted with the standard absorbance and concentrations and was used to read the concentration of tPSA in the unknown specimen.

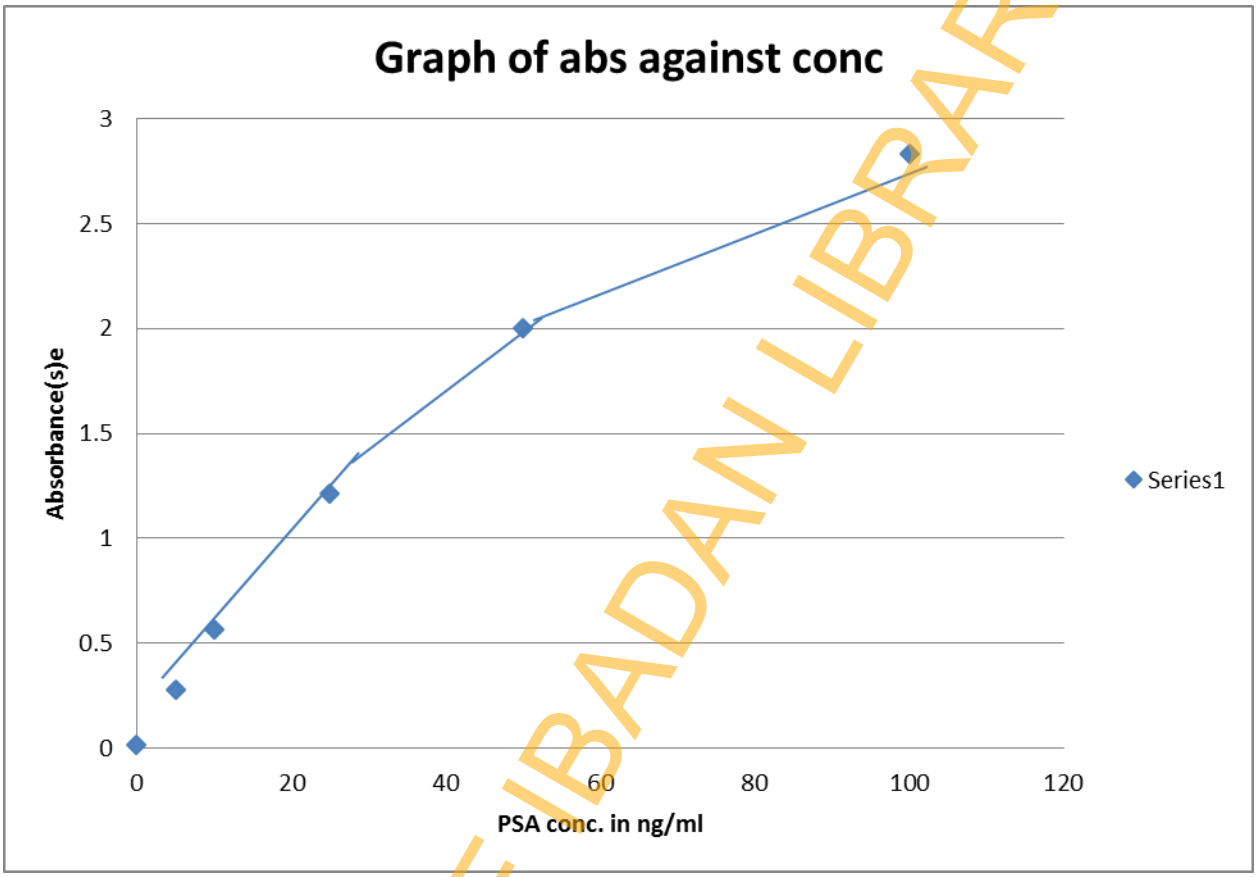


Fig. 3. 8 Dose response graph for tPSA using standard concentration and absorbance.

This graph served as quality control and validation for the assay.

3.18.2 Determination of free PSA by the method of Chen *et al*., (1995)

Free PSA was determined using ELISA (Accubind ELISA Microwells, Monobind Inc. Lake Forest, USA)

Principle

The essential reagents required for an immuno-enzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitopes recognition, in excess, and native antigen. In the procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of Streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction takes place between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex.

Simultaneously, the complex is deposited to the well through the high affinity reaction of Streptavidin and biotinylated antibody.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration (fPSA) of an unknown can be ascertained.

Procedure

1. The microplates' wells were formatted for each serum samples, controls and standards.
2. 50µl of the appropriate serum sample, control or standard were pipetted into the assigned wells
3. 100µl of the fPSA enzyme reagent was added to each well
4. The microplate was swirled gently for 20 minutes to mix and then covered
5. Incubation was done for 60 minutes at room temperature
6. The contents of the well were discarded by aspiration and washing was carried out using autowasher
7. 100µl of working substrate solution was added to each well
8. Incubation was done for 15 minutes at room temperature

9. 50µl of stop solution was added to each well and was mixed gently
10. The absorbance was read at 450nm in a microplate reader.

Calculation

A dose response curve was plotted using the absorbance and concentration values of the standard; the concentration of each sample was ascertained from the curve which also serves as a quality control measure.

3.18.3 Calculation of percentage fPSA

$$\% \text{ fPSA} = \frac{[\text{fPSA}]}{[\text{tPSA}]} \times \frac{100}{1}$$

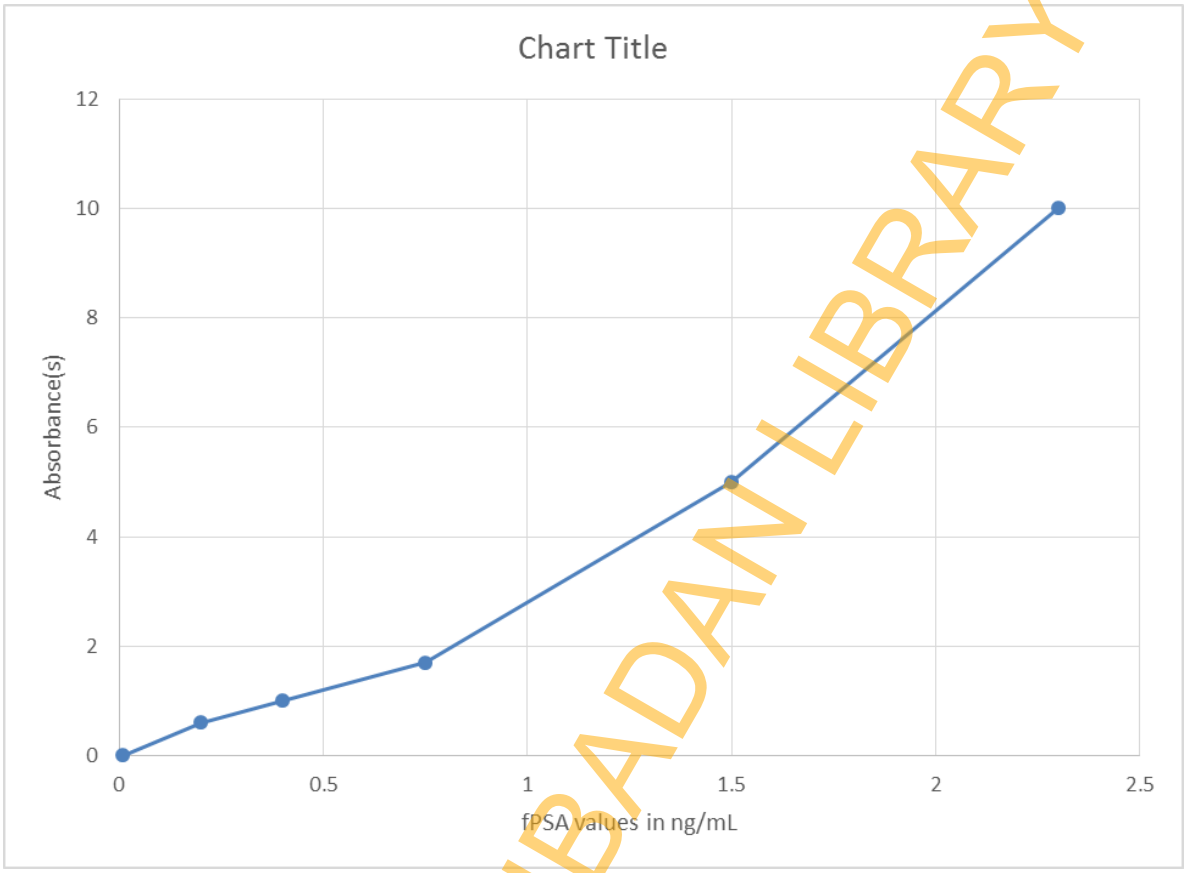


Fig. 3.9 Dose-response validation curve for fPSA

3.18.3 Determination of alpha fetoprotein (AFP)

Alpha fetoprotein was determined using Calbiotech AFP ELISA kit, Spring Valley, Canada, (Lee *et al*., 1991)

Principle

Endogenous AFP in sample binds to the antigenic site of the biotinylated Anti-AFP antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of the Peroxidase (HRP) conjugated Anti-AFP antibody reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies, labelled with Biotin and HRP. Unbound protein excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of colour developed is directly proportional to the concentration of AFP in the samples. A standard curve is prepared relating colour intensity to the concentration of the AFP.

Procedure

Prior to assay, reagents were allowed to stand at room temperature. All reagents were gently mixed before use.

1. The desired number of coated strips were placed into the holder
2. 25 µl of AFP standards, control and samples were pipetted into the wells.
3. 100 µl of Anti-AFP-Biotin Reagent was add to all wells and mix for 20 seconds.
4. The plate covered and incubated for 30 minutes at room temperature
5. Liquid was removed from all wells. Wells were washed using autowasher
6. 100 µl of the Anti-AFP- Enzyme conjugate was added to all wells. It was covered and incubated for 30 minutes.
7. Liquid was removed from all wells. Washing was done using autowasher and excess liquid was blotted on absorbent paper towels.
8. 100 µl of TMB substrate was added to all wells
9. Incubation was done for 15 minutes at room temperature
10. 50 µl of stop solution was added to all wells. The plate was shaken gently to mix solution.
11. Absorbance was read on ELISA Reader at 450nm within 15 minutes after adding the stop solution.

Calculation

The standard curve was constructed by plotting the absorbance for the AFP standards (vertical axis) versus the AFP standard concentrations in ng/ml (horizontal axis) on a linear graph paper and the best curve was drawn through the points, the absorbance for controls and each unknown sample was read from the curve. Record the value for each control and sample.

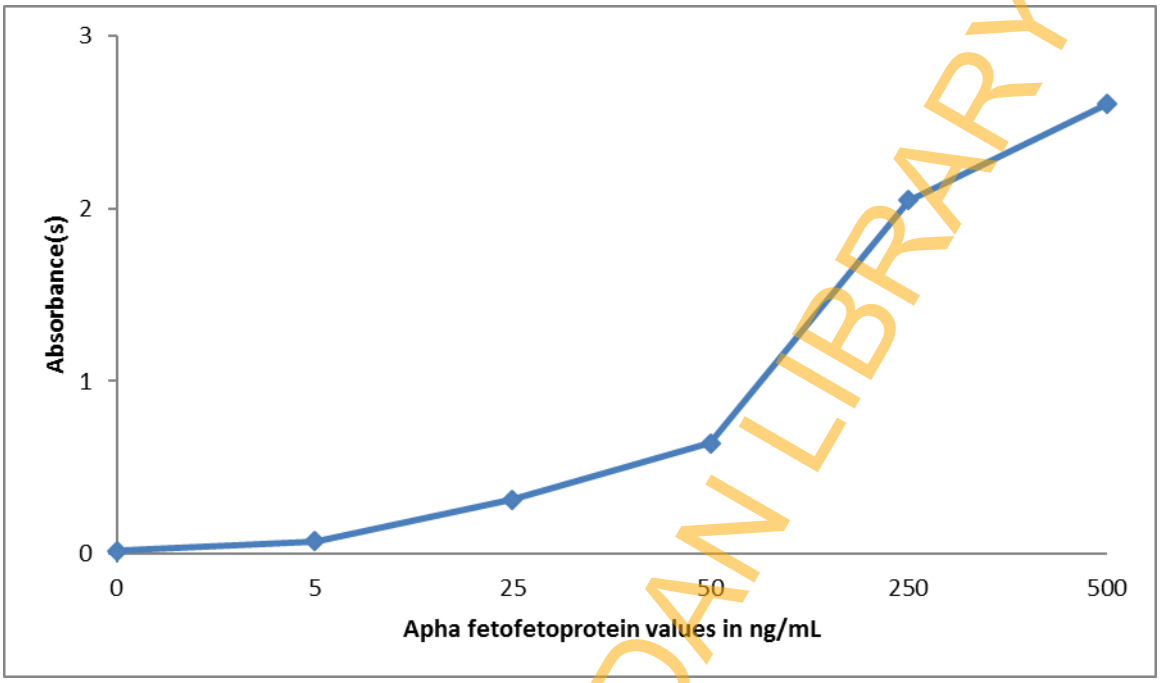


Fig 3.10 Standard validation curve for ELISA AFP determination

3.20 Statistical Analysis

Statistical analyses including descriptive statistics were carried out using the Statistical Package for Social Scientists (SPSS) version 21.0 (IBM, USA). All values were expressed as Mean ± Standard Error of the Mean; and in percentage values where necessary.

The One-way Analysis of Variance (ANOVA) was used to compare means in the three study groups of participants.

Sources of differences in means were indicated by the Post Hoc Analysis and were shown using superscripts of letters A, B and C; with similar superscripts indicating that there was no difference in mean values observed, while dissimilar superscripts indicate statistically different mean values.

Comparisons of skewed categorical data were performed by using the non-parametric Kruskal Wallis test.

Correlation (parametric/non parametric) analysis was used to evaluate the strength of relations between two or more variables among the parameters studied in the three groups.

Chi square analysis was used for comparison of means for categorical (non-replicated) variables.

With a two-sided probability, $p<0.05$ was considered significant.

CHAPTER 4

RESULTS

Demographic, anthropometric and baseline health indices of e-waste exposed and unexposed participants

Demographic characteristics and anthropometric indices of the study groups: e-waste workers, environmentally exposed participants and the unexposed controls are shown in table 4.1 below. From the observations, mean age in the three study groups was not significantly different. Sex frequency indicated that a total of 100% of the participants in each group were males and states of origin implied Nigeria as the nationality of all participants. The states of origin of the participants included seven (7) states in Western Nigeria (Oyo, Ogun, Ekiti, Lagos, Osun, Ondo and Kwara); five (5) states in Eastern Nigeria (Imo, Anambra, Akwa Ibom, Abia, and Enugu); two (2) states in South- South Nigeria (Edo and Delta); and one (1) state in Central Nigeria (Kogi). Among the e-waste workers, Imo (n = 116), Oyo (n = 49) and Edo (n = 43) had higher number of participants compared with other states. Body mass index (BMI) of e-waste workers (23.65 ± 0.56 kg/m^2) was higher but not significantly different from values obtained for environmentally exposed participants (21.27 ± 0.41kg/m^2) and the unexposed controls (21.96 ± 0.45 kg/m^2).

Findings from baseline health indices (table 4.2) showed fasting plasma glucose in e-waste workers (92.47 ± 1.71 mg/dL) was higher but not significantly different compared with the environmental group (88.40 ± 2.4mg/dL) and the unexposed controls (90.81 ± 2.4mg/dL), (p=0.061). Alcohol and tobacco use was considerably similar across the three groups of participants. Mean systolic blood pressure did not significantly vary across the three groups (p>0.05), diastolic blood pressure in environmentally exposed participants (86.21 ± 1.98mmHg) was however significantly higher than e-waste workers (75.53 ± 1.77mmHg) and unexposed group (70.34 ± 1.67mmHg), (p= 0.044). Nutritional indictors show the inclusion of carbohydrate, Proteins, Fat and oil (CPFO) in daily staple food; in addition to observed intake of vitamin-rich supplements among e-waste workers (43.31%); environmentally exposed group (46.67%); and unexposed group (48.85%).

Table 4.1 Demographic and anthropometric indices of electronic waste exposed and unexposed participants in all study locations

Observation (Mean ± SEM, %)	**E-waste workers (n=381)**	**Environmentally exposed participants (n=120)**	**Unexposed (controls) (n=131)**
Age (years) (p=0.069, F=2.78)	37.83 ± 1.37	34.60 ± 1.10	35.43 ± 1.77
Sex (Frequency)	Male Participants =100%	Male Participants =100%	Male Participants =100%
Nationality	Nigeria (100%)	Nigeria (100%)	Nigeria (100%)
States of Origin	Imo n=116 (2.5%) Oyo n= 49 Edo n= 43 Osun n=33 Delta n=31 Anambra n=24 Akwa Ibom n=21 Ogun n=21 Lagos n=13 Ekiti n=11 Abia n=10 Enugu n=9 Kwara n=3	Oyo n= 24 Lagos n=15 Kwara n=11 Edo n= 11 Delta n=11 Enugu n=10 Anambra n=9 Ekiti n=8 Osun n=7 Ondo n=5 Ogun n=4 Abia n=3 Imo n=3 Kogi n=1 Akwa Ibom n=1	Edo n= 35 Oyo n= 23 Lagos n=15 Osun n=14 Ogun n=12 Delta n=11 Anambra n=7 Ondo n=5 Imo n=3 Abia n=2 Akwa Ibom n=1 Kogi n=1
Mean Height (metre)	1.73 ± 0.01	1.76 ± 0.05	1.74± 0.07
Mean Weight (kg)	70.50 ± 1.56	65.90 ± 0.97	66.49± 1.51
Mean Body Mass Index (BMI)(kg/m^2)	23.56 ± 0.51	21.27 ± 0.41	21.96 ± 0.45

Keys:
n = number of participants (applies to all result tables)
% = percentage (applies to all result tables)
SEM =Standard error of the mean (applies to all result tables)

Table 4.2 Basic health and nutritional indices of electronic waste exposed and unexposed participants in all study locations

Observation (Mean ± SEM, %)	**E-waste workers (n=381)**	**Environmentally exposed (participants n=120)**	**Unexposed (controls) (n=131)**
Fasting plasma Glucose (mg/dL)	92.47 ± 1.71 *(5.1±0.10 mmol/L)	88.40 ± 2.40 (4.9±0.03mmol/L)	90.81 ± 2.40 (5.0 ± 0.04mmol/L) **(p = 0.061,F=9.32)***
Tobacco Use	Occasional: n = 81 (21.26%) Frequent: n = 0 (0.00%) Passive users: n 300 (78.74%)	Occasional: n = 29 (24.17%) Frequent: n = 0 (0.00%) Passive users: n = 91 (75.83%)	Occasional: n = 35 (26.72%) Frequent: n = 0 (0.00%) Passive users:n=96 (73.28%)
Alcohol Use	Occasional: n =73 (19.16%) Non-users: n= 308 (80.84%)	Occasional: n = 24 (20.0%) Non-users: n = 96 (80 %)	Occasional: n = 29 (22.14%) Non: n = 102 (77.86%)
Mean Blood Pressure (mmHg)	Systolic: 125.23 ± 2.15 Diastolic: 75.53^{B} ± 1.77	116.32 ± 2.02 86.21^{A} ± 1.98	119.84 ± 1.90 (p=0.07; F=8.34 70.34^{B} ± 1.67(p=0.044 ; F=3.14)
Nutritional Indicators			
▪ Inclusion of $^{\partial}$CPFO in Daily Staple Food	Yes: n=381 (100%) Non: n = 0 (0%)	Yes: n = 120 (100%) Non: n = 0 (0%)	Yes: n = 131 (100%) Non: n = 0 (0%)
▪ Intake of Supplements (Vitamin-rich Drugs)	Yes: n =165 (43.31%) Non: n = 216 (56.69%)	Yes: n= 56 (46.67%) Non: n =64 (53.33%)	Yes: n = 64 (48.85%) Non: n = 67 (51.15%)
▪ Specified Supplements	Iron-rich Supplements Vitamin-rich Supplements (Vitamin C)	Vitamin-rich Supplements (Multivitamins)	Vitamin-rich Supplements (Multivitamins)

Keys:
$^{\partial}$CPFO: Carbohydrates, Proteins, Fats and Oil
* Converted fasting plasma glucose in mmol/L (S.I. units)
n = number of responses

Risk awareness indicators and exposure pattern of e-waste exposed and unexposed participants all study locations

Table 4.3 below shows risk awareness levels and indicators among e-waste exposed and unexposed participants. It was observed that 248 (64.09%) of e-waste workers, 94 (78.3%) of environmentally exposed participants (EEPs), and 102 (77.86%) of unexposed controls lacked basic awareness of health hazards associated with WEEE exposure. Among the 133 (34.91%) of e-waste workers with basic health risk awareness, 6 (1.57%) specified catarrh/cough; 18 (4.72%) specified electrocution; while 17 participants (4.46%) stated impaired vision; 6 (1.57%) stated hypertension; and the largest proportion, 334 participants (87.68%) indicated uncertain responses as per condition known to them to be associated with WEEE exposure. Assessing the desire for health risk study on e-waste exposure, 95.28% of e-waste workers; 100% of the environmentally exposed participants; and 100% of unexposed participants were desirous of the conduct of the study. Practically all participants [e-waste workers (96.85%); environmentally exposed (92.6%) and unexposed controls (92.7%)] indicated willingness and granted consent to participate in the toxicological study. The assessment of methods of disposal of unwanted WEEE by e-waste workers revealed that the largest proportion (44.36%) is disposed of in local refuse dump; while another 22.31% of the WEEE is handled by commercial waste managers. More so, 11.02% of the unwanted WEEE is sold to waste scavengers and 22.31% is disposed of through undisclosed methods.

Occupational and environmental exposure pattern of study participants are indicated in Table 4.4 below. Assessing the nature of exposure, it was observed that both occupational and environmental WEEE exposures were high for e-waste workers; environmental WEEE exposure was moderate for the environmentally exposed group, but for the unexposed group, environmental WEEE exposure was minimal, while occupational exposure was not indicated. A duration of exposure of $\geq$ 5 years and exposure frequency $\geq$ 6hours/day; 6days/week (9360 hours in any 5 year duration) was observed with both e-waste workers and the environmentally exposed group. Routes of exposure observed in e-waste workers entailed all body cavities, viz, eyes, oral route, nasal cavity, and skin (dermal absorption). These observations were similar in EEPs, apart from the exposure through oral cavity which was significantly lower.

In addition, the proportion of e-waste workers that uses PPE such as apron, hand gloves and facemasks while working was barely 10.24% while non – PPE users constituted the

majority (89.76%) of the studied population. Among the PPE users, 10.24% used aprons and scarcely used hand gloves and the rest of the workers neither used neither face masks nor nose masks in the course of performing daily work tasks.

Table 4.3 Risk awareness indicators in e-waste exposed and unexposed participants in all study locations

Observation	E-waste workers (n=381)	Environmentally exposed participants (n=120)	Unexposed (controls) (n=131)
Level of Awareness of E-waste Health Consequences	-Basic Awareness: n = 133 (34.91%) -No Basic Awareness: n = 248 (64.09%)	-Basic Awareness: n = 26 (21.7%) -No Basic Awareness: n = 94 (78.3%)	-Basic Awareness: n = 29 (22.14%) -No Basic Awareness: n = 102 (77.86%)
Conditions Specified by Participants to be Associated with WEEE Exposure	Catarrh/Cough: n=6 (1.57%) Electrocution: n=18 (4.72%) Impaired Vision: n=17 (4.46%) Hypertension: n=6 (1.57%) Uncertain Response: n=334 (87.68%)	No Valid Response	No Valid Response
Desire for Health Risk Study on E-Waste Exposure	Desirous (Yes): n=363 (95.28% Non Desirous (No/indifferent): n =18; 4.72%	Desirous (Yes) = 100%	Desirous (Yes) = 100%
Willingness to participate in E-Waste Toxicological Study (By Granting Informed Consent)	Willing (Yes): n = 369 (96.85%) No response: n =12; (3.15%)	Willing (Yes) = 92.6% Not Willing (No) = 7.4%	Willing (Yes) = 92.7% Not Willing (No) = 7.3%
Methods of Disposal of Unwanted E-Waste	Commercial Waste Managers: n=85 (22.31%) Local Refuse Dump: n=169 (44.36%) Sold to Scavengers (Malams): n=42 (11.02%) Undisclosed Methods: n=85 (22.31%)	Not Applicable	Not Applicable

Keys:
WEEE= Waste electrical and electronic equipment
EEPs = Environmentally exposed participants

Table 4.4 Occupational and environmental exposure pattern of study participants

Observation	E-waste workers (n=381)	Environmentally exposed participants (n=120)	Unexposed (controls) (n=131)
Nature of exposure	Occupational / Environmental	Environmental	No occupational exposure
Duration of Exposure to E-waste Chemicals	≥ 5.0 years	≥ 5.0 years	Nil
Frequency of Exposure to E-waste Chemicals	≥ 6 hours/day; 6 days/week (9360 hours in 5 years)	≥ 6 hours/day; 6 days/week (9360 hours in 5 years)	Nil
Medium of Exposure:			
Direct	Hands, eyes, **oral cavity**, **nasal cavity**, dermal absorption	Hands, nasal cavity, dermal absorption	Hands, nasal cavity, dermal absorption
Indirect	Environmental (high)	Environmental (Moderate)	Environmental (Minimal)
% Using PPE while Working	Users [(n = 39) 10.24 %] Non-users [(n = 342) 89.76%]	Not Applicable	Not Applicable
Specified Protective Devices Used by E-Waste Workers	Apron (10.24%) Hand gloves (scarcely) Face/Nose mask (Nil)	Not Applicable	Not Applicable

Key:
PPE = Personal Protective Equipment

Blood levels of the toxic metals in e-waste exposed and unexposed participants in all study locations

Table 4.5 shows blood levels in toxic metals in all participants (e-waste workers, environmentally exposed and unexposed controls) in all three locations. Comparison of the parameters between the groups using post hoc test showed levels of key toxic metals (Al, V, Cr, Ni, Mo, Cd, Sn, Sb, Hg, Tl and Pb) in e-waste workers were significantly higher compared with the environmentally exposed and unexposed groups ($P < 0.05$). In the environmentally exposed group, levels of As was higher (1.0 ± 0.006µmol/L) compared with e-waste workers (0.73 ± 0.08 µmol/L) and unexposed group (0.22±0.02µmol/L). Moreso, blood levels of all toxic metals were lower in the unexposed group compared with the environmentally exposed group; except for which was insignificantly higher in unexposed group (0.62 ± 0.08µmol/L) compared with EEP (0.06 ± 0.02µmol/L).

Comparison of blood levels of toxic metals in all e-waste workers considered by location (Table 4.6), showed that almost all toxic metals (Al, V, Cr, N, As, Mo, Sn and Sb) levels were higher in Lagos e-waste workers compared with the environmental and unexposed groups ($p<0.048$). However Cd and Tl levels, as well as Hg and Pb levels were respectively higher in Ibadan and Benin e-waste workers when compared with other groups ($p\leq0.048$).

Comparison of blood levels of toxic metals in the environmentally exposed. participants by location (Table 4.7) showed that Al, V, N, As, Mo, Cd, Sn, Sb and Pb levels were high in Lagos e-waste workers compared with Ibadan environmental group (with Cr level alone higher); and Benin environmental participants (With higher Hg and Tl levels).

Table 4.8 shows blood levels of toxic metals in all unexposed participants by location. In Lagos unexposed participants Al, N, As, Sn and Sb levels were significantly higher compared with Ibadan and Benin unexposed populations ($p \leq 0.047$). Levels of Cr and Cd were comparatively higher in Ibadan unexposed group while Hg, Tl, Mo and Ph levels were significantly raised in Benin unexposed group compared with Lagos and Ibadan.

Table 4.5 Blood levels of toxic metals in e-waste exposed and unexposed participants in all study locations

Toxic metals (S.I. units)	Mean ± SEM			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
^{27}Al (µmol/L)	0.97 ^{A} ±0.14	0.60^{B} ±0.02	0.62 ^{B} ±0.08	3.44	0.033
^{51}V (nmol/L)	8.65 ^{A} ±0.60	5.79 ^{B} ±0.21	5.52 ^{C} ±0.10	27.77	0.000
^{52}Cr (nmol/L)	517.70 ^{A} ±32.34	433.32 ^{B} ±13.23	22.27^{C} ±1.44	83.12	0.000
^{60}Ni (nmol/L)	84.26 ^{A} ±19.26	77.26 ^{A} ±8.02	26.81 ^{B} ±4.69	3.06	0.048
^{75}As (µmol/L)	0.73 ^{B} ±0.08	1.00^{A} ±0.06	0.24 ^{C} ±0.02	23.12	0.000
^{100}Mo (nmol/L)	40.80 ^{A} ± 8.18	26.36 ^{B} ±1.19	13.64 ^{C} ±0.80	4.24	0.015
^{111}Cd (nmol/L)	12.66 ^{A} ±0.74	3.85 ^{B} ±0.11	0.83 ^{C} ±0.12	104.74	0.000
^{118}Sn (nmol/L)	14.57 ±6.47	8.82 ±0.78	2.14 ±0.48	1.30	0.274*
^{121}Sb (nmol/L)	6.65 ±2.53	3.56±1.06	0.15±0.04	2.23	0.108*
^{202}Hg (nmol/L)	17.99 ^{A} ±0.93	9.07 ^{B} ±0.24	3.79^{C} ±0.30	84.27	0.000
^{205}Tl (nmol/L)	1.11 ^{A} ±0.08	0.55 ^{B} ±0.03	0.16 ^{C} ±0.01	63.27	0.000
^{208}Pb (µmol/L)	2.49^{A}± 0.88	2.0^{B}±0.28	0.40^{C}±0.02	48.69	0.000

Keys:

SEM = Standard Error of Mean

* = P > 0.05 (not significant)

S.I. = International System of Units (applies to all result tables)

Table 4.6 Blood levels of toxic metals in all e-waste workers in the different study locations

Toxic metals	E-waste Workers				
	Benin-MiIA (n=83)	**Ibadan-MoIA (n=120)**	**Lagos-HiIA (n=178)**	***F* value**	***P* value**
^{27}Al (µmol/L)	0.89^{A} ±0.01	0.75^{B} ±0.24	1.54^{A} ±0.42	2.63	0.074
^{51}V (nmol/L)	4.48^{C} ±0.07	8.30^{B} ±0.81	15.22^{A} ±1.70	28.20	0.000
^{52}Cr (nmol/L)	507.04^{B} ±13.81	428.51^{B} ±24.97	698.64^{A} ±129.28	5.43	0.005
^{60}Ni (nmol/L)	57.66^{B}±1.07	44.87^{B} ±2.89	199.45^{A} ±83.89	5.47	0.005
^{75}As (µmol/L)	0.65^{B} ±0.01	0.50^{B} ±0.40	1.30^{A} ±0.32	8.99	0.000
^{100}Mo (nmol/L)	26.77^{B} ±0.71	26.46^{B} ±4.41	87.21^{A} ±33.28	5.14	0.007
^{111}Cd (nmol/L)	13.93^{A} ±0.51	16.79^{A} ±1.41	3.08^{B} ±0.78	34.57	0.000
^{118}Sn (nmol/L)	3.16^{B} ±0.13	2.29^{B} ±0.51	58.53^{A} ±29.81	6.55	0.002
^{121}Sb (nmol/L)	6.02 ±0.19	4.87 ±1.37	10.93 ±11.02	0.43	0.649
^{202}Hg (nmol/L)	30.76^{A} ±0.48	10.65^{B} ±1.41	11.12^{B} ±0.86	111.37	0.000
^{205}Tl (nmol/L)	1.18^{A} ±0.05	1.44^{A} ±0.16	0.44^{B} ±0.06	17.01	0.000
^{208}Pb (µmol/L)	29.22^{A} ±0.30	22.47^{B} ±1.49	23.04^{B} ±2.30	6.54	0.002

Keys:

MiIA = Mild e-waste impact area

MoIA = Moderate e-waste impact area

HiIA = High e-waste impact area

Summary (elements higher by lacations)

Lagos = 8 (Al, V, Cr, Ni, As, Mo, Sn, Sb)

Ibadan = 2 (Cd, Tl)

Benin =2 (Hg, Pb)

Table 4.7 Blood levels of Toxic metals in all environmentally exposed participants in the different study locations

Toxic metals	Environmentally exposed			*F* value	*P* value
	Benin-MiIA (n=30)	**Ibadan-MoIA (n=38)**	**Lagos-HiIA (n=52)**		
^{27}Al (µmol/L)	0.57^{B}±0.06	0.54 ^{B} ±0.01	0.69 ^{A} ±0.02	4.38	0.015
^{51}V (nmol/L)	4.11 ^{C} ±0.13	5.97 ^{B} ±0.33	7.21 ^{A} ±0.35	29.31	0.000
^{52}Cr (nmol/L)	309.46 ^{C} ±4.28	533.05 ^{A} ±16.13	460.31 ^{B} ±25.38	47.34	0.000
^{60}Ni (nmol/L)	38.09 ^{B} ±0.54	7.08 ^{C} ±0.27	201.80 ^{A} ±4.51	1810.10	0.000
^{75}As (µmol/L)	0.69 ^{B} ±0.02	0.55 ^{B} ±0.01	1.86 ^{A} ±0.09	199.64	0.000
^{100}Mo (nmol/L)	31.02 ^{B} ±0.59	10.89 ^{C} ±0.38	38.02 ^{A} ±1.13	350.41	0.000
^{111}Cd (nmol/L)	4.14 ^{B} ±0.08	2.49 ^{C} ±0.13	4.85 ^{A} ±0.12	115.53	0.000
^{118}Sn (nmol/L)	2.93 ^{B} ±0.16	2.05 ^{B} ±0.13	14.76 ^{A} ±1.51	59.47	0.000
^{121}Sb (nmol/L)	3.09 ^{B} ±0.12	2.92 ^{B} ±0.10	4.83 ^{A} ±0.12	86.68	0.000
^{202}Hg (nmol/L)	11.34 ^{A} ±0.44	8.47 ^{B} ±0.35	7.89 ^{B} ±0.26	25.76	0.000
^{205}Tl (nmol/L)	0.88 ^{A} ±0.05	0.26 ^{C} ±0.00	0.48 ^{B} ±0.01	100.42	0.000
^{208}Pb (µmol/L)	13.22±0.50	20.02±0.61	28.18±9.91	2.34	0.102

Keys:

MiIA = Mild e-waste impact area

MoIA = Moderate e-waste impact area

HiIA = High e-waste impact area

Summary (elements higher by locations)

Lagos = 9 (Al, V, Ni, As, Mo,Cd. Sn, Sb, Pb)

Ibadan = 1 (Cr)

Benin =2 (Hg, Tl)

Table 4.8 Blood levels of toxic metals in all unexposed participants in the different study locations

Toxic metals	Unexposed participants			*F* value	*P* value
	Benin-MiIA (n=41)	**Ibadan-MoIA (n=50)**	**Lagos-HiIA (n=40)**		
^{27}Al (µmol/L)	$0.48^{B} \pm 0.01$	$0.39^{B} \pm 0.01$	$0.97^{A} \pm 0.22$	5.88	0.004
^{51}V (nmol/L)	$1.82^{A} \pm 0.08$	$3.13^{B} \pm 0.08$	$1.02^{C} \pm 0.03$	125.35	0.000
^{52}Cr (nmol/L)	$10.25^{C} \pm 0.34$	$38.95^{A} \pm 1.23$	$16.64^{B} \pm 1.96$	127.62	0.000
^{60}Ni (nmol/L)	$4.98^{B} \pm 0.13$	$18.86^{B} \pm 0.68$	$56.60^{A} \pm 12.59$	13.47	0.000
^{75}As (µmol/L)	$0.21^{B} \pm 0.01$	$0.12^{C} \pm 0.00$	$0.38^{A} \pm 0.03$	41.39	0.000
^{100}Mo (nmol/L)	$17.03^{A} \pm 0.46$	$9.28^{B} \pm 0.30$	$14.69^{A} \pm 2.28$	9.87	0.000
^{111}Cd (nmol/L)	0.48 ± 0.01	1.10 ± 0.27	0.91 ± 0.21	2.64	0.077
^{118}Sn (nmol/L)	1.02 ± 0.04	2.03 ± 0.37	3.36 ± 1.37	2.03	0.137
^{121}Sb (nmol/L)	$0.01^{B} \pm 0.00$	$0.19^{A} \pm 0.01$	$0.23^{A} \pm 0.11$	3.17	0.047
^{202}Hg (nmol/L)	$6.01^{A} \pm 0.34$	$1.02^{C} \pm 0.04$	$4.34^{B} \pm 0.58$	42.13	0.000
^{205}Tl (nmol/L)	$0.18^{A} \pm 0.01$	$0.12^{B} \pm 0.01$	$0.16^{A} \pm 0.01$	10.04	0.000
^{208}Pb (µmol/L)	$4.86^{A} \pm 0.11$	$2.96^{B} \pm 0.15$	$4.25^{A} \pm 0.51$	9.69	0.000

Keys:

MiIA = Mild e-waste impact area

MoIA = Moderate e-waste impact area

HiIA = High e-waste impact area

Summary (elements higher by locations)

Lagos = 5 (Al, Ni, As, Sn, Sb)

Ibadan = 3 (V, Cr, Cd)

Benin =4 (Hg, Tl, Mo, Pb)

Hand wash levels of toxic and essential metals in e-waste exposed and unexposed participants in all study locations

As indicated in table 4.9, hand wash levels of toxic metals (Al, V, Cr, As, Mo, Sb, Sn, Tl, Ni, Cd and Pb) were significantly higher in e-waste workers compared with the environmental and unexposed groups ($p < 0.005$). Hand wash Hg level in the environmental group (0.78 ± 0.14nmol/L) and e-waste group (0.66 ± 0.09 nmol/L) were statistically similar ($p > 0.05$), and both values were significantly higher than the unexposed group (0.33 ± 0.07 nmol/L) ($p= 0.010$).

Hand wash levels of the essential metals (Mg, Mn and Co) were similar in both the occupationally and environmentally exposed groups but were both different significantly from the unexposed participants. Furthermore, hand wash levels of the essential metals (Mg, Mn and Co) were similar in the occupationally and environmentally exposed groups ($p>0.05$); but both were significantly different ($p<0.05$) when compared with the unexposed groups. Hand wash level of Cu was not significantly different in the environmentally and unexposed groups but was significantly lower in both groups compared the e-waste group ($p<0.05$). Levels of Zn and Se in hand wash water of e-waste workers (2.27±0.24 nmol/L; 0.15±0.09 µmol/L); environmental participants (1.77±0.29; 0.09±0.06 µmol/L) and unexposed group (1.62±0.35 nmol/L; 0.02±0.01 µmol/L) respectively, did not differ significantly ($P>0.05$). Comparison of hand wash levels of toxic and essential metals in all e-waste workers in Benin, Lagos and Ibadan (Table 4.10) showed that there was no significant differences in the three locations, with the exception of Cu, which was significantly higher in Lagos e-waste workers ($p = 0.037$) compared with Benin and Ibadan workers.

Comparison of hand wash levels of toxic and essential metals in all environmentally exposed participants (Table 4.11) showed that there was no statistically significantly difference in the metal levels in the three study locations ($p> 0.05$).

In Table 4.12, hand wash levels of toxic and essential metals in unexposed controls are compared in all study locations. Levels of V were significantly different in unexposed participants in the three study locations ($p = 0.032$). Cr levels in Lagos and Benin unexposed groups were both higher and significantly different from Ibadan unexposed group ($P = 0.037$). Hand wash Mn, Co and Ni were significantly higher in Lagos

unexposed participants compared with Ibadan and Benin unexposed groups ($p<0.001$). Other metal levels (Mg, Al, Zn, Cu, As, Se, Mo, Cd, Sn, Sb, Hg, Tl and Pb) were not significant different in the unexposed populations of the different study groups.

Toxic and essential metals levels in pre-wash distilled water compared with hand wash water in e-waste exposed and unexposed participants

Table 4.13 shows pre-wash and hand wash metals levels in the study groups. In pre-wash water, levels of most metals (Mg, Al, V, Cr, Co, Zn, Cu, As, Se, Sn, Sb and Pb) were below instrumental detection limit (LOD) or at most approximately zero (0), and among these, the levels of toxic metals were highly significant lower in the pre-wash water compared with e-waste exposed, environmentally exposed and unexposed hand wash water samples ($P<0.01$). Exceptionally, the pre-wash water level of Hg (0.38 nmol/L), Tl (0.06nmol/L), Cd (0.36 nmol/L), Se (0.33 µmol/L), Cu (-0.01 µmol/L) and Zn (-0.02 nmol/L) showed no statistical difference ($P>0.05$) when compared with the three hand wash groups.

