SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA, LEGON

OCCUPATIONAL EXPOSURES AND EPIGENETICS ALTERATION AMONG ELECTRONIC WASTE WORKERS AT AGBOGBLOSHIE, GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF PHD IN PUBLIC HEALTH DEGREE

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DECLARATION

I, Ibrahim Issah, hereby declare that this thesis is the result of my own original research, toward the award of Doctor of Philosophy in Public Health, except for areas where specific references have been made and duly acknowledged. I also affirm that the studies reported in this document were carried out by me under the supervision of my team of academic supervisors. Lastly, I declare that this work has not been submitted, either in part or in whole, to any other institution for an award of a degree.

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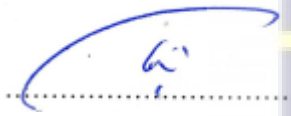
(Student)



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(Co-supervisor)

DEDICATION

To my dear wife and our lovely children, Aidan Baiwah, Najmah Hankuri, and Aiman Dadinkai, you are the best support group.

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LIST OF ABBREVIATIONS

%TDNA	Percent Tail DNA
5mC	5-methyl Cytosine
8-OHdG	8-hydroxy-2' -deoxyguanosine
ANOVA	Analysis of Variance
BMI	Body Mass Index
CA	Chromosomal Aberration
CDC	Center for Disease Control and Prevention
CpG	Cytosine Phosphate Guanine dinucleotide
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
ELIZA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
E-WASTE	Electronic Waste
GEOHealth	Global Environmental and Occupational Health
GM	Geometric Mean
HEI	Health Effect Institute
ICPMS	Inductively Couple Plasma Mass Spectrometry
IQR	Interquartile Range
LASSO	Least Absolute Shrinkage and Selection Operator
LINE-1	Long Interspersed Nucleotide Element-1
LMIC	Low and Middle Income Countries
LUMA	LUminometric Methylation Assay
MESTI	Ministry of Environment Science Technology and Innovation
MN	Micronucleus

MOH/GHS	Ministry of Health/Ghana Health Service
NHANES	National Health and Nutrition Examination Survey
NOS	Newcastle-Ottawa Scale
OLS	Ordinary Least Squares
OPC	Optical Particle Counter
OTM	Olive Tail Moment
PAH	Polycyclic Aromatic Hydrocarbon
PBL	Peripheral Blood Lymphocytes
PCR	Polymerase Chain Reaction
PM	Particulate Matter
$PM_{2.5}$	Particulate Matter with aerodynamic diameter ≤ 2.5 µm
PM_{10}	Particulate Matter with aerodynamic diameter ≤ 10 µm
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
REML	Restricted Maximum Likelihood
SAM	S-adenosyl methionine
SD	Standard deviation
SMD	Standardize Mean Difference
TEs	Trace Elements
TET	Ten-Eleven Translocation enzyme
TL	Tail Length
TM	Tail Moment
UFP	Ultra Fine Particle
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

Apoptosis - The process of programmed cell death

Bisulfite conversion of DNA – The Incubation of a target DNA with sodium bisulfite which results in conversion of unmethylated cytosine residues into uracil, leaving the methylated cytosines unchanged.

Chromosomal aberration - Chromosomal abnormalities occur when there are either deviations in the total number of chromosomes than an individual has or when there is missing, extra or rearranged genetic material on one particular chromosome

Comet assay - Comet assay or single cell gel electrophoresis assay is a relatively cheap, simple, rapid, and sensitive method used to assess DNA strand breaks in human cells associated with occupational and environmental exposure to genotoxic agents

CpG Island - Stretches of DNA 500–1500 bp long with a GC content greater than 55%

DNA damage - A change in the chemical structure and sequence of DNA

DNA methylation – The covalent addition of a methyl group to the 5' position of cytosine to form 5-methyl cytosine

Epigenetics - The study of heritable and potentially reversible gene expression changes that do not involve structural alterations in the DNA sequence, such as mutations

E-waste - Any discarded, obsolete, or broken electrical or electronic devices or products nearing the end of their useful life

Genotoxic agent - A genotoxic agent is a chemical or another agent that damages cellular DNA, resulting in mutations or cancer

Meta-analysis - A statistical technique for combining the results from several similar studies

Micronucleus - Micronucleus (MN) is formed when a whole chromosome or a lost chromosomal fragment is not included in the main daughter nuclei during mitosis

PM_{10} - Inhalable particles that can penetrate the lungs

$PM_{2.5}$ - Respirable particles that can enter the alveoli

Repetitive elements - Repetitive elements are DNA sequences that occur multiple times in the human genome

Telomere - A telomere is the end of a chromosome

ABSTRACT

Background: The techniques used in the informal recycling of e-waste, particularly in low- and middle-income countries (LMICs), are unsophisticated and rudimentary without safeguards for the health and safety of humans and the environment. Particulate matter (PM), including toxic chemical components in the form of metallic and organic compounds are generated and released into the environment during informal e-waste recycling activities. Available data suggests that PM and metals are among the most important risk factors for developing many chronic diseases such as cardiovascular diseases, neurological diseases, reproductive toxicity, renal dysfunction, autoimmune diseases and cancers. Due to the deleterious effects of PM and metals on human health, as well as elevated levels detected in occupational environments, there is a need to determine the intermediate health outcomes associated with pollutants exposure before the onset of clinical occupational disease. Epigenetic modification such as DNA methylation are highly suspected as an intermediary between environmental and occupational exposures and adverse health outcomes. Although research has been carried out on the adverse health effects of e-waste recycling in Ghana and elsewhere, there is still little published data examining the effects of metals and PM on DNA methylation in occupationally exposed populations especially those in the informal sector such as e-waste recyclers.

Objective: The objective of this work was to examine the effects of personal particulate matter exposure and a mixture of metals on global DNA methylation among e-waste recyclers and a reference population.

Methods: This study made use of biological samples and exposure data collected during the first round of a parent/larger GEOHealth II study - a longitudinal study. One hundred (100) male e-waste workers and fifty-one (51) male non-e-waste workers serving as a reference

population were recruited at baseline. The participants provided survey data and blood samples for measurements of concentrations of metals as well as DNA methylation analysis. The methylation levels of long interspersed nucleotide repetitive elements-1 (LINE-1) was measured by pyrosequencing bisulfite-converted DNA from whole blood as a proxy for global DNA methylation. Personal $PM_{2.5}$ and PM_{10} were measured over a 4-hour work-shift using real-time particulate matter monitors incorporated into a backpack and worn by study participants (e-waste workers and reference population). The concentrations of selenium (Se), zinc (Zn), manganese (Mn), cadmium (Cd) and lead (Pb) were measured in blood using inductively coupled plasma mass spectrometry (ICPMS). Descriptive statistics were used to determine differences in participant's characteristics. Multiple linear regression model with robust standard errors (SE) from ordinary least squares (OLS) was used to evaluate the associations between PM and metals exposure on the one hand and LINE-1 DNA methylation on the other hand. Further, ccorresponding interaction terms were incorporated into the regression model to determine possible modification effect of selected toxic metals (Cd and Pb) on DNA methylation caused by essential metals (Mn, Se and Zn) concentrations. Lastly, a further sensitivity analysis using different variants of the outcome model (robust and cross-fit partialling-out least absolute shrinkage and selection operator (LASSO) linear regression models) were performed to compare with the results of the OLS with robust SEs.

Results: Personal median concentrations of $PM_{2.5}$ and PM_{10} were significantly higher among the e-waste workers than the reference population ($PM_{2.5}$: median (interquartile range) 77.32(34.08) $\mu g/m^3$ vs 34.88 (16.55) $\mu g/m^3$, $p < 0.001$ and PM_{10}: median (interquartile range) 210.21 (93.32) $\mu g/m^3$ vs 121.92 (82.93) $\mu g/m^3$, $p < 0.001$, respectively). Overall, metals (Cd, Mn, and Se) were significantly higher in the reference group (geometric mean: Cd = 0.8 µg/L, Mn = 14.7 µg/L, and Se = 190.5 µg/L) than those in the e-waste worker group (geometric mean: Cd = 0.6 µg/L, Mn = 11.4 µg/L, and Se = 147 µg/L). Only Pb was significantly higher

in the e-waste workers (geometric mean: Pb = 79.6 µg/L) compared to the reference group (geometric mean: Pb = 37.7 µg/L). There was no significant difference in LINE-1 methylation among the e-waste workers and the reference group (85.16 ± 1.32% vs 85.17 ± 1.11%, p=0.950). In the linear regression models controlling for confounders, the associations between $PM_{2.5}$ and PM_{10}, and LINE-1 DNA methylation were not statistically significant among the e-waste workers ($\beta_{PM2.5}$ = 0.004; 95% CI; -0.001, 0.010, *p = 0.114*), and (β_{PM10} = 0.002; 95% CI; -0.001, 0.005, *p = 0.088*), respectively and reference population. For metals exposure, the OLS results of multiple metals showed a significant inverse association between Zn and the LINE-1 DNA methylation among only the e-waste workers (β_{Zn} = -1.180, 95% CI: -2.199, -0.161, *p = 0.024*) which corresponds to a 0.012 decrease in LINE-1 DNA methylation (95% CI: -0.022, -0.002, *p = 0.024*) for a 1% increase in Zn concentration. The linear regression results from OLS with robust SEs and those of the sensitivity analysis yielded similar estimates of the beta-coefficients. Potential interactions between toxic and essential metals on global DNA methylation were observed.

Conclusion: In conclusion, the high concentration of breathing zone PM and the body burden of metals detected in both the e-waste workers and reference population in Ghana shows the elevated levels of air pollutants in urban Ghana, particularly the capital city, Accra. Overall, PM concentration did not show significant association with LINE-1 DNA methylation in both the e-waste workers and the reference population. However, for metals exposure, increased blood zinc levels showed a significant decrease in LINE-1 methylation only among the e-waste workers. The results of this study further revealed that alteration of DNA methylation by toxic metals could be modified due to the concentration of essential metals. The alteration of LINE-1 methylation by metals could serve as an early epigenetic marker for future adverse health outcomes in e-waste workers and other workers with similar exposure. Therefore, effective

interventions to improve occupational safety for e-waste recycling workers are urgently needed.

PREFACE

Publications Related to This Work

Issah I, Arko-Mensah J, Rozek LS, Zarins KR., Agyekum TP, Dwomoh D, Basu N, Batterman S, Robins TG , Fobil JN Global DNA (LINE-1) methylation is associated with lead exposure and certain job tasks performed by electronic waste workers. International Archives of Occupational and Environmental Health.