Table 4.9 Hand wash levels of toxic and essential metals in e-waste exposed and unexposed participants

Toxic metals (S.I. units)	All Participants			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
^{24}Mg (mmol/L)	0.43^{A}± 0.08	0.29 ^{AB} ±0.06	0.14 ^{B} ±0.04	5.91	0.005
^{27}Al (µmol/L)	4.71^{A} ±0.96	4.63^{A} ±0.98	1.61^{B} ±0.75	3.83	0.030
^{51}V (nmol/L)	60.33^{A} ±11.11	57.59^{A} ±10.25	21.19^{B} ±9.53	4.49	0.017
^{52}Cr (nmol/L)	4442.64^{A} ±1114.78	4123.55^{A} ±1069.13	785.47^{B} ±169.29	5.10	0.010
^{55}Mn (nmol/L)	4122.73^{A} ±398.02	3561.87 ^{A} ±451.64	1228.93^{B} ±391.21	13.71	0.000
^{59}Co (nmol/L)	103.39^{A} ±11.43	100.89^{A} ±12.95	28.99^{B} ±6.42	15.77	0.000
^{60}Ni (nmol/L)	2631.53^{A} ±438.71	2381.07^{A} ±460.34	368.35^{B} ±141.64	10.86	0.000
^{64}Zn (nmol/L)	2.27±0.24	1.77±0.29	1.62±0.35	1.33	0.235
^{65}Cu (µmol/L)	2.87 ^{A} ±0.41	1.68^{B} ±0.22	1.10^{B} ±0.25	8.84	0.001
^{75}As (µmol/L)	0.57 ^{A} ±0.15	0.49 ^{A} ±0.16	0.12^{B} ±0.03	3.59	0.036
^{78}Se (µmol/L)	0.15±0.09	0.09±0.06	0.02±0.01	0.97	0.307
^{100}Mo (nmol/L)	19.35 ^{A} ±1.97	15.92 ^{A} ±2.12	4.97 ^{B} ±0.87	18.54	0.000
^{111}Cd (nmol/L)	92.01 ^{A} ±18.23	75.71^{B} ±17.73	19.79^{C} ±3.44	6.54	0.003
^{118}Sn (nmol/L)	378.89 ^{A} ±74.03	344.55 ^{A} ± 62.14	57.80 ^{B} ±8.46	9.91	0.000
^{121}Sb nmol/L	196.05 ^{A} ±22.51	168.49 ^{A} ±24.61	74.56 ^{B}±24.20	7.17	0.002
^{202}Hg (nmol/L)	0.66 ^{A} ±0.09	0.78 ^{A} ±0.14	0.33 ^{B} ±0.07	5.10	0.010
^{205}Tl (nmol/L)	5.51 ^{A} ±1.00	4.33 ^{AB} ±0.82	2.07 ^{B} ±0.57	4.58	0.016
^{208}Pb (µmol/L)	2.36 ^{A} ±0.31	1.97 ^{A} ±0.31	0.88 ^{B} ±0.15	8.46	0.001

Keys: Abbreviations and units as previously defined

Table 4.10 Hand wash levels of toxic and essential metals in e-waste workers in all study locations

Toxic metals	E-waste workers			*F* value	*P* value
	Benin-MiIA (n=83)	**Ibadan-MoIA (n=120)**	**Lagos-HiIA (n=178)**		
^{24}Mg (mmol/L)	0.44 ±0.14	0.39±0.14	0.46±0.15	0.05	0.955
^{27}Al (µmol/L)	4.59±1.84	4.77±1.81	4.77±1.75	0.00	0.997
^{51}V (nmol/L)	62.42±21.11	64.42±20.75	54.14±20.10	0.07	0.933
^{52}Cr (nmol/L)	4179.1±2147.3	4706.4±2082.3	4442.5±2016.7	0.02	0.984
^{55}Mn (nmol/L)	3958.4±676.0	4120.0±722.5	4289.8±818.9	0.05	0.951
^{59}Co (nmol/L)	105.1±19.07	105.2±23.63	99.88±21.08	0.02	0.980
^{60}Ni (nmol/L)	2622.6±822.6	2630.6±810.2	2641.4±834.9	0.00	1.000
^{64}Zn (nmol/L)	2.19±0.47	2.00±0.46	2.63±0.37	0.54	0.596
^{65}Cu (µmol/L)	2.34^{B}±0.64	2.00^{B}±0.46	4.27^{A}±0.63	4.41	0.037*
^{75}As (µmol/L)	0.55±0.29	0.52±0.29	0.65±0.26	0.06	0.946
^{78}Se (µmol/L)	0.008±0.004	0.006±0.002	0.44±0.25	3.04	0.085
^{100}Mo (nmol/L)	17.85±3.74	18.81±3.81	21.39±3.26	0.28	0.778
^{111}Cd (nmol/L)	70.43±29.67	89.45±38.02	116.14±30.01	0.49	0.624
^{118}Sn (nmol/L)	291.2±99.49	378.6±122.2	466.82±169.9	0.43	0.660
^{121}Sb nmol/L	181.9±70.31	188.1±39.64	218.1±44.09	0.22	0.806
^{202}Hg (nmol/L)	0.52±0.14	0.57±0.15	0.88±0.18	1.58	0.246
^{205}Tl (nmol/L)	4.62±1.53	4.72±1.50	7.18±2.22	0.66	0.534
^{208}Pb (µmol/L)	2.17±0.62	2.35±0.58	2.55±0.49	0.11	0.897

Keys: Abbreviations and units as previously defined

MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area
P<0.05 = Significant
P>0.05= Not Significant

Table 4.11 Hand wash levels of toxic and essential metals in environmentally exposed participants in all study locations

	Environmentally Exposed Participants				
Toxic metals	**Benin-MiIA (n=30)**	**Ibadan-MoIA (n=38)**	**Lagos-HiIA (n=52)**	***F* value**	***P* value**
^{24}Mg (mmol/L)	0.25±0.06	0.37±0.15	0.2±0.07	0.49	0.624
^{27}Al (µmol/L)	3.96±1.88	4.95±1.81	4.97±1.77	0.10	0.906
^{51}V (nmol/L)	47.92±13.3	60.42±21.41	64.42±20.75	0.21	0.815
^{52}Cr (nmol/L)	2919.50±843.90	4360.2±2162.90	5090.9±2449.70	0.22	0.731
^{55}Mn (nmol/L)	3488.0±747.10	3950.0±806.90	3247.6±932.10	0.18	0.834
^{59}Co (nmol/L)	84.22±19.69	109.2±23.91	109.22±26.42	0.38	0.694
^{60}Ni (nmol/L)	2356.00±777.40	2506.6±845.20	2280.6±948.60	0.02	0.982
^{64}Zn (nmol/L)	1.55±0.28	2.19±0.80	1.59±0.34	0.45	0.646
^{65}Cu (µmol/L)	1.59±0.28	1.85±0.47	1.62±0.45	0.12	0.888
^{75}As (µmol/L)	0.49±0.29	0.50±0.29	0.49±0.29	0.01	0.999
^{78}Se (µmol/L)	0.01±0.007	0.052±0.03	0.21±0.17	0.99	0.399
^{100}Mo (nmol/L)	17.43±3.74	13.41±4.16	16.93±3.65	0.32	0.730
^{111}Cd (nmol/L)	75.64±35.79	91.23±40.19	60.27±16.89	0.23	0.801
^{118}Sn (nmol/L)	346.6±110.8	336.4±109.70	350.6±127.30	0.00	0.996
^{121}Sb nmol/L	148.6±42.07	182.1±55.24	174.7±37.19	0.15	0.863
^{202}Hg (nmol/L)	0.58±0.15	0.58±0.13	1.17±0.32	2.48	0.126
^{205}Tl (nmol/L)	3.66±0.81	5.21±2.05	4.13±1.35	0.29	0.757
^{208}Pb (µmol/L)	2.20±0.64	1.57±0.28	2.12±0.66	0.38	0.689

Keys: Abbreviations and units as previously defined

MiIA = Mild e-waste impact area

MoIA = Moderate e-waste impact area

HiIA = High e-waste impact area

Table 4.12 Hand wash levels of toxic and essential metals in unexposed participants in all study locations

	Unexposed participants (Controls)				
Toxic metals	**Benin-MiIA (n=41)**	**Ibadan-MoIA (n=50)**	**Lagos-HiIA (n=40)**	***F* value**	***P* value**
^{24}Mg (mmol/L)	0.19±0.09	0.10±0.05	0.12±0.07	0.38	0.690
^{27}Al (µmol/L)	2.81±2.12	0.88±0.75	1.13±0.49	0.62	0.553
^{51}V (nmol/L)	42.18±25.39	2.12±0.88	19.28±10.25	1.62	0.032
^{52}Cr (nmol/L)	917.7^{A}±276.3	227.6^{B}±111.8	1211.1^{A}±290.9	4.41	0.037
^{55}Mn (nmol/L)	163.6±86.94	1532.0±938.1	1991.2±517.2	2.35	0.021
^{59}Co (nmol/L)	19.240^{B}±5.49	15.42^{B}±9.17	52.32^{A}±10.63	5.44	0.021
^{60}Ni (nmol/L)	61.0^{B}±12.47	33.86^{B}±15.98	1010.2^{A}±235.9	16.54	0.000
^{64}Zn (nmol/L)	2.26±0.88	1.00±0.30	1.59±0.43	1.12	0.358
^{65}Cu (µmol/L)	0.98±0.16	1.13±0.77	1.17±0.11	0.05	0.954
^{75}As (µmol/L)	0.11±0.06	0.09±0.03	0.16±0.05	0.57	0.581
^{78}Se (µmol/L)	0.05±0.04	0.004±0.002	0.02±0.02	0.67	0.529
^{100}Mo (nmol/L)	3.65±0.99	3.99±1.21	7.27±1.88	2.00	0.178
^{111}Cd (nmol/L)	11.26±2.76	18.99±4.43	29.11±7.53	2.86	0.096
^{118}Sn (nmol/L)	54.20±10.85	60.74±15.26	58.48±19.89	0.04	0.957
^{121}Sb nmol/L	38.52±8.85	139.17±66.54	46.0±8.59	2.06	0.170
^{202}Hg (nmol/L)	0.23±0.08	0.41±0.17	0.33±0.08	0.59	0.570
^{205}Tl (nmol/L)	1.85±0.89	1.94±1.02	2.42±1.26	0.08	0.921
^{208}Pb (µmol/L)	0.79±0.09	0.56±0.12	1.28±0.37	2.55	0.120

Keys: Abbreviations and units as previously defined
MiIA = Mild e-waste impact area

MoIA = Moderate e-waste impact area

HiIA = High e-waste impact area

P>0.05 – Not Significant.

Table 4.13 Toxic and essential metal levels in pre-wash distilled water compared with hand wash water of e-waste exposed and unexposed participants

Toxic and essential metals	E-waste Exposed Handwash (n = 381)	Environmental Exposed Handwash (n = 120)	Unexposed Handwash (n = 131)	Pre- wash	Chi-Square	P-value
^{24}Mg (mmol/L)	0.43	0.29	0.14	0	1.75	0.417
^{27}Al (µmol/L)	4.71	4.63	1.61	0	1.5	0.472
^{51}V (nmol/L)	60.33	57.59	21.19	-0.2	20.82	0.000
^{52}Cr (nmol/L)	4442.64	4123.55	785.47	-1.04	2633.84	0.000
^{55}Mn (nmol/L)	4122.73	3561.87	1228.93	0.68	5082.72	0.000
^{59}Co (nmol/L)	103.39	100.89	28.99	-0.88	45.76	0.000
^{60}Ni (nmol/L)	2631.53	2381.07	368.35	4.11	4072.4	0.000
^{64}Zn (nmol/L)	2.27	1.77	1.62	-0.02	0.93	0.100
^{65}Cu (µmol/L)	2.87	1.68	1.1	-0.01	1.00	0.607
^{75}As (µmol/L)	0.57	0.49	0.12	-2.18	3.50	0.174
^{78}Se (µmol/L)	0.15	0.09	0.02	0.04	0.33	0.564
^{100}Mo (nmol/L)	19.35	15.92	4.97	0.20	8.15	0.017
^{111}Cd (nmol/L)	92.01	75.71	19.79	0.36	45.62	0.000
^{118}Sn (nmol/L)	378.89	344.55	57.81	0.02	238.56	0.000
^{121}Sb nmol/L	196.05	168.49	74.56	-0.1	54.838	0.000
^{202}Hg (nmol/L)	0.66	0.78	0.33	0.38	0.00	0.100
^{205}Tl (nmol/L)	5.51	4.33	2.07	0.06	2.00	0.368
^{208}Pb (µmol/L)	2.36	1.97	0.88	-0.20	0.40	0.819

Keys: Abbreviations and units as previously defined

Pre-wash = Distilled water analyzed before hand washing

P<0.001= highly significant

P>0.05= not significant

Levels of inorganic micronutrients and antioxidant metals in e-waste exposed and unexposed participants in all study locations

Levels of inorganic micronutrients and antioxidant metals in e-waste exposed and unexposed participants in all study locations are shown in table 4.14, 4.15, 4.16 and 4.17 below. Comparison of parameters among the groups using post hoc test (Table 4.14) showed that in e-waste workers and the unexposed groups, Mg levels were similar but significantly greater than the environmentally exposed participants. Levels of Mn varied significantly across the groups (e-waste workers > environmentally exposed > unexposed). The levels of Zn, Se, Cr and Co levels in e-waste exposed group were significantly lower compared with unexposed group [Zn, Cr and Co ($p<0.001$); Se ($p<0.05$)]. Conversely, levels of Mn and Cu were significantly higher in the two e-waste exposed group compared with the unexposed, ($p<0.05$).

Considered by location (table 4.15) Se, Zn and Mn levels in Lagos e-waste workers were significantly higher than Ibadan and Benin e-waste workers ($p<0.05$). There was no significant difference sin Se, Zn and Mn levels between Benin and Ibadan e-waste workers levels of Mg, Cr and Co did not differ significantly across the three groups ($p>0.05$).

In the environmentally exposed group (Table 4.16), Mg, Mn, Zn and Cu levels were significantly lower in the Benin participants compared with Lagos and Ibadan participants ($P<0.05$) levels of Cr in the environmentally exposed group were significantly different in Benin, Ibadan and Lagos in reducing order, ($p = 0.001$). The level of Co was significantly higher in Ibadan environmental group compared with Benin and Lagos participants ($p = 0.037$).

Table 4.17 shows that Mg and Se levels were significantly higher in Lagos unexposed group than unexposed group than Ibadan, but was not significantly different from Benin unexposed group. No significant difference was observed in Mn level between Ibadan and Lagos unexposed group, however, Benin unexposed group had significantly lower Mn than both groups ($p = 0.015$). Co level in Benin and Ibadan unexposed groups did not differ significantly, the levels were however significantly higher compared with Lagos unexposed group ($p<0.000$).

Table 4.14 Inorganic micronutrients and antioxidant metals in e-waste exposed and unexposed participants in all study locations

Parameters	Mean ± SEM			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
^{24}**Mg** (mmol/L)	0.79 ^{A} ±0.01	0.75 ^{B} ±0.01	0.82 ^{A} ±0.02	6.09	0.003
^{55}**Mn** (nmol/L)	219.93 ^{A} ±16.18	159.01 ^{B} ±3.94	88.67 ^{C} ±5.41	23.30	0.000
^{64}**Zn** (µmol/L)	10.68 ^{B} ±0.16	10.80 ^{B} ±0.19	13.35 ^{A} ±0.31	42.95	0.000
^{65}**Cu** (µmol/L)	21.65 ^{A} ±0.70	20.98 ^{A} ±0.53	16.46^{B} ±0.39	24.96	0.000
^{78}**Se** (µmol/L)	1.48 ^{C} ±0.03	1.53^{C} ±0.03	1.59^{A} ±0.02	4.19	0.016
^{52}**Cr** (nmol/L)	111.44^{B} ±7.14	119.99^{B} ± 3.21	200.33^{A}±13.45	30.37	0.000
^{59}**Co** (nmol/L)	3.37^{B}± 0.28	4.18^{A}±0.11	4.75^{A}±0.22	10.50	0.000

Keys: Abbreviations and units as previously defined

P<0.001= highly significant

P>0.05= not significant

SEM = Standard Error of Mean

Table 4.15 Inorganic micronutrients and antioxidant metals in e-waste workers in all study locations

Parameters	E-waste Workers			*F* value	*P* value
	Benin-MiIA (n=83)	Ibadan-MoIA (n=120)	Lagos-HiIA (n=178)		
^{24}Mg (mmol/L)	0.78±0.02	0.78±0.01	0.81±0.01	2.11	0.126
^{55}Mn (nmol/L)	217.74 ^{B} ±1.72	175.83 ^{B} ±4.38	304.47 ^{A} ±68.05	4.96	0.008
^{64}Zn (µmol/L)	10.19^{B}±0.24	10.42 ^{B} ±0.28	11.72 ^{A} ±0.26	9.00	0.000
^{65}Cu (µmol/L)	23.84 ^{A} ±1.51	18.91 ^{AB} ±0.81	21.94 ^{A} ±0.89	4.69	0.011
^{78}Se (µmol/L)	1.52 ^{B} ±0.04	1.54 ^{B} ±0.04	1.71 ^{A} ±0.03	7.23	0.001
^{52}Cr (nmol/L)	113.08 ±4.21	98.53 ±6.48	123.64 ±19.45	1.09	0.339
^{59}Co (nmol/L)	3.52 ±0.22	3.04 ±0.21	3.58 ±0.76	0.37	0.689

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significant

SEM: Standard Error of the Mean
MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area

Summary (elements higher by locations)
Benin-MiIA: Cu (1)
Ibadan-MoIA: Nil (0)
Lagos-HiIA: Mg, Zn, Se, Cr, Co (5)

Table 4.16 Inorganic micronutrients and antioxidant metals in environmentally exposed participants in all study locations

Parameters	Environmentally Exposed			*F* value	*P* value
	Benin-MiIA (n=30)	Ibadan-MoIA (n=38)	Lagos-HiIA (n=52)		
^{24}**Mg** (mmol/L)	0.69^{B}±0.02	0.80^{A} ±0.01	0.78^{A} ±0.01	10.61	0.000
^{55}**Mn** (nmol/L)	109.55^{C} ±2.70	191.18^{A} ±3.47	183.15^{B} ±2.12	261.93	0.000
^{64}**Zn** (µmol/L)	10.39^{B} ±0.27	10.26^{B} ±0.35	11.79^{A} ±0.30	7.56	0.001
^{65}**Cu** (µmol/L)	18.89^{B} ±1.00	22.49^{A} ±0.75	21.81^{A} ±0.89	4.81	0.010
^{78}**Se** (µmol/L)	1.49±0.04	1.52±0.04	1.59±0.05	1.46	0.238
^{52}**Cr** (nmol/L)	134.06^{A} ±4.08	112.86^{B} ±5.51	109.21^{C} ±6.37	7.00	0.001
^{59}**Co** (nmol/L)	4.02^{B} ±0.15	4.58^{A} ±0.19	3.96^{B} ±0.21	3.41	0.037

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significantNote:
MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area

Summary (metal levels higher by location)
Benin-MiIA: Cr (1)
Ibadan-MoIA: Mg, Cu, Co (3)
Lagos-HiIA: Zn, Se (2)

Table 4.17 Inorganic micronutrients and antioxidant metals in unexposed participants in all study locations

Parameters	Unexposed (Controls)			*F* value	*P* value
	Benin-MiIA (n=42)	Ibadan-MoIA (n=50)	Lagos-HiIA (n=40)		
^{24}**Mg** (mmol/L)	0.82 ^{A} ±0.01	0.74 ^{B} ±0.03	0.89 ^{A} ±0.04	6.16	0.003
^{55}**Mn** (nmol/L)	67.17 ^{B} ±1.67	103.89 ^{A} ±2.38	94.05 ^{A} ±15.76	4.38	0.015
^{64}**Zn** (µmol/L)	14.04 ±0.36	13.11 ±0.69	13.09 ±0.37	0.87	0.421
^{65}**Cu** (µmol/L)	17.69 ±0.82	16.64 ±0.52	15.36 ±0.66	2.99	0.055
^{78}**Se** (µmol/L)	1.48 ^{A} ±0.03	1.36 ^{B} ±0.05	1.60 ^{A} ±0.05	7.17	0.001
^{52}**Cr** (nmol/L)	241.50 ±30.12	175.84±18.38	185.06±19.10	2.30	0.106
^{59}**Co** (nmol/L)	6.22 ^{A} ±0.24	5.62 ^{A} ±0.42	2.78 ^{B} ±0.13	42.28	0.000

Keys: Abbreviations and units as previously defined

P<0.001= highly significant

P>0.05= not significant

MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area
Summary (elements higher by locations)
Benin-MiIA: Zn, Cu, Cr, Co (4)
Ibadan-MoIA: Mn (1)
Lagos-HiIA: Mg, Se (2)

Toxic and essential metal interactions in e-waste exposed and unexposed participants

Toxic and essential metal interactions in e-waste workers, environmentally exposed and unexposed participants are indicated in table 4.18, 4.19 and 4.20 respectively.

As shown in table 4.18, among the e-waste workers, significant inverse correlation relationships were observed between Zn and Al ($r = 0.26$; $p = 0.06$); Zn and Ni ($r = -0.28$, $p = 0.005$); Zn and Cr ($r = -0.28$, $p = 0.004$); Zn and As ($r = -0.19$, $p = 0.047$); Zn and Mo ($r = -0.26$, $p = 0.008$); and between Hg and Zn ($r = -0.34$, $p = 0.000$). Similarly, Se vs. Hg ($r = -0.31$, $P = 0.002$); Se vs. Pb ($r = -0.22$, $p = 0.025$) and Mo vs. Cu ($r = -0.27$, $p = 0.006$) were observed as significant associations.

As shown in table 4.19, a significant positive correlation was observed between Mg and V ($r = 0.25$, $p = 0.010$); Mg and Cr ($r = 0.26$, $p = 0.009$); and Mg and Mn ($r = 0.41$, $p = 0.000$) in the environmentally exposed group. Conversely, Mg vs. Ni ($r = -0.25$, $p = 0.011$), and Mg vs. Hg ($r = -0.44$, $p = 0.000$) were significantly inversely correlated.

Within the environmentally exposed group, it was further observed that Zn had a weak positive but significant correlations with Al, Ni, Mo, Cd and Sn,($r < 0.5$, $p<0.05$); and Zn was significantly inversely correlated with Tl ($r = -0.29$, $p= 0.006$). Cu showed a significant positive correlation with Al ($r = 0.24$, $p = 0.015$) and Mn ($r = 0.25$, $p = 0.009$), but inversely correlated with Hg ($r = -0.27$, $p = 0.005$). Weak positive but significant correlation values was observed between Se and V, Ni and As ($r <0.5$, $P<0.05$). Inverse significant correlation was observed between Cr and Mn, Sn and Hg ($r = -0.39$, $p = 0.000$; $r = -0.22$, $p = 0.032$; $r = -0.29$, $p = 0.003$) respectively. The association of Co with Ni, Mo, Cd, Hg showed weak inverse and significant relations ($r <-0.5$, $P<0.05$), but Co was positively correlated with Pb ($r = 0.22$, $p = 0.031$).

Interactions of toxic and essentials metals in the unexposed group (Table 4.20) showed that Mg was inversely and significantly correlated with V, Cr, and Mn ($r \leq -0.5$, $p \leq 0.01$); but was significantly positive correlated with As, Hg, Tl and Pb ($r \geq 0.5$ for As and Hg and <0.5 for Tl and Pb; $p<0.05$). Cu was inversely correlated with Ni ($r = -0.32$, $p = 0.002$); while Cr was significantly positively correlated with As, Hg, and Tl ($r \leq 0.4$, $p<0.01$).

Co Vs V, Cr and Ni showed inverse significant correlation ($r < -0.5$ for V and Cr, $r >-0.59$ for Ni; $p<0.01$). In addition, Co correlated with V, Cr, Ni and Sb showing significant inverse correlation ($r <-0.5$ for V, Cr and Sb; $P<0.01$); ($r = -0.59$; $p = 0.004$ for Ni). Correlation of Co with Mo showed weak positive and significant correlation, ($r = 0.29$, $P = 0.004$). Correlation of other parameters were not significant.

Table 4.18 Toxic and essential metals interactions in all e-waste workers

Toxic vs. Essential Metals		Mg (mmol/L)	Zn (µmol/L)	Cu (µmol/L)	Se (µmol/L)	Cr (nmol/L)	Co (nmol/L)
^{27}Al (µmol/L)	*r* value	0.01	-0.26**	-0.12	-0.04	0.01	0.11
	p value	0.931	**0.006**	0.249	0.736	0.952	0.305
^{51}V (nmol/L)	*r* value	0.15	0.13	0.04	0.01	-0.20	-0.11
	p value	0.131	0.194	0.68	0.9	0.052	0.263
^{52}Cr (nmol/L)	*r* value	-0.09	-0.28**	-0.13	-0.18	-0.22*	0.15
	p value	0.367	**0.004**	0.183	0.074	**0.038**	0.165
^{55}Mn (nmol/L)	*r* value	-0.12	-0.19	-0.22*	-0.19	0.09	0.11
	p value	0.222	0.054	**0.025**	0.068	0.411	0.294
^{60}Ni (nmol/L)	*r* value	-0.12	-0.28**	0.02	-0.18	-0.14	0.19
	p value	0.211	**0.005**	0.844	0.077	0.184	0.068
^{75}As (umol/L)	*r* value	0.05	-0.19*	-0.05	-0.09	-0.17	0.02
	p value	0.592	**0.047**	0.636	0.383	0.103	0.889
^{100}Mo (nmol/L)	*r* value	-0.04	-0.26**	-0.27**	-0.18	-0.04	-0.13
	p value	0.705	**0.008**	**0.006**	0.081	0.741	0.221
^{111}Cd (nmol/L)	*r* value	-0.12	-0.07	-0.15	-0.09	-0.004	0.04
	p value	0.217	0.507	0.142	0.406	0.967	0.678
^{118}Sn (nmol/L)	*r* value	-0.07	-0.16	-0.16	-0.12	-0.09	-0.18
	p value	0.502	0.102	0.11	0.23	0.37	0.097
^{121}Sb (nmol/L)	*r* value	0.01	-0.08	-0.06	0.13	-0.11	-0.16
	p value	0.889	0.435	0.568	0.206	0.312	0.139
^{202}Hg (nmol/L)	*r* value	-0.16	-0.34**	-0.15	-0.31**	-0.12	0.09
	p value	0.104	**0.000**	0.128	**0.002**	0.242	0.357
^{205}Tl (nmol/L)	*r* value	-0.13	0.02	0.08	0.02	-0.04	-0.12
	p value	0.182	0.846	0.448	0.814	0.737	0.258
^{208}Pb (µmol/L)	*r* value	-0.15	-0.15	-0.06	-0.22*	0.05	0.13
	p value	0.134	0.118	0.541	**0.036**	0.619	0.226

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.19 Toxic and essential metals interactions in all environmentally exposed participant

Toxic vs. Essential Metals		Mg (mmol/L)	Zn (µmol/L)	Cu (µmol/L)	Se (µmol/L)	Cr (nmol/L)	Co (nmol/L)
^{27}Al (µmol/L)	*r* value	0.03	0.22*	.235*	0.02	0.03	0.06
	p value	0.744	0.028	0.015	0.825	0.789	0.565
^{51}V (nmol/L)	*r* value	0.25*	0.18	0.13	0.21*	-0.10	0.14
	p value	0.01	0.08	0.18	0.03	0.322	0.17
^{52}Cr (nmol/L)	*r* value	0.26**	0.01	0.13	0.07	-0.12	0.00
	p value	0.009	0.895	0.167	0.461	0.231	0.972
^{55}Mn (nmol/L)	*r* value	0.41**	0.09	0.25**	0.09	-0.39**	0.12
	p value	0.000	0.367	0.009	0.387	0.000	0.242
^{60}Ni (nmol/L)	*r* value	-0.25*	0.36**	-0.07	0.22*	0.08	-0.24*
	p value	0.011	0.000	0.497	0.024	0.420	0.016
^{75}As (umol/L)	*r* value	-0.16	0.31**	0.00	0.22*	-0.02	-0.13
	p value	0.112	0.002	0.965	0.025	0.877	0.200
^{100}Mo (nmol/L)	*r* value	-0.14	0.27**	-0.06	0.10	0.06	-0.23*
	p value	0.157	0.006	0.530	0.330	0.566	0.019
^{111}Cd (nmol/L)	*r* value	-0.19	0.28**	-0.10	0.16	0.03	-0.25*
	p value	0.062	0.005	0.315	0.102	0.736	0.010
^{118}Sn (nmol/L)	*r* value	0.13	0.24*	0.09	0.08	-0.22*	-0.01
	p value	0.195	0.015	0.344	0.425	0.032	0.958
^{121}Sb (nmol/L)	*r* value	-0.04	-0.14	-0.02	0.04	0.14	0.06
	p value	0.726	0.172	0.862	0.688	0.174	0.582
^{202}Hg (nmol/L)	*r* value	-0.44**	0.08	-0.27**	0.03	0.29**	-0.19*
	p value	0.000	0.440	0.005	0.763	0.003	0.047
^{205}Tl (nmol/L)	*r* value	0.18	-0.29**	0.15	0.06	-0.04	0.27**
	p value	0.075	0.006	0.127	0.528	0.724	0.006
^{208}Pb (µmol/L)	*r* value	0.14	0.05	0.09	-0.05	-0.07	0.22*
	p value	0.09	0.610	0.363	0.614	0.503	0.031

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.20 Toxic and essential metal interactions in unexposed participants

Toxic vs. Essential Metals		Mg (mmol/L)	Zn (µmol/L)	Cu (µmol/L)	Se (µmol/L)	Cr (nmol/L)	Co (nmol/L)
^{27}Al (µmol/L)	*r* value	0.15	-0.05	0.11	-0.03	0.04	-0.03
	p value	0.147	0.596	0.285	0.779	0.689	0.78
^{51}V (nmol/L)	*r* value	-0.45**	-0.20	-0.16	-0.03	-0.22	-0.32**
	p value	0.001	0.089	0.187	0.784	0.069	0.007
^{52}Cr (nmol/L)	*r* value	-0.36**	-0.18	-0.08	-0.02	-0.22*	-0.27**
	p value	0.000	0.068	0.459	0.858	0.034	0.009
^{55}Mn (nmol/L)	*r* value	-0.26**	-0.03	-0.05	-0.11	0.04	-0.09
	p value	0.009	0.752	0.663	0.268	0.679	0.365
^{60}Ni (nmol/L)	*r* value	0.06	-0.03	-0.32**	-0.004	-0.04	-0.59**
	p value	0.556	0.793	0.002	0.969	0.673	0.001
^{75}As (umol/L)	*r* value	0.47**	0.08	-0.18	0.01	0.27**	-0.18
	p value	0.000	0.41	0.073	0.955	0.009	0.086
^{100}Mo (nmol/L)	*r* value	0.24*	0.16	0.10	-0.18	0.09	0.29**
	p value	0.02	0.103	0.322	0.074	0.413	0.004
^{111}Cd (nmol/L)	*r* value	-0.01	0.13	-0.03	-0.19	0.16	-0.02
	p value	0.913	0.211	0.801	0.059	0.122	0.864
^{118}Sn (nmol/L)	*r* value	0.05	-0.11	0.01	-0.08	0.068	0.004
	p value	0.641	0.287	0.945	0.458	0.517	0.967
^{121}Sb (nmol/L)	*r* value	-0.19	-0.11	0.04	0.18	-0.17	-0.24*
	p value	0.066	0.265	0.72	0.07	0.112	0.019
^{202}Hg (nmol/L)	*r* value	0.69**	0.11	-0.08	0.02	0.28**	0.13
	p value	0.000	0.28	0.43	0.857	0.006	0.196
^{205}Tl (nmol/L)	*r* value	0.23*	0.11	0.01	-0.17	0.39**	0.17
	p value	0.024	0.286	0.959	0.098	0.000	0.095
^{208}Pb (µmol/L)	*r* value	0.24*	0.08	0.11	-0.03	0.11	0.18
	p value	0.019	0.408	0.304	0.808	0.292	0.072

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Antioxidant vitamins in e-waste exposed and unexposed participants

Comparison of levels of antioxidant vitamins in all study participants (Table 4.21) showed that levels of vitamin A in environmentally exposed participants was higher and significantly different compared with e-waste workers and the unexposed groups, ($p = 0.045$); and post hoc test showed that the difference observed in vitamin A level between e-waste workers and unexposed group was not significant. Vitamin C level was lower but not significantly different in e-waste workers compared with unexposed groups, whereas, its level in environmentally exposed participants was significantly higher compared with other two groups ($p = 0.000$). Vitamin E level varied significantly amongst the three participants groups, viz, unexposed > e-waste workers > environmentally exposed, ($p < 0.001$).

Compared by location, levels of antioxidant vitamins in the three participants groups are summarized in Table 4.22. Vitamin A level in Ibadan e-waste workers was significantly higher compared with Lagos and Benin e-waste workers ($p = 0.017$). Vitamin C levels in the e-waste workers across the three study locations did not vary significantly. Vitamin E levels in Benin and Ibadan e-waste workers did not vary significantly, but were significantly higher compared with Lagos e-waste workers.

Comparison of vitamin levels in environmentally exposed group in three study sites showed that vitamin A levels were statistically similar in Benin and Lagos environmental groups, but were higher and significantly different from Ibadan environmental participants ($p<0.001$). Vitamin C and E did not significantly vary by location in the environmentally exposed group ($p>0.05$).

In the unexposed control, vitamin A in Ibadan participants was higher and significantly different from Benin and Lagos participants ($p <0.001$). Vitamins C and E in the unexposed control did not significantly vary by location ($p>0.05$)

Table 4.21 Antioxidant vitamins levels in e-waste exposed and unexposed participants

Antioxidant Vitamins	All Participants			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
Vitamin A (µmol/L)	2.08 ^{B} ±0.04	2.20 ^{A} ±0.04	1.97 ^{B} ±0.09	3.12	0.045
Vitamin C (µmol/L)	88.75 ^{B} ±3.33	116.31 ^{A} ±203	92.19 ^{B} ±1.68	41.147	0.000
Vitamin E (µmol/L)	8.91 ^{C} ±0.19	8.05 ^{B} ±0.18	12.36 ^{A} ±0.34	77.78	0.000

Keys: Abbreviations and units as previously defined

P<0.001= highly significant

P>0.05= not significant

Table 4.22 Antioxidant vitamin levels in e-waste workers, environmentally exposed and unexposed (control) in all study locations

Antioxidant vitamins	E-waste Workers				
	Benin-MiIA (n=83)	**Ibadan-MoIA (n=120)**	**Lagos-HiIA (n=178)**	***F* value**	***P* value**
Vitamin A (µmol/L)	2.01 ^{B} ±0.04	2.27 ^{A} ±0.09	2.05 ^{B} ±0.06	4.20	0.017
Vitamin C (µmol/L)	88.15 ±3.69	91.13 ±4.85	87.85 ±8.94	0.08	0.927
Vitamin E (µmol/L)	9.69 ^{A} ±0.27	9.15 ^{A} ±0.24	7.96 ^{B} ±0.37	8.63	0.000
	Environmentally Exposed				
Antioxidant vitamins	**(n=30)**	**(n=38)**	**(n=52)**		
Vitamin A (µmol/L)	2.32 ^{A} ±0.08	1.85 ^{B} ±0.07	2.36 ^{A} ±0.08	11.49	0.000
Vitamin C (µmol/L)	120.04 ±1.13	115.79 ±5.73	112.87 ±3.60	1.16	0.316
Vitamin E (µmol/L)	7.92 ±0.19	8.21 ±0.51	8.08 ±0.30	0.20	0.821
	Unexposed (Controls)				
Antioxidant vitamins	**(n=42)**	**(n=50)**	**(n=40)**		
Vitamin A (µmol/L)	1.34 ^{B} ±0.07	3.04 ^{A} ±0.18	1.68 ^{B} ±0.06	66.95	0.000
Vitamin C (µmol/L)	93.27 ±1.67	94.12 ±3.77	89.47 ±2.75	0.75	0.473
Vitamin E (µmol/L)	12.78 ±0.24	12.73 ±0.92	11.69 ±0.60	1.14	0.321

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significant
MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area

Correlation of toxic metals and antioxidant vitamins in e-waste exposed and unexposed participants.

Correlation of toxic metals and antioxidant vitamins in e-waste workers (Table 4.23) showed that vitamin A correlated significantly and positively with V and Cd ($r = 0.28$, $p = 0.001$; $r = 0.23$, $p = 0.010$) respectively. Vitamin C vs. Cd was significant ($r = 0.20$, $P = 0.037$); while vitamin E vs. Sn and Hg were significantly positively correlated ($r = 0.23$, $p = 0.012$; ($r = 0.20$, $p = 0.030$) respectively. Inverse significant correlation was observed between Vitamin E and Tl ($r = -0.19$, $p = 0.036$).

Relationships observed between toxic metals and antioxidant vitamins in the environmental exposed participants (Table 4.24) showed that vitamin A correlated positively and significantly with Ni, As, Mo, Cd, Sn and Tl ($r < 0.4$, $p < 0.05$). Conversely, vitamin C was inversely correlated with As ($r = -0.21$, $p = 0.029$). Other parameters were not significantly correlated.

In the unexposed group, interaction of toxic metals with antioxidant vitamins (Table 4.25) revealed that V, Cr, Ni and Sb were positively and significantly correlated with vitamin A ($r \leq 0.5$, $p < 0.05$); while Hg inversely correlated with vitamin A level ($r = -0.26$, $p = 0.10$).

In addition, vitamin C and E correlated significantly and positively with Hg and As respectively ($r < 0.5$, $p \leq 0.01$).

Table 4.23 Correlation of toxic metals and antioxidant vitamins in e-waste workers

Toxic metals vs. Antioxidant vitamins		Vitamin A (µmol/L)	Vitamin C (µmol/L)	Vitamin E (µmol/L)
^{27}Al (µmol/L)	*r value*	0.01	0.11	0.15
	p value	0.961	0.274	0.089
^{51}V (nmol/L)	*r value*	0.28**	0.02	-0.01
	p value	**0.001**	0.815	0.910
^{52}Cr (nmol/L)	*r value*	0.02	-0.05	0.13
	p value	0.804	0.585	0.141
^{60}Ni (nmol/L)	*r value*	-0.02	-0.02	0.09
	p value	0.855	0.861	0.345
^{75}As (µmol/L)	*r value*	0.04	-0.11	0.02
	p value	0.685	0.267	0.868
^{100}Mo (nmol/L)	*r value*	0.05	-0.13	0.02
	p value	0.607	0.17	0.858
^{111}Cd (nmol/L)	*r value*	0.23*	0.201*	0.02
	p value	**0.010**	**0.04**	0.793
^{118}Sn (nmol/L)	*r value*	-0.09	0.04	0.23*
	p value	0.289	0.695	**0.012**
^{121}Sb (nmol/L)	*r value*	0.04	-0.02	-0.01
	p value	0.668	0.862	0.937
^{202}Hg (nmol/L)	*r value*	-0.09	-0.04	0.20*
	p value	0.294	0.705	**0.030**
^{205}Tl (nmol/L)	*r value*	-0.04	-0.17	-0.19*
	p value	0.686	0.08	**0.036**
^{208}Pb (µmol/L)	*r value*	-0.02	-0.01	-0.11
	p value	0.799	0.901	0.241

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
Significant p values in *bolds*

Table 4.24 Correlation of toxic metals and antioxidant vitamins in environmentally exposed participants

Toxic metals vs. Antioxidant vitamins		Vitamin A (µmol/L)	Vitamin C (µmol/L)	Vitamin E (µmol/L)
^{27}Al (µmol/L)	*r* value	-0.07	-0.06	0.07
	p value	0.462	0.565	0.449
^{51}V (nmol/L)	*r* value	-0.11	-0.09	-0.06
	p value	0.264	0.333	0.566
^{52}Cr (nmol/L)	*r* value	-0.18	-0.07	0.07
	p value	0.054	0.482	0.441
^{60}Ni (nmol/L)	*r* value	0.29**	-0.10	0.03
	p value	**0.002**	0.317	0.747
^{75}As (umol/L)	*r* value	0.23*	-0.21*	-0.06
	p value	**0.019**	**0.029**	0.544
^{100}Mo (nmol/L)	*r* value	0.36**	-0.12	-0.05
	p value	**0.000**	0.226	0.629
^{111}Cd (nmol/L)	*r* value	0.36**	-0.02	-0.04
	p value	**0.000**	0.847	0.679
^{118}Sn (nmol/L)	*r* value	0.20*	-0.02	-0.03
	p value	**0.038**	0.830	0.726
^{121}Sb (nmol/L)	*r* value	0.09	-0.09	-0.05
	p value	0.389	0.355	0.636
^{202}Hg (nmol/L)	*r* value	0.11	0.01	-0.10
	p value	0.270	0.995	0.308
^{205}Tl (nmol/L)	*r* value	0.28**	0.12	-0.01
	p value	**0.003**	0.227	0.957
^{208}Pb (µmol/L)	*r* value	-0.08	0.02	-0.12
	p value	0.402	0.833	0.204

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
Significant p values in bold

Table 4.25 Correlation of toxic metals and antioxidant vitamins in unexposed participants

Toxic metals vs. Antioxidant vitamins		Vitamin A (µmol/L)	Vitamin C (µmol/L)	Vitamin E (µmol/L)
^{27}Al (µmol/L)	*r* value	0.10	-0.17	0.07
	p value	0.324	0.086	0.481
^{51}V (nmol/L)	*r* value	0.33**	0.04	-0.09
	p value	0.004	0.75	0.447
^{52}Cr (nmol/L)	*r* value	0.20*	-0.03	0.00
	p value	0.04	0.801	0.999
^{60}Ni (nmol/L)	*r* value	0.46**	-0.12	-0.15
	p value	0	0.222	0.125
^{75}As (umol/L)	*r* value	0.081	-0.002	0.26**
	p value	0.418	0.984	0.007
^{100}Mo (nmol/L)	*r* value	-0.124	0.10	-0.11
	p value	0.208	0.289	0.283
^{111}Cd (nmol/L)	*r* value	0.166	.210*	-0.115
	p value	0.097	0.035	0.25
^{118}Sn (nmol/L)	*r* value	-0.013	-0.07	-0.01
	p value	0.896	0.513	0.936
^{121}Sb (nmol/L)	*r* value	0.33**	-0.05	-0.11
	p value	0.001	0.632	0.279
^{202}Hg (nmol/L)	*r* value	-0.26**	0.31**	0.05
	p value	0.01	0.002	0.608
^{205}Tl (nmol/L)	*r* value	-0.128	-0.01	0.16
	p value	0.212	0.914	0.114
^{208}Pb (µmol/L)	*r* value	-0.089	0.11	-0.12
	p value	0.371	0.289	0.238
	p value	0.495	0.707	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Association of essential metals with antioxidant vitamins in e-waste exposed and unexposed participants in the different study locations

Correlation of essential metals and antioxidant vitamin levels in e-waste workers, environmentally exposed and unexposed groups are presented on tables 4.26, 4.27 and 4.28 respectively. Vitamin A in e-waste workers correlated positively and significantly with Zn and Se (r=0.22, 0.23; p= 0.022; 0.024) respectively. A similar observation was seen between vitamin C and Co; and Vitamin E and Mn, but inverse significant correlation was observed between Vitamin E and Se.

In the environmentally exposed group (table 4.27), inverse significant correlation was observed between Vitamin A and Mn; Cu; as well as between Vitamin C and Mn, ($r \leq -0.20$, $p < 0.05$).

The unexposed group (Table 4.28) showed significant positive relations between Vitamin A and Zn, Vitamin C and Mn, and an inverse significant association between Vitamin A and Co.

Table 4.26 Correlation of essential metals and antioxidant vitamins in e-waste workers

Essential metals vs. antioxidant vitamins		Vitamin A (µmol/L)	Vitamin C (µmol/L)	Vitamin E (µmol/L)
^{24}Mg (mmol/L)	*r* value	0.06	0.00	-0.09
	p value	0.57	0.986	0.368
^{55}Mn (nmol/L)	*r* value	0.01	0.13	0.18*
	p value	0.956	0.181	**0.043**
^{64}Zn (umol/L)	*r* value	0.22*	0.09	-0.18
	p value	**0.022**	0.378	0.058
^{65}Cu (umol/L)	*r* value	-0.11	-0.10	0.02
	p value	0.269	0.308	0.873
^{78}Se (umol/L)	*r* value	0.23*	-0.09	-0.27**
	p value	**0.024**	0.376	**0.009**
^{52}Cr (nmol/L)	*r* value	-0.02	-0.05	0.14
	p value	0.849	0.667	0.183
^{59}Co (nmol/L)	*r* value	-0.04	0.21*	0.08
	p value	0.676	**0.046**	0.444

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.27 Correlation of essential metals and antioxidant vitamins in environmentally exposed participants

Essential metals vs. antioxidant vitamins		Vitamin A (µmol/L)	Vitamin C (µmol/L)	Vitamin E (µmol/L)
^{24}Mg (mmol/L)	*r* value	-0.11	-0.11	-0.05
	p value	0.27	0.29	0.648
^{55}Mn (nmol/L)	*r* value	-0.19*	-0.22*	0.03
	p value	**0.044**	**0.024**	0.733
^{64}Zn (umol/L)	*r* value	0.03	-0.03	0.16
	p value	0.764	0.746	0.105
^{65}Cu (umol/L)	*r* value	-0.21*	0.08	-0.01
	p value	**0.029**	0.416	0.907
^{78}Se (umol/L)	*r* value	-0.02	-0.09	-0.04
	p value	0.802	0.352	0.672
^{52}Cr (nmol/L)	*r* value	0.13	0.16	0.06
	p value	0.202	0.118	0.585
^{59}Co (nmol/L)	*r* value	-0.12	-0.03	0.06
	p value	0.236	0.774	0.578

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.28 Correlation of essential metals and antioxidant vitamins in unexposed participants

Essential metals vs. antioxidant vitamins		Vitamin A (µmol/L)	Vitamin C (µmol/L)	Vitamin E (µmol/L)
^{24}Mg (mmol/L)	*r* value	0.02	0.04	-0.04
	p value	0.816	0.679	0.704
^{55}Mn (nmol/L)	*r* value	-0.15	0.24*	-0.19
	p value	0.141	**0.013**	0.051
^{64}Zn (µmol/L)	*r* value	0.20*	-0.07	-0.16
	p value	**0.043**	0.463	0.106
^{65}Cu (µmol/L)	*r* value	-0.14	-0.10	-0.18
	p value	0.165	0.334	0.076
^{78}Se (µmol/L)	*r* value	-0.06	0.18	0.02
	p value	0.59	0.073	0.864
^{52}Cr (nmol/L)	*r* value	-0.18	-0.02	0.00
	p value	0.086	0.871	0.988
^{59}Co (nmol/L)	*r* value	-0.33**	-0.05	0.02
	p value	**0.001**	0.638	0.861

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Total and ionized calcium, and 25(OH) vitamin D levels in e-waste exposed and unexposed participants in the different study locations

As shown in table 4.29, the level of total calcium (tCa) in e-waste workers was higher but not significantly different from levels in unexposed group, but both were significantly different compared with the environmental group (p=0.000). Ionized calcium (iCa) was higher significantly in the two e-waste exposed groups compared with the unexposed population. Notably, 25(OH) vitamin D (Vit. D) level was significantly higher in e-waste workers compared with the environmental and the unexposed populations (P<0.001).

Compared by location (Table 4.30), tCa and iCa in Lagos e-waste workers were significantly higher compared with Benin e-waste workers. However, tCa did not vary significantly between Ibadan and Lagos e-waste workers, also iCa did not vary in Benin and Ibadan e-waste workers. Levels of 25(OH) Vitamin D incrementally varied significantly from Ibadan to Benin and to Lagos e-waste workers, (p=0.039). In the environmentally exposed group, Lagos and Ibadan e-waste workers had significantly higher iCa than their Benin counterparts, (p=0.001). The level of tCa was significantly higher in the Ibadan group compared with the Benin and Lagos environmental participants that did not record any significant variation. In addition, Vit. D was significantly higher in Benin environment group compared with the Lagos and Ibadan groups which did not differ significantly.

In the unexposed group, tCa, Vit. D and iCa were significantly higher in Ibadan group compared with other locations.

Association of Toxic and essential metals with total and ionized calcium, and 25(OH) vitamin D levels in e-waste exposed and unexposed participants.

The associations of toxic and essential metals with total and ionized calcium and vitamin D in all participants using Pearson correlation analysis are given in tables 4.31-4.36.

Among the e-waste workers, (tables 4.31 and 4.34), Sn and Hg correlated inversely and significantly with 25(OH) vitamin D. Also, significant inverse correlation was observed between tCa and Hg; iCa and Al, and between iCa and Pb. In addition, significant positive correlations were observed between 25(OH) vitamin D and Zn, Co, and Mg as well as between tCa and Zn, while inverse significant relationship was observed between 25(OH) vitamin D and Mn, iCa and Cu.

In the environmentally exposed participants, (tables 4.32 and 4.35), significant inverse relationships were observed between 25(OH) vitamin D and V, Cr, and between tCa and Tl, iCa and Hg, as well as iCa and Tl. 25(OH) vitamin D was positively significantly correlated with Mo, tCa, Hg, Tl, while iCa and V were similarly correlated.

Additionally, Mg and Mn were inversely significantly correlated. Mg and Mn also positively and significantly correlated with tCa, while iCa correlated positively with Mn.

In the unexposed group (Tables 4.33 and 4.36), inverse significant correlations were observed between 25(OH) vitamin D and V, Cr, Pb and Mn; while positive significant relations were detected between 25(OH) vitamin D and Ni, As, Mo, Cd, Hg, TC and Pb. Other correlations were not significant.

Table 4.29 Total and ionized Calcium, and 25(OH) vitamin D levels in e-waste exposed and unexposed participants

Parameters	All Participants			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
tCalcium (mmol/L)	2.81 ^{A} ±0.06	2.44 ^{B} ±0.08	2.64 ^{A} ±0.05	8.70	0.000*
iCalcium (mmol/L)	1.40 ^{A} ±0.03	1.39 ^{A} ±0.03	1.27 ^{B} ±0.03	6.56	0.002*
25(OH) Vitamin D (nmol/L)	164.75 ^{A} ±8.69	125.70 ^{B} ±5.44	118.14 ^{B} ±6.29	12.95	0.000*

Keys: Abbreviations and units as previously defined

P<0.001= highly significant

P>0.05= not significant

tCalcium = total calcium

iCa = ionized calcium

Table 4.30 Total and ionized Calcium, and 25(OH) vitamin D levels in e-waste workers, environmentally exposed and unexposed in the different study locations

Parameters	E-waste Workers				
	Benin-MiIA (n=83)	**Ibadan-MoIA (n=120)**	**Lagos-HiIA (n=178)**	***F* value**	***P* value**
tCalcium (mmol/L)	2.58^{B} ±0.05	2.84^{A} ±0.13	2.98^{A} ±0.12	18.14	0.000
iCalcium (mmol/L)	1.39^{B} ±0.03	1.32^{B} ±0.03	1.51^{A} ±0.10	18.14	0.000
25(OH) Vitamin D (nmol/L)	155.54^{B} ±2.64	94.89^{C} ±8.36	215.67^{A} ±19.27	3.32	0.039
	Environmentally Exposed				
Parameters	**(n=30)**	**(n=38)**	**(n=52)**	***F* value**	***P* value**
tCalcium (mmol/L)	2.36^{B} ±0.06	3.08^{A} ±0.21	2.53^{B} ±0.08	9.11	0.000
iCalcium (mmol/L)	1.27^{B} ±0.02	1.50^{A} ±0.07	1.42^{A} ±0.04	7.31	0.001
25(OH) Vitamin D (nmol/L)	171.02^{A} ±2.84	61.63^{B} ±4.17	141.45^{B} ±9.08	132.87	0.000
	Unexposed (Controls)				
Parameters	**(n=42)**	**(n=50)**	**(n=40)**	***F* value**	***P* value**
tCalcium (mmol/L)	2.44^{B} ±0.05	2.78^{A} ±0.14	2.23^{B} ±0.08	4.92	0.000
iCalcium (mmol/L)	1.27^{B} ±0.03	1.48^{A} ±0.09	1.14^{C} ±0.02	14.33	0.000
25(OH) Vitamin D (nmol/L)	122.86^{A} ±4.62	141.79^{A} ±24.41	97.36^{B} ±5.11	3.82	0.024

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significant
tCalcium = total calcium
iCa = ionized calcium
MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area

Table 4.31 Correlation of toxic metals, total and ionized calcium, and 25(OH) vitamin D in e-waste workers

Correlated Parameters		25(OH) vitamin D (nmol/L)	tCa (mmol/L)	iCa (mmol/L)
^{27}Al (µmol/L)	*r* value	-0.01	-0.02	-0.18*
	p value	0.912	0.861	0.047
^{51}V (nmol/L)	*r* value	0.05	0.07	-0.07
	p value	0.567	0.398	0.445
^{52}Cr (nmol/L)	*r* value	-0.16	-0.06	0.11
	p value	0.085	0.447	0.2
^{60}Ni (nmol/L)	*r* value	-0.14	-0.06	-0.14
	p value	0.125	0.462	0.114
^{75}As (umol/L)	*r* value	0.05	-0.05	0.06
	p value	0.625	0.532	0.469
^{100}Mo (nmol/L)	*r* value	-0.08	-0.11	-0.03
	p value	0.368	0.201	0.71
^{111}Cd (nmol/L)	*r* value	0.08	0.13	0.07
	p value	0.403	0.115	0.454
^{118}Sn (nmol/L)	*r* value	-0.29**	-0.08	-0.16
	p value	0.001	0.331	0.074
^{121}Sb (nmol/L)	*r* value	-0.04	-0.02	-0.11
	p value	0.686	0.845	0.213
^{202}Hg (nmol/L)	*r* value	-0.34**	-0.17*	-0.12
	p value	0	0.042	0.193
^{205}Tl (nmol/L)	*r* value	-0.03	-0.02	-0.07
	p value	0.753	0.841	0.416
^{208}Pb (µmol/L)	*r* value	-0.02	-0.01	-0.24**
	p value	0.796	0.901	0.008

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.32 Correlation of toxic metals, total and ionized calcium, and 25(OH) vitamin D in environmentally exposed participants

Correlated Parameters		25(OH) vitamin D (nmol/L)	tCa (mmol/L)	iCa (mmol/L)
^{27}Al (µmol/L)	*r* value	0.01	-0.06	-0.13
	p value	0.904	0.566	0.174
^{51}V (nmol/L)	*r* value	-0.33**	0.19	0.25**
	p value	0.000	0.051	0.009
^{52}Cr (nmol/L)	*r* value	-0.49**	0.14	0.06
	p value	0.00	0.133	0.556
^{60}Ni (nmol/L)	*r* value	0.17	-0.04	0.00
	p value	0.073	0.715	0.996
^{75}As (umol/L)	*r* value	0.14	-0.02	0.14
	p value	0.136	0.817	0.144
^{100}Mo (nmol/L)	*r* value	0.43**	-0.12	-0.07
	p value	0.00	0.223	0.446
^{111}Cd (nmol/L)	*r* value	0.34**	-0.06	0.05
	p value	0.00	0.547	0.633
^{118}Sn (nmol/L)	*r* value	-0.09	0.00	0.15
	p value	0.377	0.995	0.116
^{121}Sb (nmol/L)	*r* value	-0.18	0.02	0.09
	p value	0.073	0.844	0.362
^{202}Hg (nmol/L)	*r* value	0.35**	-0.02	-.276**
	p value	0.00	0.832	0.004
^{205}Tl (nmol/L)	*r* value	0.58**	-0.24*	-0.24*
	p value	0.00	0.012	0.012
^{208}Pb (µmol/L)	*r* value	0.05	0.03	-0.05
	p value	0.624	0.772	0.592

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.33 Correlation of toxic metals, total and ionized calcium, and 25(OH) vitamin D in unexposed participants

Correlated Parameters		25(OH) vitamin D (nmol/L)	tCa (mmol/L)	iCa (mmol/L)
^{27}Al (µmol/L)	*r* value	-0.09	0.03	-0.12
	p value	0.339	0.784	0.212
^{51}V (nmol/L)	*r* value	-0.33**	0.10	0.20*
	p value	0	0.28	0.036
^{52}Cr (nmol/L)	*r* value	-0.49**	0.11	-0.02
	p value	0.00	0.245	0.849
^{60}Ni (nmol/L)	*r* value	0.41**	-0.04	0.00
	p value	0.0..	0.715	0.987
^{75}As (umol/L)	*r* value	0.32**	0.08	0.07
	p value	0.001	0.388	0.502
^{100}Mo (nmol/L)	*r* value	0.32**	0.02	0.04
	p value	0.001	0.858	0.672
^{111}Cd (nmol/L)	*r* value	0.29**	0.10	0.11
	p value	0.002	0.288	0.263
^{118}Sn (nmol/L)	*r* value	0.05	0.02	0.07
	p value	0.641	0.851	0.467
^{121}Sb (nmol/L)	*r* value	-0.19	0.10	0.04
	p value	0.064	0.337	0.683
^{202}Hg (nmol/L)	*r* value	0.32**	0.08	-0.27**
	p value	0.001	0.393	0.005
^{205}Tl (nmol/L)	*r* value	0.64**	-0.14	-0.17
	p value	0	0.131	0.073
^{208}Pb (µmol/L)	*r* value	-0.21*	0.06	0.02
	p value	0.028	0.55	0.83

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.34 Correlation of essential metals, total and ionized calcium, and 25(OH) vitamin D in e-waste workers

Correlated Parameters		25(OH) Vitamin D nmol/L	tCa mmol/L	iCa mmol/L
^{24}Mg mmol/L	*r* value	0.24*	0.04	0.05
	p value	0.016	0.706	0.637
^{55}Mn (nmol/L)	*r* value	-0.23*	-0.02	-0.06
	p value	0.014	0.844	0.515
^{64}Zn (µmol/L)	*r* value	0.23*	0.21*	0.04
	p value	0.016	0.028	0.691
^{65}Cu (µmol/L)	*r* value	0.09	-0.09	-0.23*
	p value	0.389	0.373	0.022
^{78}Se (µmol/L)	*r* value	-0.07	0.10	-0.01
	p value	0.483	0.36	0.91
^{52}Cr (nmol/L)	*r* value	0.03	-0.10	0.14
	p value	0.798	0.333	0.2
^{59}Co (nmol/L)	*r* value	0.36**	-0.07	-0.08
	p value	0.00	0.535	0.448

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.35 Correlation of essential metals, total and ionized calcium, and 25(OH) vitamin D in environmentally participants

Correlated Parameters		25(OH) Vitamin D (nmol/L)	tCa (mmol/L)	iCa (mmol/L)
^{24}Mg mmol/L	*r* value	-0.32**	0.24*	0.10
	p value	0.001	0.015	0.324
^{55}Mn (nmol/L)	*r* value	-0.54**	0.21*	0.25**
	p value	0.00	0.03	0.01
^{64}Zn (µmol/L)	*r* value	0.03	-0.15	0.15
	p value	0.779	0.146	0.135
^{65}Cu (µmol/L)	*r* value	-0.16	0.18	0.03
	p value	0.097	0.057	0.772
^{78}Se (µmol/L)	*r* value	0.03	0.13	0.19
	p value	0.746	0.183	0.051
^{52}Cr (nmol/L)	*r* value	0.17	-0.08	-0.12
	p value	0.093	0.46	0.219
^{59}Co (nmol/L)	*r* value	-0.10	0.00	-0.05
	p value	0.324	0.997	0.618

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.36 Correlation of essential metals, total and ionized calcium, and 25(OH) vitamin D in unexposed participants

Correlated Parameters		25(OH) Vitamin D (nmol/L)	tCa (mmol/L)	iCa (mmol/L)
^{24}Mg (mmol/L)	*r* value	0.01	-0.05	-0.05
	p value	0.94	0.612	0.656
^{55}Mn (nmol/L)	*r* value	-0.48**	0.08	0.18
	p value	0.00	0.422	0.056
^{64}Zn (µmol/L)	*r* value	0.13	-0.10	0.14
	p value	0.197	0.297	0.147
^{65}Cu (µmol/L)	*r* value	0.12	0.11	0.06
	p value	0.256	0.292	0.538
^{78}Se (µmol/L)	*r* value	0.10	0.30**	-0.07
	p value	0.313	0.002	0.495
^{52}Cr (nmol/L)	*r* value	0.05	-0.03	-0.10
	p value	0.616	0.805	0.341
^{59}Co (nmol/L)	*r* value	0.01	-0.12	0.08
	p value	0.965	0.258	0.416

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Enzymatic antioxidant biomarkers in e-waste exposed and unexposed participants in the different study locations

As presented in table 4.37, the activity of superoxide dismutase (SOD) was markedly and significantly decreased in e-waste workers (EW) compared with environmentally exposed participants (EEEP), and with unexposed participants (control), (P<0.001).

Catalase (CAT) in UC was significantly higher compared with EPP, while its activity in EPP was significantly higher compared with EWW (P<0.001). Likewise, glutathione peroxidase (GPx) activity was significantly higher in UC than EEP, while EEP was equally significantly higher than EWW (P<0.001). Glutathione reductase (GR) was higher significantly in EEP (P<0.001) but was not significantly different between EWW and UC.

Compared by location, (table 4.38), SOD was lowest in Lagos e-waste workers and also varied incrementally and significantly from Ibadan to Benin (P=0.000). CAT did not vary by location between Lagos and Ibadan among the e-waste workers, but were both significantly decreased compared with Benin participants (P<0.001). Activity of GPx was similar in Benin and Ibadan e-waste workers, and both were significantly higher than the activity obtained in the Lagos group. Activity of GR in Benin and Lagos e-waste workers did not vary significantly but were lower significantly compared with Ibadan participants. GGT activity among e-waste workers did not vary by location (P>0.05).

Among the environmentally exposed group, GPx, and GR activities were significantly higher in Ibadan e-waste workers compared with their Benin and Lagos counterparts which did not differ significantly. CAT varied significantly across the three locations, while SOD was similar between Benin and Ibadan but was significantly varied compared with Lagos group.

In the unexposed group, SOD and CAT varied significantly by location, (Benin>Ibadan>Lagos). GPx levels were similar in Benin and Ibadan but were varied compared with Lagos.

Association of essential and toxic metals with enzymatic antioxidants in e-waste exposed and unexposed participants

Among the e-waste exposed group (tables 4.39 and 4.42), positive significant correlations were observed between SOD and Zn, SOD and Mn, CAT and Mn, and between GPx and Mn, while significant inverse correlations were observed between SOD and Se, CAT and Se, GPx and Cu, ($r \leq 0.56$, $p<0.05$).

SOD and CAT correlated inversely and significantly with V. Also, SOD and CAT correlated inversely and significantly with V; while SOD alone correlated positively and significantly with Cr, Ni, AS, Sn, and Hg ($r \leq 0.65$, $p \leq 0.02$). Positive and significant correlations were observed between CAT and Cr, Ni, Hg; between GPx and Cr, Ni, As, Sn, Sb, Hg, and Pb, as well as between GR and V, Mo and Sn.

Among the environmentally exposed group (Tables 4.40 and 4.43), inverse significant correlations were observed between SOD and Zn ($r = 0.41$, $p=0.000$); CAT and Zn ($r = 0.29$ $P=0.003$); CAT and Mg ($r = -0.22$, $p=0.030$) and between CAT and Mn ($r = -0.74$, $p = 0.000$). Additionally, significant positive correlations were observed between GPx and Mn; GR and Co, Mg and Mn.

Notably, SOD correlated inversely and significantly with the toxic metals: Al, V, Ni, As, Mo, Cd, Sn, and Sb, $r = -0.25$, $p<0.01$). In the same vein, CAT and AL, V, Cr, Ni, As, Sn, Sb and Pb recorded significant inverse correlations ($r \geq -0.20$, $p<0.039$). GPx and Ni, As, Mo, Cd, Hg, and Tl showed significant inverse correlations. However, GPx and Cr showed positive and significant associations.

Correlation of parameters among the unexposed group (Table 4.41), revealed that essential metals complementarily associated with most antioxidant enzymes in that SOD versus Cu, Cr, and Co; as well as CAT versus Cu and Co; in addition to GR versus Zn and Mn associations were positive and significant. However, CAT versus Mg and Mn; as well as GR vs. Co recorded inverse significant correlations.

Associations in respect to toxic metals and enzymatic antioxidants were largely antagonistic in that SOD inversely and significantly correlated with Al, V, Cr, Ni, As and Sb. Similarly, CAT versus Al, V, Ni and As; GPx versus Al and GPx versus As correlated inversely and significantly. However, significant positive correlations were observed between SOD and Mo, Hg, Tl and Pb; as well as between CAT and Hg, Tl; GR versus Mo, Ni and AS.

Table 4.37 Enzymatic antioxidant biomarkers in e-waste exposed and unexposed participants

Enzymatic antioxidants	All Participants			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
Superoxide dismutase (µmol/min/mL)	140.00 ^{C} ±6.32	188.31 ^{B} ±8.79	328.48 ^{A} ±20.44	58.17	0.000
Catalase (µmol/min/mL)	122.83 ^{B} ±3.54	123.84 ^{B} ±2.77	215.74 ^{A} ±4.55	210.72	0.000
Glutathione peroxidase (µmol/min/mL)	31.41 ^{C} ±0.84	33.46 ^{B} ±0.46	39.90 ^{A} ±0.26	63.25	0.000
Glutathione reductase (U/g/dL)	0.31 ^{B} ±0.01	0.87 ^{A} ±0.07	0.26 ^{B} ±0.01	49.33	0.000

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significant

Table 4.38 Enzymatic antioxidant biomarkers in e-waste exposed and unexposed participants in the different study locations

Parameters	E-waste Workers			*F* value	*P* value
	Benin-MiIA (n=83)	**Ibadan-MoIA (n=120)**	**Lagos-HiIA (n=178)**		
Superoxide dismutase (µmol/min/mL)	220.03^{A} ±2.27	188.90^{B} ±0.79	69.83^{C} ±1.95	1739.07	0.000
Catalase (µmol/min/mL)	178.04^{A} ±0.62	98.19^{B} ±0.99	101.31^{B} ±1.86	1220.29	0.000
Glutathione peroxidase (µmol/min/mL)	32.89^{A} ±0.90	41.17^{A} ±0.34	22.11^{B} ±0.33	279.93	0.000
Glutathione reductase (U/g/dL)	0.23^{B} ±0.01	0.39^{A} ±0.01	0.28^{B} ±0.00	200.29	0.000
	Environmentally Exposed				
	(n=30)	**(n=38)**	**(n=52)**		
Superoxide dismutase (µmol/min/mL)	233.11^{A} ±1.98	280.77^{A} ±1.23	60.86^{B} ±0.95	6660.37	0.000
Catalase (µmol/min/mL)	161.84^{A} ±3.02	119.45^{B} ±0.64	91.34^{C} ±0.85	392.13	0.000
Glutathione peroxidase (µmol/min/mL)	30.30^{B} ±0.34	38.89^{A} ±0.45	30.12^{B} ±0.48	140.47	0.000
Glutathione reductase (U/g/dL)	0.29^{B} ±0.00	2.00^{A} ±0.04	0.24^{B} ±0.00	2211.94	0.000
	Unexposed (Controls)				
	(n=42)	**(n=50)**	**(n=40)**		
Superoxide dismutase (µmol/min/mL)	577.65^{A} ±20.76	301.80^{B} ±4.42	121.55^{C}±0.96	374.93	0.000
Catalase (µmol/min/mL)	271.12^{A} ±2.36	218.78^{B} ±2.22	160.22^{C}±2.84	486.27	0.000
Glutathione peroxidase (µmol/min/mL)	40.69^{A} ±0.38	40.81^{A} ±0.32	38.11^{B} ±0.43	16.01	0.000
Glutathione reductase (U/g/dL)	0.22^{B} ±0.01	0.71^{A} ±0.07	0.19^{B} ±0.01	39.02	0.001

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significant
MiIA = Mild e-waste impact area; MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area

Table 4.39 Association of essential metals with enzymatic antioxidants in e-waste workers

Parameters correlated		SOD (µmol/min/mL)	CAT (µmol/min/mL)	GPx (µmol/min/mL)	GR (u/g/dL)
^{24}Mg (mmol/L)	*r* value	-0.14	-0.09	0.03	0.08
	p value	0.171	0.353	0.73	0.407
^{55}Mn (nmol/L)	*r* value	0.56**	0.31**	0.57**	0.11
	p value	**0.001**	**0.001**	**0.000**	0.254
^{64}Zn (µmol/L)	*r* value	0.29**	-0.15	-0.02*	-0.01
	p value	**0.002**	0.12	**0.023**	0.9
^{65}Cu (µmol/L)	*r* value	0.17	0.02	0.001	-0.14
	p value	0.094	0.808	0.988	0.164
^{78}Se (µmol/L)	*r* value	-0.26*	-0.21*	-0.22*	0.06
	p value	**0.012**	**0.039**	**0.032**	0.576
^{52}Cr (nmol/L)	*r* value	-0.06	0.02	-0.14	-0.14
	p value	0.569	0.869	0.193	0.197
^{59}Co (nmol/L)	*r* value	-0.06	0.08	-0.07	0.00
	p value	0.606	0.463	0.516	0.978

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.40 Association of essential metals with enzymatic antioxidants in environmentally exposed participants

Correlated parameters		SOD (umol/min/mL)	CAT (umol/min/mL)	GPx (umol/min/mL)	GR (u/g/dL)
^{24}Mg (mmol/L)	*r* value	0.06	-0.22*	0.19	0.37**
	p value	0.543	**0.030**	0.056	**0.000**
^{55}Mn (nmol/L)	*r* value	-0.18	-0.74**	.422**	0.57**
	p value	0.059	**0.000**	**0.000**	**0.000**
^{64}Zn (µmol/L)	*r* value	-0.41**	-0.29**	-0.11	-0.09
	p value	**0.000**	**0.003**	0.294	0.372
^{65}Cu (µmol/L)	*r* value	-0.05	-0.18	0.02	0.16
	p value	0.648	0.059	0.86	0.1
^{78}Se (µmol/L)	*r* value	-0.14	-0.18	-0.05	-0.08
	p value	0.138	0.057	0.612	0.397
^{52}Cr (nmol/L)	*r* value	0.11	0.40**	-0.12	-0.17
	p value	0.289	0.00	0.227	0.102
^{59}Co (nmol/L)	*r* value	0.15	0.04	0.10	0.23*
	p value	0.130	0.688	0.319	**0.020**

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.41 Association of essential metals with enzymatic antioxidants in unexposed controls

Correlated parameters		SOD (umol/min/mL)	CAT (umol/min/mL)	GPx (umol/min/mL)	GR (u/g/dL)
^{24}Mg (mmol/L)	*r* value	-0.09	-0.21*	-0.21*	0.22*
	p value	0.373	**0.038**	**0.041**	**0.039**
^{55}Mn (nmol/L)	*r* value	-0.18	-0.20*	0.02	-0.16
	p value	0.083	**0.046**	0.84	0.121
^{64}Zn (µmol/L)	*r* value	0.12	0.01	0.05	0.21*
	p value	0.262	0.908	0.617	**0.045**
^{65}Cu (µmol/L)	*r* value	0.22*	0.33**	0.15	-0.04
	p value	**0.039**	**0.001**	0.14	0.674
^{78}Se (µmol/L)	*r* value	-0.16	-0.07	-0.01	0.01
	p value	0.132	0.524	0.933	0.929
^{52}Cr (nmol/L)	*r* value	0.20*	0.03	-0.11	-0.07
	p value	**0.050**	0.747	0.315	0.522
^{59}Co (nmol/L)	*r* value	0.58**	0.60**	0.05	-0.25*
	p value	**0.000**	**0.000**	0.606	**0.019**

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.42 Association of toxic metals with enzymatic antioxidants in e-waste workers

Correlated parameters		SOD (µmol/min/mL)	CAT (µmol/min/mL)	GPx (µmol/min/mL)	GR (/µg/dL)
^{27}Al (µmol/L)	*r* value	0.15	0.12	0.15	0.04
	p value	0.117	0.233	0.136	0.7
^{51}V (nmol/L)	*r* value	-0.35**	-0.23*	-0.39**	0.21*
	p value	**0.000**	**0.016**	**0.000**	**0.023**
^{52}Cr (nmol/L)	*r* value	0.43**	0.27**	0.51**	0.04
	p value	**0.000**	**0.004**	**0.000**	0.655
^{60}Ni (nmol/L)	*r* value	0.44**	0.25*	0.41**	0.03
	p value	**0.000**	**0.010**	**0.000**	0.777
^{75}As (umol/L)	*r* value	0.33**	0.17	0.25**	0.08
	p value	**0.000**	0.079	**0.010**	0.377
^{100}Mo (nmol/L)	*r* value	0.03	-0.02	-0.05	0.19*
	p value	0.724	0.811	0.595	**0.041**
^{111}Cd (nmol/L)	*r* value	0.02	0.03	0.15	0.09
	p value	0.82	0.753	0.126	0.318
^{118}Sn (nmol/L)	*r* value	0.30**	0.08	0.30**	0.22*
	p value	**0.002**	0.402	**0.002**	**0.020**
^{121}Sb (nmol/L)	*r* value	-0.14	-0.09	-.200*	-0.11
	p value	0.143	0.384	**0.041**	0.239
^{202}Hg (nmol/L)	*r* value	0.65**	0.36**	0.62**	0.14
	p value	**0.000**	**0.000**	**0.000**	0.137
^{205}Tl (nmol/L)	*r* value	-0.13	-0.07	-0.13	-0.05
	p value	0.192	0.488	0.192	0.582
^{208}Pb (µmol/L)	*r* value	0.15	0.08	0.26**	0.08
	p value	0.132	0.385	**0.008**	0.425

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.43 Association of toxic metals with enzymatic antioxidants in environmentally exposed group

Correlated parameters		SOD (µmol/min/mL)	CAT (µmol/min/mL)	GPx (µmol/min/mL)	GR (µ/g/dL)
^{27}Al (µmol/L)	*r* value	-0.25**	-0.22*	-0.04	0.05
	p value	**0.008**	**0.017**	0.644	0.586
^{51}V (nmol/L)	*r* value	-.358**	-.490**	0.18	0.07
	p value	**0.001**	**0.000**	0.061	0.484
^{52}Cr (nmol/L)	*r* value	0.03	-0.48**	0.40**	0.20*
	p value	0.763	**0.000**	**0.000**	**0.041**
^{60}Ni (nmol/L)	*r* value	-0.98**	-0.60**	-0.50**	0.02
	p value	**0.001**	**0.000**	**0.000**	0.867
^{75}As (umol/L)	*r* value	-0.83**	-0.53**	-0.36**	-0.07
	p value	**0.001**	**0.000**	**0.000**	0.485
^{100}Mo (nmol/L)	*r* value	-0.72**	-0.07	-0.68**	0.01
	p value	**0.000**	0.475	**0.000**	0.894
^{111}Cd (nmol/L)	*r* value	-0.64**	-0.09	-0.53**	-0.06
	p value	**0.000**	0.374	**0.000**	0.502
^{118}Sn (nmol/L)	*r* value	-0.51**	-0.45**	-0.11	0.02
	p value	**0.000**	**0.000**	0.25	0.875
^{121}Sb (nmol/L)	*r* value	-0.58**	-0.53**	-0.18	0.03
	p value	**0.000**	**0.000**	0.07	0.788
^{202}Hg (nmol/L)	*r* value	0.15	0.41**	-0.28**	-0.05
	p value	0.124	**0.000**	**0.004**	0.581
^{205}Tl (nmol/L)	*r* value	-0.03	0.51**	-0.34**	-0.11
	p value	0.73	**0.000**	**0.000**	0.238
^{208}Pb (µmol/L)	*r* value	-0.16	-0.20*	-0.06	0.12
	p value	0.108	**0.039**	0.539	0.223

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.44 Association of toxic metals with enzymatic antioxidants in unexposed group

Correlated parameters		SOD (µmol/min/mL)	CAT (µmol/min/mL)	GPx (µmol/min/mL)	GR (µ/g/dL)
^{27}Al (µmol/L)	*r* value	-0.23*	-0.24*	-0.28**	-0.02
	p value	**0.024**	**0.017**	**0.005**	0.867
^{51}V (nmol/L)	*r* value	-0.60**	-0.77**	0.16	-0.16
	p value	**0.000**	**0.000**	0.192	0.179
^{52}Cr (nmol/L)	*r* value	-0.37**	-0.156	.215*	-0.03
	p value	**0.000**	0.114	**0.028**	0.737
^{60}Ni (nmol/L)	*r* value	-0.37**	-0.52**	-0.19	0.01
	p value	**0.000**	**0.000**	0.051	0.958
^{75}As (umol/L)	*r* value	-0.25*	-0.45**	-0.37**	-0.09
	p value	**0.014**	**0.000**	**0.000**	0.351
^{100}Mo (nmol/L)	*r* value	0.26*	0.07	0.06	0.25**
	p value	**0.010**	0.485	0.517	**0.009**
^{111}Cd (nmol/L)	*r* value	-0.20	-0.11	0.01	-0.03
	p value	0.059	0.258	0.929	0.742
^{118}Sn (nmol/L)	*r* value	-0.17	-0.05	0.02	0.10
	p value	0.093	0.658	0.836	0.337
^{121}Sb (nmol/L)	*r* value	-0.27**	-0.05	0.02	0.03
	p value	**0.009**	0.645	0.842	0.774
^{202}Hg (nmol/L)	*r* value	0.28**	0.24*	-0.05	0.04
	p value	**0.006**	**0.017**	0.653	0.673
^{205}Tl (nmol/L)	*r* value	0.29**	0.26*	-0.16	0.11
	p value	**0.004**	**0.011**	0.115	0.308
^{208}Pb (µmol/L)	*r* value	.219*	0.14	-0.08	0.14
	p value	**0.034**	0.169	0.442	0.171

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Selected oxidative stress biomarers in e-waste exposed and unexposed population in the different study locations

Tables 4.45 and 4.46 show summary results of biomarkers of oxidative stress in e-waste exposed and unexposed population in different study locations.

From table 4.45, malondialdehyde (MDA) level was highest in e-waste workers compared with environmentally exposed and unexposed populations, with the lowest level recorded for the unexposed group. There was a high significant difference across the three groups tested ($P<0.01$). Similarly, uric acid (UA), total glutathione (tGSH) and total bilirubin (TBil) were also highly significantly different ($P<0.01$) across the three groups studied, with UA level being highest in the e-waste workers and lowest in unexposed population; but tGSH was however highest in unexposed population and lowest in the e-waste workers.

Furthermore, unexposed population had highest value of albumin (Alb) followed by environmentally exposed population then the e-waste workers which had the lowest. TBil level was highest in e-waste workers but lowest in environmentally exposed populations while conjugated bilirubin (CBil) was highest in e-waste workers, and lowest in unexposed group.

Considered by location (table 4.46), MDA levels in Lagos e-waste workers recorded the highest value, followed by Benin and Ibadan. Total GSH level was lowest in Lagos e-waste workers and its levels varied significantly across locations. Uric acid, Tbil and Cbil did not vary by location among the e-waste workers. Alb was highest in Lagos e-waste workers but was not significantly different compared with Benin, while both were significantly higher in same group ($p< 0.001$).

In the environmental group, MDA level was highest in the Benin e-waste workers and its level varied significantly across the three locations. Uric acid levels in Benin and Ibadan e-waste workers were similar but significantly varied compared with the Lagos location where the lowest value was obtained. Total GSH was similar between Ibadan and Lagos locations, and the levels were significantly higher than the Benin location. Total Bilirubin did not vary by locations, while Cbil and Alb. levels were higher in both Benin and Lagos compared with Ibadan location, in which the lowest value was obtained.

In the unexposed group, MDA level was higher in Benin compared with Ibadan and Lagos, while uric acid varied significantly across the different locations, with Ibadan recording the highest value. Total GSH was highest in Benin, lowest in Lagos and moderately low in

Ibadan. Tbil level did not vary with location. Cbil and Alb Levels were similar in Benin and Lagos, but were significantly higher compared with Ibadan location ($p < 0.001$).

Correlation of toxic metals with selected biomarkers of oxidative stress in e-waste exposed and unexposed participants

Tables 4.47, 4.48 and 4.49 show the interrelationships among selected biomarkers of oxidative stress and toxic metals in the study participants.

Among the occupationally exposed participants (Table 4.47), inverse significant correlations were observed between MDA and Sn, Hg; tGSH versus Tl, as well as between UBil and Tl. Positive significant correlations were observed between UBil and Al, Cr, Ni, Sn, and Hg, while other correlations were not significant.

Among the environmentally exposed group, (table 4.48), MDA versus Mo and Tl; as well as tGSH versus V, Cr, and Sn showed positive significant associations ($r \geq 0.20$, $p \leq 0.038$)

However, inverse significant associations were observed with UA and Ni, As, Cd and Sb; as well as between tGSH and Hg and Tl. Alb was weakly significantly correlated with Sn, and inversely correlated with Hg.

Associations among the unexposed population (Table 4.49) revealed that MDA and V, Cr, Ni, and Sb, correlated inversely and significantly, whereas MDA positively and significantly correlated with Hg and Tl. VA correlated significantly positively with V and Cr, and significantly negatively with V, Cr, Mo, Hg, Tl, and Pb.

In addition, UBil correlated universal, with V, Ni, and As, but positively with Mo and Hg.