Issah I, Arko-Mensah J, Rozek LS, Zarins KR., Agyekum TP, Dwomoh D, Batterman S, Robins TG , Fobil JN Association between global DNA methylation (LINE-1) and occupational particulate matter exposure among informal electronic-waste recyclers in Ghana. International Journal of Environmental Health Research

Issah I, Arko-Mensah J, Agyekum TP, Dwomoh D, Fobil JN Electronic waste exposure and DNA damage: A systematic review and meta-analysis. Reviews on Environmental health

Issah I, Arko-Mensah J, Rozek LS, Zarins KR., Agyekum TP, Dwomoh D, Basu N, Batterman S, Robins TG, Fobil JN Association between toxic and essential metals in blood and global DNA methylation among electronic waste workers in Agbogbloshie, Ghana. Environmental Science and Pollution Research

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

There are well documented public health concerns arising from the high production volumes of electrical and electronic waste (e-waste), especially in low-to-middle-income countries (LMICs) (Alabi et al., 2012; Baldé et al., 2017; Orlins & Guan, 2016; Robinson, 2009; Song & Li, 2014a). The composition of electrical and electronic equipment (EEE) in the general waste stream presents a challenge in the management and disposal because they are simultaneously a source of recoverable precious materials (especially metals) as well as a wide spectrum of toxic contaminants (Alabi & Bakare, 2017; Amankwaa, Adovor Tsikudo, & Bowman, 2017; Bakhiyi et al., 2018; Dias, Bernardes, & Huda, 2019; Fowler, 2017). Therefore, environmentally less polluting recycling processes are required to recover valuable materials while protecting humans and the environment from undue chemical exposures (Ikhlayel, 2017). This obviously represents serious challenges, since recycling activities in developing countries including Ghana are mostly informal and rudimentary. Currently, there are no truly and properly engineered e-waste recycling facilities in developing countries that are the primary recipients and processors of these wastes (Ikhlayel, 2018).

Given the high unemployment rates in developing countries (Nattrass & Seekings, 2018), many youths, and other groups, engage in the informal collection and recycling of e-waste to earn a living. The informal e-waste recovery work in LMICs where proper regulation and controls are lax or absent, and worker protection is often inadequate involves the manual dismantling of e-waste using basic tools or sometimes with bare hands with no protective equipment to retrieve reusable components. Crude methods such as the burning of e-waste material are widely used as the quickest way to recover valuable metals. Such methods result in the formation and release of multiple toxic chemicals into the environment, including human carcinogens such as

polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Imran et al., 2017; Yang et al., 2020a), dioxin-like compounds (DLCs) (Dai et al., 2020), or polychlorinated biphenyls (PCBs) (Wittsiepe et al., 2015), and volatile organic compounds (VOCs) (Lin et al., 2021) .

E-waste workers and individuals who live near e-waste recycling sites are directly exposed to these air pollutants mainly through inhalation, dermal contact, and ingestion through food and/or water (Perkins et al., 2014; Song & Li, 2015). Several studies have reported massive contamination of e-waste recycling sites by metals and many organic pollutants in India (Awasthi, Zeng, & Li, 2016; Singh, Thind, & John, 2018), China (Xu et al., 2015), Nigeria (Alabi, Adeoluwa, & Bakare, 2019; Ohajinwa et al., 2018), and Ghana (Feldt et al., 2014; Kwarteng et al., 2020; Laskaris et al., 2019; Lin et al., 2021; Srigboh et al., 2016; Takyi et al., 2021; Tue et al., 2016; Wittsiepe et al., 2017b; Wittsiepe et al., 2015). Exposures resulting from informal sector e-waste recovery have been associated with adverse health outcomes, including adverse effects on reproductive health, thyroid function, lung function, growth, and changes in cell function (Grant et al., 2013).

The high importation of second-hand electrical and electronic products into Ghana in recent years has resulted in a significant increase in recycling and dumping of e-waste. E-waste recycling has been a source of employment opportunity for hundreds of young men in Accra (Amankwaa, Bowman, & Tsikudo, 2016), especially young men who migrated from the northern part of Ghana. The Agbogbloshie e-waste recovery site is the main center in Ghana for processing e-waste. These recyclers, who are often young men are a particularly vulnerable group because the Agbogbloshie e-waste site is considered one of the largest, busiest and harshest informal recycling sites worldwide (Srigboh et al., 2016). The workers are usually involved in multiple tasks and work exclusively in the open using rudimentary tools with little or no use of personal protective equipment. The recycling process itself involves the manual

dismantling of old or end-of-use electronic and electrical equipment to retrieve reusable components. A significant activity at the e-waste recycling site involves open-air burning of electrical cables of all sizes in pits to retrieve oxidized copper wires with flammable materials such as styrofoam recovered from old discarded fridges as fuel. This burning activity results in the release of a mixture of toxic chemicals such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and oxides of metals, including toxic metals into the ambient environment. Recent studies revealed elevated ambient particulate matter (PM) levels (Kwarteng et al., 2020; Laskaris et al., 2021) and VOCs (Lin et al., 2021) at the Agbogbloshie e-waste site over background levels. Several other studies have documented high concentrations of PAHs, chlorinated and brominated dioxin-related compounds (DRCs) and dioxin-like polychlorinated biphenyls (DLPCBs), polybrominated diphenyl ethers (PBDEs) and metals in surface soil samples from the Agbogbloshie e-waste recycling site in Ghana (Akortia et al., 2017; Daso, Akortia, & Okonkwo, 2016; Tue et al., 2016; Tue et al., 2017). Studies have also reported high levels of PAH-derived metabolites (Feldt et al., 2014) and heavy metals (Srigboh et al., 2016; Wittsiepe et al., 2017b) in workers' blood and urine.

In Ghana, there are no proper e-waste recycling facilities or industry standards (Asante et al., 2012). The agency responsible for the regulation of the management of hazardous waste, including e-waste, is the Environmental Protection Agency (EPA). This agency functions under the auspices of the Ministry of Environment, Science, Technology and Innovation (MESTI) and has regulatory responsibilities. The regulation regarding the management of e-waste is contained in the hazardous and electrical waste control and management act, 2016 (Act 917) (Government of Ghana, 2016). The main objective of this act is "to provide for the control, management and disposal of hazardous waste, electrical and electronic waste and for related purposes" (Government of Ghana, 2016). Noteworthy in the Act with regards to e-waste management is the introduction of an electrical and electronic waste advanced eco-levy.

Importers of used electrical and electronic equipment register with EPA and pay the advanced eco-levy to the government of Ghana through METSI and EPA. The equipment then goes through inspection in line with the Basel convention using an export verification portal (DailyGuide, 2019). This is to prevent waste from arriving into the country under the guise of used items. Furthermore, funds generated from the advance eco-levy will be used to provide support for the construction and maintenance of e-waste recycling or treatment plants and to support research into methods of e-waste preservation, prevention and control (Government of Ghana, 2016). In addition, Ghana ratified the Basel convention on the control of the transboundary movement of hazardous waste and its disposal to regulate the importation of e-waste into the country, but like many other developing countries, e-waste in-flow continues unabated due to the lack of financial commitment and political will to effectively enforce these regulations (Amankwah-Amoah, 2016).

1.2 Problem statement

Metals are toxicologically important compounds that are ubiquitous in the environment and are largely used in electrical and electronic equipment (Woo, Lee, & Lim, 2016). End of life electrical and electronic equipment (e-waste) are increasingly recognized as a serious, worldwide public health concern due to advances in technology without adequate infrastructure to recycle the generated e-waste (Krishnamoorthy et al., 2018; Kumar & Singh, 2014). The techniques used in the informal recycling of e-waste, particularly in lower- and middle-income countries (LMICs), are basic and primitive, with little or no regard for the health and safety of humans and the environment (Lau et al., 2014). Recyclers often use basic tools such as a hammer, chisel and occasionally screwdrivers and spanners to dismantle and separate the different components (Wang et al., 2010) and a long metal rod to rotate/flip burning items such as insulated wires and circuit boards of various sizes (Acquah et al., 2019; Gullett et al., 2007). Particulate matter (PM), including toxic chemical components in the form

of metallic and organic compounds, are generated and released into the environment during informal e-waste recycling activities (Fowler, 2017).

There is ample scientific evidence to show that PM and metals are among the most important risk factors for many chronic diseases such as cardiovascular diseases, neurological diseases, reproductive toxicity, renal dysfunction, autoimmune diseases and cancers (Hu, 2002b; Lim et al., 2019; Rzymski et al., 2015; Shi, Jing, & Xi, 2019). Due to the deleterious effects of PM and metals on human health, in addition to their elevated levels in occupational environments (Baloch et al., 2020; Kwarteng et al., 2020), there is a growing interest to determine the intermediate health outcomes associated with pollutants exposure before the onset of clinical occupational disease (Leelapongwattana & Bordeerat, 2020; Salemi et al., 2017). These intermediate health outcomes are particularly useful information that may provide guidance for the implementation of preventive strategies in populations occupationally exposed to a mixture of chemicals such as those generated through e-waste recycling. Epigenetic modification such as DNA methylation is highly suspected as an intermediary between environmental and occupational exposures and adverse health outcomes (Chervona, Arita, & Costa, 2012; Stein & Davis, 2012).

Several researchers have reported that occupational exposure to toxic metals and PM could induce global DNA methylation changes in occupational settings, although these results are largely limited to a single metal. These include hexavalent chromium in a chromate plating facility (Wang et al., 2012), lead in battery plant workers Li et al. (2013), lead in automotive battery factory workers (Devóz et al., 2017), $PM_{2.5}$ in welders (Fan et al., 2014), and PM_{10} in steel workers (Tarantini et al., 2009). However, there are still data gaps addressing methylation among occupationally exposed populations in the informal sector (Braun et al., 2016) or the level, how and the extent to which essential elements or toxic metals or their interactions affect

DNA methylation (Vidal et al., 2015). In addition, previous studies examined the effects of one chemical at a time on health outcomes. These studies, while useful, do not reflect the reality of exposure to pollutant mixtures in environmental and occupational fields (Deng et al., 2019). Finally, to the best of my knowledge, no previous study has examined the relationship between pollutants exposure and epigenetic alteration among e-waste workers in Ghana. There is, therefore, a justifiable interest to examine the joint and potential interactive effects of different pollutants on DNA methylation among e-waste workers at Agbogbloshie. In addition, studies of this may provide insights about the latency period between chemical exposures and the onset of clinical disease and thus, inform the development of efficient prevention strategies for workers and people living near e-waste sites with uncontrolled exposures and further inform policymakers to strengthen regulation involving the safe disposal of e-waste in Ghana.

1.3 Conceptual framework

Informal e-waste recycling at Agbogbloshie release hazardous pollutants (stressors) such as PM and metals. Other sources of PM and metals at Agbogbloshie may include vehicular emissions, burning of refuse pile, outdoor biomass use for commercial cooking, and suspended road dust. These stressors are emitted into various compartments (air, water, food, etc.) and then transported into the environment, which accumulates waiting to interact with a receptor (e.g. worker). E-waste workers come into contact (exposed) with these stressors (PM and metals) mainly through inhalation, ingestion, and/or dermal contact. Factors that contributes to the level of internal exposure (dose) include the intensity (concentration) of the stressors, duration of exposure, and workers activity.

Continuous exposure to toxic chemicals could result in epigenetic modification that alters the way the DNA expresses its information without changing the DNA sequence. The most studied epigenetic modification is DNA methylation, which occurs when a methyl group (CH_{3}-) is added to the 5' position of cytosine (5-methylcytosine, 5-mC) (Portela & Esteller, 2010). The

process of methylation is catalyzed by DNA methyltransferases (DNMTs), which transfers methyl group from the universal methyl donor, S-adenosyl methionine (SAM) to cytosine (Brocato & Costa, 2013). Occupational and environmental pollutants may therefore alter DNA methylation through the alteration of methylation pathways by direct action on the function of DNMTs and ten-eleven translocation (TET) enzymes, or alteration in the availability of SAM (Ruiz-Hernandez et al., 2015). These alterations may result in genome-wide/global methylation alterations such as increased or decreased methylation.