Table 4.45 Selected biomarkers of oxidative stress in Nigerian e-waste exposed and unexposed populations

Biomarkers	Mean ± SEM			*F* value	*P* value
	E-waste workers (n=381)	Environmental exposed (n = 120)	Unexposed (n=131)		
Malondialdehyde (nmol/dL)	59.02 ^{A} ±0.93	43.70 ^{B} ±0.52	37.41 ^{C} ±0.49	273.80	0.000*
Uric acid (mmol/L)	0.57 ^{A} ±0.01	0.55 ^{A} ±0.08	0.49 ^{B} ±0.10	27.12	0.000*
Total Glutathione (µmol/L)	3.96 ^{C} ±0.91	4.44 ^{B} ±0.07	5.41 ^{A} ±0.09	81.17	0.000*
Albumin (g/dl)	47.17±0.25	48.55±0.31	48.76±0.38	8.92	0.079
Total Bilirubin (µmol/L)	10.57^{A}±0.37	8.71^{B}±0.52	9.10^{B}±0.70	4.02	0.019*
Conjugated Bilirubin (µmol/L)	6.29±0.24	5.82±0.31	5.32±0.30	2.43	0.090

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P>0.05= not significant
*= significant at p≤ 0.05

Table 4.46 Selected biomarkers of oxidative stress in Nigerian e-waste exposed and unexposed populations compared by locations

Biomarkers	E-waste workers			*F* value	*P* value
	Benin-MiIA	**Ibadan-MoIA**	**Lagos-HiIA**		
Malondialdehyde (nmol/dL)	59.27^{A}±0.76	53.57^{B} ±2.18	63.24^{A} ±1.28	10.76	0.000
Uric acid (mmol/L)	0.56 ±0.01	0.59 ±0.02	0.57 ±0.01	0.70	0.497
Total Glutathione (µmol/L)	3.97^{B} ±0.99	4.81^{A} ±0.11	3.18^{C} ±0.80	72.20	0.000
Total bilirubin (µmol/L)	10.39±0.65	10.72±0.53	10.61±0.63	0.08	0.928
Conjugated bilirubin (µmol/L)	6.14±0.44	5.14±0.41	6.38±0.42	2.44	0.090
Albumin(g/L)	47.63^{A} ±0.34	40.92^{B} ±0.59	46.94^{A} ±0.47	56.69	0.000
Environmental Participants					
Malondialdehyde (nmol/dL)	46.32^{A} ±0.91	40.22^{C} ±0.66	43.90^{B} ±0.81	13.31	0.000
Uric acid (mmol/L)	0.55^{A} ±0.01	0.57^{A} ±0.01	0.51^{B} ±0.01	8.76	0.000
Total Glutathione (µmol/L)	3.84^{B} ±0.79	4.72^{A} ±0.10	4.76^{A} ±0.13	24.85	0.000
Total bilirubin (µmol/L)	8.71±0.91	9.13±0.56	8.71±0.91	0.09	0.912
Conjugated bilirubin (µmol/L)	5.80^{A} ±0.50	3.89^{B} ±0.33	5.82^{A} ±0.54	5.47	0.005
Albumin(g/L)	46.56^{A} ±0.58	38.91^{B} ±0.80	48.55^{A} ±0.54	74.97	0.000
Unexposed participants					
Malondialdehyde (nmol/dL)	41.59^{A} ±0.58	34.33^{B} ±0.74	36.83^{B} ±0.75	24.65	0.000
Uric acid (mmol/L)	0.40^{C} ±0.02	0.56^{A} ±0.01	0.48^{B} ±0.01	33.41	0.000
Total Glutathione (µmol/L)	6.11^{A} ±0.14	5.15^{B} ±0.09	4.82^{C} ±0.14	29.84	0.000
Total bilirubin (µmol/L)	8.71 ±0.91	9.13 ±0.56	8.71 ±0.91	0.09	0.912
Conjugated bilirubin(µmol/L)	5.32^{A} ±0.54	3.55^{B} ±0.30	5.47^{A} ±0.52	6.83	0.002
Albumin(g/L)	46.56^{A} ±0.58	38.91^{B} ±0.80	48.55^{A} ±0.54	74.97	0.000

Table 4.47 Correlation of toxic metals with oxidative stress biomarkers in e-waste workers

Correlated parameters		MDA (nmol/dL)	Uric acid (mmol/L)	tGSH (µM/L)	Albumin (g/L	Total bilirubin (µmol/L)	Unconjugated bilirubin (µmol/L)
^{27}Al	*r* value	-0.02	0.04	-0.04	0.07	0.14	0.21*
(µmol/L)	*p value*	0.821	0.722	0.57	0.324	0.053	**0.025**
^{51}V	*r* value	0.09	-0.04	0.04	0.00	-0.01	-0.14
(nmol/L)	*p value*	0.338	0.654	0.62	0.967	0.915	0.146
^{52}Cr	*r* value	-0.13	0.07	0.01	0.13	0.05	0.24**
(nmol/L)	*p value*	0.19	0.473	0.946	0.078	0.463	**0.010**
^{60}Ni	*r* value	-0.14	0.02	0.01	0.02	0.01	0.28**
(nmol/L)	*p value*	0.153	0.849	0.906	0.832	0.85	**0.003**
^{75}As	*r* value	0.01	0.07	0.12	0.02	-0.01	0.17
(umol/L)	*p value*	0.898	0.499	0.117	0.758	0.937	0.071
^{100}Mo	*r* value	0.06	0.09	0.01	-0.03	-0.04	0.10
(nmol/L)	*p value*	0.551	0.355	0.857	0.708	0.574	0.295
^{111}Cd	*r* value	0.10	0.02	-0.09	0.00	0.01	0.04
(nmol/L)	*p value*	0.309	0.867	0.2	0.982	0.948	0.687
^{118}Sn	*r* value	-0.21*	0.00	-0.05	0.04	-0.04	0.29**
(nmol/L)	*p value*	**0.029**	0.967	0.518	0.619	0.626	**0.001**
^{121}Sb	*r* value	0.04	-0.08	-0.06	-0.06	-0.05	-0.09
(nmol/L)	*p value*	0.651	0.438	0.444	0.404	0.529	0.346
^{202}Hg	*r* value	-0.28**	0.02	-0.01	-0.03	0.03	0.50**
(nmol/L)	*p value*	**0.003**	0.876	0.919	0.646	0.642	**0.000**
^{205}Tl	*r* value	0.01	-0.01	-0.18*	-0.07	-0.03	-0.20*
(nmol/L)	*p value*	0.917	0.959	**0.012**	0.344	0.7	0.035
^{208}Pb	*r* value	-0.12	-0.05	0.08	-0.08	-0.01	0.14
(µmol/L)	*p value*	0.196	0.627	0.271	0.277	0.884	0.149

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.48 Correlation of toxic metals with oxidative stress biomarkers in environmentally exposed participants

Correlated parameters		MDA (nmol/dL)	Uric acid (mmol/L)	tGSH (µM/L)	Albumin (g/L)	Total bilirubin (µmol/L)	Unconjugated bilirubin (µmol/L)
^{27}Al (µmol/L)	*r* value	-0.09	-0.02	0.02	0.01	-0.02	-0.03
	p value	0.337	0.803	0.837	0.919	0.805	0.734
^{51}V (nmol/L)	*r* value	-0.12	-0.15	0.20*	0.03	0.06	-0.09
	p value	0.196	0.111	0.035	0.737	0.51	0.362
^{52}Cr (nmol/L)	*r* value	-0.16	-0.04	.266**	0.00	0.09	-0.08
	p value	0.088	0.697	**0.005**	0.981	0.33	0.439
^{60}Ni (nmol/L)	*r* value	0.15	-0.29**	0.14	-0.06	0.03	0.04
	p value	0.114	**0.002**	0.139	0.565	0.774	0.67
^{75}As (umol/L)	*r* value	0.09	-0.20*	0.18	-0.04	0.06	0.03
	p value	0.369	**0.033**	0.064	0.715	0.536	0.78
^{100}Mo (nmol/L)	*r* value	0.30**	-0.17	-0.15	-0.02	-0.04	-0.04
	p value	0.001	0.075	0.114	0.862	0.717	0.672
^{111}Cd (nmol/L)	*r* value	0.17	.190*	-0.13	0.11	0.02	0.01
	p value	0.076	**0.045**	0.184	0.263	0.8	0.929
^{118}Sn (nmol/L)	*r* value	-0.05	-0.05	.200*	0.30*	0.12	-0.08
	p value	0.61	0.595	**0.038**	**0.017**	0.209	0.414
^{121}Sb (nmol/L)	*r* value	-0.03	-0.21*	0.19	0.17	-0.18	-0.08
	p value	0.787	**0.035**	0.051	0.095	0.081	0.401
^{202}Hg (nmol/L)	*r* value	0.16	0.12	-0.30**	-0.22*	0.14	-0.07
	p value	0.092	0.215	**0.002**	**0.020**	0.142	0.501
^{205}Tl (nmol/L)	*r* value	0.21*	0.07	-0.41**	-0.02	0.04	0.10
	p value	0.027	0.49	**0.000**	0.815	0.709	0.282
^{208}Pb (µmol/L)	*r* value	0.03	-0.06	0.07	-0.12	-0.04	0.00
	p value	0.782	0.552	0.466	0.225	0.66	0.973

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.49 Correlation of toxic metals with oxidative stress biomarkers in unexposed group

Correlated parameters		MDA (nmol/dL)	Uric acid (mmol/L)	tGSH (µM/L)	Albumin (g/L)	Total bilirubin (µmol/L)	UC bilirubin (µmol/L)
^{27}Al (µmol/L)	*r* value	-0.10	-0.01	0.14	0.10	-0.04	-0.12
	p value	0.336	0.956	0.16	0.314	0.723	0.226
^{51}V (nmol/L)	*r* value	-0.44**	0.40**	0.10	-0.05	-0.05	-0.27*
	p value	**0.000**	**0.001**	0.411	0.692	0.702	**0.022**
^{52}Cr (nmol/L)	*r* value	-0.35**	0.48**	-0.07	-0.04	-0.02	-0.14
	p value	0.00	**0.001**	0.475	0.702	0.83	0.169
^{60}Ni (nmol/L)	*r* value	-0.21*	0.02	0.12	0.14	0.00	-0.22*
	p value	**0.035**	0.835	0.233	0.175	0.999	**0.024**
^{75}As (µmol/L)	*r* value	0.00	-0.12	0.09	0.14	0.08	-0.22*
	p value	0.971	0.239	0.391	0.169	0.417	**0.023**
^{100}Mo (nmol/L)	*r* value	0.24*	-0.23*	0.01	0.14	0.17	0.23*
	p value	**0.015**	**0.021**	0.93	0.143	0.076	0.019
^{111}Cd (nmol/L)	*r* value	-0.17	0.10	-0.15	-0.10	-0.06	-0.08
	p value	0.085	0.301	0.129	0.319	0.543	0.418
^{118}Sn (nmol/L)	*r* value	-0.18	0.17	0.09	-0.06	-0.07	-0.10
	p value	0.078	0.1	0.395	0.541	0.476	0.317
^{121}Sb (nmol/L)	*r* value	-0.27**	0.18	0.00	.202*	-0.10	-0.08
	p value	**0.007**	0.083	0.985	**0.045**	0.318	0.453
^{202}Hg (nmol/L)	*r* value	0.39**	-0.43**	-0.09	-0.12	-0.12	0.20*
	p value	**0.000**	**0.001**	0.393	0.251	0.232	**0.049**
^{205}Tl (nmol/L)	*r* value	0.22*	-0.22*	0.04	-0.05	-0.05	0.14
	p value	**0.029**	0.034	0.725	0.632	0.641	0.169
^{208}Pb (µmol/L)	*r* value	0.12	-0.16	-0.04	-0.02	-0.18	0.10
	p value	0.248	0.113	0.685	0.822	0.076	0.332

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Liver function indices in e-waste exposed and unexposed participants in different study locations

The risk assessment indices of liver damage in e-waste exposed and unexposed participants in different study locations are indicated in tables 4.50, 4.51, 4.52 and 4.53.

Among participants' groups (table 4.50), TBil. Level was highest in e-waste workers and lowest in environmentally exposed group. CBil and UBil were both highest in e-waste workers. However, CBil was lowest in unexposed group while UBil was lowest in environmentally exposed group. Total protein, Alb, Glob as well as CBil did not vary significantly across the exposed and unexposed groups ($p>0.05$).

Notably, ALT, AST, ALP and GGT showed decrease in values from highest to lowest across e-waste, environmentally exposed and unexposed groups respectively, except in AST/ALT ratio where unexposed had the highest value (0.99 ± 0.05) but lowest in environmentally exposed population (0.69 ± 0.02).

Across the different study locations, TBil was not significantly different ($P > 0.05$) for the 3 different locations in the e-waste worker's group. However, TBil was highest in Ibadan e-waste workers (Table 4.51) but lowest in Ibadan unexposed population (Table 4.53).

CBil was highest in Lagos in e-waste workers (Table 4.51) but lowest in unexposed population of Ibadan (Table 4.53). In the environmentally exposed and unexposed groups, CBil at the different study locations were significantly different across board, ($p < 0.05$)

UBil was highest in unexposed group in Lagos (Table 4:53) but not significantly different from the environmentally exposed group ($p>0.05$) (Table 4.52) with exception of the e-waste workers (Table 4.51) where it was significantly different ($p<0.05$) and lowest values also recorded.

Total protein was highest in unexposed group in Ibadan (Tab. 4.53) and lowest in environmentally exposed group in Ibadan (Tab 4.52).

Alb, Glob, ALT, AST, AST / ALT, ALP and GGT generally had high significant difference ($p< 0.05$) across the study locations and amongst the three populations studied, (Table 4.51, 4.52 and 4.53).

Notably, GGT was highest in e-waste workers in Lagos (Tab 4.51), but was not significantly varied by location. In the environmental and unexposed groups (tables 4.52 and 4.43 respectively), GGT was higher in Ibadan participant compared with Benin and Lagos which did not vary significantly ($p=0.108$).

Table 4.50 Liver function indices in e-waste exposed and unexposed participants

Parameters	Mean ± SEM			*F* value	*P* value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
Total bilirubin (µmol/L)	10.54^{A}±0.37	8.71^{B}±0.52	9.10^{B}±0.70	4.02	0.019
Conjugated bilirubin (µmol/L)	6.29 ±0.24	5.82 ±0.31	5.32 ±0.30	2.43	0.090
Unconjugated bilirubin (µmol/L)	*4.23^{A} ± 0.20	3.01^{B} ± 0.39	3.80^{A} ± 0.52	4.58	0.011
Total protein (g/L)	69.30±0.49	70.93±0.58	69.72 ±0.80	8.93	0.132
Albumin(g/L)	47.17 ± 0.25	48.55 ± 0.31	48.76 ± 0.38	8.92	0.079
Globulin (g/L)	22.03 ± 0.50	22.39 ± 0.54	20.96 ± 0.67	1.50	0.225
ALT (U/L)	40.99^{A}±1.43	34.61^{B} ±1.17	19.15^{C}±0.62	75.75	0.000
AST (U/L)	39.72^{A} ± 0.85	23.95^{B} ± 0.90	18.98^{C} ± 0.83	163.15	0.000
*AST/ALT (De Ritis ratio)	0.97^{A} ± 0.04	0.69^{B}±0.02	0.99^{A}±0.05	34.30	0.000
ALP (U/L)	76.56^{A} ± 1.78	64.16^{B} ± 2.14	38.84^{C}±2.04	91.24	0.000
GGT(U/L)	24.32^{A} ±0.92	16.35^{B}±0.70	10.83^{C} ± 0.43	74.20	0.000

Keys: Abbreviations and units as previously defined

P<0.001= highly significant

P<0.05- Significant

P>0.05= not significant

Different superscript letters across the rows indicates that the mean at a treatment is significant from other.

Table 4.51 Liver function indices in e-waste workers in all study locations

Risk assessment indices	E-Waste Workers			F-value	Significant
	Benin-MiIA (n=83)	Ibadan-MoIA (n=120)	Lagos-HiIA (n=178)		
Total bilirubin (µmol/L)	10.39±0.65	10.72±0.53	10.61±0.63	0.08	0.928
Conjugated bilirubin (µmol/L)	6.14±0.44	5.14±0.41	6.38±0.42	2.44	0.090
Unconjugated bilirubin (µmol/L)	4.23^{B} ±0.38	5.58^{A} ±0.29	4.23^{B} ±0.34	5.29	0.006
Total protein (g/L)	69.30±0.83	70.75±0.58	69.30±0.83	1.21	0.299
Albumin(g/L)	47.63^{A} ±0.34	40.92^{B} ±0.59	46.94^{A} ±0.47	56.69	0.000
Globulin (g/L)	21.34^{B} ±0.84	29.67^{A} ±0.47	22.37^{B} ±0.88	36.44	0.000
Alanine aminotransferase (U/L)	24.40^{C} ±1.30	33.14^{B} ±2.86	49.29^{A} ±2.26	31.84	0.000
AST (U/L)	37.36^{A} ±1.42	22.41^{B} ±2.09	40.90^{A} ±1.49	33.58	0.000
AST/ALT (De Ritis ratio)	1.69^{A} ±0.08	0.89^{B} ±0.08	0.91^{B} ±0.04	42.40	0.000
ALP (U/L)	60.21^{B} ±.75	88.60^{A} ±2.27	84.73^{A} ±2.03	30.46	0.000
GGT(U/L)	19.51±1.27	25.16±3.89	26.73±1.66	2.25	0.108

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P<0.05- Significant
P>0.05= not significant
Different superscript letters across the rows indicates that the mean at a treatment is significant from other.
Benin-MiIA
Ibadan-MoIA
Lagos-HiIA

Table 4.52 Liver function indices in environmentally exposed participants in all study locations

Risk Assessment Indices	Environmentally Exposed			F-value	Significant
	Benin-MiIA (n=30)	**Ibadan-MoIA (n=38)**	**Lagos-HiIA (n=52)**		
Total bilirubin (µmol/L)	8.71±0.91	9.13±0.56	8.71±0.91	0.09	0.912
Conjugated bilirubin (µmol/L)	5.80^{A} ±0.50	3.89^{B} ±0.33	5.82^{A} ±0.54	5.47	0.005
Unconjugated bilirubin(µmol/L)	4.90±0.68	5.25±0.41	4.40±0.58	0.11	0.898
Total protein (g/L)	68.43±1.03	68.18±0.94	70.93±1.01	2.60	0.079
Albumin(g/L)	46.56^{A} ±0.58	38.91^{B} ±0.80	48.55^{A} ±0.54	74.97	0.000
Globulin (g/L)	22.39^{B} ±0.95	29.27^{A} ±0.42	24.29^{B} ±0.75	24.20	0.000
ALT (U/L)	34.61^{A} ±2.04	25.32^{B} ±3.20	37.41^{A} ±3.03	4.64	0.011
AST (U/L)	23.95^{A} ±1.58	15.49^{B} ±1.64	26.25^{A} ±2.53	9.35	0.000
AST/ALT	0.69^{A} ±0.04	0.61^{B} ±0.09	0.70^{A} ±0.04	5.19	0.007
ALP (U/L)	45.86^{C} ±3.33	97.52^{A} ±4.21	73.30^{B} ±3.08	52.43	0.000
GGT(U/L)	14.57^{B} ±0.76	22.39^{A} ±1.88	17.25^{B} ±0.86	7.87	0.001

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P<0.05- Significant
P>0.05= not significant
Different superscript letters across the rows indicates that the mean at a treatment is significant from other.
Benin-MiIA
Ibadan-MoIA
Lagos-HiIA

Table 4.53 Liver function indices in unexposed participants in all study locations

Parameters	Unexposed (Controls)			F-value	Significant
	Benin-MiIA (n=42)	**Ibadan-MoIA (n=50)**	**Lagos-HiIA (n=40)**		
Total bilirubin (µmol/L)	9.08±1.16	8.58±0.47	9.11±1.26	0.11	0.898
Conjugated bilirubin (µmol/L)	5.32^{A} ±0.54	3.55^{B} ±0.30	5.47^{A} ±0.52	6.83	0.002
Unconjugated bilirubin(µmol/L)	5.71 ±0.93	4.87 ±0.36	5.73 ±0.89	0.55	0.578
Total protein (g/L)	68.59^{B} ±1.22	116.47^{A} ±1.37	70.26^{B} ±1.46	423.66	0.000
Albumin(g/L)	48.64^{A} ±0.60	39.48^{B} ±0.65	48.83^{A} ±0.68	72.81	0.000
Globulin (g/L)	19.95^{B} ±1.12	28.63^{A} ±0.37	21.45^{B}±1.19	29.97	0.000
ALT (U/L)	18.00^{A} ±1.24	7.86^{B} ±0.42	19.55^{A} ±0.99	57.23	0.000
AST (U/L)	23.54^{A} ±1.34	9.20^{C} ±0.42	16.59^{B} ±1.34	51.32	0.000
AST/ALT	1.39^{A} ±0.06	1.26^{A} ±0.06	0.91^{B} ±0.08	12.97	0.000
ALP (U/L)	38.74^{B} ±3.64	69.29^{A} ±5.17	39.16^{B} ±3.51	16.69	0.000
GGT(U/L)	10.36^{B} ±0.86	16.85^{A} ±1.33	11.01^{B} ±0.67	11.64	0.000

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P<0.05- Significant
P>0.05= not significant
Different superscript letters across the rows indicates that the mean at a treatment is significant from other.
Benin-MiIA
Ibadan-MoIA
Lagos-HiIA

Correlation of Toxic metals with liver function indices in all study participants

Association of toxic metals and Livers function parameters are shown in tables 4.54, 4.55 and 4.56.

In the occupationally exposed group, non-significant relationships were observed between toxic metals vs. TBil and toxic metals vs. UBil. Alb was significantly inversely correlated with Tl, while total protein was inversely correlated with Sn ($r = -0.16$, $p = 0.024$). Glob vs Sn was also inversely significantly related. Notably, ALT was inversely significantly correlated with AC, Cr, Ni, As, Mo, Cd, Sn, Sb, Hg and Pb; however, ALT association with V was positive and significant.

Remarkably also, inverse significant relationships were observed between ALP and Cr, Sn, Sb and Hg, as well as between GGT and Ni, Sb, Hg and Pb.

Among the environmentally exposed group, (Table 4.55), TBil correlated significantly with Sn ($r = 0.23$, $P = 0.017$) and inversely with Hg ($r = -0.22$, $P = 0.020$). ALP correlated positively and significantly with V, Cr, Ni, As and Sb, but inversely with Tl. GGT and Cr were positively and significantly.

Correlation of parameters among unexposed group (Table 4.56) revealed that TBil correlated positively and significantly with Sb ($r = 0.2$, $P < 0.05$), while AST inversely correlated with Ni and directly correlated with Tl, both associations were significantly. ALP and Sb association were direct and significant while ALP and Hg were inverse and significant. GGT was positively and significantly correlated with Mo, ($r = 0.25$, $P = 0.009$).

Correlation of Essential metals with liver function indices in all participants

Tables 4.57, 4.58 and 4.59 show the correlation relationships between essential metals and liver damage risk indices in the different groups of participants among the e-waste workers group (table 4.57), ALT correlated inversely and significantly with Mn, and positively with Zn. ALP and Mn as well as GGT and Cu were inversely and significantly related.

The environmentally exposed group (Table 4.58) showed that TBil vs Se; Alb vs Cr and AST vs Se were negatively and significantly correlated, while CBil vs Cr, UBil vs Cr, Co as well as ALP vs Mg and Mn were significantly positively correlated.

In the unexposed group, table 4.59 showed that Alb vs Mn, and Se; AST vs Mn; ALP vs Se; as well as GGT vs Cr presented with significant inverse associations. However, AST vs. Co; ALP vs Cr; and GGT vs Mg were positively significantly correlated.

Table 4.54 Correlation of toxic metals with liver function indices in E-waste workers

Correlated parameters		Total bilirubin (µmol/L)	CB (µmol/L)	Total protein (g/L)	Total protein (g/L)	Globulin (g/L	Globulin (g/L	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
^{27}Al (µmol/L)	*r* value	0.07	0.02	0.14	-0.08	-0.04	-0.05	-0.20**	-0.05	-0.09	-0.10
	p value	0.324	0.815	0.053	0.309	0.57	0.501	0.007	0.523	0.212	0.157
^{51}V (nmol/L)	*r* value	-0.003	0.02	-0.01	0.09	0.04	0.08	0.34**	0.11	0.13	0.13
	p value	0.967	0.809	0.915	0.215	0.62	0.251	0	0.142	0.081	0.086
^{52}Cr (nmol/L)	*r* value	0.13	0.13	0.05	-0.07	0.01	-0.05	-0.21**	-0.15*	-0.23**	-0.06
	p value	0.078	0.085	0.463	0.371	0.946	0.471	0.003	0.045	0.002	0.455
^{60}Ni (nmol/L)	*r* value	0.02	-0.01	0.01	0.08	0.01	0.06	-0.16*	-0.03	-0.14	-0.16*
	p value	0.832	0.895	0.85	0.264	0.906	0.452	0.031	0.644	0.063	0.031
^{75}As (umol/L)	*r* value	0.02	0.04	-0.01	0.02	0.12	-0.03	-0.21**	-0.02	-0.09	-0.01
	p value	0.758	0.626	0.937	0.792	0.117	0.668	0.004	0.777	0.244	0.894
^{100}Mo (nmol/L)	*r* value	-0.03	0.00	-0.04	-0.04	0.01	-0.09	-0.23**	-0.03	-0.07	-0.01
	p value	0.708	0.997	0.574	0.559	0.857	0.236	0.001	0.67	0.362	0.917
^{111}Cd (nmol/L)	*r* value	-0.002	0.02	0.01	-0.02	-0.09	0.00	-0.15*	-0.10	-0.01	0.04
	p value	0.982	0.799	0.948	0.824	0.200	0.994	0.039	0.159	0.918	0.634
^{118}Sn (nmol/L)	*r* value	0.04	0.08	-0.04	-0.16*	-0.05	-0.17*	-0.35**	-0.03	-0.15*	-0.02
	p value	0.619	0.288	0.626	0.024	0.518	0.017	0	0.645	0.038	0.747
^{121}Sb (nmol/L)	*r* value	-0.06	-0.10	-0.05	-0.06	-0.06	-0.04	-0.35**	-0.01	-0.19**	-0.21**
	p value	0.404	0.195	0.529	0.407	0.444	0.574	0	0.846	0.009	0.004
^{202}Hg (nmol/L)	*r* value	-0.03	-0.10	0.03	-0.06	-0.01	-0.06	-0.41**	-0.12	-0.36**	-0.15*
	p value	0.646	0.195	0.642	0.423	0.919	0.404	0	0.1	0	0.038
^{205}Tl (nmol/L)	*r* value	-0.07	-0.07	-0.03	-0.11	-.18*	0.00	-0.11	-0.09	-0.12	-0.02
	p value	0.344	0.32	0.7	0.146	0.012	0.979	0.134	0.217	0.1	0.809
^{208}Pb (µmol/L)	*r* value	-0.08	-0.10	-0.01	-0.05	0.08	-0.10	-0.25**	-0.01	-0.07	-0.15*
	p value	0.277	0.169	0.884	0.474	0.271	0.202	0.001	0.911	0.344	0.05

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.55 Correlation of toxic metals with liver function indices in environmentally exposed participants

Correlated parameters		Total bilirubin (µmol/L)	CB (µmol/L)	UC bilirubin (µmol/L)	Total protein (g/L)	Albumin (g/L	Globulin (g/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
^{27}Al (µmol/L)	*r* value	0.01	-0.04	-0.02	-0.07	-0.03	-0.05	-0.09	-0.13	0.10	0.05
	p value	0.919	0.653	0.805	0.49	0.734	0.581	0.333	0.169	0.274	0.586
^{51}V (nmol/L)	*r* value	0.03	0.07	0.06	0.04	-0.09	0.10	-0.09	-0.16	0.22*	0.07
	p value	0.737	0.449	0.51	0.645	0.362	0.299	0.343	0.088	0.021	0.484
^{52}Cr (nmol/L)	*r* value	-0.002	-0.03	0.09	-0.07	-0.08	-0.04	-0.03	-0.04	0.26**	.195*
	p value	0.981	0.733	0.33	0.453	0.439	0.716	0.747	0.681	0.007	0.041
^{60}Ni (nmol/L)	*r* value	-0.06	-0.12	0.03	0.05	0.04	0.03	-0.06	-0.09	0.27**	0.02
	p value	0.565	0.214	0.774	0.631	0.67	0.784	0.517	0.345	0.004	0.867
^{75}As (umol/L)	*r* value	-0.04	-0.08	0.06	0.06	0.03	0.05	-0.05	-0.06	0.19*	-0.07
	p value	0.715	0.405	0.536	0.538	0.78	0.611	0.61	0.522	0.046	0.485
^{100}Mo (nmol/L)	*r* value	-0.02	0.05	-0.04	0.09	-0.04	0.12	-0.08	-0.09	0.03	0.01
	p value	0.862	0.592	0.717	0.371	0.672	0.22	0.428	0.358	0.755	0.894
^{111}Cd (nmol/L)	*r* value	0.11	0.02	0.02	-0.02	0.01	-0.03	0.05	-0.01	-0.05	-0.06
	p value	0.263	0.832	0.8	0.801	0.929	0.744	0.616	0.962	0.581	0.502
^{118}Sn (nmol/L)	*r* value	.229*	0.15	0.12	-0.07	-0.08	-0.03	-0.01	-0.02	0.18	0.02
	p value	0.017	0.125	0.209	0.502	0.414	0.796	0.892	0.849	0.065	0.875
^{121}Sb (nmol/L)	*r* value	0.17	-0.01	-0.18	0.08	-0.08	0.14	-0.04	0.01	0.29**	0.03
	p value	0.095	0.943	0.081	0.427	0.401	0.171	0.727	0.891	0.003	0.788
^{202}Hg (nmol/L)	*r* value	-.22*	0.03	0.14	-0.04	-0.07	0.00	0.04	0.03	-0.10	-0.05
	p value	0.02	0.784	0.142	0.702	0.501	0.98	0.699	0.755	0.329	0.581
^{205}Tl (nmol/L)	*r* value	-0.02	-0.09	0.04	-0.06	0.10	-0.13	0.03	0.00	-0.35**	-0.11
	p value	0.815	0.327	0.709	0.52	0.282	0.182	0.79	0.968	0	0.238
^{208}Pb (µmol/L)	*r* value	-0.12	0.17	-0.04	-0.01	0.003	-0.01	-0.14	-0.06	0.04	0.12
	p value	0.225	0.072	0.66	0.913	0.973	0.888	0.161	0.558	0.65	0.223

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.56 Correlation of toxic metals with liver function indices in unexposed participants

Correlated parameters		Total bilirubin (µmol/L)	CB (µmol/L)	UC bilirubin (µmol/L)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
^{27}Al (µmol/L)	*r* value	0.10	-0.05	-0.04	-0.01	0.14	-0.10	0.20	0.09	0.10	-0.02
	p value	0.314	0.641	0.723	0.905	0.16	0.348	0.052	0.357	0.312	0.867
^{51}V (nmol/L)	*r* value	-0.05	0.06	-0.05	0.03	0.10	-0.02	0.03	-0.22	0.03	-0.16
	p value	0.692	0.641	0.702	0.816	0.411	0.85	0.782	0.06	0.803	0.179
^{52}Cr (nmol/L)	*r* value	-0.04	0.08	-0.02	0.04	-0.07	0.09	0.05	-0.15	0.09	-0.03
	p value	0.702	0.449	0.83	0.708	0.475	0.386	0.616	0.119	0.377	0.737
^{60}Ni (nmol/L)	*r* value	0.14	0.03	0.00	0.15	0.12	0.11	-0.02	-0.30**	-0.12	0.01
	p value	0.175	0.766	0.999	0.145	0.233	0.275	0.808	0.003	0.228	0.958
^{75}As (umol/L)	*r* value	0.14	0.01	0.08	-0.02	0.09	-0.07	0.06	-0.04	0.02	-0.09
	p value	0.169	0.944	0.417	0.862	0.391	0.483	0.536	0.702	0.808	0.351
^{100}Mo (nmol/L)	*r* value	0.14	0.03	0.17	0.05	0.01	0.06	-0.01	0.13	-0.07	0.25**
	p value	0.143	0.772	0.076	0.618	0.93	0.575	0.891	0.183	0.49	0.009
^{111}Cd (nmol/L)	*r* value	-0.1	0.01	-0.06	-0.10	-0.15	-0.04	-0.03	-0.07	-0.09	-0.03
	p value	0.319	0.943	0.543	0.302	0.129	0.698	0.79	0.461	0.383	0.742
^{118}Sn (nmol/L)	*r* value	-0.06	-0.05	-0.07	0.02	0.09	-0.02	-0.15	-0.18	0.04	0.10
	p value	0.541	0.611	0.476	0.831	0.395	0.822	0.143	0.073	0.681	0.337
^{121}Sb (nmol/L)	*r* value	0.20*	-0.08	-0.10	-0.10	0.002	-0.12	0.19	0.06	0.22*	0.03
	p value	0.045	0.436	0.318	0.336	0.985	0.241	0.065	0.536	0.032	0.774
^{202}Hg (nmol/L)	*r* value	-0.12	-0.10	-0.12	-0.06	-0.09	-0.02	0.04	0.10	-0.20*	0.04
	p value	0.251	0.304	0.232	0.56	0.393	0.825	0.69	0.304	0.046	0.673
^{205}Tl (nmol/L)	*r* value	-0.05	-0.02	-0.05	-0.06	0.04	-0.09	0.04	0.24*	0.07	0.11
	p value	0.632	0.841	0.641	0.593	0.725	0.395	0.72	0.021	0.491	0.308
^{208}Pb (µmol/L)	*r* value	-0.02	-0.05	-0.18	-0.07	-0.04	-0.07	-0.02	0.19	-0.17	0.14
	p value	0.822	0.641	0.076	0.465	0.685	0.511	0.859	0.051	0.081	0.171

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.57 Correlation of essential metals with liver function indices in e-waste workers

Correlated parameters		Total bilirubin (µmol/L)	Conjugated bilirubin (µmol/L)	UB (µmol/L)	Total protein (g/L)	Albumin (g/L	Globulin (g/L	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
^{24}Mg	*r* value	0.04	0.10	-0.03	-0.01	-0.01	0.00	-0.02	0.14	0.02	0.04
(mmol/L)	*p value*	0.68	0.319	0.731	0.96	0.922	0.988	0.873	0.163	0.884	0.696
^{55}Mn (nmol/L)	*r* value	0.05	0.07	-0.04	0.03	0.06	0.01	-0.27**	-0.02	-0.21**	-0.07
	p value	0.463	0.338	0.602	0.662	0.38	0.906	0	0.835	0.004	0.339
^{64}Zn (µmol/L)	*r* value	-0.07	-0.07	-0.05	-0.11	-0.12	-0.04	0.20*	0.14	0.01	-0.05
	p value	0.449	0.507	0.591	0.271	0.222	0.672	0.044	0.145	0.886	0.595
^{65}Cu (µmol/L)	*r* value	-0.11	-0.04	-0.14	0.00	0.06	-0.01	-0.11	-0.13	-0.06	-0.32**
	p value	0.285	0.66	0.17	0.991	0.582	0.909	0.266	0.21	0.529	0.001
^{78}Se (µmol/L)	*r* value	-0.05	-0.01	-0.08	0.09	-0.10	0.14	0.18	0.02	0.12	0.11
	p value	0.629	0.904	0.473	0.375	0.348	0.165	0.074	0.857	0.236	0.283
^{52}Cr (nmol/L)	*r* value	-0.04	-0.02	-0.04	0.14	0.01	0.13	-0.05	-0.05	0.11	-0.05
	p value	0.734	0.843	0.733	0.183	0.927	0.209	0.656	0.62	0.29	0.614
^{59}Co (nmol/L)	*r* value	-0.06	-0.11	0.01	0.00	0.11	-0.04	0.07	-0.06	0.17	0.15
	p value	0.549	0.306	0.913	0.976	0.295	0.677	0.506	0.604	0.102	0.171

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.58 Correlation of essential metals with liver function indices in environmentally exposed participants

Correlated parameters		Total bilirubin (µmol/L)	Conjugated bilirubin (µmol/L)	UB (µmol/L)	Total protein (g/L)	Albumin (g/L	Globulin (g/L	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
^{24}Mg (mmol/L)	*r* value	0.01	0.09	0.05	-0.03	-0.02	-0.02	0.12	0.11	0.35**	0.13
	p value	0.896	0.359	0.6	0.8	0.864	0.859	0.248	0.28	0	0.217
^{55}Mn (nmol/L)	*r* value	-0.04	-0.03	-0.01	0.09	-0.04	0.13	-0.02	-0.08	0.32**	0.15
	p value	0.668	0.729	0.931	0.34	0.645	0.189	0.825	0.431	0.001	0.111
^{64}Zn (µmol/L)	*r* value	-0.02	-0.19	-0.07	0.06	0.09	0.01	0.18	0.00	0.10	-0.02
	p value	0.845	0.066	0.473	0.57	0.398	0.902	0.068	0.981	0.325	0.825
^{65}Cu (µmol/L)	*r* value	-0.10	-0.12	0.07	-0.02	-0.05	0.01	0.00	0.00	0.17	0.01
	p value	0.333	0.206	0.486	0.851	0.589	0.912	0.976	0.967	0.081	0.928
^{78}Se (µmol/L)	*r* value	-.225*	-0.15	0.10	0.04	-0.03	0.06	-0.02	-.195*	0.02	0.02
	p value	0.02	0.131	0.326	0.667	0.755	0.512	0.878	0.044	0.848	0.863
^{52}Cr (nmol/L)	*r* value	0.11	.203*	.237*	-0.14	-.197*	-0.03	0.05	-0.02	-0.12	0.02
	p value	0.263	0.043	0.018	0.181	0.049	0.749	0.65	0.821	0.23	0.832
^{59}Co (nmol/L)	*r* value	-0.02	0.14	.199*	-0.04	-0.13	0.03	0.10	0.00	0.19	0.19
	p value	0.838	0.174	0.045	0.696	0.198	0.747	0.324	0.99	0.06	0.059

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.59 Correlation of essential metals with liver function indices in unexposed controls

Correlated parameters		TBil (µmol/L)	CBil (µmol/L)	UBil (µmol/L)	TProt (g/L)	Albumin (g/L)	Globulin (g/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
^{24}Mg (mmol/L)	*r* value	-0.02	0.01	-0.04	0.00	0.06	-0.04	-0.09	-0.12	0.13	0.27**
	p value	0.871	0.926	0.681	0.968	0.591	0.723	0.389	0.223	0.222	0.007
^{55}Mn (nmol/L)	*r* value	-0.04	-0.05	-0.03	-0.05	-0.20*	0.05	0.05	-0.24*	0.01	0.08
	p value	0.688	0.62	0.797	0.59	0.044	0.626	0.64	0.016	0.947	0.423
^{64}Zn (µmol/L)	*r* value	0.07	0.19	0.09	-0.01	-0.05	0.02	-0.03	0.01	0.19	0.16
	p value	0.501	0.053	0.357	0.95	0.607	0.828	0.738	0.904	0.061	0.116
^{65}Cu (µmol/L)	*r* value	-0.05	0.06	-0.02	-0.13	-0.14	-0.08	-0.14	0.05	-0.05	0.12
	p value	0.619	0.533	0.866	0.202	0.178	0.438	0.185	0.602	0.635	0.243
^{78}Se (µmol/L)	*r* value	-0.01	-0.08	-0.06	-0.19	-0.20*	-0.12	-0.10	0.05	-0.21*	0.17
	p value	0.923	0.452	0.531	0.058	0.049	0.246	0.349	0.632	0.035	0.088
^{52}Cr (nmol/L)	*r* value	0.13	-0.13	0.02	-0.14	0.04	-0.19	0.18	0.18	0.21*	-0.25*
	p value	0.207	0.211	0.834	0.186	0.727	0.069	0.086	0.092	0.047	0.015
^{59}Co (nmol/L)	*r* value	-0.09	0.07	0.09	0.04	0.15	-0.04	0.06	0.27**	-0.10	-0.14
	p value	0.399	0.47	0.388	0.734	0.145	0.676	0.578	0.007	0.332	0.164

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Selected cancer risk biomarkers: prostate – specific antigen and alpha fetoprotein in e-waste exposed and unexposed participants in the different study locations

Total prostate-specific antigen (tPSA) and alpha fetoprotein (AFP) were both significantly raised in e-waste workers compared with environmentally exposed and unexposed participants ($p = 0.050$ and 0.000) respectively (table 4.60). Free PSA (fPSA) did not vary significantly across the study participants. The values obtained for % fPSA were similar in the e-waste workers and environmental groups; both were however significantly lower compared with the unexposed population ($p=0.001$). When compared with a reference value of 25%, e-waste workers' % fPSA (31.87%) and environmentally exposed groups' %fPSA (34.04%) showed considerable closeness to 25% as against the 41.8% observed in the unexposed group.