Reduction of global methylation content has been associated with alteration in gene expression and increased genomic instability whereas increased methylation of CpG islands of specific genes silences the gene and result in loss of function (Sun et al., 2018). Therefore, abnormal methylation patterns induced by toxic chemicals could result in the development some degenerative diseases and/or indicate toxic levels of metals that lead to disease throuth multiple pathways.

Other domains of stressors that may contribute to epigenetic modifications and lead to an increased risk of disease include nutrition, and psychosocial stress (Thayer & Kuzawa, 2011). For example, nutritional status can influence epigenetic profiles by inhibiting the enzymes that catalyze DNA methylation or by influencing the dietary availability of substrates necessary for these enzymatic processes (Thayer & Kuzawa, 2011). In addition, demographic and lifestyle factors such as age, cigarette smoking and alcohol consumption may also influence the efficiency of DNA methylation (Rakyan et al., 2010; Teschendorff et al., 2010). The conceptual framework is presented in figure 1.

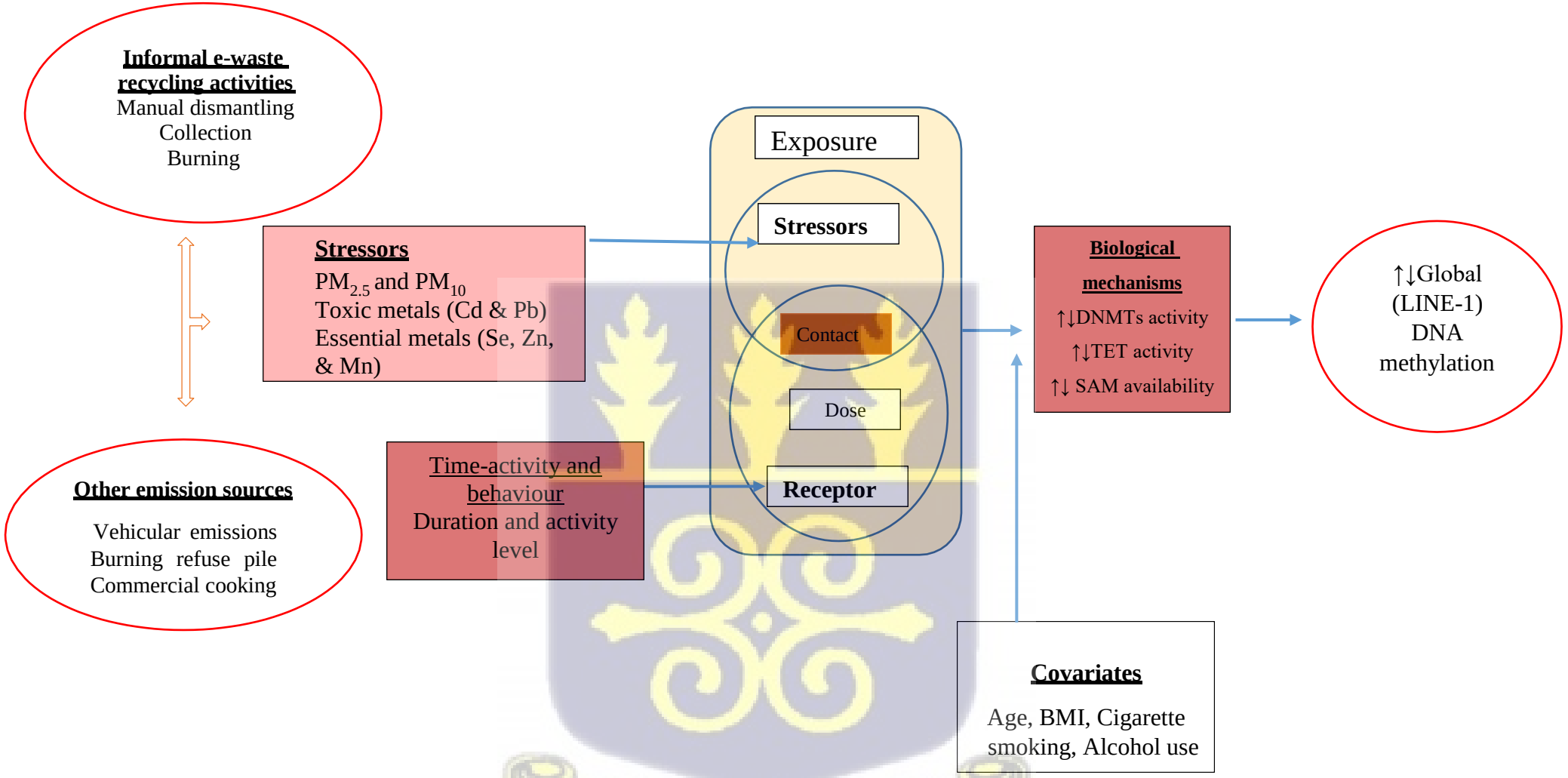


Figure 1: Conceptual framework showing linkages between PM and metals exposure and alteration of global (LINE-1) DNA methylation

1.4 Justification

The generation of millions of tons of e-waste, the lack of recycling infrastructure and the relaxed regulation on its recycling in developing countries where the e-waste ends up, highlight the need for new public health risk assessment approaches. These new risk assessment methods need to focus on early detection of human illnesses from exposure to toxicants generated from e-waste recycling as well as guide evidence-based policies on the protection of workers. Investigating into DNA damage and epigenetic modifications due to unregulated environmental pollution with toxic chemicals, especially from e-waste will be informative in such a risk assessment process. Chronic diseases such as cancer may take several years to decades to develop; therefore, there is justifiable interest in determining whether biomarkers of DNA damage and epigenetic alterations that may ultimately lead to the development cancers are associated with e-waste exposure.

The global incidence of cancer estimated by the International Agency of Research on Cancer (IARC) for 2018 stands at 18.1 million with 9.6 million estimated deaths, and lung cancer is identified as the commonest type with an estimated incidence of 11.6% and mortality of 18.4% (Bray et al., 2018). In Africa, the proportion of cancer deaths (7.3%) was estimated to be higher than the proportion of incidence cases (5.8%). This higher proportion of deaths relative to the incidence is partly attributable to the limited access to early and quality diagnosis and treatment (Bray et al., 2018). Cancer is a significant public health problem in Ghana, and its burden is increasing. According to the Ghana Cancer Registry, cancer is the fourth leading cause of death in Ghana, accounting for about 10% of all deaths (Yarney et al., 2020). The most common cancers in Ghana include breast cancer, cervical cancer, liver cancer, prostate cancer, and colorectal cancer (Laryea et al., 2014). Studies have linked environmental exposures to the burden of cancer in Africa mainly due to the fact that working conditions in Africa are likely to produce higher levels of exposure with no safety standards for the protection of the workers and the environment (McCormack & Schüz, 2012).

Chemicals exposure may damage DNA in the cell and may thus lead to the development of several diseases including cancers (da Silva, 2016; Dreval & Pogribny, 2018; Rodgers et al., 2018). Recently, considerable literature has grown around the theme of e-waste exposure and intermediate health outcomes, including DNA damage and cytogenetic alterations (Alabi, Adeoluwa, & Bakare, 2020; Neitzel et al., 2020; Ngo et al., 2020). Intermediate health outcomes such as markers of direct DNA damage (e.g. chromosomal aberrations (CA), micronuclei (MN) frequency, and comet assay parameters (tail length, tail moment, etc.) and epigenetic modifications (e.g. DNA methylation) are indicators of early biological effects, that may provide valuable insight into the mechanism by which our health is influenced by the environment as well as the appropriate levels of exposure in occupational settings. These biomarkers may provide valuable information in designing effective preventive interventions among e-waste workers and other workers with similar exposures, and findings, if adverse will also help shape policy on e-waste recycling.

1.5 Objectives

1.5.1 General objective

The overarching aim of this research work was to examine the association between personal particulate matter and metals (chemical) exposures on the one hand and global DNA methylation on the other among e-waste recyclers and a reference population.

1.5.2 Specific objectives

1. Assess the level of global DNA methylation among e-waste workers and a reference population
2. Quantify the breathing zone particulate matter and body burden of metals in e-waste workers and a reference population
3. Determine the association between $PM_{2.5}$ and PM_{10}, and global DNA methylation among e-waste workers and reference population
4. Assess the joint effect of co-exposure to toxic and essential metals on global DNA methylation

1.6 Research questions

1. Are there differences in global DNA methylation levels among e-waste workers a reference population?
2. Are there differences in metals measured in blood and $PM_{2.5}$ and PM_{10} measured in the breathing zone of e-waste workers and a reference population?
3. Is there an association between occupational exposure to PM (2.5 and 10) and global DNA methylation level among e-waste workers?
4. Is there a relationship between co-exposure to toxic and essential metals and global DNA methylation?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

This chapter presents a synthesis of evidence of genetic alteration that is associated with e-waste recycling, and a summary of epidemiological evidence of any association between occupational exposures to particulate matter (PM) and metals on the one hand and global DNA methylation on the other hand. It begins with a presentation of a systematic review and meta-analysis of e-waste exposure and DNA damage, which is immediately followed by a presentation of e-waste as a public health concern, and then an overview of DNA methylation. Gaps and/or limitations identified are discussed.

2.2 Electronic waste exposure and DNA damage: A systematic review and meta-analysis

2.2.1 Introduction

Although studies have reported evidence of an association between crude e-waste disposal and DNA damage, there has not been any systematic synthesis of evidence linking specifically e-waste exposure to DNA damage in human populations as yet. This systematic review was conducted to assess the evidence of genetic alteration associated with e-waste recycling to provide evidence for the development of efficient prevention strategies for workers and people living near e-waste sites with uncontrolled exposures and strengthen regulation involving the safe disposal of e-waste in general.

2.2.2 Protocol development and registration

A review protocol was developed and registered with the International prospective register of systematic reviews (PROSPERO) with registration number CRD42020201149, and it is available from https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=201149.

The systematic review/meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement checklist (Liberati et al., 2009).

2.2.3 Eligibility criteria

This review focused on observational studies on human populations exposed to e-waste disposal. Studies were included if they were original peer-reviewed publications, assessed e-waste exposure and biomarkers of DNA damage, and involved human populations, including women and children. Studies that were not original (e.g., reviews, conference proceedings, letters to the editor, and abstracts) and did not report biomarkers of DNA damage in human populations were excluded.

2.2.4 Information sources and search strategy

Articles published in English from January 2000, investigating the associations between e-waste exposure and biomarkers of DNA damage were retrieved through the following three major databases: MEDLINE (Academic Search Complete, CINAHL Complete, Education Research Complete, GreenFILE, Health Source: Nursing/Academic Edition, Library, Information Science & Technology Abstracts), ProQuest, and Scopus. The search terms used included the following keywords: ("electronic waste" OR "e-waste" OR "WEEE") AND ("DNA damage" OR "chromosomal aberration" OR "DNA strand breaks" OR "micronucl*" OR "Sister chromatid exchanges" OR "oxidative DNA damage" OR "genotox*" OR "oxidative stress"). Reference lists of selected articles were hand-searched for relevant publications that were not captured by the electronic search. The search strategy and results of the various databases are presented in the appendix (Appendix 5.1).