Considered by location (Table 4.61), Benin e-waste workers with tPSA of 12.62± 6.00 ug/L) was higher (but not significantly different) compared with Lagos and Ibadan groups ($p=0.105$). There was no significant difference in % fPSA and fPSA levels of e-waste workers across the study locations. AFP in Lagos e-waste workers was significantly elevated compared with both Benin and Ibadan e-waste workers ($p<0.001$).

In the environmentally exposed participants, tPSA, fPSA and AFP levels were not varied in Ibadan and Lagos participants, but were both significantly higher than the Benin environmental group ($p < 0.05$). Percentage fPSA values did not vary significantly by location.

Among the unexposed population, tPSA and fPSA did not vary significantly by location ($p > 0.05$). Percentage fPSA levels were higher significantly in both Ibadan and Lagos unexposed groups compared with Benin group. AFP level in Lagos unexposed group was significantly higher than Ibadan, which was equally higher than Benin unexposed group, $p < 0.001$.

Correlation of toxic and essential metals with selected cancer risk biomarkers in e-waste exposed and unexposed participants

Tables 4.62, 4.63 and 4.64 show the correlations of toxic metals with cancer risk biomarkers in e-waste workers, environmental and unexposed groups respectively.

In the e-waste workers group (table 4. 62), AFP correlated weakly and significantly with Ni and As ($r < 0.16, 0.19$; $p = 0.027, 0.007$) respectively. Other correlation relationships were not significant.

It was observed in the environmentally exposed group (Table 4.63) that tPSA and Tl, AFP and Tl correlated inversely and significantly; while AFP correlated positively and significantly with Cr and As ($r = 0.21, 0.20$; $p = 0.029, 0.042$) respectively.

In the unexposed group (table 4.64), apart from AFP and Sn which recorded a weak positive and significant association ($r = 0.35$, $p = 0.000$), all toxic metals showed non-significant correlations with cancer risk biomarkers in the unexposed group.

Correlation of essential metals with selected cancer risk biomarkers in e-waste exposed and unexposed participants

In table 4.65, essential metals were correlated with cancer risk biomarkers among e-waste workers and it was observed that tPSA and Zn as well as AFP and Mg were significantly inversely correlated, while fPSA correlated positively and significantly with Zn.

Among the environmentally exposed participants (table 4.66) tPSA and Zn, Se, Mg and Mn were significantly positively correlated while an inverse significant correlation was observed between tPSA and Cr. In addition, fPSA vs Zn and Mn were positively significantly correlated, while AFP correlated significantly with Mn ($r = 0.25$, $p = 0.004$)

From table 4.67, unexposed group, it was shown that fPSA vs Mg as well as AFP vs Co produced inverse significant associations, while AFP and Mn was positively significantly correlated.

Table 4.60 Status of selected cancer risk biomarkers: prostate-specific antigen and alpha fetoprotein in e-waste exposed and unexposed participants

Biomarkers	Mean ± SEM			F value	P value
	E-waste workers (n=381)	Environmentally exposed (n=120)	Unexposed (n=131)		
tPSA (µg/L)	7.61^{A} ±1.93	3.51^{B} ±0.27	2.80^{B} ±0.24	2.87	0.050*
fPSA (µg/L)	2.51 ±1.06	1.19 ±0.11	1.17 ±0.13	1.39	0.249
% fPSA	31.87^{B} (vs. 25%) $^{\Omega}$	34.04^{B} (vs 25%) $^{\Omega}$	41.87^{A} (vs 25%) $^{\Omega}$	18.76	0.001
AFP (µg/L)	5.62^{A} ±0.32	3.34^{B} ±0.22	3.56^{B} ±0.20	22.89	0.000

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P<0.05- Significant
P>0.05= not significant
Ω= Reference value for the ultility of % fPSA
Different superscript letters across the rows indicates that the mean at a treatment is significant from other.

Table 4.61 Status of selected cancer risk biomarkers: prostate-specific antigen and alpha fetoprotein in e-waste exposed and unexposed participants compared by locations

Parameters	E-waste worker			*F* value	*P* value
	Benin-MiIA (n=83)	Ibadan-MoIA (n=120)	Lagos-HiIA (n=178)		
tPSA (µg/L)	12.62 ±6.00	3.63±0.38	4.03±0.58	2.29	0.105
fPSA (µg/L)	6.02 ±3.32	0.86 ±0.10	0.92 ±0.11	2.58	0.079
% fPSA	28.98 ±3.22	26.41± 2.58	40.81±9.26	1.81	0.167
AFP (µg/L)	3.35^{B} ±0.34	3.82^{B} ±0.37	8.86^{A} ±0.58	45.38	0.000
	Environmentally exposed				
	(n=30)	**(n=38)**	**(n=52)**		
tPSA (µg/L)	2.50^{B} ±0.76	3.83^{A} ±0.10	4.17^{A} ±0.29	3.62	0.029
fPSA (µg/L)	0.71^{B} ±0.22	1.33^{A} ±0.13	1.53^{A} ±0.20	5.19	0.007
% fPSA	31.24 ±3.34	34.90± 3.3	35.89±3.65	0.50	0.605
AFP (µg/L)	2.51^{B} ±0.23	3.66^{A} ±0.45	3.86^{A} ±0.41	3.69	0.027
	Unexposed participants				
	(n=42)	**(n=50)**	**(n=42)**		
tPSA (µg/L)	2.14 ±4.78	3.02±0.19	3.06±0.24	1.41	0.248
fPSA (µg/L)	0.96 ±0.43	1.27 ±0.13	1.22 ±0.14	0.49	0.617
% fPSA	30.05^{B} ±4.02	43.39^{A} ± 2.66	39.18^{A} ±3.45	4.18	0.017
AFP (µg/L)	2.27^{C} ±0.26	3.38^{B} ±0.39	4.48^{A} ±0.32	11.54	0.000

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P<0.05- Significant
P>0.05= not significant
Different superscript letters across the rows indicates that the mean at a treatment is significant from other
MiIA = Mild e-waste impact area
MoIA = Moderate e-waste impact area
HiIA = High e-waste impact area

Table 4.62 Correlation of toxic metals with selected cancer risk biomarkers in e-waste workers

Correlated parameters		tPSA (µg/L)	fPSA (µg/L)	AFP(µg/L)
^{27}Al (µmol/L)	*r* value	0.03	0.02	0.06
	p value	0.707	0.836	0.417
^{51}V (nmol/L)	*r* value	-0.05	-0.06	0.14
	p value	0.533	0.451	0.053
^{52}Cr (nmol/L)	*r* value	0.01	0.03	0.04
	p value	0.893	0.739	0.54
^{60}Ni (nmol/L)	*r* value	-0.01	0.00	0.16*
	p value	0.862	0.958	0.027
^{75}As (umol/L)	*r* value	0.00	0.02	0.19**
	p value	0.961	0.77	0.007
^{100}Mo (nmol/L)	*r* value	0.02	0.01	-0.01
	p value	0.812	0.912	0.883
^{111}Cd (nmol/L)	*r* value	-0.06	-0.07	-0.03
	p value	0.441	0.411	0.65
^{118}Sn (nmol/L)	*r* value	0.00	0.01	0.10
	p value	0.956	0.887	0.173
^{121}Sb (nmol/L)	*r* value	0.02	0.03	0.07
	p value	0.823	0.743	0.318
^{202}Hg (nmol/L)	*r* value	0.08	0.09	-0.08
	p value	0.312	0.242	0.268
^{205}Tl (nmol/L)	*r* value	0.01	0.00	-0.13
	p value	0.859	0.985	0.066
^{208}Pb (µmol/L)	*r* value	0.02	0.03	0.07
	p value	0.839	0.747	0.327

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.63 Correlation of toxic metals with selected cancer risk biomarkers in environmentally exposed participants

Correlated parameters		tPSA (µg/L)	fPSA (µg/L)	AFP(µg/L)
^{27}Al (µmol/L)	*r* value	-0.06	-0.02	0.07
	p value	0.501	0.8	0.494
^{51}V (nmol/L)	*r* value	0.11	0.17	0.18
	p value	0.24	0.085	0.063
^{52}Cr (nmol/L)	*r* value	0.14	0.12	0.21*
	p value	0.142	0.222	0.029
^{60}Ni (nmol/L)	*r* value	0.06	0.14	0.13
	p value	0.559	0.145	0.171
^{75}As (umol/L)	*r* value	0.08	0.15	0.20*
	p value	0.421	0.114	0.042
^{100}Mo (nmol/L)	*r* value	-0.09	-0.04	0.06
	p value	0.362	0.667	0.559
^{111}Cd (nmol/L)	*r* value	-0.08	-0.04	0.03
	p value	0.378	0.649	0.797
^{118}Sn (nmol/L)	*r* value	0.09	0.18	0.00
	p value	0.371	0.059	0.973
^{121}Sb (nmol/L)	*r* value	0.14	0.18	0.03
	p value	0.161	0.069	0.752
^{202}Hg (nmol/L)	*r* value	-0.02	-0.05	-0.09
	p value	0.806	0.593	0.383
^{205}Tl (nmol/L)	*r* value	-0.20*	-0.17	-0.19*
	p value	0.033	0.081	0.042
^{208}Pb (µmol/L)	*r* value	0.05	-0.03	-0.01
	p value	0.643	0.738	0.909

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.64 Correlation of toxic metals with selected cancer risk biomarkers in unexposed controls

Correlated parameters		tPSA (µg/L)	fPSA (µg/L)	AFP(µg/L)
^{27}Al (µmol/L)	*r* value	-0.03	-0.05	-0.03
	p value	0.808	0.607	0.787
^{51}V (nmol/L)	*r* value	0.12	0.16	0.07
	p value	0.304	0.185	0.578
^{52}Cr (nmol/L)	*r* value	0.06	0.03	0.05
	p value	0.558	0.74	0.616
^{60}Ni (nmol/L)	*r* value	0.03	0.01	0.13
	p value	0.75	0.929	0.205
^{75}As (umol/L)	*r* value	0.00	-0.10	0.10
	p value	0.974	0.318	0.303
^{100}Mo (nmol/L)	*r* value	0.00	0.06	-0.02
	p value	0.977	0.522	0.832
^{111}Cd (nmol/L)	*r* value	-0.06	-0.03	-0.05
	p value	0.579	0.8	0.599
^{118}Sn (nmol/L)	*r* value	0.09	0.03	0.35**
	p value	0.356	0.785	0.00
^{121}Sb (nmol/L)	*r* value	-0.04	-0.03	-0.04
	p value	0.727	0.771	0.663
^{202}Hg (nmol/L)	*r* value	-0.16	-0.18	-0.10
	p value	0.107	0.071	0.335
^{205}Tl (nmol/L)	*r* value	-0.13	-0.11	-0.10
	p value	0.203	0.308	0.342
^{208}Pb (µmol/L)	*r* value	-0.09	-0.07	-0.02
	p value	0.354	0.462	0.883

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.65 Correlation of essential metals with selected cancer risk biomarkers in e-waste workers

Correlated parameters		tPSA (µg/L)	fPSA (µg/L)	AFP(µg/L)
^{24}Mg (mmol/L)	*r* value	0.08	0.07	-0.21*
	p value	0.402	0.498	0.036
^{55}Mn (nmol/L)	*r* value	0.03	0.01	0.02
	p value	0.707	0.939	0.793
^{64}Zn (µmol/L)	*r* value	-0.24*	0.20*	-0.15
	p value	0.013	0.039	0.129
^{65}Cu (µmol/L)	*r* value	-0.09	-0.18	-0.07
	p value	0.392	0.079	0.498
^{78}Se (µmol/L)	*r* value	0.14	0.07	-0.01
	p value	0.191	0.475	0.894
^{52}Cr (nmol/L)	*r* value	-0.05	0.00	-0.17
	p value	0.634	0.973	0.105
^{59}Co (nmol/L)	*r* value	-0.09	0.05	0.07
	p value	0.408	0.659	0.502

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.66 Correlation of essential metals with selected cancer risk biomarkers in environmentally exposed participants

Correlated parameters		tPSA (µg/L)	fPSA (µg/L)	AFP(µg/L)
^{24}Mg (mmol/L)	*r* value	0.25*	0.06	0.13
	p value	0.013	0.528	0.184
^{55}Mn (nmol/L)	*r* value	0.29**	0.27**	0.25**
	p value	0.002	0.004	0.008
^{64}Zn (µmol/L)	*r* value	0.38**	0.33**	-0.08
	p value	0.000	0.001	0.422
^{65}Cu (µmol/L)	*r* value	0.11	0.15	-0.01
	p value	0.247	0.133	0.941
^{78}Se (µmol/L)	*r* value	0.21*	0.17	-0.01
	p value	0.027	0.078	0.961
^{52}Cr (nmol/L)	*r* value	-0.30**	-0.15	-0.02
	p value	0.003	0.137	0.88
^{59}Co (nmol/L)	*r* value	-0.01	-0.05	-0.16
	p value	0.935	0.618	0.11

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.67 Correlation of essential metals with selected cancer risk biomarkers in unexposed group

Correlated parameters		tPSA (µg/L)	fPSA (µg/L)	AFP(µg/L)
^{24}Mg (mmol/L)	*r* value	-0.18	-0.22*	-0.03
	p value	0.07	0.032	0.806
^{55}Mn (nmol/L)	*r* value	0.03	-0.01	0.21*
	p value	0.761	0.929	0.037
^{64}Zn (µmol/L)	*r* value	-0.07	-0.01	-0.04
	p value	0.517	0.931	0.7
^{65}Cu (µmol/L)	*r* value	-0.07	-0.02	-0.16
	p value	0.522	0.879	0.12
^{78}Se (µmol/L)	*r* value	0.17	0.17	0.10
	p value	0.096	0.09	0.324
^{52}Cr (nmol/L)	*r* value	-0.13	-0.16	-0.07
	p value	0.203	0.124	0.477
^{59}Co (nmol/L)	*r* value	-0.18	-0.16	-0.30**
	p value	0.082	0.123	0.003

**Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Levels of wild-type p53 and selected biomarkers of genotoxity in e-waste exposed and unexposed participants in all study locations

The levels of wild-type p53 and selected biomarkers of genotoxity in e-waste exposed and unexposed participants and in the different study locations are presented in tables 4.68 and 4.69. Unexposed population showed a significantly higher level of wild-type tumour suppressor protein (wt-p53) (0.73 ± 0.12 ng/ml) compared with levels observed in e-waste workers (0.45 ± 0.05 ng/mL), while the lowest level was observed in the environmentally exposed group (0.41 ± 0.04 ng/mL), (p= 0.011).

Although the levels of 8-Hydroxy-2'-deoxyguanosine (8 – OHdG) appears higher in e-waste workers than in environmentally exposed group and lowest in unexposed group (Tab. 4.68), there was no significant difference across the groups (p=0.222).

In Addition, levels of 8-Oxoguanine-DNA glycosylase (OGG_1), was highest in a significant proportion in environmentally exposed group and lowest in unexposed group. When compared, OGG_1 levels was not significantly varied between the group of e-waste workers and unexposed controls (p=0.042).

At the different study locations (Tab. 4. 69), wt-p53, 8-OHdG, and OGG_1 levels in e-waste workers and the environmentally exposed participants did not vary significantly by location (p≥ 0.240). It is noteworthy that evidence of damage (8-OHdG levels) and wt-p53 expression were comparatively moderately higher in Lagos e-waste workers than Benin and Ibadan, while OGG_1 level was comparatively lowest in Lagos, however, these differences were not significant (p>0.05). Similarly, 8-OHdG level was comparatively moderately higher in Lagos environmental group than Benin and Ibadan, while OGG_1 level was also comparatively lowest in Lagos. Wt-p53 levels in Benin and Lagos were similarly higher than Ibadan. In the unexposed group, 8-OHdG levels and wt-p53 expression were comparatively higher in Lagos e-waste workers than Benin and Ibadan, OGG_1 level was however significantly higher (p=0.026) in Benin unexposed group compared with Lagos and Ibadan where the levels did not vary significantly (p>0.05).

With respect to micronuclei frequency, minimum and maximum observation in e-waste exposed and unexposed participants, frequency of micronucleated polychromatic erythrocytes (MnPCE)/1000PCE in e-waste workers (22.70 ± 0.15) was significantly higher compared with the environmentally exposed participants (4.17 ± 0.28), which in turn was significantly higher than the lowest frequency (0.99 ± 0.76) observed in the

unexposed population, ($p < 0.001$). The maximum MnPCE/1000PCE observed in the participants' groups were: e-waste workers, 34; environmentally exposed, 7; and unexposed, 3; while the minimum MnPCE/1000 PCE were; e-waste workers 15, environmentally exposed 1, and unexposed, 0.

Table 4.68 Levels of wild-type p53 and selected biomarkers of genotoxicity in e-waste exposed and unexposed participants

Genotoxicity Biomarkers	All Participants				
	E-waste workers	Environmentally exposed	Unexposed	*F* value	*P* value
WT-p53 (ng/mL)	0.45^{B}±0.05	0.41^{B} ±0.04	0.73^{A} ±0.12	4.57	0.011
8-OHdG (pg/mL)	127.82±18.29	126.53±14.01	95.95±5.60	1.51	0.222
OGG_1 (ng/mL)	0.24^{B} ±0.03	0.35^{A} ±0.04	0.23^{B} ±0.05	3.20	0.042
MnPCE/1000PCE	22.70^{A} ± 0.15	4.17^{B} ± 0.28	0.99^{C} ± 0.76	620.80	0.000
Minimum MnPCE/1000PCE	15.00	1.00	0.00	-	-
Maximum MnPCE/1000PCE	34.00	7.00	3.00	-	-

Keys: Abbreviations and units as previously defined

P<0.001= highly significant

P<0.05- Significant

P>0.05= not significant

Different superscript letters across the rows indicates that the mean at a treatment is significant from other.

Table 4.69 Levels of wild-type p53 and selected genotoxicity biomarkers in e-waste exposed and unexposed participants compared by locations

Biomarkers	E-waste Workers			*F* value	*P* value
	Benin-MiIA	**Ibadan-MoIA**	**Lagos-HiIA**		
WT-p53 (ng/mL)	0.44 ±0.11	0.32±0.04	0.55±0.09	1.45	0.240
8-OHdG (pg/mL)	119.99 ±27.74	122.66± 37.28	141.22±32.83	0.14	0.872
***OGG$_1$** (ng/mL)	0.25±0.02	0.28±0.03	0.21 ±0.07	0.41	0.668
	Environmentally Exposed participants				
Biomarkers	**Benin-MiIA**	**Ibadan-MoIA**	**Lagos-HiIA**	***F* value**	***P* value**
wtP53 (ng/mL)	0.45 ±0.09	0.31±0.03	0.45±0.07	1.01	0.367
8-OHdG (pg/mL)	113.84 ±8.16	113.12± 23.27	132.01±35.06	0.19	0.824
***OGG$_1$** (ng/mL)	0.39±0.02	0.38±0.12	0.30 ±0.03	0.52	0.594
	Unexposed participants				
Biomarkers	**Benin-MiIA**	**Ibadan-MoIA**	**Lagos-HiIA**	***F* value**	***P* value**
wtP53 (ng/mL)	0.77 ±0.22	0.60±0.11	0.85±0.29	0.36	0.697
8-OHdG(pg/mL)	86.11 ±7.62	98.01± 10.58	102.08±10.20	0.72	0.490
OGG$_1$ (ng/mL)	0.41^{A}±0.12	0.20^{B} ±0.03	0.12^{B} ±0.06	3.79	0.026

Keys: Abbreviations and units as previously defined
P<0.001= highly significant
P<0.05- Significant
P>0.05= not significant
*Kruskal-Wallis Nonparametric test : OGG_1 (p = 0.000; X^2 =18.63) (E-waste workers)
*Kruskal-Wallis Non-parametric Analysis: OGG_1 (p = 0.003; X^2 =11.37) (Environmentally exposed)
MiIA = Mild e-waste impact area

MoIA = Moderate e-waste impact area

HiIA = High e-waste impact area

Correlation of essential metals with wild-type p53 and selected genotoxicity biomarkers in e-waste exposed and unexposed participants

Tables 4.70, 4.71 and 4.72 show the correlation of essential metals with genotoxicity biomarkers in e-waste workers, environmentally exposed and unexposed groups respectively.

Among the e-waste workers' group, significant inverse correlations were observed between wt-p53 and Mn, OGG_1 and Zn, OGG, and Se; while wt-p53 and Zn correlated positively and significantly. Notably, Zn levels complimentarily increased with wt-p53 levels in the e-waste workers group.

In the environmentally exposed group (Table 4.71), Mn and wt-p53 were significantly positively correlated, while 8 – OHdG was inversely positively correlated with Mn.

It was further observed among the unexposed group (Table 4.72) that 8 – OHdG vs Mn and Cr; as well as OGG, vs Cu and Co were positively significantly correlated, while wt-p53 negatively significantly correlated with Mn.

Tables 4.70, 4.71 and 4.72 present the correlation of MnPCE frequency with essential metals in e-waste workers, environmentally exposed and unexposed groups. Se was inversely significantly correlated with MnPCE in the e-waste workers group ($r = -0.4$, $p=0.043$). No significant correlation was detected among parameters in the environmentally exposed and the unexposed groups.

Table 4.70 Correlation of wild-type p53 and selected genotoxicity biomarkers with essential metals in e-waste workers

Correlated parameters		wtP53 (ng/mL)	8-OHdG (pg/mL)	OGG 1 (ng/mL)	MnPCE /1000PCE
^{24}Mg (mmol/L)	*r* value	0.02	-0.05	0.01	-0.09
	p value	0.832	0.623	0.96	0.632
^{55}Mn (nmol/L)	*r* value	-0.12**	0.02	0.27	-0.16
	p value	0.227	0.855	0.004	0.413
^{64}Zn (µmol/L)	*r* value	.020*	0.04	-0.20*	-0.30
	p value	0.04	0.682	0.038	0.113
^{65}Cu (µmol/L)	*r* value	0.08	0.03	-0.08	-0.26
	p value	0.444	0.76	0.445	0.171
^{78}Se (µmol/L)	*r* value	0.11	0.09	-0.31**	-0.37*
	p value	0.298	0.395	0.002	0.043
^{52}Cr (nmol/L)	*r* value	0.10	-0.11	-0.03	-0.03
	p value	0.35	0.284	0.795	0.895
^{59}Co (nmol/L)	*r* value	0.13	-0.05	0.20	0.18
	p value	0.207	0.634	0.058	0.33

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.71 Correlation of wild-type p53 and selected genotoxicity biomarkers with essential metals in environmentally exposed participants

Correlated parameters		wtP53 (ng/mL)	8-OHdG (pg/mL)	OGG_1 (ng/mL)	MnPCE /1000PCE
^{24}Mg (mmol/L)	*r* value	-0.03	-0.03	-0.08	0.24
	p value	0.797	0.77	0.414	0.211
^{55}Mn (nmol/L)	*r* value	0.05**	-0.17**	-0.25	0.26
	p value	0.666	0.079	0.009	0.164
^{64}Zn (µmol/L)	*r* value	-0.06	-0.07	-0.08	-0.06
	p value	0.586	0.507	0.412	0.747
^{65}Cu (µmol/L)	*r* value	-0.06	-0.04	-0.06	-0.12
	p value	0.575	0.68	0.564	0.521
^{78}Se (µmol/L)	*r* value	-0.21	-0.01	0.05	0.26
	p value	0.064	0.94	0.649	0.171
^{52}Cr (nmol/L)	*r* value	-0.04	0.12	0.15	0.01
	p value	0.699	0.256	0.126	0.999
^{59}Co (nmol/L)	*r* value	0.05	0.14	-0.05	-0.18
	p value	0.669	0.168	0.587	0.353

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.72 Correlation of wild-type p53 and selected genotoxicity biomarkers with essential metals in unexposed controls

Correlated parameters		Wtp53 (ng/mL)	8-OHdG (pg/mL)	OGG $_1$ (ng/mL)	MnPCE /1000PCE
^{24}Mg (mmol/L)	*r* value	0.12	-0.14	-0.05	0.24
	p value	0.256	0.202	0.658	0.211
^{55}Mn (nmol/L)	*r* value	-0.06**	0.10**	-0.17	0.03
	p value	0.573	0.375	0.091	0.858
^{64}Zn (µmol/L)	*r* value	0.06	-0.11	-0.11	-0.06
	p value	0.591	0.343	0.268	0.747
^{65}Cu (µmol/L)	*r* value	0.00	-0.09	0.20*	-0.12
	p value	1	0.421	0.044	0.521
^{78}Se (µmol/L)	*r* value	0.09	-0.02	-0.09	0.26
	p value	0.409	0.831	0.374	0.171
^{52}Cr (nmol/L)	*r* value	-0.05	0.23*	0.09	0.02
	p value	0.634	0.037	0.366	0.999
^{59}Co (nmol/L)	*r* value	0.04	-0.01	0.32**	-0.18
	p value	0.684	0.919	0.001	0.353

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Correlation of toxic metals with wild-type p53 and selected genotoxicity biomarkers in e-waste exposed and unexposed participants.

Among the e-waste workers group (table 4.73), OGG_1, correlated positively and significantly with Al, As, Mo and Hg, but inversely with V. Wt-p53 correlated inversely and significantly wit Ni, Mo, and Sn, while 8-OHdG correlated positively and significantly with As. Other correlations were not significant.

In the environmentally exposed participants (table 4.74), OGG_1, correlated directly and significantly with both Hg and Tl.

From Table 4.75, correlations among the unexposed group showed that wt-p53 correlated inversely and significantly with V, while OGG_1 correlated inversely and significantly with V and Ni but positively with Mo. In addition, 80HdG correlation positively and significantly with Sb and Tl ($r = 0.23, 0.25$; $p = 0.033, 0.024$) respectively.

Tables 4.73, 4.74 and 4.75 present the correlation of MnPCE frequency with toxic metals in e-waste workers, environmentally exposed and unexposed groups. Inverse and positive correlations were observed among the parameters but the associations were not statistically significant ($p > 0.05$).

4.73 Correlation of toxic metals with wild-type p53 and selected genotoxicity biomarkers in e-waste workers

Correlated parameters		Wt-p53 (ng/mL)	8-OHdG (pg/mL)	OGG_1 (ng/mL)	MnPCE /1000PCE
^{27}Al (µmol/L)	*r* value	-0.225	0.013	0.05	0.05
	p value	0.023	0.9	0.804	0.804
^{51}V (nmol/L)	*r* value	-.004	.096	-0.15	-0.15
	p value	0.967	0.351	0.438	0.438
^{52}Cr (nmol/L)	*r* value	-0.248	-0.044	0.07	0.07
	p value	0.011	0.666	0.70	0.70
^{60}Ni (nmol/L)	*r* value	-.227*	-.064	0.13	0.13
	p value	0.021	0.532	0.51	0.51
^{75}As (umol/L)	*r* value	-0.081	-.200**	0.11	0.11
	p value	0.416	0.05	0.559	0.559
^{100}Mo (nmol/L)	*r* value	-.268**	-0.205	0.09	0.09
	p value	0.006	0.044	0.646	0.646
^{111}Cd (nmol/L)	*r* value	-0.342	-0.143	-0.19	-0.19
	p value	0	0.163	0.305	0.305
^{118}Sn (nmol/L)	*r* value	-.226**	-0.05	0.03	0.03
	p value	0.022	0.628	0.868	0.868
^{121}Sb (nmol/L)	*r* value	.009	0.199	-0.21	-0.21
	p value	0.928	0.051	0.264	0.264
^{202}Hg (nmol/L)	*r* value	-.179	-.140	0.18	0.18
	p value	0.071	0.173	0.337	0.337
^{205}Tl (nmol/L)	*r* value	-0.092	0.017	0.10	0.10
	p value	0.354	0.866	0.601	0.601
^{208}Pb (µmol/L)	*r* value	.084	0.141	0.16	0.16
	p value	0.4	0.168	0.386	0.386

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4.74 Correlation of toxic metals with genotoxicity biomarkers in environmentally exposed group

Correlated parameters		Wt-p53 (ng/mL)	8-OHdG (pg/mL)	OGG_1 (ng/mL)	MnPCE /1000PCE
^{27}Al (µmol/L)	*r* value	0.14	-0.152	-0.309	-0.309
	p value	0.206	0.121	0.097	0.097
^{51}V (nmol/L)	*r* value	-.077	-.129	-0.01	-0.01
	p value	0.487	0.188	0.95	0.95
^{52}Cr (nmol/L)	*r* value	0.028	-0.161	-0.25	-0.25
	p value	0.804	0.1	0.18	0.18
^{60}Ni (nmol/L)	*r* value	-.024	-.099	0.09	0.09
	p value	0.829	0.315	0.63	0.63
^{75}As (umol/L)	*r* value	-.112	-0.087	-0.06	-0.06
	p value	0.313	0.38	0.76	0.76
^{100}Mo (nmol/L)	*r* value	-.048	-.049	-0.21	-0.21
	p value	0.667	0.62	0.26	0.26
^{111}Cd (nmol/L)	*r* value	-0.035	-.018	-0.11	-0.11
	p value	0.754	0.853	0.58	0.58
^{118}Sn (nmol/L)	*r* value	.011	-0.051	0.09	0.09
	p value	0.925	0.604	0.62	0.62
^{121}Sb (nmol/L)	*r* value	-.143	-0.229	-0.25	-0.25
	p value	0.198	0.021	0.18	0.18
^{202}Hg (nmol/L)	*r* value	-.116	.191	0.24	0.24
	p value	0.295	0.051	0.19	0.19
^{205}Tl (nmol/L)	*r* value	-0.058	.109	0.03	0.03
	p value	0.603	0.27	0.88	0.88
^{208}Pb (µmol/L)	*r* value	-0.055	-.011	0.04	0.04
	p value	0.619	0.909	0.85	0.85

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4.75 Correlation of toxic metals with genotoxicity biomarkers in unexposed controls

Correlated parameters		Wt-p53 (ng/mL)	8-OHdG (pg/mL)	OGG_1 (ng/mL)	MnPCE /1000PCE
^{27}Al (µmol/L)	*r* value	0.077	.030	0.07	0.07
	p value	0.462	0.787	0.728	0.728
^{51}V (nmol/L)	*r* value	-.237**	.08	-0.03	-0.03
	p value	0.045	0.53	0.859	0.859
^{52}Cr (nmol/L)	*r* value	-.056	0.149	-0.09	-0.09
	p value	0.593	0.175	0.637	0.637
^{60}Ni (nmol/L)	*r* value	.016	.066	0.037	0.037
	p value	0.879	0.553	0.847	0.847
^{75}As (umol/L)	*r* value	0.075	-.089	-0.09	-0.09
	p value	0.474	0.42	0.634	0.634
^{100}Mo (nmol/L)	*r* value	.121	-.061	-0.17	-0.17
	p value	0.25	0.584	0.357	0.357
^{111}Cd (nmol/L)	*r* value	-.068	0.04	-0.27	-0.27
	p value	0.517	0.718	0.152	0.152
^{118}Sn (nmol/L)	*r* value	-0.023	-0.027	0.07	0.07
	p value	0.829	0.81	0.699	0.699
^{121}Sb (nmol/L)	*r* value	0.037	.233**	-0.12	-0.12
	p value	0.728	0.033	0.517	0.517
^{202}Hg (nmol/L)	*r* value	0.01	-.109	-0.06	-0.06
	p value	0.926	0.324	0.77	0.77
^{205}Tl (nmol/L)	*r* value	-0.031	.247**	-0.11	-0.11
	p value	0.768	0.024	0.567	0.567
^{208}Pb (µmol/L)	*r* value	-0.008	-.028	-0.11	-0.11
	p value	0.939	0.803	0.56	0.56

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

CHAPTER 5

DISCUSSION

Waste electrical and electronic equipment (WEEE) have been reported to contain more than one thousand toxic chemicals (Puckett *et al.*, 2002). Sources of exposure to e-waste have been classified into three sectors, namely informal recycling, formal recycling, and exposure to hazardous e-waste compounds remaining in the environment (Grant *et al.*, 2013). Exposure through informal and formal recycling can be considered as occupational exposure to hazardous chemicals present in WEEE, and the informal recycling (crude management) adequately describes the mode of exposure of the occupationally exposed participants enlisted for this study. As a fallout of this crude and environmentally unsound methods, the environment in the high e-waste-impact areas is not spared from contamination by the WEEE-borne toxicants (Osibanjo and Nnoron, 2007; Nnorom *et al.*, 2009), a justification that necessitated the inclusion of environmentally exposed participants in the present study.

Baseline health indicators and the anthropometric indices of the participants' groups in this study indicated that the participants were apparently healthy individuals in agreement the purposive sampling techniques as well as the inclusion and exclusion criteria that were used. The demographic characteristics: age and sex frequency, and the anthropometric index (BMI) were considerably similar in all groups, ruling out their effects on the outcome of the data obtained. Furthermore, the demographic consideration of the states of origin revealed that easterners appear to be more involved with WEEE/EEE informal management/ reprocessing as the proportion of e-waste workers from eastern Nigerian states were highest in number (180 participants, representing 47.2%); while western Nigerian population of e-waste workers in the present study was 130 participants (representing 34.1%) and 74 participants (19.4%) from the South-South part were mainly from the Niger-Delta region.

Nutritional contribution to cancer prevention and risk of cancer development has been documented (Adejuwon *et al.*, 2014; Anetor, 2016). The Considerably similar nutritional status observed in the three groups of participants in this study is pertinent to the exclusion of confounding influence of carbohydrate, proteins, fats and oil; as well as micronutrients and food supplements in-take on data obtained in this study.

Alcohol and tobacco use as predisposing factors to some pathologic states and cancer risk has been documented (Anand *et al.*, 2011; Kushi *et al*., 2012). There was however a fairly similar pattern of alcohol and tobacco use in the e-waste exposed and unexposed groups of the present study.

The risk awareness indicators and exposure pattern analysis in this study revealed that 248 (64.09%) of e-waste workers, 94 (78.3%) of environmentally exposed participants (EEPs), and 102 (77.86%) of unexposed controls lacked basic awareness of health hazards associated with WEEE exposure. Among the 133 (34.91%) of e-waste workers with basic health risk awareness, 6 (1.57%) specified catarrh/cough; 18 (4.72%) specified electrocution; while 17 participants (4.46%) stated impaired vision; 6 (1.57%) stated hypertension; and the largest proportion, 334 participants (87.68%) indicated uncertain responses as per condition known to them to be associated with WEEE exposure. The implication of these observations is that this lack of basic awareness may be responsible for the workers' poor attitude to the use of personal protective equipment. Also, the non-inclusion of cancer as one of the conditions suspected or known to be associated with WEEE exposure shows a demonstrated ignorance about the association of e-waste-borne chemicals with pathologic states, of which cancer is prominent. This obversation appears worrisome and calls for serious occupational health education among Nigerians involved in this and similar vocations. Moreover, these observations (from the risk awareness indicators) agree with the high desire for the conduct of health risk (toxicological) study on e-waste exposure indicated by the participants of the present study; viz, 95.28% of e-waste workers; 100% of the environmentally exposed participants; and 100% of unexposed participants being desirous of the conduct of the study, as the survey ascertained.

The analysis of the methods of disposal of unwanted WEEE by e-waste workers as observed in this study raises some public health concerns. As seen, the largest proportion of unwanted WEEE (44.36%) is disposed of in local open refuse dumps; while another 22.31% is collected by commercial waste managers and disposed of unprocessed. An additional 11.02% of the unwanted WEEE is sold to waste scavengers and 22.31% is disposed of through undisclosed methods. Overall, all listed methods of disposal are not technologically driven, and as such are environmentally unsafe and unfriendly. This has been previously reported and the alarm of the potential public health hazard raised in China (Leung *et al*., 2008) and Nigeria (Osibanjo and Nnoron, 2007; Nnorom *et al*., 2009).

Assessing the nature of exposure/exposure pattern of participants in this study, it was observed that both occupational and environmental WEEE exposures were high for e-waste workers; environmental WEEE exposure was moderate for the environmentally exposed group, while for the unexposed group, environmental WEEE exposure was minimal and there was no indication for occupational exposure. A duration of exposure of ≥ 5 years and exposure frequency ≥ 6 hours/day; 6 days/week (indicating 9360 hours in any 5 year duration) was observed with both e-waste workers and the environmentally exposed group. Routes of exposure observed in e-waste workers entailed all body cavities, viz, eyes, oral cavity, nasal cavity, and the skin (dermal absorption). These observations were similar in EEPs, apart from the exposure through oral cavity which was significantly lower.

In addition, the proportion of e-waste workers that used personal protective device (PPE) such as apron, hand gloves and facemasks while working was barely 10.24% while non – PPE users constituted the majority (89.76%) of the studied population. Among the PPE users, 10.24% used aprons and scarcely used hand gloves and the rest of the workers neither used face masks nor nose masks in the course of performing daily work tasks.

Our observations in the present study suggest an occupational practice that enhances exposure to WEEE-borne toxic and potentially carcinogenic metals and chemicals through almost all body cavities, particularly due to the unsafe occupational lifestyle of e-waste workers which shows high level of primitiveness and near zero safety practices. The outlook portrayed by these data corroborates the observations of Igharo *et al*. (2014) in Benin, a single-location pilot work designed as a preliminary part of this study (appendix X).

Electronic waste exposure has been linked to risk of metal toxicity. In direct contact with e-waste, elevated body burden of heavy metals (Huo *et al*., 2007) and persistent toxic substances (Bi *et al*., 2007) in children and e-waste workers, respectively, at Guiyu, China (Leung *et al*., 2008) have been reported. E-waste toxic components have been reported to bio-accumulate in plants and sea foods from Nigerian water bodies (Nnorom, *et al.*, 2009), which suggests evidence of food chain transfer.

In the present study, the observed significantly raised blood levels (body burden) of key toxic metals in e-waste workers and environmentally exposed group compared with the unexposed (control) may be linked to the observed chronic exposure; and this burden may

have been further exacerbated by the low risk awareness and exposure pattern detected and earlier described.

The irregular or outright non-use of PPE (hand gloves in this instance) observed in this study would have significantly raised the ingestion of loads of metal-borne particles through the hand-to-mouth route of exposure among the e-waste workers. The comparatively elevated hand wash metal levels in the e-waste workers may substantiate this assertion.

Considered by location, the comparison of blood levels of toxic metals in all e-waste workers which showed that eight toxic metals (Al, V, Cr, Ni, As, Mo, Sn and Sb) were higher in Lagos e-waste workers compared with their Ibadan and Benin counterparts agrees with the report that very high volumes of e-waste are received and reprocessed in the Lagos metropolis compared with other locations in Nigeria. In the same vein, in the environmentally exposed participants, difference by location was also remarkable as Al, V, Ni, As, Mo, Cd, Sn, Sb and Pb levels were high in Lagos environmental group compared with Ibadan (with Cr level alone higher) and Benin (with higher Hg and Tl levels) participants respectively. In the unexposed group, Al, N, As, Sn and Sb levels in Lagos unexposed participants were significantly higher compared with Ibadan and Benin unexposed populations.

The volume of e-waste received and generated in Lagos is reportedly substantial, and comparatively, it is the largest e-waste high impart area in Nigeria. The significantly higher exposure burden in the Lagos e-waste exposed and unexposed participants observed in this study may be attributed to this fact.

Comparison of hand wash levels of toxic and essential metals in all e-waste workers in Benin, Lagos and Ibadan showed that there were no significant differences in the three locations, with the exception of Cu, which was significantly higher in Lagos e-waste workers compared with Benin and Ibadan workers. The hand wash levels of toxic and essential metals in all environmentally exposed participants showed that there was no statistically significant difference in the metal levels in the three study locations. The metals (Mg, Al, Zn, Cu, As, Se, Mo, Cd, Sn, Sb, Hg, Tl and Pb) were not significantly different in the unexposed populations of the different study groups.

Levels of Zn and Se in hand wash water of e-waste workers; environmental participants and unexposed group respectively, did not differ significantly, ruling out a possible contribution to the level of these genome protective elements from this source.

In the present study, the ingestion of metals via the hand-to-mouth route, amongst others, appear to contribute significantly to the high body burden of toxic metals in the e-waste group as evidenced in the high levels of metals in hand wash samples of both e-waste workers and the environmental group.

Overall, with the observed general upward trend in the blood and hand wash levels of toxic metals in the exposed populations of this study, it appears evident that there is a systemic build-up of toxic metals in the exposed populations and this may lead to multi-element metal accumulation in the body. The effects of this may be the establishment of synergistic metal toxicity as a predisposing factor to chronic, acute and sub-acute pathologic conditions associated with heavy metal toxicity, of which the risk of cancer development is prominent.

In the mechanisms of cancer development, the involvement of chemical toxicants and oxidative stress, as well as oxidative DNA damage have been documented (Elst, 2007; Valavanidis *et al.*, 2009 Terada, 2012; Anetor *et al.*, 2013). E-waste contains approximately 1,000 chemicals; including mercury, lead oxide, cadmium, and polyvinyl chloride, some of which are carcinogenic, and listed as restricted hazardous substances. Metal-induced oxidative damage is a known mechanism of carcinogenesis (Mudgal *et al*., 2010), and this may be a basis for the prediction of cancer risk in chronic occupational exposure in the Nigeria e-waste workers enrolled into this study.