2.2.5 Study selection

The study selection was conducted in 2 phases. In phase 1, two reviewers independently screened titles and abstracts of publications retrieved from the electronic databases and hand

searches. Publications that did not meet the inclusion criteria were excluded. After phase 1 screening, 32 publications advanced to phase 2, full-text screening. In phase 2, the same reviewers independently examined the full-text publications for inclusion. Any discrepancies between reviewers were resolved by a third reviewer. Studies that were excluded at this stage are presented in supplementary table of results (Appendix 5.2) with reasons. Finally, a total of 20 publications met the inclusion criteria.

2.2.5.1 Diagrammatic representation of study selection

The flowchart representing the process of study selection is presented in Figure 2. In the initial search of 3 electronic databases, a total of 822 articles were retrieved. Duplicates of 106 were identified and removed, with a total of 717 articles making it to the title and abstract screening stage. After the title and abstract screening, a total of 685 articles were excluded, allowing 32 articles to advance to the full-text screening stage.

Out of the 32 full-text articles screened, 12 were excluded either because they were not original articles (5), did not meet the inclusion criteria (3), were retracted articles (1) or shared the same population and outcome with another study already included (3). A total of 20 publications were included in this review, of which seven studies were within the occupational setting, and the rest were ecological studies. Seven of the 20 studies included in this systematic review were included in the meta-analysis.

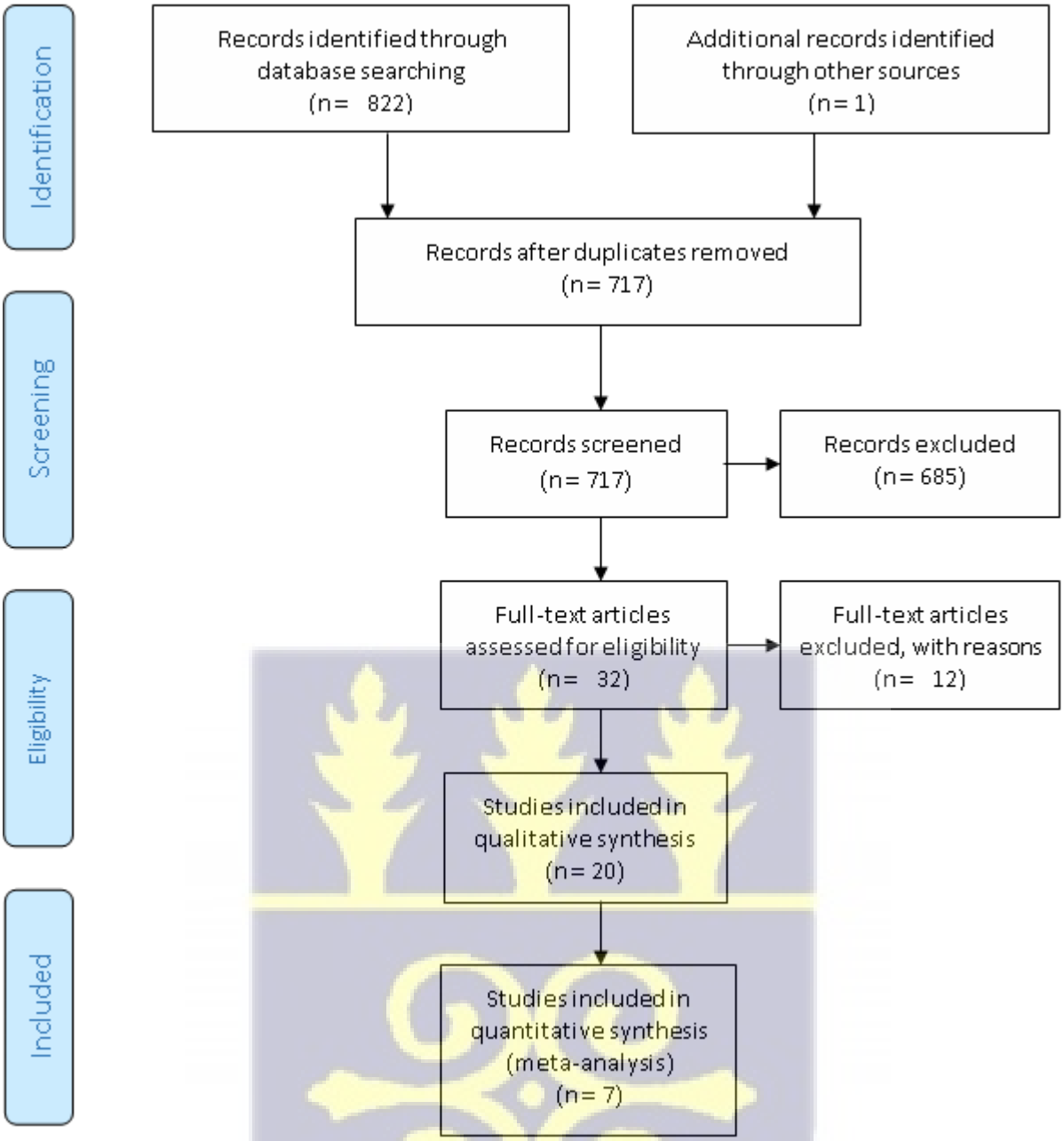


Figure 2: PRISMA flow chart illustrating the process of selecting the studies included in the systematic review

2.2.6 Data extraction

The type of data extracted from each of the selected studies were both qualitative and quantitative. The qualitative data include author and year of publication, details of study design (exposure setting and population), the country where the study was conducted, and methods details (samples type, targeted chemicals, outcome measures). The quantitative data extracted

included the sample size for each study, and the main findings expressed as means and standard deviations if applicable (Table 1). Data extraction was performed by two reviewers. One reviewer extracted the data, and the second reviewer compared the extracted data with the original report.

2.2.7 Risk of bias (quality) assessment

The risk of bias (methodological quality) of each included study was assessed using the modified version of the Newcastle-Ottawa Scale (NOS) for cross-sectional studies developed by Elyasi et al. (2015) to ensure that the conclusions and findings of the reviews were based on the best available evidence. The tool was adjusted to include exposure assessment and a comparison group.

The score for each cross-sectional study was calculated based on four categories: group selection (three items), comparability (two items), exposure measurement (1 item), and outcome measurement (one item). The items in the first two categories, 'group selection' and 'comparability', were awarded a maximum of 2 stars and 1 star for items in the remaining categories. The NOS score ranged from 0 (lowest grade) to 10 (highest grade). Studies were considered high qualityif they were scored above the median (Hermont et al., 2014), that is, > 5 in this review. The risk of bias assessment was performed by two reviewers. Any discrepancies were resolved by discussion between the 2 reviewers or by the intervention of a third independent reviewer. The NOS tool can be found in Appendix 5.3.

2.2.8 Statistical analysis

Nine out of the 20 included studies used Micronuclei (MN) frequency assay to measure the risk of DNA damage associated with e-waste exposure. Seven of these studies were included in the meta-analysis. One study (Alabi et al., 2020), which measured MN in exfoliated buccal cells, was excluded from the meta-analysis because it was considered an outlying study that

significantly affected the meta-analysis results. The other excluded study was conducted in the Philippines by Berame et al. (2020), which did not report the mean and standard deviation (SD) values. Studies that reported medians and interquartile range (IQR) or range (Chen et al., 2010; Wang et al., 2011; Yuan et al., 2008) were converted to means and SD using the formulas provided for different sample sizes by Wan et al. (2014).

The effect size was calculated using standardized mean differences (SMD) since the studies were conducted in different settings (ecological or occupational). Heterogeneity was determined using Cochran's χ^2 test and quantified using the I^2 test. The null hypothesis for heterogeneity was that all studies share a common mean difference for MN frequency. The I^2 describes the percentage of differences across studies attributed to heterogeneity rather than chance (Higgins et al., 2003). An I^2 value of 25% is considered low heterogeneity, a value between 50% and 75% is moderate, and a value above 75% is considered high heterogeneity (Higgins et al., 2003).

The random effect meta-analysis model with restricted maximum likelihood (REML) method (Raudenbush, 2009) was used to calculate the overall SMD and its 95% confidence interval (CI). The REML method performs well with small number of studies and produces an unbiased estimate of the between study variability owing to differences in study designs and interlaboratory reproducibility. The REML assumes normal distribution of the random study effect sizes (Kontopantelis & Reeves, 2012). Forest plots were used to present the results of the meta-analysis.

To further explore heterogeneity between studies, subgroup analyses were performed based on study setting, i.e., whether studies were conducted among e-waste workers vs non-e-waste workers (occupational) or studies were conducted among residents of an e-waste exposed town/village vs residents in a neighboring town/village without e-waste exposure

(ecological), and quality, (high quality vs low quality) as determined by the NOS for cross-sectional studies. All statistical analyses were conducted using Stata version 16.1 (StataCorp LLC, College Station, TX, USA).

2.2.9 Findings of the systematic review and meta-analysis

2.2.9.1 Study characteristics

2.2.9.1.1 Design and site

All 20 studies included in this review were cross-sectional studies. The majority of the studies (15 of 20) were conducted in China, and the remaining five studies were conducted in Nigeria (Alabi et al., 2020), Palestine (Khlaif & Qumsiyeh, 2017), Thailand (Neitzel et al., 2020), Vietnam (Ngo et al., 2020), and the Philippines (Berame et al., 2020).

2.2.9.1.2 Populations studied

A total of seven out of the 20 studies included in the review were conducted in occupational settings; the remaining 13 studies targeted people resident in e-waste exposed towns (ecological studies). Only four of the ecological studies targeted children (Ngo et al., 2020; Xu et al., 2018) and neonates (Li et al., 2008; Ni et al., 2014); the remaining nine studies involved adult populations. A total of 17 of the 20 studies included a comparator group. The comparator groups were mostly residents of non-e-waste recycling towns with no history of e-waste exposure. Two of the occupational-related studies (Wang et al., 2018; Yuan et al., 2008) recruited age- and sex-matched farmers as control groups. The sample sizes of the studies included in this review ranged between 48 (He et al., 2015) and 377 (Wang et al., 2010). The majority of the studies (16 of 20) had both male and female participants, three studies had all-male participants (Sheng et al., 2008; Wang et al., 2018; Yu et al., 2018), and 1 study did not describe gender breakdown (Khlaif & Qumsiyeh, 2017). Tables 1 and 2 provide a summary of the study characteristics of the included studies.

Table 1: Summary results of previous occupational studies including exposure groups, study location, samples used, and key findings

Author	Exposure setting	Exposed group	Country	Samples type	Exposure	Outcome	Main findings
Alabi et al. (2020)	Occupational	Scavengers (95) vs Control group (104)	Nigeria	Blood, Buccal Cells	Pb, Ni, Cd, and Cr	Micronuclei, Binucleated cells, Pycnosis, Condensed chromatin, Karyorrhesis, Lobbed nuclei	Micronuclei: mean (168.04 vs 3.23, p<0.01), Binucleated cells: (42.20 vs 0.08, p<0.01), Pycnosis: (26.02 vs 0.00, p<0.01), Condensed chromatin: (13.72 vs 0.01, p<0.01), Karyorrhesis: (29.47 vs 0.00, p<0.01), Lobbed nuclei: (35.29 vs 0.00, p<0.01).
Berame et al. (2020)	Occupational	e-waste recyclers (40) vs controls (52)	Philippines	Buccal cells	NR	Micronuclei frequency	E-waste workers had increased micronuclei compared to the control group.
Neitzel et al. (2020)	Occupational	Informal recycling (120)	Thailand	Urine and blood	Pb, Cd, Mn	8-hydroxy-2'-deoxyguanosine (8-OHdG)	Men who reported working >48 hours/week had significantly (p=0.045) higher levels of 8-OHdG compared to men working ≤48 hours/week
Sheng et al. (2008)	Occupational	Informal recycling (64)	China	Dust, hair, and urine	PCDD/Fs, PBDEs, and PCBs	8-OHdG	Pre- vs postworkshift 8-OHdG: mean (range): 6.4 (0.64-95.74) vs 24.55 (0.37-343.17) µmol/mol creatinine, p<0.05.
Wang et al. (2011)	Occupational	Informal recycling (48) Vs Controls (56)	China	TSP, blood and urine	Pb, Cu, and Cd	Micronuclei in binucleated cells	Micronuclei, median (range): median 4·0% (2·0–7·0) vs 1·0% (0·0–2·0), p<0·01. Positive correlation between blood lead and micronuclei in binucleated cells (r=0·245, p<0·01).

Table 1: Continued

Author	Exposure setting	Exposed group	Country	Samples type	Exposure	Outcome	Main findings
Wang et al. (2018)	Occupational	Informal recycling (146) vs farmers (121)	China	Blood and semen	PCBs, Pb, Cu Zn, Ca, Mg, Fe and Se	Chromosomal aberration, micronuclei, and DNA damage (DNA TDNA%, T.M. and OTM)	Chromosomal aberration (%): (8.01 vs 1.80), micronuclei (%): (26.30 vs 4.52), comet assay (greater DNA damage in exposed than in control group), P_{all} <0.001. duration of exposure is associated with C.A., CBMN, and DNA damage
Yuan et al. (2008)	Occupational	Informal recycling (23) vs farmers (26)	China	Blood and urine	PBDEs	Micronuclei, 8-OHdG	Micronuclei, median (range): 5(0–96) vs 0.00 (0–5.00), p<0.001), 8-OHdG, mean±SD: 69.04 ± 222.2 vs 229.97 ± 210.1) µmol/mol of creatinine, p=0.200). Working with e-waste is associated with increased Micronuclei frequencies OR, 38.85; 95%CI (1–1358.71), p=0.044

Abbreviations: Pb-lead, Cd-cadmium, Ni-nickel, Cr-chromium, Mn-manganese, Cu-copper, Zn-zinc, Ca-calcium, Mg-magnesium, Fe-iron, Se-selenium, Hg-mercury PBDEs-polybrominated diphenyl ethers, PCDD/F- Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, PCBs- Polychlorinated Biphenyls, OH-PAHs- hydroxylated polycyclic aromatic hydrocarbons, TSP-total suspended particles, 8-OHdG- 8-Hydroxy-2′-deoxyguanosine, UCB-umbilical cord blood, DNA- Deoxyribonucleic acid, %TDNA-% tail DNA, TM-tail moment, OTM-olive tail moment, CBMN-cytokinetic block micronuclei CA-chromosomal aberration, MNed BNC- micronucleated binucleated cells, NR-not reported, SD-standard deviation

Table 2: Summary of previous ecological studies, including exposure groups, study location, samples used, and key findings

Author	Exposure setting	Exposed group	Country	Sample type	Exposure	Outcome	Main findings
Chen et al. (2010)	Ecological: exposed town vs control town	Population (n=138) 58 vs 80	China	Blood	NR	micronucleated binucleated cells	MNed BNC frequency: (median: 4.0%, IQR: 2.0–7.0%) vs (median: 1.0%, IQR: 0.0–2.0%, $P < 0.01$
He et al. (2015)	Ecological: exposed town vs control town	Population (n=48) 23 vs 25	China	Blood	POPs	ROS and micronucleus rate	Micronucleus rate: (16.74 ± 4.17‰) vs (7.8 ± 1.13‰), p<0.05
Khlaif and Qumsiyeh (2017)	Ecological: exposed town vs control town	Population (n=61) 45 vs 16	Palestine	Blood	N.R.	Total chromosome aberrations (CA), tail length relative to tail plus nucleus' length (TL/TL + NL)	Total chromosome aberrations (CA): mean±SD (4.84±2.9 vs 0.75±0.931, p<0.001). Comet assay: (TL/TL + NL) mean±SD (0.7088± 0.5595 vs 0.520 ± 0.0498, p<0.001
Li et al. (2014)	Ecological: proximity group vs remote group	Population (n=58) 30 vs 28	China	Blood	Ca, Cu, Fe, Pb, Mg, Se, and Zn	Micronucleus rate	Micronucleus rate: (18.27% vs. 7.32%, p<0.05)
Li et al. (2008)	Ecological: exposed town vs control town	Neonates (n=302) 200 vs 102	China	UCB	Cr	comet assay (injury rate (tailing rate) and the lengths of tail)	Cell injury rate (%): 33.20 vs 10.70, p<0.01, Length of tails (%): 4.49 vs 2.09, p<0.01
Lin (2013)	Ecological: exposed town vs two control towns (20 and 40km from the exposed town)	Puerperae (n=320) 227 vs 93	China	Placenta	Cd, Pb	placental telomere length	Placental telomere length: negative correlation with placental Cd concentration (r = -0.138, p = 0.013).

Table 2: Continued

Author	Exposed setting	Exposed group	Country	Sample type	Exposure	Outcome	Main findings
Liu et al. (2009)	Ecological: exposed towns vs control towns	Population (n= 201) 171 vs 30	China	Blood	NR	chromosomal aberrations, micronucleus, DNA percentage in the comet tail (TDNA%), tail moment (TM), and Olive tail moment (OTM)	CA rates (%): (5.50 vs 1.70, p<0.001), micronuclear rates (%): (16.92 vs 3.47, p<0.001), comet assay (mean±SD): TDNA% (4.27±0.32 vs 1.18±0.13), TM (0.53±0.09 vs 0.05±0.01), OTM (0.82±0.09 vs 0.19±0.02), p_{all} <0.001.
Lu et al. (2016)	Ecological: exposed towns vs control towns	Population (n=176) 130 vs 46	China	Urine	OH-PAHs	8-OHdG	8-OHdG, GM order: e-waste area>rural reference>urban reference (16.2>12.3>11.6). Positive association between 8-OHdG and $\sum_{10}$OH-PAHs in e-waste participants ((β = 0.349; 95% CI: -0.210, 0.488; p <0.001)
Ngo et al. (2020)	Ecological: exposed town vs control town	Children (8-14 years) (n=80) 40 vs 40	Vietnam	Blood	Pb, Cd, Cr, Ni, and As	Comet (Tail Length (µm), Olive Tail Moment (µm), and %Tail DNA	Comet assay: Tail length (2.07 ± 0.41µm vs 1.78 ± 0.59, p<0.001), Olive Tail Moment (0.16 ± 0.04µm vs 0.14 ± 0.03, p<0.001), Tail DNA (2.67 ± 0.42% vs 2.22 ± 0.40, p<0.001). Blood arsenic correlated with Tail length (r=0.244, p < 0.05) and Olive Tail Moment (r=0.231, p < 0.05)
Ni et al. (2014)	Ecological: exposed town vs control town	Neonates (n=201) 126 vs 75	China	UCB	Pb, Cd, Cr, and Ni	8-OHdG	UCB plasma 8-OHdG: (median: 179.77 ng/mL vs 159.00 ng/mL, P = 0.028). 8-OHdG correlated with Cd (r = 0.235, P = 0.001), Cr (r = 0.214, P = 0.002), and Ni (r = 0.314, P <0.001)

Table 2: continued

Author	Exposed setting	Exposed group	China	Sample type	Exposure	Outcome	Main findings
Wang et al. (2010)	Ecological: exposed towns vs control town	Population (n=377) 286 vs 91	China	Blood and urine	Cu, Fe	8-OHdG	8-OHdG in males (mean±SD): (7.75±14.39 vs 9.73±7.39, p<0.01) µmol/mol creatinine. Blood ferrous associated negatively with 8-OHdG (β=-0.215, p=0.037)
Xu et al. (2018)	Ecological: exposed town	Preschool children (n=118)	China	Blood and urine	Pb, Cd, and Hg	8-OHdG	8-OHdG, median (range): 407.79 (152.05–876.26) ng/g creatinine. Higher Pb and Hg exposure are associated with higher 8-OHdG
Yu et al. (2018)	Ecological: exposed town vs control town	Population (n=57) 32 vs 25	China	House dust, and semen	PBDEs	Tail DNA%, OTM, and apoptosis rate	Comet assay (mean±SD): tail DNA% (57.88 ± 6.08 vs 33.55 ± 6.99, p<0.001), OTM (12.15 ± 2.52 vs 5.14 ± 4.86, p<0.001) TUNEL assay: apoptosis rate (32 ± 19 vs 20 ± 8, p= 0.037

Abbreviations: Pb-lead, Cd-cadmium, Ni-nickel, Cr-chromium, Mn-manganese, Cu-copper, Zn-zinc, Ca-calcium, Mg-magnesium, Fe-iron, Se-selenium, Hg-mercury PBDEs- polybrominated diphenyl ethers, PCDD/F- Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, PCBs- Polychlorinated Biphenyls, OH-PAHs- hydroxylated polycyclic aromatic hydrocarbons, TSP-total suspended particles, 8-OHdG- 8-Hydroxy-2′-deoxyguanosine, UCB-umbilical cord blood, DNA- Deoxyribonucleic acid, %TDNA-% tail DNA, TM-tail moment, OTM-olive tail moment, CBMN-cytokinetic block micronuclei CA-chromosomal aberration, MNed BNC- micronucleated binucleated cells, NR-not reported, SD-standard deviation

2.2.9.2 Presentation of key findings of previous studies

Of the 20 studies included, six (6) biomarkers of DNA damage (micronuclei, comets assay, 8-OHdG, telomere length, apoptosis rate and chromosomal aberrations) were measured in seven (7) different biological matrices (buccal cells, blood, umbilical cord blood (UCB), placenta, urine and semen) (Appendix 5.4). Telomere length (n=1) and apoptosis rate (n=1) were the least biomarkers measured, while the micronuclei frequency rate (n=9) was the most commonly measured biomarker. Finally, whereas 6 studies measured more than one biomarker, the remaining 14 studies measured one biomarker each. Most studies used blood (n=9) and urine (n=7) as biological matrices in which DNA damage was measured.