The levels of toxic metals in human body fluids and tissues represent an important indicator of the health status. A growing amount of data provide evidence that toxic and carcinogenic metals are capable of interacting with nuclear proteins and DNA bases and cause oxidative deterioration of this biological macromolecule (Valko *et al.*, 2005), resulting in genetic mutation and molecular changes, which are often transmissible. Metal-mediated generation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis (Valko *et al.*, 2005). At the molecular level, heavy metals are capable of activating cells and triggering signaling pathways, induced by targeting a number of cellular regulatory proteins or signaling proteins participating in cell growth, apoptosis, cell cycle regulation, DNA repair mechanism, and cellular differentiation (Valko *et al*., 2005). The overall effects of this

action is the loss of regulatory growth mechanisms in cells, initiating uncontrollable cell proliferation and autonomous cell growth, which is the hall mark of carcinogenesis. The observed elevated body burden of toxic metals such as lead, chromium, cadmium, arsenic as well as mercury in both occupationally and environmentally exposed participants is corroborated by the previous observations made by Ademuyiwa, *et al.*, (2005) and Orisakwe, *et al.*, (2007) who reported similar pattern of higher concentrations of toxic chemicals in occupationally exposed subjects compared to the non-exposed subjects, but contrary to the observations of Ogunsola, *et al.*, (1994) and Mehdi, *et al.*, (2000) who observed that the non-occupationally exposed subjects had higher concentrations of some toxic metals compared to the occupationally exposed subjects in their study.

Nutritionally, some metals, such as copper, zinc, selenium and iron e.t.c, are essential to human health and play critical roles and specific functions, for example, in the effective functioning of enzyme systems catalyzing various biochemical reactions in vivo. However, these metals regarded as essential have the potential to turn toxic and become harmful at very high levels of exposure and when intake exceeds physiologic requirements, a reflection of a very basic tenet of toxicology, "the dose makes the poison", pioneered by Paracelsus (Borzelleca, 2000; Hu, 2002). Conversely, metals such as beryllium, Gold and Silver e.t.c, are regarded as xenobiotic, in that they serve no useful functions in human physiological state, even worst, as in the case of metals such as lead, mercury, cadmium, arsenic and hexavalent chromium, which are toxic even at low concentrations (Llobet *et al.*, 2003).

Cadmium, for instance, have been reported by several authors to be nephrotoxic, cardicotoxic and hepatoxic (Ercal *et al.*, 2001; Goyer *et al.*, 2004). One of the mechanisms involving cadmium induced renal damage includes the formation of cadmium-metallothionein complex that filters into the proximal tubules and eventually degrades in the kidney, leading to the accumulation of cadmium in the kidney (Ercal *et al.*, (2001), because the body lacks an effective excretory mechanism for cadmium excretion. The accumulated cadmium in turn impair kidney functions, resulting in increased renal excretion of calcium and low molecular weight proteins such as albumin and beta$_2$-microglobulin (Hu, 2002), establishing proteinuria in the exposed subjects. Adams *et al.*, (1979) observed that once proteinuria have been established, it persist and progresses even after a change in occupational exposure. Furthermore, cadmium interferes with bone

metabolism and calcium transport through the mechanism of molecular mimicry (Goyer *et al.,* 2004). The result of this is bone resorption and softness of bone tissue, making the bones prone to fracture and initiates the onset of pathologic conditions such as osteoarthritis, osteoporosis, osteomalacia and various bone disorders. Cadmium has a cumulative effect and long term storage in the body, with an average half-life of 17 to 30 years in the bone, a characteristic feature that further potentiates its ability to exert its toxic effects on target organs. Additionally, cadmium through its antagonistic effects on copper and iron in antioxidant enzymes such as superoxide dismutase, can mediate the generation of free radical species through the Fenton reaction, which can cause oxidative stress in cells and establish DNA damage (Valko *et al.,* 2005). Specific diseases have been linked to cadmium toxicity, e.g Itai-tai disease, first observed in Japan, was reported to be a disease of Cadmium toxicity.

It may be suggested therefore, that e-waste workers in this study may be faced with the risk of developing pathologic conditions associated with cadmium toxicity.

Mercury, which is also WEEE-borne, is known to be toxic through different mechanisms. All forms of mercury have toxic effects in a number of organs, especially in the kidneys (Zalups, 2000), and the central nervous system is particularly susceptible to mercury toxicity (Neustadt and Pieczenik, 2007). In the body, methylmercury is mainly, if not exclusively, bound to the sulfur atom of thiol ligands. It is rapidly absorbed and distributed to target organs and it is metabolized to inorganic mercury prior to elimination via feces, but the rate of conversion is slow. In the liver and kidney, it is rapidly converted to inorganic mercury and stored as divalent mercury cation. This, together with the fact that the human body has no effective mechanism of excreting mercury, therefore means that mercury continues to accumulate in the body throughout life (Neustadt and Pieczenik, 2007).

Mercury is also known to be involved in metal-induced oxidative stress (Valko *et al.*, 2005). Copper is a cofactor for super oxide dismutase (SOD), unavailability of copper causes decrease SOD activity. Involvement of mercury (a component of e-waste) in oxidative stress may be due to its ability to displace copper from its binding site. It has been suggested that mercury increases intracellular copper by increasing influx from extracellular medium which could particularly increase oxidative stress in cells (Boveris *et al*., 1972). The higher body burden of mercury in e-waste workers observed in this study compared to the unexposed group may be a reflection of occupational exposure among e-

waste workers.

Copper alone, for instance is seriously linked with carcinogenesis (Valko *et al.*, 2005). Essential metal ions play important roles in biological enzyme systems, and without their presence in trace or ultratrace amounts many biochemical reactions involving enzyme systems requiring this co-factors will not take place. However, they become toxic to cells when their concentrations surpass optimal levels. Catalytic copper, because of its mobilization and redox activity, is believed to play a central role in the formation of reactive oxygen species (ROS), such as singlet oxygen (O^{2-}*) and hydroxyl (*OH) radicals, that bind avidly to DNA, and cause damage to DNA by breaking the DNA strands and producing DNA adducts or modifying the nuclear bases leading to carcinogenesis.

Mercury-mediated oxidative stress and through the mechanism of metal-metal interaction (Hg-Cu), increase in blood Hg may portend deleterious biochemical and chronic pathologic changes in the Nigerian e-waste exposed population studied.

Arsenic is a complex metal that forms a variety of compounds, either inorganic or organic. Organic arsenic compounds like arsenobetaine, arsenocholine, arsenosugars and tetramethylarsonium salts contain carbon and are mainly found in sea-living organisms, however occasionally they can be found in species living on land. Inorganic forms of arsenic, such as Arsenite and Arsenate are generally known to be more toxic.

Arsenic is a known carcinogen and has been well-documented in a number of studies (Waalkes *et al.*, 2004a; Valko *et al.*, 2005). Exposure to arsenic has been linked with a risk of developing tumours of the lung, skin, liver, bladder, and kidney (Waalkes *et al.*, 2004a). Chronic arsenic exposure also causes a markedly elevated risk for developing a number of cancers, most notably skin cancer, cancers of the liver, blood vessels (angiosarcoma), lung, bladder, and possibly the kidney and colon (Hu, 2002).

This elevated blood arsenic level may pose some risks to the e-waste populations with respect to cancer aetiology.

In addition, as part of its mechanism of action and toxicity, arsenic interferes with mitochondrial adenosine triphosphate (ATP) production (Ercal *et al.*, 2001; Goyer *et al.*, 2004). Once absorbed into the body, it is rapidly redistributed into body tissues (Goyer *et al.*, 2004). After its distribution, arsenic penetrates into cells and inhibits cellular energy production through mechanisms dependent on the element's oxidation state. Arsenate has the same oxidation state as inorganic phosphate (p^{5+}) and shares many chemical properties

with it and can substitute for P^{5+} in glycolysis (Ercal *et al*., 2001; Goyer *et al*., 2004). In this pathway, arsenic-poisoned enzymes produce 1-arseno-3-phosphoglycerate rather than the usual 1, 3-bisphosphoglycerate. 1-Arseno-3-phosphoglycerate is unstable and hydrolyzes spontaneously. Glycolysis proceeds so that ATP continues to be produced. However, the arsenic-oxygen bond is significantly weaker than the phosphorus-oxygen bond, and 1-arseno-3-phosphoglycerate hydrolysis yields dramatically less energy. Arsenate can also inhibit the conversion of pyruvate (by poisoning the enzyme complex, pyruvate dehydrogenase) into acetyl-CoA, blocking the Krebs cycle and therefore resulting in further loss of ATP (Ercal *et al*., 2001; Goyer *et al*., 2004). This implies a possibility of disordered metabolism and energy utilization with WEEE-borne arsenic exposure.

Lead is a major component of e-waste and is often used by WEEE workers in the course of soldering electrical circuit systems together. Lead is a cumulative toxicant that affects multiple organ systems in the body, including the neurological, haematological, gastrointestinal, cardiovascular, immune and renal systems (Dietert *et al*., 2004). This study demonstrates a highly significant difference between blood lead levels in the occupationally exposed participants compared with the unexposed group. This observation may portend risks of lead related pathophysiological disorders among e-waste workers. Lead is known to induce renal tumours, reduce cognitive development, and increase blood pressure and cardiovascular diseases risk for adults (Harmanescu *et al*., 2011). At the gastrointestinal level, the absorption and distribution of lead is dependent on the nutritional status of an individual. In the presence of micronutrients such as calcium and iron, the absorption of lead will be markedly decreased due to competitive binding to transport mechanism (Hennig *et al*., 2007; Hernandez-Avila *et al*., 2003). Lead has critical effects on heme and hemoglobin synthesis and changes the morphology and survival of red blood cells (Flora *et al*., 2008). Activities of aminolevulinic acid dehydratase (ALAD), a cytosolic sulfhydryl enzyme in this pathway is most sensitive to lead insult. Lead also inhibits the activities of ferrochelatase in the last step of heme synthesis. The overall effects are the development of anaemia and decreased haematocrit. ALAD level increases and accumulates in the blood and further potentiates the toxic effects of lead (Ercal *et al*., 2001).

In addition, the observed levels of hexavalent chromium in exposed groups may pose some risk for metal induced carcinogenesis. Trivalent (Cr III) and hexavalent (Cr VI)

chromium are thought to be the most biologically significant compounds of chromium. Cr III is an essential dietary mineral in low doses while Chromium Cr VI is toxic even at low concentration (Stern, 1982). Chromium carcinogenicity was first identified over a century ago and Cr VI compounds were among the earliest chemicals to be classified as carcinogens (Stern, 1982; Bright *et al*., 1997). Through the mechanisms of biological reductants such as glutathione, ascorbate and lipoic acid; and generation of free radicals involving the Fenton reaction and activation of transcription factors, chromium can cause various degrees of DNA damage and exerts its genotoxic effects (Valko *et al.,* 2005), this alone may exercerbate cancer risk among the e-waste exposed group.

The decreased levels of inorganic micronutrients and antioxidant metals (Mg, Zn, Se, Cr and Co) observed in the e-waste groups in the present study may indicate that their role in cancer prevention may not be optimal in the exposed participants. These micronutrients have genome-protective roles (Anetor, 2016). Also, in a study by Bai *et al*. (2016) the essential metals Zinc, Selenium, and Strontium has been reported to protect against chromosome damage caused by polycyclic aromatic hydrocarbons exposure. Their study showed evidence that Zn, Se, and Sr played protective roles in reducing chromosome damage, and that these effects were modified by polycyclic aromatic hydrocarbon exposure levels. These findings added potential evidence for the preventive effects of Zn, Se, and Sr against carcinogenesis in human subjects.

By implication, optimal levels of essential metals in the e-waste exposed participants would have offered some protection from cancer risk, however, the findings of the present study indicate a depression of these genome-protective metals in the exposed populations, a condition which could favour pathologic states that may predispose to cancer development.

Since the toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic or essential metals, studies addressing the chemical interactions between trace elements are gaining more attention (Bebe and Panemangalore, 1996; López-Alonso *et al*., 2004). These interactions probably indicate that mineral balance in the body is regulated by important homeostatic mechanisms in which toxic elements compete with the essential metals, even at low levels of metal exposure. The knowledge of these correlations may be essential to understand the kinetic interactions of metals and their implications in the trace metal metabolism (Chimielnicka *et al*., 1983; López-Alonso *et al*., 2004).

In this study, interaction of some toxic and essential metals in the e-waste exposed population showed an antagonism that may biochemically reduce the nutrigenomic (genome protective) potential and biological essentiality of the essential metals. As shown in the results, among the e-waste workers, significant inverse correlation relationships were observed between Zn and Al; Zn and Ni; Zn and Cr; Zn and As; Zn and Mo; and between Hg and Zn. Similarly, Se vs. Hg; Se vs. Pb and Mo vs. Cu were significant inverse associations. The inverse correlations suggest biochemical antagonism between essential metals and toxic metals. The existence of this is unfavorable as the biological nutritional effects or role of essential metals may be compromised in the e-waste exposed populations.

In contrast, interactions of toxic and essentials metals in the unexposed group showed that Mg was inversely and significantly correlated with V, Cr, and Mn; but was significantly positively correlated with most toxic metals such as As, Hg, Tl and Pb. Also, Cr was significantly positively correlated with the toxic metals: As, Hg, and Tl, in addition to Co with Mo also showed weak positive and significant correlation.

Cd, Pb, Hg, and Al are toxic metals that may interact metabolically with nutritionally essential metals. Iron deficiency increases absorption of Cd, Pb, and Al. Pb interacts with Ca in the nervous system to impair cognitive development. Cd and Al interact with Ca in the skeletal system to produce osteodystrophies while Pb replaces Zn on heme enzymes and Cd replaces Zn on metallothionein (López-Alonso *et al.*, 2004). Selenium protects from mercury and methylmercury toxicity. Al interacts with Ca in bone and kidneys, resulting in aluminum osteodystrophy. Ca deficiency along with low dietary Mg may contribute to aluminum-induced degenerative nervous disease (Goyer, 1997).

In this study, though Co vs. V, Cr vs. Ni as well as Co vs. Cr, Ni and Sb showed inverse significant correlations, as earlier outlined, most interactions among toxic and essential metals in the unexposed group by comparison, appear more complementary and protective.

Vitamins A, C and E are knows to have antioxidant properties; and antioxidant vitamins from supplements and natural sources have been enlisted to be useful in cancer prevention (Palace, 1999; IOM, 2000; Bjelakovic *et al.*, 2008; Bjartell *et al.*, 2012).

In the present study, Comparison of levels of antioxidant vitamins shows that levels of vitamin A in environmentally exposed participants was higher and significantly different compared with e-waste workers and the unexposed groups, and the post hoc test showed that the difference observed in vitamin A level between e-waste workers and unexposed

group was not significant. Vitamin C level was lower but not significantly different in e-waste workers compared with unexposed groups, whereas, its level in environmentally exposed participants was significantly higher compared with other two groups. Vitamin E level varied significantly amongst the three participants groups, viz, unexposed > e-waste workers > environmentally exposed. This suggests a considerable low levels of both vitamins E and C in the e-waste groups, and by implication, depressed antioxidant status from the contributions of these antioxidant vitamins.

Oxidative processes have been implicated in both initiation and promotion of cancer. Likewise, animal studies have revealed effects of the antioxidant nutrients on both processes. Vitamin E exhibits antioxidant properties by acting as a lipid-soluble free radical scavenger in cell membranes. Thus, vitamin E may be involved in both initiation and promotion stages. Among the other potentially anti-carcinogenic effects of vitamin E are its ability to inhibit formation of the carcinogenic chemical nitrosamine from nitrites in some foods, and its ability to promote immune system function (van Poppel and van den Berg, 1997; IOM, 2000).

Vitamin C (ascorbic acid) also acts as an antioxidant, and through its ability to scavenge free radicals, it may have protective effects on biopolymers such as DNA. Like vitamin E, vitamin C may be protective for both initiation and promotion of carcinogenesis. Also, like vitamin E, it is thought to prevent formation of nitrosamine (by converting nitrite to nitrous oxide) and to influence immune system function (van Poppel and van den Berg, 1997; IOM, 2000). Vitamin C has also been reported to affect liver enzymes responsible for detoxification and transformation of carcinogens, Coenzyme Q10 (also termed ubiquinone and ubidecarenone) is an endogenously synthesized chemical (called a quinone) that is also obtained through food intake (van Poppel and van den Berg, 1997; IOM, 2000). In addition to its role as an electron carrier in the mitochondrial electron transport chain, it can also function as a soluble antioxidant (Overvad *et al.*, 1999). Also, its role as an antioxidant (possibly in conjunction with vitamin E) and as a free radical scavenger, and other roles have been proposed for Q10 (Overvad *et al.*, 1999). These include acting as a nonspecific stimulant for the immune system, (Hattersley,1996) and playing a role in membrane stabilization, prostaglandin metabolism, inhibition of intracellular phospholipases, and stabilization of calcium-dependent slow channels. Decreased levels of Q10 have been noted with aging and in such disorders as congestive heart failure, cardiomyopathy, cancer, hypertension, Parkinson's disease, spontaneous abortion, male

infertility, chronic hemodialysis, and periodontal disease (Overvad *et al.*, 1999). Vitamins C and E and coenzyme Q10 have been implicated in a variety of potential anti-carcinogenic processes.

Epidemiological studies have suggested an inverse correlation between cancer development and dietary consumption of vitamin A. Pharmacological concentrations of vitamin A decreased the incidence of chemically induced experimental tumours (van Poppel and van den Berg, 1997; IOM, 2000). Natural and synthetic retinoids have been demonstrated to inhibit the growth and the development of different types of tumours, including skin, breast, oral cavity, lung, hepatic, gastrointestinal, prostatic, and bladder cancers (Niles, 2000; Altucci and Gronemeyer 2001; Arrieta *et al.*, 2010; Bryan *et al.*, 2011; Siddikuzzaman *et al.*, 2011). Moreover, the addition of retinoic acid or synthetic retinoids to human cancer cell lines or human tumour xenografts in nude mice result in growth arrest, apoptosis, or differentiation (Niles, 2000). It is noteworthy that natural retinoids act as chemotherapeutic agents for the treatment of acute promyelocytic leukemia (APL). Moreover, vitamin A reduced the induction of carcinoma of the stomach by polycyclic hydrocarbons (Shibata *et al.*, 2014) and vitamin A-deficient rats were more susceptible to induction of colon tumours by aflatoxin B than normal animals (Rogers *et al.*, 1973). Genomic functions of the retinoids are mediated via their nuclear DNA-binding receptors, RARs, and RXRs, which regulate gene transcription through recruitment of corepressors and coactivators. Natural and synthetic retinoids have been used as potential chemotherapeutic or chemopreventive agents because of their differentiation, antiproliferative, proapoptotic, and antioxidant effects (Clarke *et al.*, 2004; Ribeiro *et al.*, 2014).

Evidence that supplements of these substances have the ability to prevent some types of cancers has been reported, (Li, 1993). In the e-waste exposed groups, the observed use of some vitamin-rich supplements (listed in table 4.) may have exerted a modifying effects on the vitamin reserve, as vitamins A and C levels were considerably similar in the participants and across the locations.

Correlation of toxic metals and antioxidant vitamins in e-waste workers showed that vitamin A correlated significantly and positively with V and Cd. Vitamin C vs. Cd was significant while vitamin E vs. Sn and Hg were significantly positively correlated. Inverse significant correlation was observed between Vitamin E and Tl. Relationships observed between toxic metals and antioxidant vitamins in the environmental exposed participants

showed that vitamin A correlated positively and significantly with Ni, As, Mo, Cd, Sn and Tl. Conversely, vitamin C was inversely correlated with As. In the unexposed group, interaction of toxic metals with antioxidant vitamins revealed that V, Cr, Ni and Sb were positively and significantly correlated with vitamin A while Hg inversely correlated with vitamin A level.

From the observations in this study interaction of essential metals with antioxidant vitamins appear biochemically complementary as correlation of essential metals and antioxidant vitamin levels in e-waste workers showed that vitamin A in e-waste workers correlated positively and significantly with Zn and Se. A similar observation was seen between vitamin C and Co; and Vitamin E and Mn, but inverse significant correlation was observed between Vitamin E and Se. In the environmentally exposed group, inverse significant correlation was observed between Vitamin A and Mn; Cu; as well as between Vitamin C and Mn. The unexposed group showed significant positive relations between Vitamin A and Zn, Vitamin C and Mn, and an inverse significant association between Vitamin A and Co.

Status of vitamin D and calcium metabolism in our study participant is salient owing to their roles in signal transduction in relation to the stages of cancer development. Vitamin D and calcium are metabolically interrelated and highly correlated dietary factors. Experimental studies have shown their anti-carcinogenic effects due to their participation in regulating cell proliferation, differentiation, and apoptosis in normal and malignant cells (Mantel *et al*., 2000; Welsh, 2004).

In the present study, the level of total calcium (tCa) in e-waste workers was higher but not significantly different from levels in unexposed group, but both were significantly different compared with the environmental group. Ionized calcium (iCa), which is the physiologically active calcium, was higher significantly in the two e-waste exposed groups compared with the unexposed population. Notably, 25(OH) vitamin D level was significantly higher in e-waste workers compared with the environmental and the unexposed populations.

The biologically active form of vitamin D (25(OH) 2D) exerts its effects mainly through binding to nuclear vitamin D receptor (VDR) and further binding to specific DNA sequences, namely vitamin D response elements (Colston *et al*., 1989; Saez *et al*., 1993; Mantel *et al*., 2000; Welsh, 2004). Through this genomic pathway, 1, 25(OH) 2 D

modulates expression of specific genes in a tissue-specific manner. Experimental studies have shown that 1, 25(OH)2 D can inhibit cellular proliferation, induce differentiation and apoptosis, and inhibit angiogenesis in normal and malignant breast cells (Colston *et al*., 1989; Saez *et al*., 1993; Mantel *et al*., 2000; Welsh, 2004). In rodent models, high intake of vitamin D has been shown to suppress high-fat diet-induced epithelial hyper-proliferation and tumorigenesis of the mammary gland (Jacobson *et al*., 1989; Xue *et al*., 1999). In addition, a non-genomic pathway of 1, 25(OH) 2 D has been shown, in which 1, 25(OH) 2 D interacts largely with membrane VDR to exert its biological effects by altering intracellular calcium channels (Mehta and Mehta, 2002). However, the involvement of this pathway in carcinogenesis and cancer prevention is not clearly defined. Two distinct pathways of vitamin D biosynthesis and action have been proposed in mammary carcinogenesis, one involving 1, 25 (OH) 2D and the other involving 25 (OH) D (Welsh *et al*., 2003; Welsh, 2004). In the endocrine pathway, circulating 1, 25 (OH) 2D reaches the breast tissue to exert its anti-carcinogenic effect. The other pathway is the autocrine/paracrine pathway, in which circulating 25(OH) D reaches the breast tissue and is further catalyzed to 1, 25(OH) 2D by the 1-a-hydroxylase in the breasts. The locally produced 1, 25 (OH) 2D may bind to VDR and therefore regulate cell proliferation, differentiation, and apoptosis (Welsh *et al*., 2003).

On the other hand, the importance of calcium in carcinogenesis derives from its participation in regulating cell proliferation, differentiation, and apoptosis (Whitefield *et al*., 1979; Mathiasen *et al*, 2002; Sergeev, 2004). Increasing the concentration of calcium decreased cell proliferation and induced differentiation of mammary cells in experimental studies (McGath and Soule, 1984; Xue *et al*., 1999;; Russo and Russo, 2001). In rodent models, high intake of calcium has been shown to suppress high-fat diet-induced epithelial hyperproliferation of the mammary gland and mammary tumorigenesis induced by 7, 12-dimethylbenzo (a) anthracene (Jacobson *et al*., 1989; Xue, 1999). Evidence is available that calcium at least partially exerts its anti-carcinogenic effects through vitamin D. For example, calcium is one of the key mediators of apoptosis induced by vitamin D compounds in breast cancer cells (Mathiasen *et al*., 2002).

The mechanisms of calcium and vitamin D action in control of neoplastic cell growth are fundamental to their established role the carcinogenesis process. For one, because Ca^{2+}/ the extracellular calcium –sensing receptor (CaR); and VDR/1,25$(OH)_2D_3$ signaling interact positively in growth control of cancer cells, it can be expected that an adequate vitamin D

status is required to achieve the benefits of high calcium intake and *vice versa*. In fact, there is evidence from epidemiological as well as interventional studies that optimal reduction of cancer risk can be achieved only by a high intake of both calcium and vitamin D. For example, in a study on the effect of vitamin D and calcium supplementation on recurrence of colorectal adenomas, Grau *et al.* (2006) found that calcium supplementation was only effective in patients if their serum 25(OH) D values were normal. Conversely, high 25(OH) D levels were associated with a reduced risk of adenoma recurrence only among those on calcium supplements. Holt *et al.* (2006) gave adenomatous polyp patients high doses of supplemental calcium in combination with vitamin D. After six months of treatment they observed a significant reduction in the rate of polyp formation that was accompanied by an increase in expression of apoptotic markers. Similar results were reported recently by Fedirko *et al.* (2009). Cho *et al.* (2004) concluded from an analysis of pooled primary data from 10 cohort studies with a follow-up of more than half a million individuals for 6-16 years, that optimal risk reduction for colorectal cancer necessitates high intake levels of both vitamin D and calcium.

The CaR is an essential part of an intricate network of calcium signaling pathways that control normal and cancer cell growth (Rodland, 2004; Tfelt-Hansen and Brown, 2005; Capiod *et al.*, 2007; Roderick and Cook, 2004). G proteins, also known as guanine nucleotide-binding proteins, are a family of proteins that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to its interior (Hurowitz, 2000). Depending on cell-specific coupling to appropriate G-proteins, activation of the CaR by elevated extracellular Ca^{2+} reduces the rate of cellular proliferation as in human colon carcinoma (Kallay *et al*., 2003; Chakrabarty *et al*., 2005) or ovarian surface epithelial cells (Bilderback *et al*., 2002), but may also stimulate cell growth as in malignant Leydig cells (Tfelt-Hansen *et al.*, 2005) and protect from apoptosis, for example, in prostate cancer cells (Lin *et al*., 1998).

From the above, the optimal levels of tCa, iCa and Vitamin D in the e-waste exposed population may offer some forms of defense against the severity of existing and undiagnosed cancers (if any exists) or reduce the risk of cancer development in the e-waste exposed participants in this study. However, the observed antagonistic effects of toxic metals on calcium ions and vitamin D may unfavourably modulate these expected biological beneficial roles in the e-waste exposed populations in this study.

The associations of toxic and essential metals with total and ionized calcium and vitamin D in all participants using Pearson correlation analysis are given in in the results. Among the e-waste workers, Sn and Hg correlated inversely and significantly with 25(OH) vitamin D. Also, significant inverse correlation was observed between tCa and Hg; iCa and Al, and between iCa and Pb. In addition, significant positive correlations were detected between 25(OH) vitamin D and Zn, Co, and Mg as well as between tCa and Zn, while inverse significant relationship was observed between 25(OH) vitamin D and Mn, iCa and Cu. In the environmentally exposed participants, significant inverse relationships were observed between 25(OH) vitamin D and V, Cr, and between tCa and Tl, iCa and Hg, as well as iCa and Tl. 25(OH) vitamin D was positively significantly correlated with Mo, Ca, Hg, Tl, while iCa and V were similarly correlated. Additionally, Mg and Mn were inversely significantly correlated. Mg and Mn also positively and significantly correlated with tCa, while iCa correlating positively with Mn.

Metal-induced oxidative damage is a known mechanism of carcinogenesis (Mudgal *et al*., 2010). In this study, the enzymatic antioxidant biomarkers considered were superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and gamma-glutamyltransferase (GGT), while the non-enzymatic biomarkers of oxidative stress were malondialdehyde (MDA), total glutathione (tGSH), uric acid (UA), albumin (ALB), total bilirubin (TB) and conjugated bilirubin (CB).

Oxidative stress indicators as reflected by the enzymatic antioxidant biomarkers (CAT, SOD and GPx) were significantly reduced in e-waste workers compared with the unexposed human population. The differences in the activities of CAT, SOD, and GPx between the e-waste groups were highly statistically significant. GR activity was reduced in exposed group compared with unexposed group, the difference was however statistically insignificant.

Taken separately, the activity of SOD was markedly and significantly decreased in e-waste workers compared with the environmental; and with the unexposed controls. CAT in unexposed controls was significantly higher compared with the environmental group, and its activity this group was significantly higher compared with e-waste workers. Likewise, GPx activity was significantly higher in unexposed controls than the environmental group, which was equally significantly higher than occupationally exposed group. GR was higher significantly in environmental group, but was not significantly different between e-waste

workers and the unexposed. GGT was significantly raised in e-waste workers compared with unexposed and the environmental groups, with unexposed recording the lowest activity.

To further assess the influence of antioxidant enzymes on the metal-induced oxidative stress status in the three study populations, essential and toxic metals were separately correlated with enzymatic antioxidants. Among the e-waste exposed group (tables 4.39 and 4.42), positive significant correlations were observed between SOD and Zn, SOD and Mn, CAT and Mn, and between GPx and Mn, while significant inverse correlations were observed between SOD and Se, CAT and Se, GPx and Cu. SOD and CAT correlated inversely and significantly with V. SOD and CAT correlated inversely and significantly with V; while SOD alone correlated positively and significantly with Cr, Ni, AS, Sn, and Hg. Positive and significant correlations were observed between CAT and Cr, Ni, Hg; between GPx and Cr, Ni, As, Sn, Sb, Hg, and Pb, as well as between GR and V, Mo and Sn. Among the environmentally exposed group (Tables 4.40 and 4.43), inverse significant correlations were observed between SOD and Zn; CAT and Zn; CAT and Mg and between CAT and Mn. Additionally, significant positive correlations were observed between GPx and Mn; GR and Co, Mg and Mn. Notably, SOD correlated inversely and significantly with the toxic metals: Al, V, Ni, As, Mo, Cd, Sn, and Sb. In the same vein, CAT and AL, V, Cr, Ni, As, Sn, Sb and Pb recorded significant inverse correlations. GPx and Ni, As, Mo, Cd, Hg, and Tl showed significant inverse correlations. However, GPx and Cr, as well as GGT and V showed positive and significant associations. Correlation of parameters among the unexposed group (Table 4.41), revealed that essential metals complementarily associated with most antioxidant enzymes in that SOD versus Cu, Cr, and Co; as well as CAT versus Cu and Co; in addition to GR versus Zn and Mn associations were positive and significant. However, CAT versus Mg and Mn; as well as GR vs. Co recorded inverse significant correlations.

Associations in respect to toxic metals and enzymatic antioxidants were largely antagonistic in that SOD inversely and significantly correlated with Al, V, Cr, Ni, As and Sb. Similarly, CAT versus Al, V, Ni and As; GPx vs Al and GPx vs As correlated inversely and significantly. However, significant positive correlations were observed between SOD and Mo, Hg, Tl and Pb; as well as between CAT and Hg, Tl; GR versus Mo, GGT versus Al, Ni and AS.

In the mechanism of metal-induced oxidative stress, metals are classified into two: redox-active and redox-inactive metals. Redox active metals include; Fe, Cu, Cr, Mn and other transition metals. Redox inactive metals include; Pb, Cd, Hg, As. Both of these groups deplete cells' major antioxidants by different mechanisms. Redox-active metals are able to undergo fenton-like reaction to exacerbate oxidative stress while redox-inactive metals deplete antioxidants especially thiol-containing antioxidants and enzymes (Ercal *et al.*, 2001).

By the exposure pattern observed in this study, it can be inferred that the e-waste workers and the environmental group were frequently exposed to both redox-active and redox-inactive metals reported to be present in WEEE, it is therefore believed that oxidative stress can occur through any of the above mechanisms. Redox-inactive metals can directly interfere with the activity of glutathione reductase (GR) due to the presence of disulphide bond in its structure. Other antioxidant enzymes which remove peroxides superoxide radicals including glutathione peroxidase (GPx), Catalase (CAT) and superoxide dismutase (SOD) are also potential target of metals such as lead, which is a component of WEEE and also often used by WEEE workers for soldering. GR catalyzes the reduction of glutathione disulphide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell (Meister, 1988; Ercal *et al.*, 2001).

Glutathione plays a key role in maintaining proper function and preventing oxidative stress in human cells. It can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H_2O_2), a dangerously reactive species within the cell. GR does not play a central role as an antioxidant enzyme per say, it only catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). However, another antioxidant; alpha lipoic acid (ALA) has been shown to recycle GSH in the same way GR catalyzes the reduction of GSSG. Dihydroxyl lipoic acid (DHAL) has been shown to be more potent than ALA. DHAL was reported to recycle oxidized ascorbate, glutathione, coenzyme Q and vitamin E to their active forms as antioxidants (Queiroz, 1998). DHAL is responsible for the ability of ALA to increase intracellular GSH level; and of importance is its ability to regenerate GSH (Lynch, 1999; Chelikani *et al.*, 2004). This function of ALA might have preserved GR activity since they appear to work synergistically to prevent oxidative stress; hence the GR

level in these exposed participants was not significantly depleted unlike other antioxidant enzymes activities observed in this study.

Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al*., 2004). It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Superoxide dismutases are important in antioxidant defense in nearly all cells exposed to oxygen, they are proteins co-factored with copper and zinc, or manganese, iron, or nickel. In biological systems, the main reactions of superoxides are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (approximately 10^5 $M^{-1}s^{-1}$ at pH 7). SOD is necessary because superoxide reacts with sensitive and critical cellular targets to cause pathological condition. Reduced SOD activity is observed in oxidative damage (Heinrich *et al*., 2006).

GPx is a selenium-dependent enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GPx requires selenium for its activity, but in lead toxicity; lead may form a complex with selenium, resulting in decreases in GPx activity decreases (Whanger, 1992). This may account for the reduced activity of GPx in the e-waste workers studied. Treatment of human fibroblasts with sodium arsenite slightly decreases GPx activity and significantly decreased CAT activity; as reported by lee *et al.*, 2011. Notably, the low Se levels in the e-waste exposed groups of this study may account for the low GPx activity observed. Also, lead exposure may account for the significant reduction of SOD activities in exposed groups. Animal studies demonstrated reduced erythrocyte SOD activity in lead-exposed rats (Adler *et al*., 1993; Poderoso *et al*., 1996).

WEEE-borne Cd as a carcinogen and an inducer of oxidative damage (Mudgal *et al*., 2010) has been reported to affect the activities of antioxidant enzymes especially CAT and SOD. Administration of Cd-acetate to liver and kidney in-vitro caused inhibition of SOD activity, and also lead to increased lipid peroxidation was found in these tissues (Chelikani *et al*., 2004).

Copper is a cofactor for SOD, unavailability of copper causes decrease in SOD activity. Involvement of mercury (a component of e-waste) in oxidative stress may be due to its

ability to displace copper from its binding site. It has been suggested that mercury increases intracellular copper only by increasing influx from extracellular medium which could particularly increase oxidative stress (Boveris *et al*., 197; Chelikani *et al*., 2004).

In addition, quite recently, gamma-glutamyltransferase (GGT) has been documented as a predictive biomarker of cellular antioxidant inadequacy and disease risk (Koenig and Seneff, 2015). Gamma-glutamyltransferase (GGT) is a well-established serum marker for alcohol-related liver disease. However, GGT's predictive utility applies well beyond liver disease: elevated GGT is linked to increased risk to a multitude of diseases and conditions, including cardiovascular disease, diabetes, metabolic syndrome (MetS), and all-cause mortality (Koenig and Seneff, 2015).

A comprehensive review by Whitfield in 2001 described GGT in its traditional role as a marker of liver dysfunction, bile duct conditions, and alcohol consumption. Some generalized or summary of medical and scientific literature still describe GGT in those terms. However, Whitfield had already extended that description to include elevated GGT in association with risk of coronary heart disease, type-II diabetes (T2D), and stroke (Whitfield, 2001). Although gamma-glutamyl compounds include antioxidants, inflammatory molecules, drug metabolites, and neuroreactive compounds (Wickham, 2011), the major function of GGT is enabling metabolism of glutathione and glutathionylated xenobiotics. However, elevated GGT levels, as noted by Whitfield and others, contribute to prooxidant activity, particularly in the presence of iron or copper (Corti *et al*., 2009; Milnerowics *et al.,* 2014). When GGT levels are elevated, damage to red blood cell membranes can occur causing the release of these potentially toxic transition metals, which can further result in chain, prooxidant reactions (Aberkane *et al*., 2002). Increased levels of prooxidation can lead to downstream cell, tissue, and DNA damage caused by oxidative and nitrosative stress and the generation of deleterious reactive oxygen species or nitric oxide (ROS or NO)(Stefano and Kream, 2015). This combination of factors is observed with increasing frequency in many chronic diseases. Other investigators have added many newly identified GGT-related diseases and conditions to a rapidly growing list that recently was modified by Sreeram *et al*. (2015) to even include GGT as a marker for oxidative stress in periodontal disease.

The antioxidant deficiency observed in the e-waste groups seem to be well justifiably flagged off by the elevated GGT levels also observed in the same participants.This

prooxidant activity exerted by GGT may further exacerbate the establishment of oxidative stress in the exposed groups of this study.

Taken together, the lower levels of key enzymatic antioxidants coupled with the raised level of GGT in the e-waste exposed groups may portend higher risk of cancer development should the trend continue.

Considering non-enzymatic biomarkers of oxidative stress, this study demonstrates significantly raised non enzymatic oxidative stress indicators (MDA and UA) in exposed groups compared with the unexposed population. Metal toxicity exacerbates oxidative stress which causes different pathological processes including lipid peroxidation. Malondialdehyde (MDA) is a product of lipid peroxidation and provides a means of accessing the extent of lipid peroxidation. Many studies have confirmed lipid peroxidation secondary to heavy metal toxicity. In the work of Yiin *et al.*, (1999), lipid peroxidation was accessed by MDA analysis; and it was observed that lipid peroxidation increases in lead toxicity. MDA concentration was found to increase in liver and kidney after cadmium exposure (Shaikh *et al*., 1994).

The significantly raised MDA concentration in the e-waste exposed group in this study directly reflects increased lipid peroxidation and oxidative damage, a mechanism that contributes to carcinogenesis.

Serum uric acid (UA) has been shown to have antioxidant activity. Ames *et al*., (1981), demonstrated that urate reduces the oxo-haem oxidant formed by peroxidation reaction with haemoglobin, and protects erythrocytes from peroxidative damage which leads to lysis. Also, Urate was reported to be as effective an antioxidant as ascorbate in scavenging hydroxyl radicals (Daun *et al*., 2002; Amaro *et al*., 2007).

Furthermore, this study demonstrates that UA level in the exposed participants was significantly higher than levels obtained in unexposed participants, despite the evidence of oxidative stress in the exposed groups. Acting as systemic antioxidant, UA level is expected to have been lowered in oxidative stress due to depletion as an antioxidant (Chartterjea and Shinde, 2012). This elevation of UA in the exposed participants enrolled in this study may be due to the effect of reactive oxygen species on DNA or may be attributed to toxic metal- induced renal dysfunction. Uric acid, being an end product of

purine (Adenosine and Guanosine) metabolism; adenosine and guanosine are released from DNA in their triphosphate forms (Chartterjea and Shinde, 2012).

In-vitro study in plants exposed to various environmental stresses such as salinity has been shown to enhance DNA degradation. Oxidative attack on DNA results in deoxyribose oxidation, strand breakage, removal of nucleotides, variety of modification in the organic bases of the nucleotides and DNA-protein crosslink (Liu *et al*., 2000). The increased release of adenosine and guanosine phosphates from damaged DNA may be responsible for the elevated UA level in the exposed participants of this study.

In another light, uric acid is excreted by the kidney; thus renal dysfunction may also cause elevated UA. Cadmium is a known nephrotoxicant and heavy metal toxicity has been shown to cause renal damage (Daun *et al*., 2002; Amaro *et al*., 2007).

ALB has been shown to exert specific antioxidant function due to its multiple ligand-binding capacities and free-radical trapping properties, both closely related to its structure (Oehl and Stauber, 2007). In this study, the mean ALB concentration in the exposed groups was less than, but not significantly different from unexposed participants. This observed difference may be attributed to the depletion of ALB due to its antioxidant properties. Also, since metal toxicity affects liver function; the slight decrease may be due to reduced production by the liver.

Bilirubin has been reported to perform antioxidant function. Bilirubin when bound to human albumin and at concentration present in normal human plasma protects albumin-bond linoleic acid from peroxyl radical-induced oxidation in-vitro (Stoker *et al*., 1987). Stoker *et al* (1987) also showed that one mole of albumin-bound bilirubin can scavenge two moles of peroxyl radicals and that small amount of plasma bilirubin is sufficient to prevent oxidation of albumin-bound fatty acids as well as of the protein itself.