2.2.9.2.1 Micronuclei frequency

Micronuclei (MN) frequency has been widely used as a biomarker to investigate DNA damage in human populations exposed to genotoxic agents (Migliore et al., 2011). In this review, nine studies (Alabi et al., 2020; Berame et al., 2020; Chen et al., 2010; He et al., 2015; Li et al., 2014; Liu et al., 2009; Wang et al., 2011; Wang et al., 2018; Yuan et al., 2008) utilized MN frequency rates as a biomarker of DNA damage associated with e-waste exposure. Five out of the nine studies (Alabi et al., 2020; Berame et al., 2020; Wang et al., 2011; Wang et al., 2018; Yuan et al., 2008) targeted occupationally exposed groups, and the remaining 4 were ecological studies. Almost all the studies were conducted in China except for two (Alabi et al., 2020) and (Berame et al., 2020), which were done in Nigeria and the Philippines, respectively.

In the Nigerian study, Alabi et al. (2020) evaluated micronuclei frequency among teenage e-waste scavengers in the Alaba International market, a major electronic waste dumpsite in Lagos. The results show that the average MN in exfoliated buccal cells in the scavenging e-waste workers (n=95) was significantly higher than that in the control group (n=104) (168.04 vs 3.23, $p<0.001$). Further analysis of other cytogenic alterations, such as markers of cell proliferation (cells with condensed chromatin and binucleated chromatin) and parameters of

cell death (pyconostic and karyorrhective cells), were all significantly higher in the Alaba group than in the control group (all $p<0.01$). Similarly, Berame et al. (2020) measured MN frequency in the buccal epithelium of e-waste recyclers and controls in Payatas, the Philippines. The results showed a significant increase in the number of micronuclei in e-waste workers than controls ($p = 0.00$) (Berame et al., 2020).

All the studies conducted in China reported consistently higher MN in e-waste-exposed populations than in control groups. Wang et al. (2011) and Chen et al. (2010) reported similar results between e-waste-exposed populations and control groups. The median MN frequency was 4% in Guiyu e-waste workers (n=48) compared to 1% in the control group (n=56) ($p<0.05$) (Wang et al., 2011). Similarly, Chen et al. (2010) evaluated MN frequency in residents of an e-waste site and reported a higher median MN frequency (4%) in the e-waste residents than in the control group (1%), $p<0.01$).

In a study of male e-waste recyclers by Wang et al. (2018), a significant increase in the frequency of MN was observed in those with occupational exposures compared with those with no occupational exposures (26.30 vs 4.52%, $p<0.001$). In addition, e-waste workers were classified into exposure durations (≤3, 3-6, and >6 years) to investigate the relationship between e-waste exposure and MN frequency. The results showed a significant positive association between MN and duration working with e-waste (Wang et al., 2018). The results from earlier studies (He et al., 2015; Li et al., 2014; Liu et al., 2009; Yuan et al., 2008) all demonstrated a strong and consistent association between e-waste exposure and MN frequency rates. Overall, these studies consistently indicated a link between e-waste exposure and MN frequency in exfoliated buccal cells and peripheral blood lymphocytes (PBLs).

2.2.9.2.2 Chromosomal aberrations

Three studies examined the association between e-waste exposure and DNA damage using chromosomal aberration (CA) as a biomarker. All three studies showed significantly higher CA frequency among the e-waste exposed group compared to the control groups. Wang et al. (2018) examined DNA damage in peripheral blood lymphocytes among e-waste workers (n=146) and a control group (121) in China. The results of this study showed that the total CA in the e-waste workers was approximately 5-fold higher than that in the control group (8.01% vs 1.8%, $p<0.001$). The duration of e-waste exposure was also positively correlated with CA. However, no significant difference in CA was observed between smoking e-waste workers and nonsmoking workers ($p>0.05$). Similarly, according to Liu et al. (2009), individuals (n=171) recruited from three e-waste polluted villages in northern China had significantly increased levels of total CA than those recruited (n=30) from a neighbouring village with no e-waste exposure (5.50% vs 1.70%, $p<0.001$). The third study by Khlaif and Qumsiyeh (2017) also found a significant mean difference in total CA among the e-waste-exposed group compared to a control group in Palestine (4.83% vs 0.75%, $p<0.001$).

2.2.9.2.3 Comet assay parameters

Six studies (Khlaif & Qumsiyeh, 2017; Li et al., 2008; Liu et al., 2009; Ngo et al., 2020; Wang et al., 2018; Yu et al., 2018) utilized the comet assay to measure DNA damage associated with e-waste disposal. One study (Li et al., 2008) examined the tail injury rates and tail length (TL) of neonates born in Guiyu compared to those born in Chaonan. Tail injury rates and TL were observed to be significantly higher in the Guiyu group than in the control group (33.20 vs 10.7, $p<0.05$) and (4.49 vs 2.09, $p<0.01$), respectively. The measured umbilical cord blood chromium (UCB Cr) level, which was higher in the exposed neonates, correlated positively with DNA damage parameters (tail injury rate and tail length). Similarly, in a recent study in Vietnam, DNA damage in blood cells given as TL, olive tail moment (OTM) and % tail DNA

(%TDNA) were measured in children who resided in an e-waste polluted village and children from a control village. The mean ± standard deviation TL, OTM, and %TDNA of 2.07 ± 0.41, 0.16 ± 0.04, and 2.67 ± 0.42 respectively, in the e-waste exposed children were higher than those in children from a control village with 1.78 ± 0.59, 0.14 ± 0.03, and 2.22 ± 0.40 respectively (p_{all} <0.001) (Ngo et al., 2020).

Liu et al. (2009) examined DNA damage in residents of e-waste processing sites in China. The results showed that the averages of %TDNA (4.27 vs 1.18), TM (0.53 vs 0.05), and OTM (0.82 vs 0.19) were significantly higher in the e-waste-exposed group than in the control group. Among the exposed group, females had a higher degree of DNA damage than their male counterparts. Another study performed by Khlaif and Qumsiyeh (2017) found significantly increased DNA damage parameters (tail and nucleus lengths) in an e-waste-exposed population compared with controls in Palestine.

Yu et al. (2018) also examined TL and %TDNA in blood lymphocytes of men recruited from e-waste dismantling areas in south China to assess semen quality associated with e-waste exposure. The results of this study showed that the average %TDNA and OTM of 57.88 vs 33.55, p<0.001 and 12.3 vs 5.14, p<0.001 respectively, were significantly higher in the exposed group (n=32) than in the control group (n=25). The results indicate a higher risk of infertility in the e-waste-exposed group. Similarly, Wang et al. (2018) found significantly higher levels of DNA damage (represented by %TDNA, TM and OTM) in spermatozoa and lymphocytes of e-waste-exposed men than in those of the controls. Again, comparing 146 adult men directly and actively involved in e-waste processing with 121 adult vegetable farmers who resided approximately 50 km away with no history of e-waste exposure, Wang et al. (2018) reported the duration of exposure to be significantly positively associated with %TDNA and TM of both

spermatozoa and lymphocytes and showed that the e-waste workers were at increased risk of DNA damage compared to the vegetable farmers.

2.2.9.2.4 Oxidative DNA damage

Oxidative DNA damage threatens genome stability and has been implicated in the pathogenesis of chronic diseases, including cancers (Dai et al., 2019; El Hassani et al., 2019). 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is the primary product of oxidative DNA damage and is used as a biomarker of genome stability associated with genotoxic exposure (Miglani et al., 2019). In this review, seven studies (Lu et al., 2016; Neitzel et al., 2020; Ni et al., 2014; Sheng et al., 2008; Wang et al., 2010; Xu et al., 2018; Yuan et al., 2008) examined plasma or urine 8-OHdG as a biomarker of DNA damage associated with e-waste exposure.

The results of these studies were contradictory. Three studies (Ni et al., 2014; Wang et al., 2010; Yuan et al., 2008) did not find a significant difference between the e-waste-exposed population and the control group. Ni et al. (2014) did not find any significant difference in UCB plasma concentration of 8-OHdG in neonates born in Guiyu and those born in a control town (median: 162.9 ng/mL vs 153.69 ng/mL, p=0.117). However, neonates whose mothers engaged in e-waste recycling activities had higher UCB plasma concentrations of 8-OHdG than neonates to mothers who were non-occupationally exposed (median: 179.77 ng/mL vs 159.00 ng/mL, p=0.028). In addition, blood Cd, Cr, and Ni were significantly positively associated with UCB plasma 8-OHdG concentration. In contrast, Wang et al. (2010) found higher urinary 8-OHdG in the non-occupationally exposed group than in the occupationally exposed group (mean creatinine levels: 3.78 vs 3.55 nmol/mol p<0.01). Yuan et al. (2008) also reported a statistically insignificant difference in urinary 8-OHdG among 23 e-waste workers and 26 farmers (mean creatinine levels: 69.04 vs 229.97 µmol/mol p=0.200) in China.

Lu et al. (2016) examined the urinary concentration of 8-OHdG in people living in and around e-waste dismantling facilities (n=130) and in reference populations from rural (24) and urban (22) areas in China. They reported that urinary 8-OHdG concentrations were in the following order: e-waste dismantling area (GM: 16.2 µg/g Cre) > rural reference area (GM: 12.3 µg/g Cre) > urban reference area (GM: 11.6 µg/g Cre). Another study in Thailand found an association between the duration of e-waste exposure and urinary level 8-OHdG. The study found significantly higher urinary 8-OHdG in men who worked ≥ 48 hours/week than in those who worked ≤ 48 hours/week (Neitzel et al., 2020). Similarly, Sheng et al. (2008) found a sharp increase in the urinary level 8-OHdG from the preworkshift (6.40 ± 1.64 µmol/mol) to the postworkshift (24.55 ± 5.96 µmol/mol), $p<0.05$. The rise in postworkshift urinary 8-OHdG levels was attributed to oxidative stress on workers during their work time processing e-waste. Xu et al. (2018) also found elevated blood Pb and Hg levels in preschool children living in e-waste-exposed towns to be significantly associated with urinary 8-OHdG concentration.

2.2.9.2.5 Telomere length

One study (Lin, 2013) examined 227 placentas of healthy puerperae from Guiyu and 93 placentas from a control group to assess placenta telomere length associated with e-waste exposure. The results showed that placental Cd concentration was negatively correlated with placental telomere length ($r = -0.138$, $p = 0.013$) and was observed to be correlated in a dose-dependent manner.

2.2.9.2.6 Apoptosis rate

Regarding the apoptosis rate as a biomarker of DNA damage associated with e-waste exposure, Yu et al. (2018) found a significant increase in the apoptosis rate in spermatozoa of residents of e-waste-exposed towns compared to a control group (32 ± 19% vs 20 ± 8%, $p= 0.037$).

2.2.9.3 Risk of bias (quality) assessment

All studies included in this review were cross-sectional. The risk assessment scores ranged between 3-7 (maximum of 10). Of the 20 studies, 12 studies (Alabi et al., 2020; He et al., 2015; Li et al., 2008; Lin, 2013; Liu et al., 2009; Ngo et al., 2020; Ni et al., 2014; Wang et al., 2010; Wang et al., 2011; Wang et al., 2018; Yu et al., 2018; Yuan et al., 2008) scored above the median score of 5 and were considered high quality. The appraisal details are summarized in (Appendix 5.3).

2.2.9.4 Estimate of variance across studies and pooled outcomes

Of the nine studies that utilized MN assay to measure DNA damage, seven were combined for the meta-analysis. Despite high heterogeneity observed between studies (I^2 = 95.81 %), the meta-analysis showed a significantly higher MN frequency among the e-waste exposed population than the control population with an overall SMD of 2.30 (95% CI: 1.36, 3.24, p-value < 0.001) (Figure 3). Potential publication bias was not explored due to the limited number of studies (<10 studies) included in the meta-analysis.

In a subgroup analysis considering only ecological studies, four studies were included in the analysis. The pooled estimate of the SMD was 1.68 (95% CI: 0.85, 2.51, p < 0.001), with high variability between studies (I^2 = 90.94%) (Figure 4). Considering only studies conducted among e-waste workers (occupational), only three studies were included in the analysis. The combined SMD showed that e-waste recyclers had higher MN than controls (SMD: 3.09 (95% CI: 1.53, 4.66, p < 0.001) (Figure 4). In a further sensitivity analysis stratified by study quality, five studies adjudged "high quality" by the NOS confirmed that MN frequency was higher among the e-waste exposed group compared to the controls (MSD: 2.80, 95% CI: 1.79, 3.81, p < 0.001) (Figure 5). Only two studies were considered "low quality" and did not show a significant difference in MN frequency between the two groups (p = 0.35).

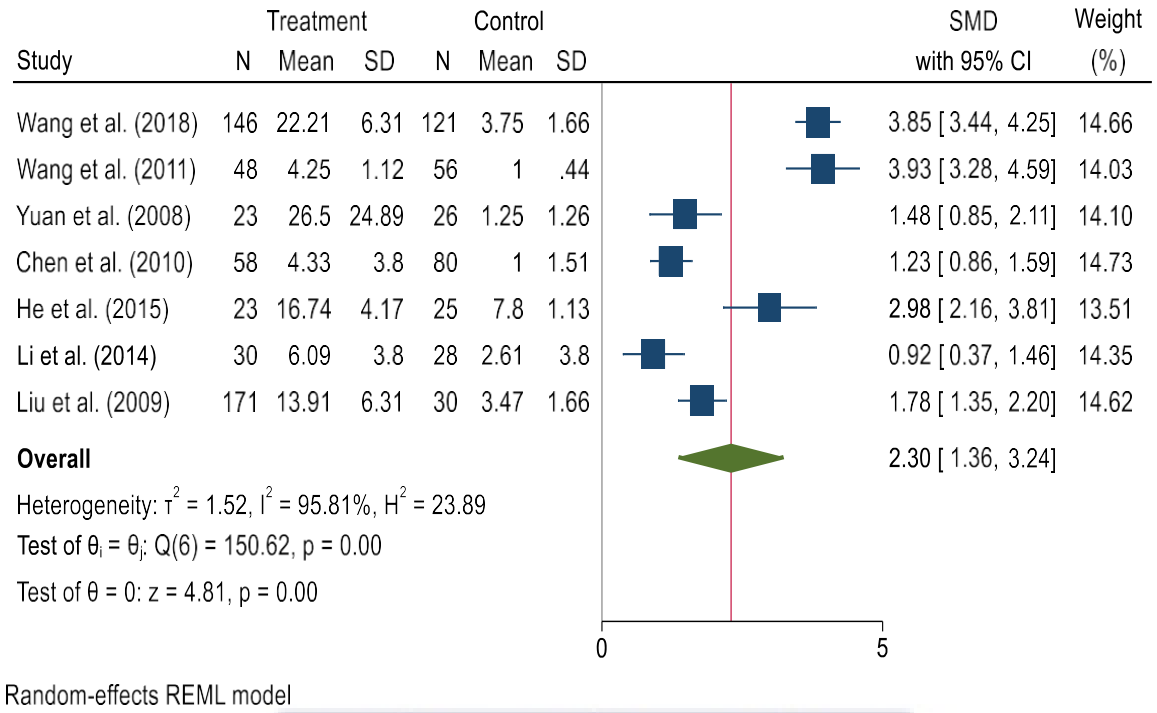


Figure 3: Standardized mean difference of micronuclei frequency for e-waste exposed population vs control.

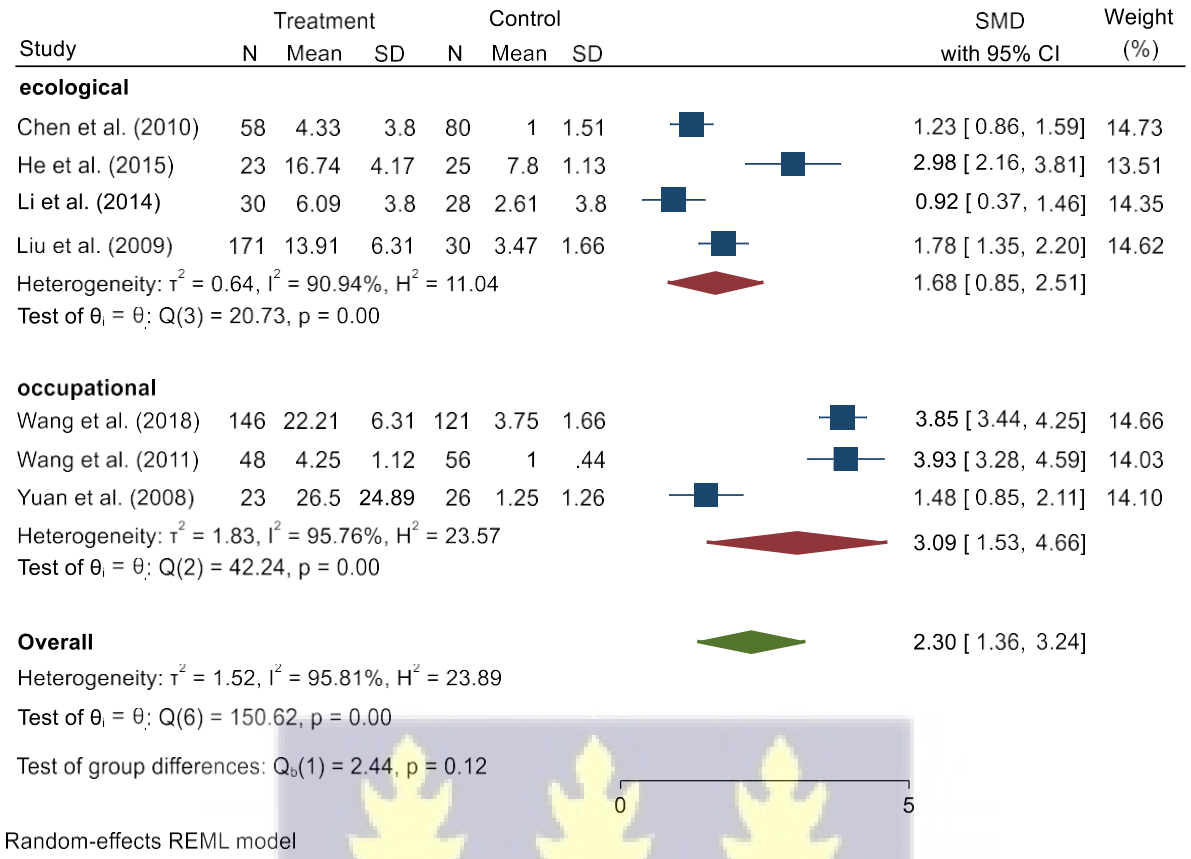


Figure 4: Sub-group analysis by study setting

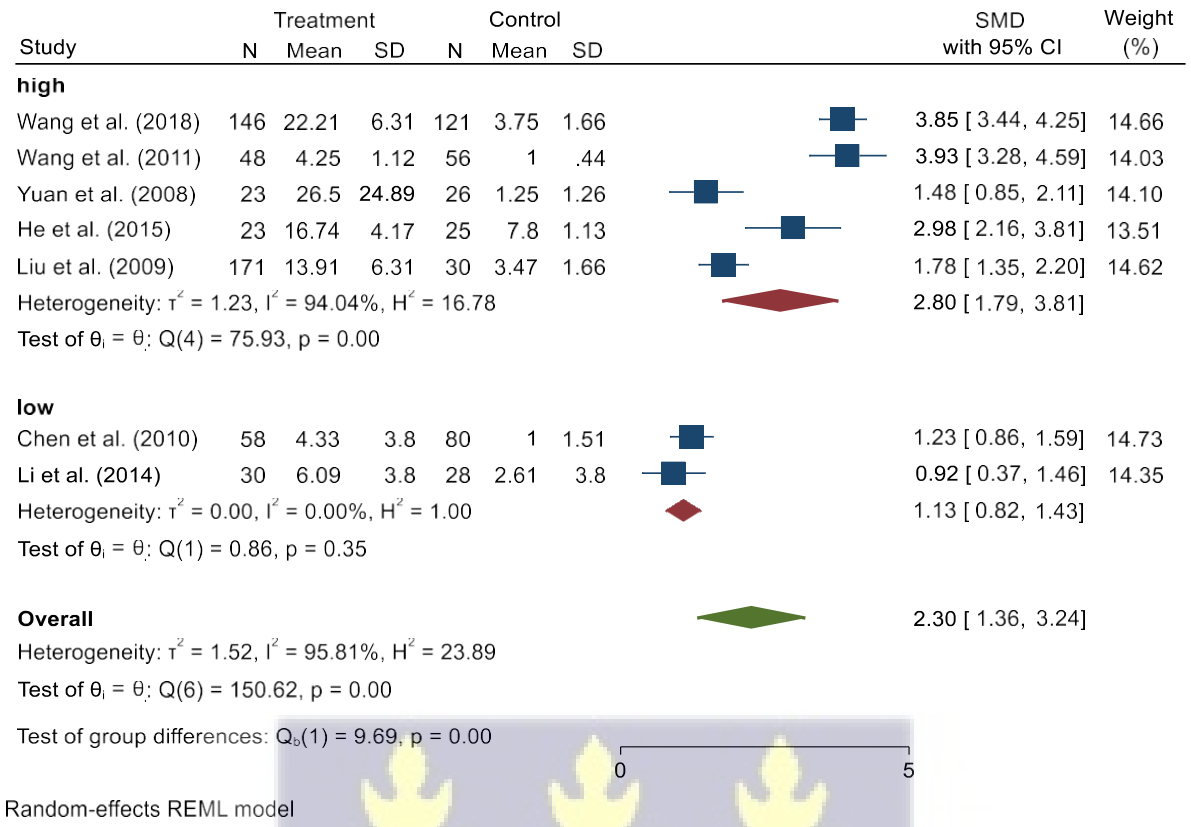


Figure 5: Sub-group analysis by study quality

2.2.10 Discussion

To the best of my knowledge the systematic review with meta-analysis is the first to specifically assess the risk of DNA damage associated with e-waste processing/disposal in human populations. The review identified and evaluated 20 studies that investigated associations between e-waste exposure and various biomarkers of DNA damage. Nine of these studies measured DNA damage by MN assay of which seven were deemed combinable for meta-analysis. The review provides ample evidence of the deleterious effects of e-waste processing on DNA integrity, as evaluated through well-known DNA damage biomarker assays.