In this study, the mean values for Total bilirubin (TB) was significantly higher in e-waste workers compared with the other two groups, and Conjugated bilirubin (CB) level was slightly higher (not significantly different) in e-waste workers compared with other groups. Increase in both TB and CB in the exposed participants may be as a result of haemolytic processes which may be secondary to erythrocyte membrane lipid peroxidation. It has been reported that some metals such as gold, mercury, copper and lead (also part of WEEE) cause lipid peroxidation (Ribarov and Benov, 1981). Increased release of haemoglobin

from haemolysis of erythrocytes explains the higher values of the two forms of bilirubin in the exposed participants.

Inferentially, the present study suggests that there is evidence of increased oxidative stress and lipid peroxidation in the exposed groups (particularly in occupational group) studied and this may predispose to or tacitly predict high risk of cancer development. The mechanism for this predisposition may be complex and multifactorial but related to the effects of oxidative damage and lipid radicals on biological systems. Lipid radicals which are products of lipid peroxidation can diffuse through membranes, thus modifying the structure and function of the membrane and resulting in the loss of cell integrity and homeostasis. In addition, lipid peroxides may interact with cellular DNA and cause the formation of DNA-MDA adducts (Chaudhary *et al*., 1994). Proteins are also easily attacked by reactive oxygen species (ROS) directly or indirectly through lipid peroxidation. Protein radicals can be rapidly transferred to other sites within the protein infrastructure, this can result in further modification of enzyme activity (stimulation or inhibition) (Bellomo *et al*., 1983). Changes to receptor proteins and gap junction proteins may also modify signal transfer in cells. In selective cases alteration of protein structure may allow the target protein to be further attacked by proteinases (Davies, 1986). Thus, protein oxidative damage can result in the modifications in structure, enzyme activity, and signaling pathways. Activation of transcription factors is an important signaling pathway for the regulation of gene transcription by ROS (Storz and Polla, 1996). Transcription factors regulate the transcription of genes involved in the development, growth, and aging of cells. Nuclear factor kappa B and AP-1, by direct oxidation and phosphorylation, are two transcription factors that are modulated by oxidative stress. ROS can cause activation of AP- 1 as well as new synthesis of AP-1 (Kerr *et al*., 1992). By activating AP-1, ROS can stimulate cell proliferation and the persistent proliferation due to chronic oxidative stress would make the rate of cell production to exceed the rate of apoptosis. This is a hallmark of cancer development and malignancy, resulting in subsequent tumour.

Furthermore, ROS or their byproduct of lipid peroxidation, MDA, through direct reaction with DNA forms oxidative DNA adducts. The presence of oxidative DNA adducts generated by chemical carcinogens suggest an interactive role of ROS in initiation of cancer. ROS, therefore, can have multiple effects in the initiation stage of carcinogenesis by mediating carcinogen activation, causing DNA damage, and interfering with the repair

of the DNA damage (Klaunig *et al*., 1998). Cancer results when defective DNA molecules are incorporated into the DNA of developing cells during cell proliferation.
In summary, the present study demonstrates that the oxidative stress arising from increased lipid peroxidation and lowered antioxidant defenses observed in the studied population of e-waste workers is associated with chronic occupational exposure to e-waste chemicals and may be a predictive mechanism for chemical carcinogenesis in Nigerian e-waste workers.

Liver disease has been reported as a risk factor for cancer (Wogan, 2000, ACS 2012). The liver plays an important role in sex hormone metabolism by making binding proteins that carry the hormones in the blood. These binding proteins affect the hormones' activity. Men with severe liver disease such as cirrhosis have relatively low levels of androgens and higher estrogen levels. They have a higher rate of benign male breast growth (gynecomastia) and also have an increased risk of developing breast cancer (ACS, 2012).

The role of liver in xenobiotics metabolism is worthy of note. Enzymes located in the endoplasmic reticulum of liver cells protect against an accumulation of lipid-soluble exogenous and endogenous compounds by converting them to water-soluble metabolites which can be easily excreted by the kidney. But only few drugs possess suitable groups which are conjugated with glucuronic or sulfuric acid. Most compounds have to be hydroxylated first. For this purpose the endoplasmic reticulum has at its disposal an enzymatic system, completely unspecific, which activates molecular oxygen for the oxidation of lipid-soluble compounds. This takes place at a cytochrome, P450, which is available abundantly in the endoplasmic membranes abundantly. The oxidation rate, however, is extremely slow and dependent on the chemical configuration of the compound and on genetically determined differences of the protein moiety of the enzyme. Since more specific enzymes located in liver cells metabolize most of the endogenous compounds, such as steroids, at a much higher rate, the slow hydroxylation by the unspecific endoplasmic enzyme does not play an important role in their conversion to inactive compounds. Because of the lack of specificity of this enzyme, drugs compete for the binding sites if high concentrations of several drugs are present in the liver cells. A slower metabolism of these drugs with less affinity is the result. Metabolism of drugs by this enzyme system leads sometimes to more active and toxic compounds which produce liver injury, e.g. carbon tetrachloride. Drug metabolism is inhibited only in severe hepatitis, and exceptionally in liver cirrhosis (Remmer, 1970)

In the present study, risk assessment indices of liver damage in e-waste exposed and unexposed participants in different study locations were determined and indicated in tables 4.50, 4.51, 4.52 and 4.53. Among participants' groups, TBil level was highest in e-waste workers and lowest in environmentally exposed group. CBil and UBil were both highest in e-waste workers. However, CBil was lowest in unexposed group while UBil was lowest in environmentally exposed group. Total protein was highest in environmentally exposed than in unexposed and e-waste workers while Alb and Glob were alternately higher in unexposed group and environmentally exposed group respectively but lowest in e-waste workers and unexposed group, with exception of CBil, total protein, Alb and Glob, other parameters showed high significant difference in results recorded for the three different populations studied. Notably, ALT, AST, ALP and GGT showed decrease in values from highest to lowest across e-waste, environmentally exposed and unexposed groups respectively, except in AST/ALT ratio where unexposed had the highest value but lowest in environmentally exposed population. This indicates that the enzymatic biomarkers of liver damage were significantly elevated in the e-waste groups than the unexposed group.

Some metals are known to bio-accumulate in the liver and interfere with the integrity of liver function by their hepatotoxic effects (Dufour *et al.*, 2000; Sthanadar, 2013). Assessment of the associations of toxic metals and liver function parameters in the study participants is highlighted in this study.

In the occupationally exposed group, non-significant relationships were observed between toxic metals and TBil; and between toxic metals and UBil. However, Alb was significantly inversely correlated with Tl, while total protein was inversely correlated with Sn. Glob vs. Sn was also inversely significantly related. Notably, ALT was inversely significantly correlated with Cr, Ni, As, Mo, Cd, Sn, Sb, Hg and Pb; however, ALT association with V was positive and significant. Remarkably also, inverse significantly relationships were observed between ALP and Cr, Sn, Sb and Hg, as well as between GGT and Ni, Sb, Hg and Pb. Among the environmentally exposed group, TBil correlated significantly with Sn and inversely with Hg. ALP correlated positively and significant with V, Cr, Ni, As and Sb, but inversely with Tl. GGT and Cr were positively and significantly correlated. Correlation of parameters among unexposed group revealed that TBil correlated positively and significantly with Sb, while AST inversely correlated with Ni and directly correlated with Tl, both associations were significant. ALP and Sb association was direct and

significant while ALP and Hg was inverse and significant. GGT was positively and significantly correlated with Mo.

In summary, the trend towards hepatocellular damage as reflected by elevated liver enzymes in the e-waste group may be partly attributed to the direct effects of metals as such as membrane damage to hepatocytes by the accumulated metals in various hepatic tissues.

The liver's strategic location between intestinal tract and the rest of the body facilitates its maintenance of metabolic homeostasis in the body. The liver extracts ingested nutrients, vitamins, metals, drugs, environmental toxicants, and waste products of bacteria from the blood for catabolism, storage, and/or excretion into bile (Dufour *et al*., 2000).

Formation of bile is essential for uptake of lipid nutrients from the small intestine, protection of the small intestine from oxidative insults, and excretion of endogenous and xenobiotic compounds. Cholestasis is either a decrease in the volume of bile formed or an impaired secretion of specific solutes into bile, which results in elevated serum levels of bile salts and bilirubin. Hepatocytes have a rich supply of phase I enzymes that often convert xenobiotics to reactive electrophilic metabolites and of phase II enzymes that add a polar group to a molecule and thereby enhance its removal from the body. The balance between phase I and phase II reactions determines whether a reactive metabolite will initiate liver cell injury or be safely detoxified (Dufour *et al*., 2000).

Liver biomarker could correctly identify clinically advanced or minimal liver disease. Enzyme markers may serve as reliable indicators of the status of an organ. The liver enzymes like ALT, AST, ALP and GGT have very low serum concentration because they are intracellular enzymes. The ALT is found primarily in the liver with trace amount in skeletal muscles and heart. It is found in the cytoplasm and mitochondria where it is involved in protein metabolism. It leaks out of damaged tissues in hepatocellular necrosis (Dufour *et al*., 2000). It is known that AST is also a mitochondrial and cytoplasmic enzyme that catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. It also leaks out into the serum during hepatocellular necrosis (Amacher, 2002; Ozer *et al*., 2008). Both ALP and γ-GT are membrane bound glycoprotein enzymes. Their elevated plasma concentration is due to hepatobiliary injury and cholestasis (Sheehan and Haythorn, 1979; Leonard, 1984; Saukkonen *et al*., 2006; Ramaiah 2007). In the event of damage to the parenchymal cells of the liver, these enzymes leak from the intracellular compartments into the serum resulting

in elevated serum concentrations. The investigations of Tripathi and Flora, (1998), Al-Hashem *et al*., (2009) and Bashandy *et al*., (2011) showed that toxic metals (including cadmium, mercury, arsenic and others) are hepatotoxic. Mondal *et al*., (2007) observed that arsenite intoxication of rat induced hepatocyte membrane damage caused leakage of ALT, AST and ALP into circulation as well as causing focal necrosis in the liver.

The AST/ALT values (De Ritis ratio) obtained in the participants of this study were less than 2:1 (table 4.50) and revealed that the observed trend towards liver impairment may not be associated with alcoholism. This observation is supported by the work of Moussavian *et al*. (1985) on influence of alcohol ingestion and liver disease. They established that an AST to ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease, particularly in the setting of an elevated gamma-glutamyl transferase.

The present study has shown a marked elevation of serum concentration of these enzymes in the e-waste exposed participants compared to the unexposed group. To a large extent, this metal-induced hepatotoxicity appear milder in the environmentally exposed group. This may have resulted from interaction of toxic metals with the parenchymal cells of the liver which may have resulted in necrosis. This is in consonance with the works of Mahour and Saxena (2008) and Lee *et al*. (2009), where marked elevation of serum activities of liver enzymes were observed in rats intoxicated with toxic metals. Elevation of serum activities of liver enzymes was also observed by Jagadeesan and SankarsamiPillai, (2007) in albino rats intoxicated with $HgCl_2$.

Hepatic functions, biosynthetic ability and integrity of hepatocytes are affected by the deleterious effects of toxic metals. In liver disorders, total protein and albumin are observed to decrease due to reduced number of hepatocytes and impaired function (Al-Hazzi, 2008; Ige *et al*., 2011; Bamidele *et al*., 2012). In liver cirrhosis, all liver synthesized proteins decrease while globulin increases due to imposed kupffer cell function and acute phase protein production.

However, the liver has significant reserve capacity to maintain protein concentration. This can only fall in extensive liver damage. Many liver proteins have long half-life. For instance, albumin has a half-life of three weeks. The rate of decreased protein synthesis in the liver depends on the type, severity and duration of liver injury. In acute hepatic dysfunction, there is little or no change in the total plasma protein concentration (Carl *et al*., 2008).

From the present study, serum concentration of albumin in Nigerians occupationally exposed to e-waste was found to be reduced but was not significantly different compared with the unexposed group. This may be due to the fact that hepatocytes which synthesize these proteins have a minimal turn-over and a lifespan of about one year (MacDonald, 1961). The liver can loss its biosynthetic function only when a larger portion has been destroyed or removed. For instance, in liver transplant, liver failure occurs when about 20-25% is left and fails to regenerate (Clavien *et al.*, 2007). Higgins and Anderson, 1931, observed that liver has the ability to regenerate itself after an assault or surgical removal of its part. This stimulated hepatocytes proliferation leads to the gradual restoration of the liver mass through a process of compensatory hyperplasia. The regeneration of liver cells from its oval (progenitor) cells could be a reason for the undetected reduction in the biosynthetic function of the liver (Margeli *et al.*, 1994; Tzirogiavinis *et al.*, 2005). In addition, the liver increases the production of positive acute phase proteins than negative acute phase proteins during inflammation of its cells.

The liver conjugates indirect bilirubin from erythrocytes destruction in the reticuloendothelial system. Bilirubin is transported to the liver bound to albumin. It is taken up by the hepatocytes which conjugate them to bilirubin diglucuronide by the action of uridyldiphosphate glucuronyl transferase enzyme. This enables the renal excretion of bilirubin. Injury to the hepatocytes results in increased total bilirubin. Diseases and assaults on the hepatocytes may reduce the conjugating function of the liver. More so, any obstruction to the bile canaliculi can as well cause an increase in serum bilirubin. Venkatesan and Sadiq (2013) observed an elevated serum bilirubin in rats exposed to mercury intoxication. In another study, using animal model, Mohamed *et al.*, (2010) observed an increased serum level of total bilirubin as a result of mercury intoxication. In vitro study done by Elias *et al.*, 2013, it was observed that cadmium caused an elevated bilirubin level along with ALT, AST, ALP and GGT.

However, in this study, the mean values for Total bilirubin alone was significantly higher in e-waste workers, and Conjugated bilirubin (CB) level was slightly higher (not significantly different) in exposed participants compared with unexposed participants. Increase in both TB and CB in the exposed subjects may be as a result of haemolytic processes which may be secondary to erythrocyte membrane lipid peroxidation. It has been reported that some metals such as gold, mercury, copper and lead (also part of WEEE) cause lipid peroxidation (Ribarov and Benov, 1981). Increased release of haemoglobin

from haemolysis of erythrocytes explains the higher values of the two forms of bilirubin in the exposed groups.

The unsubstantial differences observed in the serum albumin, globulin as well as conjugated bilirubin in the exposed participants studied compared with the unexposed may indicate that the biosynthetic capacity and biotransformation function of the liver of the e-waste populations in this study was not or has not been compromised. However, the marked elevation of serum activities of liver enzymes is an indication that the liver may be undergoing necrosis as a result of interaction of the liver cells with toxic metals in WEEE.

Prostate cancer is a leading cause of cancer-related death of men globally and it is the most common cancer in Nigerian males; having overtaken liver cancer (Jeddy-Agba *et al*., 2012; Ukah and Nwofor, 2017). However, liver cancer is the most common cause of cancer death in Nigeria (when all cancer deaths in Nigeria are considered). The most common liver malignancy in Nigeria is hepatocellular carcinoma (Jeddy-Agba *et al*., 2012; Ukah and Nwofor, 2017). Based on this, the status of total prostate-specific antigen (tPSA), free prostate-specific antigen (fPSA) and alpha fetoprotein (AFP) as cancer risk biomarkers were investigated in Nigerian e-waste workers and the environmentally exposed group.

From the results obtained it is show that tPSA and AFP were both significantly raised in e-waste workers compared with environmentally exposed and unexposed participants respectively. Free PSA (fPSA) did not vary significantly across the study participants. The values obtained for % fPSA were similar in the e-waste workers and environmental groups, both were however lower significantly compared with the unexposed population. When compared with a reference value of 25%, e-waste workers' % fPSA (31.87%) and environmentally exposed groups' %fPSA (34.04%) showed considerable closeness to 25% as against the 41.8% observed in the unexposed group, an observation that portends higher prostate cancer risk for the e-waste exposed groups as would be made clear below.

Considered by location, the tPSA of Benin e-waste workers was remarkably higher (but not statistically different) and seem to be more at risk compared with Lagos and Ibadan groups. There was no significant difference in % fPSA and fPSA levels of e-waste workers across the study locations. AFP in Lagos e-waste workers was significantly elevated compared with both Benin and Ibadan e-waste workers. This shows that Lagos e-waste workers may be more at risk of liver damage related to AFP elevation. In the environmentally exposed participants, tPSA, fPSA and AFP levels were unvaried in Ibadan and Lagos participants, but were both significantly higher than the Benin environmental

group. Percentage fPSA values did not vary significantly by location. Among the unexposed population, tPSA and fPSA did not vary significantly by location. Percentage fPSA levels were higher significantly in both Ibadan and Lagos unexposed groups compared with Benin group. AFP level in Lagos unexposed group was significantly higher than Ibadan, which was equally higher than Benin unexposed group.

In this study, toxic and essential metals were correlated with cancer risk biomarkers in e-waste exposed and unexposed participants and it was observed that AFP correlated weakly positively and significantly with Ni and As. while other correlation relationships were not significant. It was observed in the environmentally exposed group that tPSA and Tl, AFP and Tl correlated inversely and significantly; while AFP correlated positively and significantly with Cr and As. In the unexposed group, apart from AFP and Sn which recorded a weak positive and significant association, all toxic metals showed non-significant correlations with cancer risk biomarkers in the unexposed group.

Some essential metals like Zn and Se have been documented to offer anticancer protective effects in the prostate gland. In this study, Correlation of essential metals with cancer risk biomarkers in e-waster workers that tPSA and Zn as well as AFP and Mg were significantly inversely correlated, while fPSA correlated positively and significantly with Zn. This inverse correlation of tPSA and Zn in e-waste workers may be linked with the higher cancer risk

Among the environmentally exposed participants tPSA and Zn, Se, Mg and Mn were significantly positively correlated while an inverse significant correlation was observed between tPSA and Cr. In addition, fPSA vs Zn and Mn were positively significantly correlated, while AFP correlated significantly with Mn. In the unexposed group, it was shown that fPSA vs Mg as well as AFP vs Co produced inverse significant associations, while AFP and Mn was positively significantly correlated.

Metals may affect the male reproductive system directly, when they target specific reproductive organs, or indirectly, when they act on the neuroendocrine system. Prostate cancer is dependent on male sex steroid hormone for development, growth and survival. Cadmium, which is present in WEEE is a known endocrine disruptor. PSA is produced in the epithelial cells of the prostate, and can be demonstrated in biopsy samples or other histological specimens using immunohistochemistry. Disruption of this epithelium, for example in inflammation or benign prostatic hyperplasia, may lead to some diffusion of the antigen into the tissue around the epithelium, and it is also the cause of elevated blood

levels of PSA in this condition (Thompson *et al*., 2004). Normally, PSA is present in blood at low level, the desirable level of PSA that is considered normal in men is less than 4ng/mL (Thompson *et al*., 2004). Previous reports have shown that PSA level is increased in heavy metal toxicity (Waalkees, 2003; Alvarez *et al*., 2007). The significantly raised PSA concentration in the e-waste exposed groups in this study may be linked to a trend towards metal-induced oxidative stress and the associated initiation of carcinogenesis caused by the effects of heavy metals on the prostate tissue. Benign or cancerous tumor of the prostate has been reported to lead to excessive proliferation of the prostate cells including the epithelial cells of the prostate which are the site of production of PSA.

Pathological states in the prostate gland and reproductive system of the studied e-waste workers may arise from direct metal toxicity and oxidative stress related conditions. Kampa *et al*. (2008) demonstrated that testosterone induces PSA secretion by cells suc as androgen sensitive human prostate adenocarcinoma cells (LNCaP) through membrane sites different from classical androgen receptors. Testosterone is produced by leydig and sertoli cells, with excessive proliferation of leydig and sertoli cells which may arise from tumor processes caused by DNA damage, induced by oxidative stress in heavy metal toxicity (Kampa *et al*., 2008). Under this condition testosterone secretion by the leydig cells will be increased. Testosterone has been shown to increase PSA secretion (Kampa *et al*. 2008); thus increase in PSA level in the exposed participants can also be linked to this mechanism.

Another mechanism for PSA increase in the exposed population may be associated with metal-metal interaction. WEEE-borne cadmium is known to be a metabolic antagonistic of zinc in biological systems. Some case-control studies have demonstrated that the concentrations of zinc in plasma/ serum or total prostate tissue in men with Prostate cancer are lower than those in men without prostate disease or with benign prostate hyperplasia (Lekili *et al*., 1991; Zaichick *et al*., 1997). Superoxide dismutase contains zinc as a co-factor, therefore the unavailability of zinc in the prostate may result in reduced activity of SOD which will intensify oxidative stress and consequently DNA damage and tumorigenesis in the prostate gland. DNA repair mechanisms defend against exogenous insults which can lead to gene rearrangement, translocation, amplification and deletion which in turn contribute to cancer development (Pioriet and Weston, 2002). Zinc plays a vital role in these repair mechanism through zinc finger (Berg, 1990; Berdamier, 1998). Cadmium (found in WEEE) can antagonize zinc because cadmium and zinc have similar

chemical properties. It has been suggested that even small repeated low doses of cadmium could accumulate and mimic zinc, leading to the adverse effects of cadmium observed on the prostate (Sancar and Tang, 1993). The occupational lifestyle of our studied population showed near zero safety practices, a culture that predisposes the e-waste workers to toxic and carcinogenic chemicals (cadmium inclusive) in WEEE.

The percentage of free prostate-specific antigen (%fPSA) in serum has been shown to enhance the specificity of PSA testing for prostate cancer detection, and some preliminary cutoffs for its clinical use have been suggested.

Catalona *et al*., (1995) in a Prospective Multicenter Clinical Trial prescribed the use of the % fPSA to enhance differentiation of prostate cancer from benign prostatic disease. Their cohort study defined the situations in which % fPSA may be used to improve the performance of total PSA level results. The study further posited that the use of the %fPSA can reduce unnecessary biopsies in patients undergoing evaluation for prostate cancer, with a minimal loss in sensitivity in detecting cancer. A cutoff of 25% or less free PSA was recommended for patients with PSA values between 4.0 and 10.0 ng/mL and a palpably benign gland, regardless of patient age or prostate size. Cutoffs for percentage of free PSA of 25% and 22% yielded 95% and 90% sensitivity, respectively. Use of these cutoffs (i.e, performing biopsies only in patients with percentages of free PSA less than or equal to these cutoffs) could have avoided biopsies in 20% and 29%, respectively, of the patients with benign prostatic disease, as reported by the study.

The present study observed % fPSA levels that were lower and closer to the 25% cutoff mark in the e-waste groups compared with the unexposed group, even though the physiologically active fPSA concentrations did not vary statistically in the exposed and unexposed groups. This again affirms the higher prostate cancer risk in e-waste workers previously reported by Igharo *et al*., (2015) as a pilot study of the present research (attached in appendix).

The level AFP in e-waste workers was mildly elevated above values obtained for other groups and compared with the reference range in healthy population. The difference was significantly higher than the mean AFP levels in the unexposed group.

Previous report demonstrated histopathological changes in liver of fish exposed to a wide range of heavy metals (Abdel-Moneim, 2012). The value of testing for alpha-fetoprotein (AFP) for the diagnosis of primary hepatocellular carcinoma is well established (Tatarinov, 1964; Abelev and Eraiser, 1999; Abdel-Moneim, 2012). The body has limited capacity to respond to cadmium exposure, as the metal cannot undergo metabolic degradation to less toxic species and it is only poorly excreted, making long term storage (especially in the liver) a viable option for dealing with this toxic element (Abdel-Moneim, 2012). The increased AFP level in the exposed subjects of this study may be associated with the promotion of oxidative stress by liver-stored heavy metals, of which cadmium is a culprit being reportedly stored in the liver (Abdel-Moneim, 2012). The oxidative stress stimulated in the liver by the accumulated heavy metals may cause DNA damage which may exacerbate cellular proliferation in the liver. The increased level of AFP may have been attributed to this cellular proliferation.

Also, AFP reactivation in adults may result from liver regeneration, noncancerous liver diseases such as viral hepatitis or cirrhosis, primary liver or germ cell tumors and to lesser extent several forms of other epithelial malignances (Abelev and Eraiser, 1999). It is well known that the liver has regenerative ability; destruction of hepatocytes in heavy metal toxicity by oxidative stress will lead to stimulation of regeneration of hepatocytes which will consequently lead to increase in AFP as the expression of AFP gene is increased during growth and regeneration of the liver cells. This may have accounted for the higher AFP values obtained in the e-waste workers' group.

In addition, AFP has been shown to be localized in the cytoplasm of hepatocytes, thus increased destruction of hepatocytes in heavy metal toxicity will reflect an increased level of serum AFP (Abelev and Eraiser, 1999).
Thus, the rising AFP levels in the exposed participants (particularly the e-waste group) of this study may be associated with the pathobiology of metal-induced hepatotoxicity in chronic occupational exposure.

In this study, levels of 8-Hydroxy-2'-deoxyguanosine (8 – OHdG), 8-Oxoguanine-DNA glycosylase (OGG_1) and wild-type tumour suppressor protein (wt-p53) were determined to evaluate the extent of genotoxity, while frequency of micronucleated polychromatic erythrocytes (MnPCEs/1000PCE) was employed to determine the existence of chromosomal aberration in e-waste exposed and unexposed participants.

In summary, the unexposed population showed a significantly higher level of wt-p53 compared with levels observed in e-waste workers, and the lowest level was observed in the environmentally exposed group. Levels of the biomarker of oxidative DNA damage, 8 – (OH)dG was higher in e-waste workers than in environmentally exposed group and lowest in unexposed group; indicating a considerable DNA damage activities with chronic e-waste exposure. In Addition, the biomarker of DNA repair, OGG_1, was highest in a significant proportion in environmentally exposed group and lowest in unexposed group. When compared, OGG_1 levels were not significantly varied between the group of e-waste workers and unexposed controls.

At the different study locations, wt-p53, 8-OHdG, and OGG_1 levels in e-waste workers and the environmentally exposed participants did not vary significantly by location. It is noteworthy that evidence of damage (8-OHdG levels) and wt-p53 expression were comparatively moderately higher in Lagos e-waste workers than Benin and Ibadan, while OGG_1 level was comparatively lowest in Lagos. Similarly, 8-OHdG level was comparatively moderately higher in Lagos environmental group than Benin and Ibadan, while OGG_1 level was also comparatively lowest in Lagos. Wt-p53 levels in Benin and Lagos were similarly higher than Ibadan. In the unexposed group, 8-OHdG levels and wt-p53 expression were comparatively higher in Lagos e-waste workers than Benin and Ibadan, OGG_1 level was however significantly higher in Benin unexposed group compared with Lagos and Ibadan where the levels did not vary significantly.

These observations may be linked to the comparatively elevated levels of toxic metals and lower levels of some genome-protective micronutrients observed in this study for Lagos e-waste workers. From our observation in the study and the available literature (Puckett *et al*., 2002; Terada, 2012) on influx of e-waste to Nigeria, Lagos appears to be the location with the highest e-waste impact in Nigeria.

Among the e-waste workers' group, significant inverse correlations were observed between wt-p53 and Mn, OGG_1 and Zn, OGG, and Se; while wt-p53 and Zn correlated positively and significantly. Notably, Zn levels complimentarily increased with wt-p53 levels in the e-waste workers group. In the environmentally exposed group, Mn and wt-p53 were significantly positively correlated, while 8 – OHdG was inversely positively correlated with Mn. It was further observed among the unexposed group that 8 – OHdG vs Mn and Cr; as well as OGG, vs Cu and Co were positively significantly correlated, while wt-p53 negatively significantly correlated with Mn.

In multicellular organisms, p53 prevents cancer formation, thus, functions as a tumor suppressor. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. Hence p53 is classified as a tumor suppressor gene (Isobe *et al*., 1986; McBride and Merry, 1986; Bourdon *et al*., 2005). This is made possible as p53 induces aberrant cell circle arrest (between G1 and S phase of cell circle) and or through apoptosis mediated by wt-type p53 (Isobe *et al*., 1986; McBride and Merry, 1986; Bourdon *et al*., 2005).

Naturally, p53 mediates cellular stress responses; initiates DNA repair, cell circle arrest; senescence and importantly, apoptosis. If p53 is downregulated, these roles will be abrogated. In normal cell, unperturbed p53 is at low concentration being continually produced and degraded. The degradation of p53, as associated with MDM2 is itself induced by p53, to degrade it in ubiquitination system, hence level is low (Bourdon *et al*., 2005). Mutation of the p53 gene is a key event in the carcinogenesis of many types of tumours. This can occur throughout the length of the p53 gene. Anti-p53 auto-antibodies are commonly produced in response to these p53 mutations (Choudhury *et al*., 2013).

In this study, oxidative stress from reactive oxygen species and metals may activate p53, hence increase expression, but can also become inactivated or denatured by binding with metals and zinc micronutrient insufficiency (Zn, Co particularly). Moreover, mutant p53 (mp53) often do not induce MDM2 (as seen with wtp53, through the usual negative feedback), and are often able to accumulate at high concentrations. Worse, mutant p53 protect itself and can inhibits normal (wild-type) p53 (Blogosklonny, 2002). Wt-p53 gene can be damaged in cells by mutagens (chemicals, metals, radiations or viruses) leading to raised levels of mp53, hence low wtp53. It has been reported that about 50% of human tumours contain a mutation or deletion of the p53 gene. Missense point mutation of the p53 gene is seen in this 50% human cancers. These mutations impair p53 anticancer gene-inducing effects (Choudhury *et al*., 2013).

The observed downregulation of wt-p53 in the e-waste exposed populations in the present study may be attributed to the reported chemical -/mutagen-induced damage and or deletion or attenuation of wt-p53, in addition to a resultant chemical- or mutagen induced over expression of mp53 (Meplan *et al*., 1999; Olivier *et al*., 2004; Gurufi, *et al*., 2014). High levels of mp53 has been reported to downregulate wt-p53 (Blogosklonny, 2002). In addition to loss of function, mp53 has been documented to exhibit a dominant negative effect over wt-p53 and or gain of functional activity independently of the wild type protein.

Some studies (Meplan *et al*., 2000; Olivier *et al*., 2001; Garufi, *et al*., 2013) have also found out that p53 missense mutants markedly reduce the binding of the wt-p53 to the p53 responsive elements in the target genes of p21, MDM2, and PIG3. It could be drawn from the studies that mp53 exerts its dominant negative activity by abrogating the DNA-binding, and subsequently the growth suppression and function of wt-p53 (Meplan *et al*., 2000; Olivier *et al*., 2001; Garufi, *et al*., 2013).

In the present study, the low levels of wt-p53 in the e-waste exposed groups may not be attributed to the effect of mp53 (induced by toxic insults) alone, owing to disordered toxic-essential metal interaction observed in the e-waste groups, the alteration of wt-p53 metalloregulation may partly take responsibility for this. That is, the downregulation of p53 in the exposed group may be attributed to the disrupted /altered or aberration in the metalloregulatory activity of p53 function. P53 is stabilized by Zn. This may be affected by negative or unfavourable metal-metal interaction, a fallout of mixed metal toxicity.

In support of this view, Meplan *et al*., (2000) documented that in the metalloregulation of the tumor suppressor protein p53, zinc mediates the renaturation of p53 after exposure to metal chelators in vitro and in intact cells. The p53 is a transcription factor which binds DNA through a structurally complex domain stabilized by a zinc atom. Still from the study by Meplan *et al*., (2000) Zn chelation disrupted the architecture of this domain, inducing the protein to adopt an immunological phenotype identical to that of many mutant forms of p53. In their report, ^{65}Zn was used to show that incorporation of zinc within the protein was required for folding in the 'wild-type' conformation capable of specific DNA-binding. Using a cellular assay, they showed that addition of extracellular zinc at concentrations within the physiological range (5µmol) was required for renaturation and reactivation of wild-type p53. Among other divalent metals tested (Cd^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+} and Ni^{2+}), only Co^{2+} at 125µmol had a similar effect. Recombinant metallothionein (MT), a metal chelator protein, was found to modulate p53 conformation *in vitro*. In cultured cells, overexpression of MT by transfection could modulate p53 transcriptional activity.

Taken together, these results suggest that zinc binding plays a regulatory role in the control of p53 folding and DNA-binding activity. This may offer added explanation to the lowered wt-p53 in chronic e-waste exposure typified in the Nigerian e-waste workers studied.

In another perspective, cancer may result when there is imbalance between DNA repair and damage. The evaluation of this relationship was captured in this study by levels of the biomarker of oxidative DNA damage, 8 – (OH)dG which was observed to be higher in e-

waste workers than in environmentally exposed group and lowest in unexposed group; indicating a considerable DNA damage activities with chronic e-waste exposure; and the biomarker of DNA repair, OGG_1, which was highest in a significant proportion in environmentally exposed group and lowest in unexposed group. When compared, OGG_1 levels was not significantly varied between the group of e-waste workers and unexposed controls.

Heavy metal-induced oxidative DNA damage in lower animal- earthworms has been reported (Hirano, 2008). Molecular oxygen is essential for the survival of all aerobic organisms, and reactive oxygen species (ROS), which are byproducts of oxygen metabolism, are harmful for living organisms. Thus, oxygen is a double-edged sword. In fact, ROS are known to directly attack vital cellular components, including proteins, lipids, and nucleic acids. The oxidation of these molecules is associated with cellular dysfunction, leading to various biological responses, such as inflammation and apoptosis. Since ROS, such as superoxide radical (O_2^-), H_2O_2, and hydroxyl radical ($\cdot OH$), are constantly generated in vivo as byproducts of respiration, agent metabolism, or pathophysiological conditions (Dizdaroglu 1991; Ames *et al*., 1993; Breen and Murphy, 1995), it is difficult to completely prevent their harmful effects on cellular components. When ROS attack DNA, oxidized bases are frequently generated (Bohr *et al*., 2002). Among the various forms of oxidative DNA damage, 8-oxoguanine (7, 8-dihydro-8-oxoguanine, abbreviated as 8-oxo-Gua or 8-OH-Gua) has been most extensively investigated (Floyd *et al.*, 1986). Since 8-oxo-Gua is premutagenic, it has been suggested to contribute to human diseases (Shimoda *et al*., 1994; Baik *et al*, 1996). On the other hand, living organisms have repair systems for oxidative DNA damage, to preserve genetic stability. Some studies have revealed the complicated network of 8-oxo-Gua repair systems (termed as the "GO system") (Hirano, 2008). However, if unrepaired oxidative DNA damage remains in DNA, then it can induce point mutations.

This damage has been monitored in the different groups of participants in this study using 8(OH)dG. The higher level of 8(OH)dG in the e-waste groups appears to suggest ROS/ metal induced DNA damage is reconcilable with chronic occupational and environmental e-waste exposure. Even in the unexposed population, the level appear to be high as well (and not statistically varied comparatively), giving a signal of the level of exposure to genotoxicants in the various study sites of the Nigerian environment. Of course, the e-waste toxicants release is a nationwide issue in that the volume of WEEE/EEE trickles

from high impact areas like Lagos, Ibadan, Benin, Aba etc., to other Nigerian cities and towns with attendant environmental pollution.

It is known that OGG_1 is a DNA repair protein involved in the repair of the major product of DNA oxidation, the miscoding base 8-oxoguanine (8-oxo-G). OGG_1 is implicated in the first step of the base-excision repair process, removal of the 8-oxo-G damaged base from the DNA duplex, resulting in the creation of an apurinic/apyrimidinic (AP) site. Due to the slow AP-lyase reaction kinetics of OGG_1, the next step of in vivo AP-site removal may be aided by AP endonuclease (APE1) (Boiteux and Radicella, 2002; Hill *et al.*, 2001; Fortini, 2003). Polymorphism in the human OGG_1 gene is associated with the risk of various cancers such as lung and prostate cancer (Goode *et al.*, 2002).

The OGG_1 specifically repairs oxidative DNA damage, 8-oxoG being a critical mutagenic lesion (Kasprzak, 2002). OGG_1 is a bifunctional DNA glycosylase with an associated apurinic/apyrimidinic (AP) lyase activity that cleaves DNA at abasic sites via a β-elimination mechanism (Mitra *et al.*, 1997; Izumi *et al.*, 2003). OGG_1-initiated DNA base excision repair pathway (OGG_1–BER) is a multistep process, which includes lesion recognition, changes in DNA structure, insertion of the 8-oxoG-containing DNA double helix into the base-binding pocket (active site) of OGG_1, and base excision and strand cleavage (Mitra *et al.*, 1997; Bruner *et al.*, 2000; Izumi *et al.*, 2003). It has been shown that heavy metal such as cadmium toxicity causes decrease of OGG_1 activity in both mouse and human models (Zharkov *et al.*, 2002; Youn *et al.*, 2005). However, in the work of Pizzino *et al.*, (2014), they reported a significant up-regulation of OGG_1 mRNA expression in exposed compared with unexposed adolescents.

In the present study, OGG_1 levels appear to be downregulated similarly in e-waste workers and in the unexposed controls. The upregulation of its activity in the environmentally exposed participants appear to corroborate the observation of Pizzino *et al.*, (2014), they reported a significant up-regulation of OGG_1 mRNA expression in exposed compared with unexposed adolescents as earlier stated.

Overall, the repression of p53 expression coupled with its possible altered metalloregulation and the upregulation of 8-OHdG appear suggestive of a high possibility of disruption of genome protection mechanism in Nigerian e-waste population.

Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division.

MN can be induced by defects in the cell repair machinery and accumulation of DNA damages and chromosomal aberrations. A variety of genotoxic agents may induce MN formation leading to cell death, genomic instability, or cancer development (Luzhna, *et al*., 2013).

To assess the extent of chromosomal aberration with respect to e-waste chronic exposure in exposed Nigerians, this study explored the frequency of formation of MN in the peripheral blood films of the participants. The mammalian *in vitro* micronucleus test is for the detection of damage induced by toxic test substances to chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in the bone marrow and or peripheral blood cells of animals (Heddle, 1973; Schmid, 1975; Schmidt *et al*, 2012).

Findings in this study indicate that the frequency of micronucleated polychromatic erythrocytes (MnPCE)/1000PCE in e-waste workers was significantly higher compared with the environmentally exposed participants, which in turn was significantly higher than the lowest frequency observed in the unexposed population. The maximum MnPCE/1000PCE observed in the participants' groups were: e-waste workers, 34; environmentally exposed, 7; and unexposed, 3; while the minimum MnPCE/1000 PCE were; e-waste workers 15, environmentally exposed 1, and unexposed, 0.

Increase in the frequency of MNPCE indicate induced chromosomal damage (Fenech, 2011). The formation of micronuclei and binuclei in fish cells caused by their exposure to cadmium, copper and chromium was reported by Cavas *et al*. (2005), thus verifying that heavy metals have cytotoxic and genotoxic effects.

Micronuclei formation in humans has been associated with various medical conditions (Fenech, 2011). Micronuclei in spermatids may lead to infertility, while a high number of MN in lymphocytes is associated with pregnancy complications and miscarriages (Fenech, 2011). MN are one of the four main endpoints, together with chromosomal aberrations, aneuploidy, and sister chromatid exchange (SCE) in the identification of cancer initiation (Tucker and Preston, 1996; Hagmar *et al*., 2001). A large number of reports describe the correlation between MN and cancer development. A significant increase in MN in lymphocytes was shown in untreated cancer patients (Iarmarcovai *et al*., 2008b). Furthermore, healthy women with breast cancer genes (BRCA1 and BRCA2) mutations showed a higher increase in MN frequency and a higher radiation sensitivity than women without family history of breast cancer (Rothfuss *et al*., 2000; Trenz *et al*., 2003). Similar outcomes were shown in lung cancer patients with a high frequency of spontaneous MN

(Guler *et al*., 2005), as well as in patients with pleural malignant mesothelioma (Bolognesi *et al*., 2002), and adenocarcinoma patients (Karaman *et al*., 2008). Cancer-prone patients with Bloom syndrome and ataxia telangiectasia also possess a high frequency of MN in lymphocytes (Rosin and German, 1985). Analysis of European cohorts indicates that individuals with increased MN are more likely to get cancer 12–15 years after the test was performed (Bonassi *et al*., 2007).

With regard to the fate of MN in the e-waste population of this study, the role of p53 in apoptosis could have been beneficial if the levels were optimal in them. Some micronucleated cells originating from the loss of chromosomes can be eliminated by apoptosis. For instance, nocodazole, a microtubule inhibitor and blocker of cell cycle at M-phase, gives rise to aneuploid, polyploid, and micronucleated cells. It was observed that such MN-carrying cells were apoptotically eliminated through the activation of caspase-8, caspase-9, and effector caspase-3 (Decordier *et al*., 2008). Interestingly, when MCF-7 cells lacking caspase-3 were treated with nocodazole, MN induction decreased, which allowed the authors to suggest a possible role of caspase-3 in MN formation (Decordier *et al*., 2005). There is also data suggesting the reincorporation of MN into the main nucleus and the restoration of normal biological activity in the cell. Alternatively, retention of MN within the cell as an extra-nuclear entity is also possible (Leach and Jackson-Cook, 2004). Further, cells treated with colchicine, vinblastine, bleomycin, and arsenic showed a significant induction of MN and p53 (Salazar *et al*., 2009). By analogy with DNA replication in MN, DNA repair may also be compromised by micronuclear envelope trafficking abilities (Labidi *et al*., 1987). Multiple studies showed the existence of apoptotic-like DNA degradation in MN that were unable to repair DSBs (Terradas *et al*., 2009; 2012). Such MNi are expulsed from the cell and are lost forever. The effect of MN expulsion on a cell can be dual. If destroyed MNi carried extra chromosomes in the cell, then their elimination would be necessary for regaining the normal cellular status, but if the MN chromosome was complementary to the main nucleus, then the cell might lose a certain gene dosage (Terradas *et al*., 2012).

In summary, chromosomal aberration may be potentiated by chronic exposure to e-waste-borne genotoxic metals and other chemicals, and the resultant MN induction may have been exacerbated by observed repression of the activity of the 'guardian of the genome', wt-p53.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Summary

Nigeria is reported as the largest e-waste dump yard in Africa; and to date, Nigeria's e-waste management practices remain completely primitive. Most e-waste-borne metals have been previously reported to have potentials for metal-induced carcinogenesis by their direct genotoxic effects and disordered metalloregulation of gene repair mechanisms. Cancer is a global epidemic, however its burden is on the rise in the developing world with significantly high incidence, morbidity, and mortality. Cancer risk from occupational metal exposure has been documented. The present study, which focused on human populations occupationally and environmentally exposed to e-waste in the high WEEE-impact areas of Oyo, Lagos, and Edo States in Nigeria, was designed to evaluate risk of cancer development in participants with a minimum of five-year exposure duration. Apparently healthy participants with no occupation or hobby connected with e-waste served as unexposed group. Blood and hand wash samples obtained from exposed and unexposed participants were analyzed using standard laboratory procedures for levels of toxic and carcinogenic metals found in e-wastes, trace elements and antioxidant vitamins. Also determined are Calcium and Vitamin D levels, antioxidant status, oxidative stress levels and oxidative DNA damage/repair using biomarkers like 8-hydroxy-2-deoxyguanosine and 8-oxoguanine DNA glycosylase as predictors of cancer risk. Molecular analysis such as DNA Micronuclei Assay and evaluation of p53 expression was also carried out. In addition, simple biomarkers of prostate and liver cancers using Prostate Specific Antigen (PSA) test; Alpha fetoprotein and liver function tests were evaluated in the study populations.

Arising from this study, findings on the effects of chronic exposure to toxic metals in e-waste and the resultant biochemical changes, including genotoxicity and oxidative responses leading to systemic damage and risk of cancer development in Nigerian WEEE exposed populations are elucidated and documented. Previously, Osibanjo and Nnorom, (2007); Nnorom and Osibanjo, 2007; Nnorom *et al*., (2009) documented findings on the potential toxicity and bioaccumulation of e-waste-borne chemicals from animal, plant and environmental studies.

For the first time, this study presents findings and contributions to knowledge from human

population based study on the following thematic areas of e-waste occupational/environmental exposure in Nigeria:

(i) Risk awareness indicators, and occupational and environmental exposure indices in Nigerians occupationally and environmentally exposed to e-waste.

(ii) A thirteen-element profile of toxic metal body burden in e-waste exposed and unexposed Nigerians.

(iii) Organic and inorganic micronutrient profile (vitamins and essential metals) in Nigerian e-waste workers and in environmentally exposed participants.

(iv) Oxidative stress and antioxidant profile of Nigerians occupationally and environmentally exposed to e-waste.

(v) Association of total and ionized calcium, and vitamin D metabolism and signaling roles in carcinogenesis with chronic e-waste exposure in Nigerians.

(vi) Liver function and prostate health status as cancer risk indices in Nigerian e-waste workers and in the environmentally exposed group.

(vii) Alterations in the levels of genotoxicity biomarkers: wild-type tumour suppressor protein (wt-p53), DNA repair protein; 8-oxoguanine-DNA glycosylase (OGG_1), and oxidative DNA damage biomarker; 8-hydroxy-2'-deoxyguanosine (8-OHdG) in chronic occupational and environmental exposure to e-waste in Nigeria.

(viii) Frequency of induction of micronucleated polychromatic erythrocytes associated with e-waste occupational and environmental exposures in Nigeria.

(ix) In addition, development of a proposed concept model (schema) for cancer risk in Nigerian e-waste exposure (attached in appendix IX)

Conclusions

A duration of exposure of ≥ 5 years and exposure frequency ≥ 6hours/day; 6days/week (9360 hours in any 5 year duration) was observed with both e-waste workers and the environmentally exposed group. Routes of exposure observed in e-waste workers entailed all body cavities, viz, eyes, oral route, nasal cavity, and skin (dermal absorption). In addition, the proportion of e-waste workers that used PPE such as apron, hand gloves and facemasks while working was barely 10.24% while non – PPE users constituted the majority (89.76%) of the studied population. Among the PPE users, 10.24% used aprons and scarcely used hand gloves and the rest of the workers neither used face masks nor nose masks in the course of performing daily work tasks.

This study concludes that the elevated body burden of toxic metals in the e-waste exposed populations provides sufficient impression that there is a systemic build-up of these metals in the exposed populations, and that this may establish synergistic toxicity as a predisposing factor to chronic, acute and sub-acute pathologic conditions, one of which is cancer.

In addition, there is evidence of depressed antioxidants levels, and lowered genome protective micronutrients in the e-waste exposed populations which may be associated with antagonistic interaction of toxic metals with essentials metals in the exposed group.

Also, the data obtained demonstrates that the oxidative stress arising from increased lipid peroxidation and lowered antioxidant defenses observed in the e-waste groups may be associated with the chronic occupational exposure to e-waste chemicals and could be a key mechanism for oxidative stress induced chemical carcinogenesis in the Nigerian e-waste workers studied.

The optimal levels of tCa, iCa and Vitamin D in the e-waste exposed population may offer some forms of defense against the severity of existing and undiagnosed cancers (if any exists) or reduce the risk of cancer development in the e-waste exposed participants in this study. However, the antagonistic effects of toxic metals on calcium ions and vitamin D may unfavourably modulate these expected biological beneficial roles in the e-waste exposed populations in this study.

The significantly elevated cancer risk biomarkers (PSA and AFP) observed in the studied population of Nigerian e-waste workers may be associated with occupational exposure to

known carcinogens in e-waste.

From the indices of genotoxicity, the significant repression of tumour suppressor protein (wt-p53) expression, with a suspected alteration in the structural integrity of the genomic zinc finger domain as implied by the observed low zinc levels, coupled with the markedly raised oxidative DNA damage biomarker, 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the e-waste exposed populations, genome instability, a precursor of the carcinogenic process, appears suggestive.

To further fortify the findings, the observed increase in the frequency of the chromosomal damage biomarker, micronucleated polychromatic erythrocytes, in the e-waste exposed groups is predictive of significantly higher cancer risks associated with e-waste exposure in Nigeria.

Recommendations

Based on the findings of this study, the recommendations below are hereby given:

(i) National e-waste health risk surveys should be conducted periodically on occupational and environmental e-waste exposed Nigerians, considering the substantial amount of WEEE currently generated and imported into Nigeria. As part of this survey, health risk assessment may be periodically conducted for the exposed populations and accommodated as part of an established health insurance policy.

(ii) Extant regulatory frame work should be more strictly applied to control illegal and unwanted trans-boundary inflow of end-of-life and near-end-of life EEE into Nigeria

(iii) Being established that WEEE/EEE reprocessing serves as a source of employment for artisans, income generation, as well as an avenue for precious metal recovery, government agencies such as Ministries of Science and Technology, Labour and Productivity, and Commerce and Industry should get involved to organize and train WEEE/EEE artisans on formal, technology-based recycling approaches which are more environmentally friendly and less risky to health. In line with this, these government agencies and private investors may establish formal e-waste recycling plants, with e-waste shredders (appendix VII) that may be regionally sited to cater for the locally generated e-waste, a facility that is not currently available in Nigeria owing essentially to high cost of acquisition, operation and maintenance.

(iv) Workers involved in informal e-waste recycling (WEEE/EEE artisans) and management (waste disposal workers) should be periodically provided with or urged to acquire and routinely use adequate personal protective equipment, in additional to organized symposia and public enlightenments on environmental and health education on e-waste handling and disposal methods.

In line with these, data and findings from this and similar studies should be used to strengthen policy formulations, reform environmental and health education and campaigns that may lead to new precautionary measures and amelioration of the impact of e-waste on the environment, the food chain and the rising exposed human populations in Nigeria.

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APPENDIX

Appendix I: Ethical Consideration I

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)
COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN. IBADAN, NIGERIA.
Director: **Prof. A. Ogunniyi,** B.Sc(Hons), MBChB, FMCP, FWACP, FRCP (Edin), FRCP (Lond)
Tel: **08023038583, 08038094173**
E-mail: **aogunniyi@comui.edu.ng**

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Comparative Study of Occupational Exposure to E-Waste and Risk of Cancer Development in Nigerian E-Waste Recycling Workers

UI/UCH Ethics Committee assigned number: UI/EC/13/0372

Name of Principal Investigator: **Osaretin G. Igharo**

Address of Principal Investigator: Department of Chemical Pathology,
College of Medicine,
University of Ibadan, Ibadan

Date of receipt of valid application: 07/11/2013

Date of meeting when final determination on ethical approval was made: **20/03/2014**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from 20/03/2014 to 19/03/2015. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

UI/UCH EC
20 MAR 2014
APPROVED
COLLEGE OF MEDICINE, U.I.

Professor A. Ogunniyi
Director, IAMRAT
Chairman, UI/UCH Ethics Committee
E-mail: uiuchirc@yahoo.com

Appendix II: Ethical Consideration II

MINISTRY OF HEALTH
PMB 1113
KING SQUARE, BENIN CITY
EDO STATE

File no: ha.577/Vol.11/164

Igharo O. G.
Dept. Of Medical laboratory Science,
College of Medical Science,
University of Benin.

4th August, 2015

Sir,

RE: APPLICATION FOR ETHICAL CLEARANCE TO CONDUCT STUDY ON "TOXICOLOGICAL AND BIOCHEMICAL STUDIES IN WORKERS OCCUPATIONALLY EXPOSED TO WASTE ELECTRONIC AND ELECTRICAL EQUIPMENT"

I am directed to acknowledge the receipt of your request on the above subject matter.

2. Consequent upon the review of your proposal and recommendation by the State Ethical Clearance Committee, the Honourable Commissioner has approved the conduct of your Research on **"TOXICOLOGICAL AND BIOCHEMICAL STUDIES IN WORKERS OCCUPATIONALLY EXPOSED TO WASTE ELECTRONIC AND ELECTRICAL EQUIPMENT."**

3. You are to ensure confidentiality of the respondents and make available to the Ministry of Health a copy of your research findings.

4. Accept the assurances of the highest esteem of the Honourable Commissioner.

Dr. (Mrs). H. I. Eboreime,
(Director Medical Services)
For: Honourable Commissioner.

Appendix III: Informed Consent Form

This study is being undertaken by Igharo, Osaretin Godwin of the Chemical Pathology Department, University of Ibadan/University College Hospital, Ibadan.

Purpose of Research:

The purpose of the research is to evaluate the risk of cancer development in Nigerians occupationally exposed to toxic e-waste chemicals in the course of recycling waste electronics and electrical equipment (WEEE).

IRB Research approval number…….

This approval will elapse on……

In order to work efficiently and effectively, I would need your kind assistance and cooperation. Hand wash water and 10mL of blood will be carefully and aseptically drawn from you to assay for parameters in relation to the aforementioned study. The process of collecting the blood and hand wash water will take you some time and cause you a bearable discomfort, but not harm. Other measurements like height and weight will be taken.

You are free to refuse to take part in this study, and have a right to withdraw at any given time if you so choose.

I will greatly appreciate you help in taking part in this study

Consent: Now that the study has been well explained to me, and I fully understand the content of the study process, I will be willing to take part in the study.

---------- ----------

Signature / thumb print of participant/Date **Signature of interviewer/Date**

Signature/thumb print of witness/ Date

Appendix IV: Sample Size Calculation

Sample size for this study was determined using:

$n=Z^2$ P (1-P) / d^2; (Naing *et al.,* 2006)

Where n=minimum sample size

P= expected proportion or prevalence rate of the condition

Note: Incidence rate for all cancers from the Ibadan Cancer Registry was reported as 66.4 per 100,000 men, Jeddy-Agba *et al.,* 2012.

Therefore, P = 0.0664 (reported prevalence of cancer in Nigerian men)

d = precision or desired level of significance, i.e. 0.05 (choosing 95% Confidence Interval)

Z = z statistic for a level of confidence. For the level of confidence of 95%, which is conventional, Z= value is 1.96

Inputting these values, we have

$n = 1.96^2 \times 0.0664 (1-0.0664)/ 0.05^2$

n= 95.27 (95 participants)

Three hundred and seventy eighty one (381) e-waste workers, one hundred and twenty (120) environmentally exposed participants and one hundred and thirty one (131) unexposed participants were enrolled into the study.

Appendix V: Evaluation of risk awareness level and use of protective devices by weee workers in parts of South-Western Nigeria

WEEE WORKERS' QUESTIONNAIRE

PREAMBLE: This questionnaire is administered to evaluate and find out the risk Awareness Level Associated with the use of imported / faulty electronics and electrical equipment by workers in Edo, Oyo and Lagos States of Nigeria.

We assure you that all information provided will be treated with utmost confidentiality. Please kindly take out a little time to respond to the following questions.

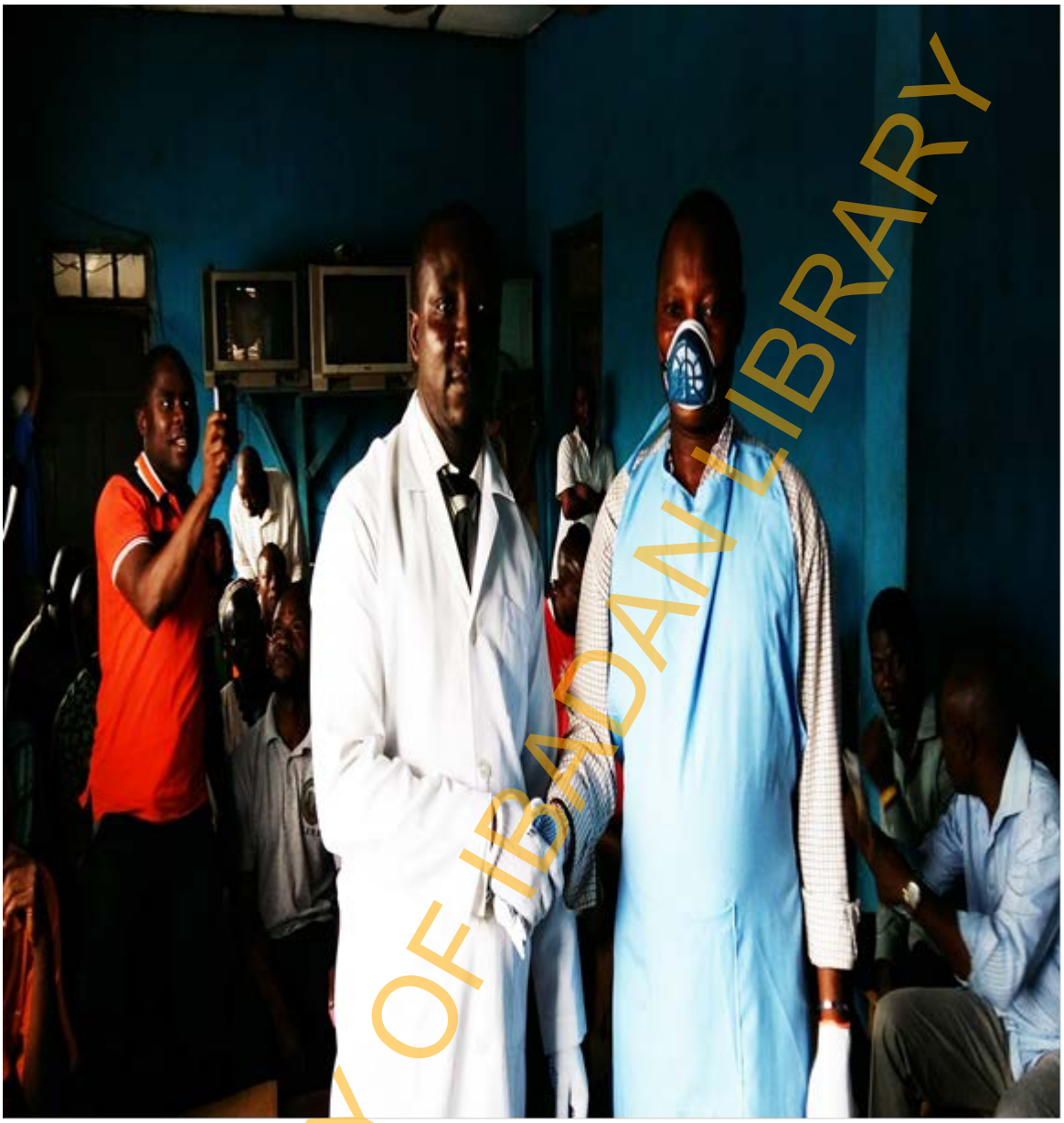
1. Age (Years)/ State of Origin : ☐ / ☐
2. Sex : Male ☐ Female ☐
3. Use of Tobacco (cigarette) : Yes ☐ No ☐ Occasionally ☐
4. Use of Alcohol: Yes ☐ No ☐ Occasionally ☐
5. Medical History (Beside Common Ailments Such as Malaria, Typhoid, Headache etc.), any other Serious Medical Conditions like liver disease, hypertension, kidney disease, ☐
   Diabetes mellitus in The Past three (3) Years? Yes ☐ No
7. If yes, Please Specify or Mention ……
8. Length of involvement with imported / Faulty electronic & electrical equipment
   1 year ☐ 2 years ☐ years ☐ 4years ☐
9. Are you aware of any health risk associated with the repair and recycling of WEEE?
   Yes ☐ No ☐
10. If Yes, Please Specify or Mention ……..
11. Do you use any protective device in the process of working? Yes ☐ No ☐
    Not often
12. If yes, Please ☐ or Mention ……..
13. Would you went a study to be done to determine Health risk associated with repair and Recycling of WEEE? Yes ☐ No ☐
14. Would you like to Participate in such study? Yes ☐ No ☐
15. Please how do you dispose your unwanted EEE……..
16. Are carbohydrates, protein, fats and oil part of your daily stable foods? Yes ☐ No ☐
    Occasionally ☐ . If yes, mention some……
17. Do you take food supplements of drugs rich in vitamins? Yes ☐ No ☐
    Occasionally ☐ . If yes, mention some: ……
18. Do you take food supplements of drugs rich in micronutrients (trace minerals)? Yes ☐ No ☐
    Occasionally ☐ . If yes, mention some: ……
19. Height and weight: Height in metre ☐ Weight in kg ☐ Body mass index ☐
20. Blood Pressure (S/D) ☐ / ☐

. Phone Contact ☐ ***Thanks for your Cooperation***!

Appendix VI: Images of Personal Protective Equipment provided to e-waste workers and sessions of demonstration of usage as well as observed WEEE disposal practices

(a) PPEs: hand glove, apron and nose mask worn as demonstration to e-waste workers (The PPEs were given to consenting e-waste workers as incentives)

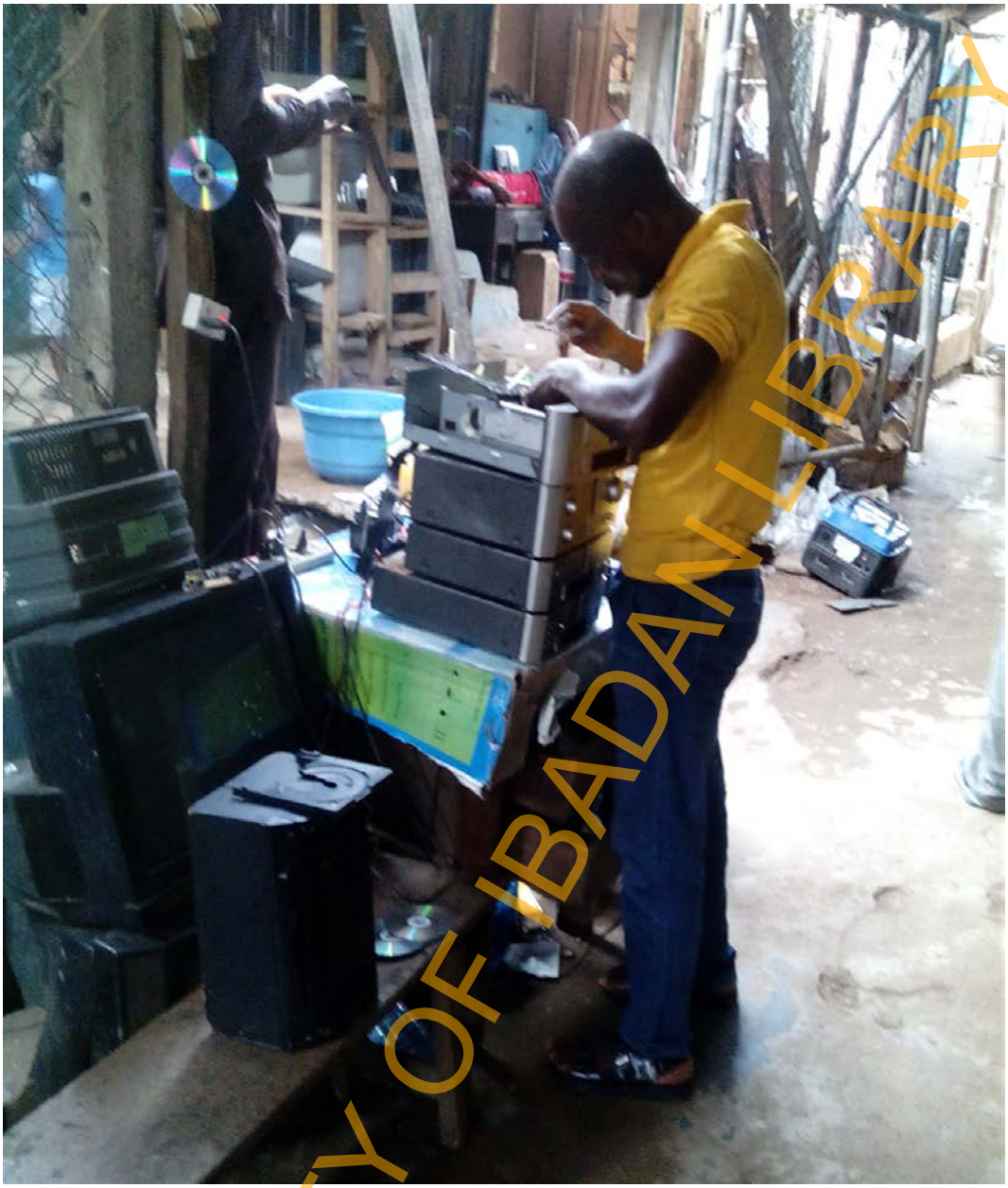
(b) A representative of the e-waste workers in Ogunpa, Ibadan, demonstrating how to put on PPEs



(c) A scene showing acceptance and willingness of e-waste workers to use PPEs after demonstration.

(d) Indiscriminate WEEE disposal practice observed in Ogunpa, Ibadan

(e) Indiscriminate WEEE disposal practice observed in New Benin Market Area, Benin City



(f) A typical WEEE worker captured on field in Ogunpa, Ibadan, working without PPEs

Appendix VII: Electronic waste shredder and shredded e-waste

Appendix VIII: Reagents, preparation of reagents and Laboratory equipment

Equipment/Reagents for ICP-MS analysis

- Sample from KEDTA is preferred
- The thermo-fisher-scientific X – II series ICP – MS (Germany)
- Hamilton microlab 500/600 auto dilutor (Microlab Safelab, UK)
- Millipore Elix/Synergy ultra – pure water supply.
- Rotanta 460 centrifuge, (Hettich zentrifugen, UK)
- 100mL acid washed volumetric flasks
- Teklab 10mL plastic sample tube 1 caps
- Adjustable pipette with acid washed tips
- Reverse osmosis/deionized water with a resistivity of >18 M Ω cm
- Concentrated nitric acid, Trace grade, fisher scientific product code: N/2272/PB17
- Triton X – 100
- Nitric acid 1% v/v diluent 10ml concentrated nitric acid to 1 litre with RODI water
- Internal standard
- Internal standard diluent: Dilute 10ml concentrated nitric acid to 1 litre with DORI water. To this, add 1mL of the internal standard solution (1500µg/L), and 1 mL 5% Triton X – 10.

Quality Control for Heavy Metals Analysis

i. Standard sample for each element was to calibrate and standardize the equipment and a graph was generated.

ii. Before being used all volumetric polyethylene (including the autosampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO_3 overnight

iii. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container

Certified reference materials (CRMs) from (Le Centre de toxicologie du Quebec) were analyzed. Each whole blood CRM was certified for a single element.

Human p53 ELISA reagents

1. 53 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-Human P53.
2. A Wash Buffer Concentrate (20X) (Item B): 25 ml of 20X concentrated solution.
3. Standard Protein (Item C): 2 vials of Human p53. 1 vial is enough to run each standard in duplicate.
4. Detection Antibody p53 (Item F): 2 vials of biotinylated anti-Human p53 (each vial is enough to assay half microplate).
5. HRP-Streptavidin Concentrate (item G): 200 µl 500X concentrated HRP-conjugated streptavidin.
6. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3,5,5-tetramethylbenzidine (1MB) in buffer solution.
7. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
8. Assay Diluent D (Item K): 15 ml of 5X concentrated buffer.
9. Assay Diluent B (Item E): 15 ml of 5X concentrated buffer.

Additional Materials used

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1 -25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Reagent Preparation

1. All reagents and samples were brought to room temperature (18 - 25°C) before use.
2. Assay Diluent D (Item K) and Assay Diluent B (Item E) were diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1 X Assay Diluent D (Item K) was used for dilution of serum and plasma samples. 1X Assay Diluent B (Item E) was used for dilution of cell culture supernatant samples. The dilution for normal serum/plasma was 2 fold.
4. Preparation of standard: Briefly, spining a vial of Item C was done. 500 µl 1X Assay Diluent D (for serum samples) or was added into Item C vial to prepare

an 80 ng/ml standard. The powder was dissolved thoroughly by a gentle mix. Pipette 300 µl 1X Assay Diluent D was dispensed into each tube. Then 80 ng/ml standard solution was used to produce a dilution series. Mixing of each tube was done thoroughly before the next transfer. 1X Assay Diluent D served as the zero standard (0 ng/ml).

5. When the Wash Concentrate (2QX) (Item B) was observed to contain visible crystals, it was warmed to room temperature and mixed gently until dissolved. 20 ml of Wash Buffer Concentrate was diluted into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
6. Briefly spin the Detection Antibody vial {item F) before use. Add 100 µl of 1X, Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80- fold with 1X Assay Diluent B (Item E) and used in step 4 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial {Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 500-fold with 1X Assay Diluent B (Item E).

For example: Briefly spin the vial (Item G} and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent B to prepare a 500-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Reagents for 8-OHdG ELISA

Reagents and Materials Provided

Reagents	Quality	Reagents	Quantity
Pre-coated, ready to use 96- well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1x20mL
Detection Reagent A	1	Assay Diluent A	1x12mL
Detector) Reagent B	1x120uL	Assay Diluent B	1xl2mL
Reagent Diluent	1 x300µL	Stop Solution	1x6mL
"MB Substrate	1x9mL	Instruction manual	1
Wash Buffer (30 x concentrate)	1x20mL		

Additional materials used but not supplied

1. Microplate reader with 450 ± 10n m filter.
2. Precision single or multi-channel pipettes and disposable lips
3. Eppendorf Tubes for diluting samples,
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution

Reagents for OGG_1 ELISA

Reagents and Materials Provided

Reagents	Quantity	Reagents	Quantity
P re-coated , ready to use 96-well strip plate	1	Plate sealer for96wells	4
Standard	2	Standard Diluent	1x20mL
Detection Reagent A	1x120uL	Assay Diluent A	1xl2tnL
Detection Reagents	1x-l20uL	Assay Diluent B	1xl2mL
TMB Substrate	1x9mL	Stop Solution	1*6mL
Wash Buffer (30 x concentrate)	1x20mL	Instruction manual	1

Materials used but not supplied

1. Microplate reader with 450 ± 10nm filter. '
2. Precision single or multi-channel pipettes and disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution

Reagent preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. Standard - Reconstitute the Standard with 1.0ml of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 20ng/mL.
3. Please firstly dilute the stock solution to 10ng/mL and the diluted standard serves as the highest standard
4. (10ng/mL). Then prepare 7 tubes containing 0.5ml Standard Diluent and use the diluted standard to produce, a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10ng/mL, 5ng/ml, 2.5ng/mL, 1.25ng/ml, 0.625ng/ml, 0.3l2ng/mL. 0.156ng/ml, and the last EP tubes with Standard Diluent is the blank as 0ng/mL
5. Detection Reagent A and Detection Reagent B - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with Assay Diluent A and G, respectively (1:100).
6. Wash Solution - Dilute 20ml. of Wash Solution concentrate (30x) with 580mL of deionized or distilled water to prepare 600ml of Wash Solution (1x).
7. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into tie vial again.

Reagents for Alpha-fetoprotein (AFP) ELISA

MATERIALS PROVIDED	96 Tests
1. Microwell coated with Strepfavidin	12x8x1
2. AFP Standard: 6 vials (ready to use)	0.5ml
3. Anti-AFP Enzyme Conjugate: 1 bottle (ready to use}	12ml
4. Anti-AFP-Biotin Reagent: 1 bottle (ready to use}	12ml
5. TMB Substrate: 1 bottle (ready to use}	12ml
6. Stop Solution: 1 bottle (ready to use)	12ml
7. 20X Wash concentrate: 1 bottle	25ml

Other materials used

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 45Qnrn
5. Absorbance paper or paper towel
6. Graph paper

Reagents Preparation

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26^0 C)

Analysis of Oxidative Stress Biomarkers

Reagents:

Trichloroacetic acid/Thiobarbituric acid (TCA/TBA) Solution, distilled water, 0.034M H_2O_2, 0.01M $KMnO_4$, Phosphate buffer (Ph = 8.0), Pyrogallol solution, 1% H_2O_2, Ellman's reagent, Standard glutathione, 5% Trichloroacetic acid solution, Adrenaline, BCG concentrate, Albumin standard.

Preparation of TCA/TBA solution

I. 63ml of concectrated Hcl was added to 37.5g TCA and 0.94g TBA in a clean beaker
II. The volume was made up to 250ml with distilled water
III. The solution was placed in water bath at 100^{0}C and stirred evenly to dissolve the particles

IV. The reagent was allowed to cool and stored in brown bottle.

0.034M H_2O_2 : 6.8ml of stock hydrogen peroxide was diluted with cold distilled water and the volume was made up to 200ml.

0.01M $KMnO_4$: 0.77g of KMnO4 salt was weigh and dissolved in 500ml of cold distilled water. 6M H_2SO_4.

Phosphate buffer (Ph = 8.0): 0.13g Na_2HPO_4, 0.019g NaH_2PO_4 and 0.8g NaCl were dissolved in 100ml distilled water.

1% H_2O_2 : 1ml concentrated H_2O_2 was diluted to 100ml with cold distilled water.

Pyrogallol solution: 5g of dehydrated pyrogallol was dissolved in 100ml of cold distilled water. This was stored in a brown bottle to prevent photoreactivity of Pyrogallol.

Ellman's reagent: 0.02g of 2-nitrobenzoic acid and 1.0g of sodium citrate salt was dissolved in 100ml distilled water.

Standard glutathione: 0.1g of the standard glutathione salt was dissolved in 100ml distilled water.

5% Trichloroacetic acid solution: 5g of TCA salt was measured and dissolved in 100ml distilled water.

Adrenaline; 0.1g of adrenaline salt was dissolved in solution of 25ml concentrated HCl and volume was made up to 100ml with distilled water.

Uric Acid Reagent Composition

Content	Concentration in the test
R1a. Buffer	
Hepes buffer	50mmol/l, pH7.0
3,5-Dichloro-2-hydroxy-benzenesulfonic acid	4mmol/l
R1b.Enzyme reagent	
4-aminophenazone	0.25mmol/l
Peroxidise	≥1000 U/l
Uricase	≥200 U/l
CAL.standard	0.592mmol/l or 9.95mg/dl

Albumin Reagent Composition

Content	initial concentration of solution
R1. BCG concentration	
Succinate buffer	75mmol/l, pH4.2
Bromocresol green	0.15mmol/l
Brij 35	
Preservative	
CAL. Standard	4.57g/dl or 45.70g/l

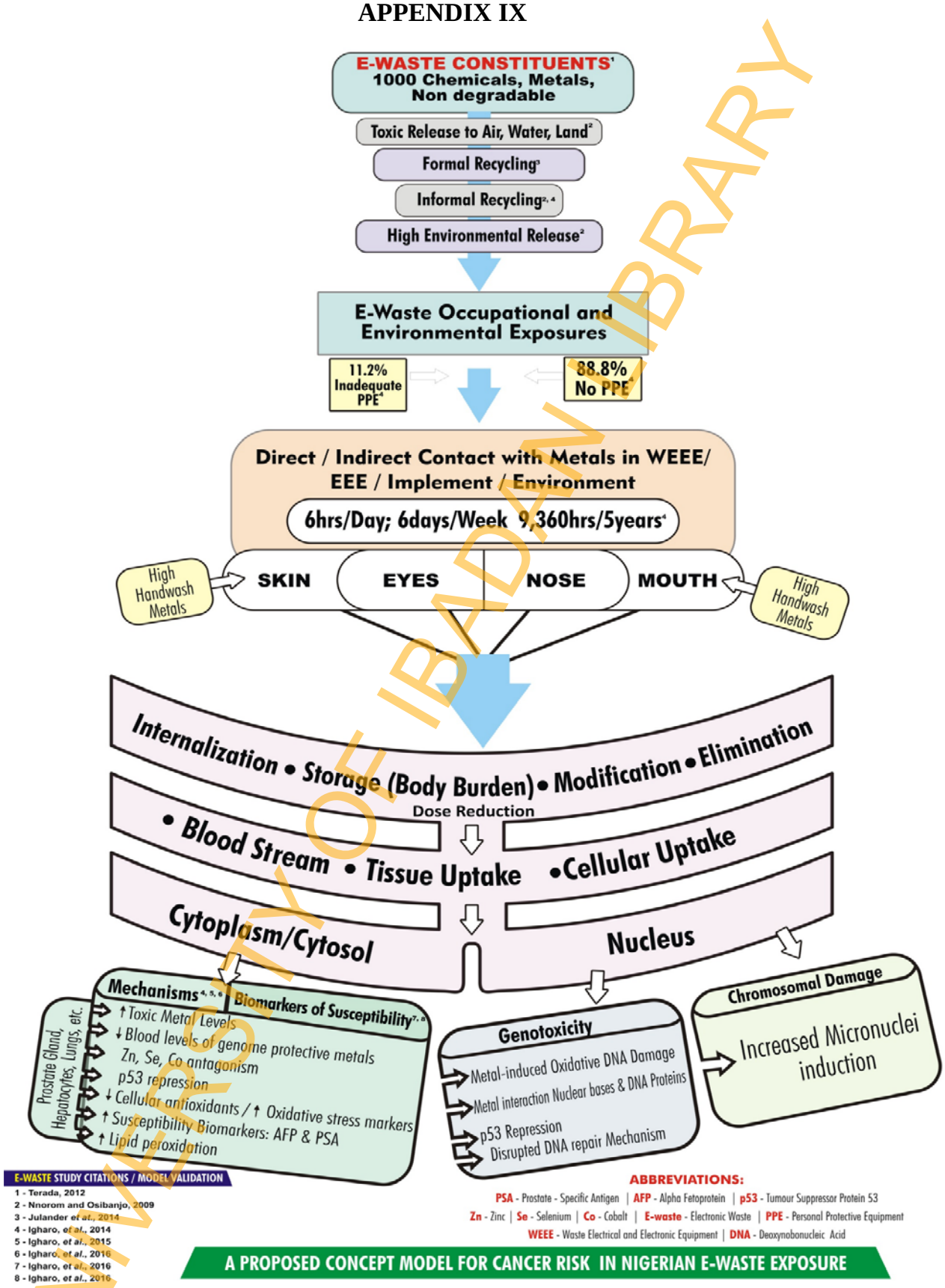
Total Protein Reagent Composition

Contents	Concentration of solutions
R1. Biuret reagent	
Sodium hydroxide	100mmol/l
Na-K-tartarate	16mmol/l
Potassium iodide	15mmol/l
Cupric sulphate	6mmol/l
R2. Blank reagent	
Sodium hydroxide	100mmol/l
Na-K-t	
Artarate	16mmol/l
CAL. Standard; Protein	59.89g/l or 5.99g/dl

Quality Control Table

ANALYTE	BOV PREC CONTROL 1		BOV PREC CONTROL 2		BOV PREC CONTROL 3	
	Reference Values	Observed Values	Reference Values	Observed Values	Reference Values	Observed Values
Uric Acid (mmol/L)	0.202	0.200	0.337	0.338	0.531	0.532
Serum Albumin (g/L)	28.9	27.0	40.1	41.2	46.1	45.9
Conjugated Bilirubin (µmol/L)	14.1	14.2	21.4	21.3	30.5	30.6
Total Bilirubin (µmol/L)	20.3	21.0	31.0	30.1	79.3	80.2
Total Protein (g/dl)	4.53	4.10	6.12	5.90	7.69	7.80

APPENDIX IX



APPENDIX X

JOURNAL ARTICLES PUBLISHED FROM THE STUDY

(1) Igharo, O.G., Anetor, J.I., Osibanjo, O.O., Osadolor, H.B., David, M.O. and Agu, K.C. (2016): Oxidative stress and antioxidant status in Nigerian e-waste workers: A cancer risk predictive study. *Brit. J Med. Res.* 13 (2) 1-11.

(2) Igharo, O.G., Anetor, J.I., Osibanjo O. O., Osadolor, H.B., Idomeh, F.A. Igiewe, W.O. and Kalikwu, O.U. (2015a) Liver Damage Risk Assessment Study in Workers Occupationally exposed to e-waste in Benin City, South-South. *Journal of Chemical Health Risks* 5 (3):155-166

(3) Igharo, O.G., Anetor, J.I., Osibanjo O. O., Osadolor, H.B., Aiyanyor, D. O. and David, O. M. (2015b): Status of Prostate Specific Antigen and Alpha Fetoprotein in Nigerian E-Waste Workers: A Cancer Risk Predictive Study. *Journal of Carcinogenesis and Mutagenesis* 6 (3):224: 1-5

(4) Igharo, O.G., Anetor, J.I., Osibanjo, O.O., Osadolor, H.B. and Dike, K.C. (2014): Toxic metal levels in Nigerian electronic waste workers indicate occupational metal toxicity associated with crude electronic waste management practices. *Biokemistri*. 26 (3) 99-105.

CONFERENCE PRESENTATIONS / ARTICLES PUBLISHED IN BOOK OF PROCEEDINGS

1. Igharo, O. G., Anetor, J. I., Osibanjo, O. and Osadolor, H.B. (2016): Downregulation of Wild-Type Tumour Suppressor Protein in Nigerian E-Waste Population: Implication for Disruption of Genome Protection Mechanism. Book of Proceedings, the 2nd University of Benin Annual Research Day Conference, 2016, University of Benin, Nigeria
2. Igharo O. G., Anetor, J. I., Osibanjo, O. O., Osadolor, H. B., Aiyanyor, D. O., Dike, K. C. and David, M. O. (2015). Occupational exposure to E-Waste and risk of cancer development: Evidence from South-South Nigeria. *Int. J. Waste Resources.* 5(2): 41. http://dx.doi.org/10.4172/2252-5211.S1.002.

Presented at the World Congress and Expo on Recycling, July 20-22, 2015 Barcelona, Spain.

3. Igharo, O. G., Anetor, J. I., Osibanjo, O., Osadolor, H.B. and Cukwuemelie, Z. U. (2016). Levels of 8-Oxoguanine-DNA Glycosylase, 8-Hydroxy-2'-Deoxyguanosine, tumour suppressor protein, toxic and essential metals in nieria electronic waste workers and risk of genome instability. Presented at 5th UNIBADAN Conference of Biomedical Research. July 12 – 15, 2016. Pg 46.

ARTICLE UNDERGOING PEER REVIEW

Igharo, O. G., Anetor, J. I., Osibanjo, O. and Ola-Davies, O.E. (2017): Exposure Assessment and Micronuclei Induction in Populations Occupationally and Environmentally Exposed to E-Waste in South-West Nigeria. *Toxicology and Industrial Health.*