Despite high heterogeneity between studies, the overall standardized mean difference (SMD) estimate showed higher MN frequency among the e-waste exposed group compared with the

control group with an effect size (SMD) of 2.30 (95% CI =1.36, 3.24). Subgroup analysis by study setting revealed that workers who directly recycle e-waste (occupational) had higher MN frequency (SMD: 3.09, 95% CI: 1.53, 4.66, $p < 0.001$) compared to occupationally unexposed individuals (SMD: 1.68, 95% CI: 0.85, 2.51, $p < 0.001$). A possible explanation for these results might be that e-waste workers in the informal sector are continuously involved in multiple tasks and work in the open using rudimentary tools with little or no use of personal protective equipment (Tue et al., 2016; Zhang et al., 2019a). This practice exposes the recyclers to higher levels of toxic chemicals compared to the general population (Song & Li, 2015). The frequency of MN is generally used as a biomarker of effect associated with exposure to genotoxic chemicals (Bolognesi & Holland, 2019; Panico et al., 2020). Therefore, chronic exposure to these toxic chemicals may result in some degree of DNA damage as explained by the increased levels of MN in e-waste recyclers compared to the non-occupational exposed group.

Even though all studies included in the meta-analysis were conducted in China and had similar exposure profiles, substantial heterogeneity still existed. The high variability between studies could be attributed to participant's characteristics such as age, gender, diet, and lifestyle factors (e.g., smoking, alcohol intake, and recreational drugs) which may influence MN frequency in peripheral blood leukocytes (PBL) (Fenech & Bonassi, 2011). Except for the study done by Li et al. (2014), which did not control for any confounders, the remaining seven studies controlled for confounding by smoking and other important risk factors through data collection and analysis. However, four studies (Liu et al., 2009; Wang et al., 2011; Wang et al., 2018; Yuan et al., 2008) that controlled for confounding by smoking did not provide quantitative data about smoking, which could result in misleading conclusions when comparing smokers and non-smokers (Fenech & Bonassi, 2011). Given the causal role of MN in cancer development (Bonassi et al., 2007) and the evidence of their increased levels shown in this review, MN could be a primary biomarker for assessing DNA damage in e-waste-exposed populations.

The review also found that other DNA damage biomarkers, including chromosomal aberrations, tail length (TL), percent tail DNA (%TDNA) tail moment (TM) and 8-OHdG showed a consistent higher frequency among the e-waste exposed group than the controls. For instance, three studies in this review assessed chromosomal aberrations (CAs) associated with e-waste exposure. Wang et al. (2018) demonstrated increased CA levels in the PBL of e-waste workers compared to controls in China. Similarly, Liu et al. (2009) showed that individuals living in e-waste polluted villages in northern China had higher CA levels than those living in a neighbouring village with no e-waste exposure. A similar study conducted in Palestine by Khlaif and Qumsiyeh (2017) reported increased CA levels in an e-waste-exposed population compared with controls. Induced chromosomal aberrations are useful biomarkers of occupational and environmental exposures to genotoxic agents and are predictive of future cancer risk (Bonassi et al., 2004; Hagmar et al., 1994). This review's results are consistent with a recent meta-analysis that concluded that occupational exposure to genotoxic agents such as benzene was associated with CA and MN frequencies (Scholten et al., 2019). Other studies have found increased frequencies of CA in foundry workers (Hasani et al., 2016) and farmers (Bianco et al., 2017).

Six studies adopted the comet assay (single-cell gel electrophoresis assay) to assess DNA damage associated with e-waste disposal. Primary comet assay measurements, including tail length (TL) and a fraction of DNA in the tail (% tail DNA), and derived indices, including tail moment (TM) (tail length x % tail DNA) and olive tail moment (OTM) (the distance between the centres of gravity of the head and the tail along the x-axis of the comet x %TDNA) (Mozaffarieh et al., 2008), were used to determine DNA damage levels associated with e-waste exposure. The comet assay is a versatile, economical, and fast technique that is widely used in biomonitoring human exposure to mutagenic agents and is considered one of the most reliable biomarkers of early biological effects (Anderson, Dhawan, & Laubenthal, 2013). However, the

available literature does not consider the comet assay to be predictive of cancer risk (Intranuovo et al., 2018). Overall, all the studies reviewed demonstrated strong and consistent relationships between e-waste exposure and DNA damage, represented by TL, %TDNA, TM and OTM, as determined by the comet assay. These results may be explained by the fact that majority of the studies were conducted in and around Guiyu, China. Guiyu is noted for informal e-waste recycling and other industrial activities contributing significantly to environmental pollution (Li et al., 2019; Song & Li, 2015). E-waste recyclers and residents may be exposed to high levels of potential clastogens and aneugens, which may damage the DNA, as observed in this systematic review's findings. Genotoxic chemicals released during e-waste recycling such as metals and other persistent organic pollutants (POPs) are suggested to induce DNA damage through direct interaction with the DNA (Alabi & Bakare, 2011). The results of this review are similar to those reported by Villarini et al. (2015) and Cayir et al. (2019), where DNA damage measured by the comet assay was higher among welders exposed to magnetics and farmers exposed to pesticides, respectively, than the general population.

Three of the seven studies (Ni et al., 2014; Wang et al., 2010; Yuan et al., 2008) that examined DNA damage using 8-OHdG did not find any significant differences between e-waste-exposed populations and the reference populations. Several factors could account for the lack of differences observed in these studies. First, Yuan et al. (2008) recruited only 49 (23 exposed and 26 controls) participants in their study. This small sample size may lack the power to detect any differences between the groups. In addition, the biological matrix used to measure 8-OHdG concentration could affect the results of these studies. For instance, urine was widely used to measure 8-OHdG in e-waste workers and controls. Although useful, urinary 8-OHdG is considered a less sensitive and accurate biomarker compared to peripheral blood leukocytes (PBL) 8-OHdG levels (Wu et al., 2017). None of the studies in this review measured 8-OHdG in PBL, representing a long-term response to oxidative stress and a more accurate measure of

the body burden of DNA damage lesions (Wu et al., 2017). However, direct involvement in e-waste recycling was consistently associated with 8-OHdG levels. For example, neonates of mothers who were directly involved in the processing of e-waste had a significantly higher umbilical cord blood (UCB) plasma 8-OHdG than neonates whose mothers were non-occupationally exposed to e-waste (Ni et al., 2014). This could be attributed to the higher concentrations of metals detected in mothers who recycle e-waste, as evidenced by 8-OHdG been positively associated with Cd, Cr, and Ni concentrations ($p_{all} < 0.05$) (Ni et al., 2014). In addition, Neitzel et al. (2020) found a significant association between increased work duration and urinary 8-OHdG concentration, whiles Sheng et al. (2008) observed a significant difference between preworkshift and postworkshift urinary levels of 8-OHdG in the urine of e-waste workers.

The current review is not without limitations. First, because most of the studies included in this review (75%) were conducted in China, results and conclusions may not be transferable to other populations. In addition, the current review cannot demonstrate a causal relationship between e-waste exposure and DNA damage since all the studies included are cross-sectional. Future studies should consider longitudinal studies that will allow researchers to evaluate the causal relationships between e-waste exposure and DNA damage by assessing factors such as temporality and dose-response relationships.

Second, most of the studies included suffered from inadequate sample sizes and non-reporting of response rates and were limited to convenience samples. Only six studies out of the 20 studies had sample sizes > 200. In addition, only two studies randomly recruited participants, and no study justified the sample sizes used or reported on the response rates. Future studies should consider robust methods that will enable the generalizability of study findings by including sample size calculations and reporting participant's response rates.

Third, to date, far too little attention has been paid to susceptible populations, such as neonates and children, with an increased risk of exposure due to extra exposure routes (breastfeeding and hand-to-mouth behaviour) and lower toxic elimination rates. Only four studies targeted neonates and children in this review. There is, therefore, the need to scale up research involving these groups of people since some of the chemicals released during e-waste recycling are known neurodevelopmental toxicants.

Finally, the use of urinary 8-OHdG concentration to measure DNA damage may not provide an adequate measure of DNA damage. Future studies should consider measuring DNA adducts from the blood, which provides an integrated measure of exposure to the chemicals of interest, their ability to escape detoxification (metabolic activation) and to be delivered to the target macromolecules in target tissues, and the efficiency of the body's DNA repair pathways (Phillips, 2005; Rundle, 2006).

2.2.11 Conclusion

Despite the limitations outlined above, the evidence from this review suggests that occupational and non-occupational exposures to e-waste are associated with an increased risk of DNA damage measured through an MN frequency and other wide range of DNA damage biomarkers. Overall, sensitive, reliable and cost-efficient assays including comet, and micronuclei assays, were used to measure DNA damage. Therefore, the findings of these studies suggest that chronic exposure to e-waste could be predictive of future cancer risk to people who directly process e-waste and residents of e-waste polluted towns. However, future studies should look beyond China and consider e-waste recyclers in other developing countries in Africa, Latin America, and South Asia to increase these findings' generalizability. In addition, other DNA modifications, including epigenetic markers such as DNA methylation, post-translational histone modifications and micro RNA frequencies, should be considered in future investigations to provide further elucidation on the mechanisms of e-waste induced health

effects. I, therefore, propose to conduct a primary study at the Agbogbloshie e-waste recycling site in Accra, Ghana, to analyze DNA methylation of long interspersed nucleotide element-1 (LINE-1) as a proxy for global DNA methylation among informal e-waste recyclers. I also intend to apply robust statistical techniques for estimating the health effects of multi-pollutant mixtures to estimate DNA methylation associated with the mixture of metals, which represent the reality of e-waste exposure other than estimating the effect of one chemical at a time.

2.3 Electrical and electronic waste (e-waste) as a public health concern

Electronic waste (e-waste) is defined as "any discarded, obsolete, or broken electrical or electronic devices or products nearing the end of their useful life" (Zeng et al., 2017). E-waste has become a global public health concern due to the continual increase in its generation, which is mainly fuelled by the high consumption of electrical and electronic equipment (EEE); short life cycles; ease of replacement; and few repair options (Ikhlayel, 2018). Approximately 53.6 million metric tons (Mt) of e-waste was generated globally in 2019 and projected to exceed 74 Mt in 2030. However, only about 17.4% of the e-waste generated in 2019 was formally collected and recycled in an environmentally friendly manner, leaving over 80% to be handled by the informal sector (Forti et al., 2020). Developing countries in Africa and Asia primarily receive this waste due to the high cost associated with formal collection and recycling (Ackah, 2017). These receiving countries ironically do not have the resources or political will to sufficiently equip the e-waste recycling infrastructure or put regulatory guidelines to deal with the increasing volumes of e-waste in a manner that will help recover valuable components and also protect human health and the environment (Fowler, 2017; Olds, 2012).

In Ghana, for example, there are no proper recycling facilities and industry standards. There is also a palpable absence of clear and specific national regulation to define, control, and forbid e-waste recycling (Liu et al., 2012). E-waste is thus seen as a crisis not only by quantity alone but also of toxic components such as lead, chromium, cadmium, and arsenic (Song & Li, 2015;

Yu et al., 2017). It is reported that in 2009, about 171,000 tons of e-waste found its way into the informal recycling sector in Ghana which is estimated to be two times greater by the year 2020 (Liu et al., 2012). Countries noted to have a high degree of e-waste contamination with inadequate management practices are; China (Guiyu and Taizhou), India (Delhi and Bengaluru), Nigeria (Lagos), and Ghana (Accra), (Xu et al., 2015).

Electrical and electronic equipment is made up of a variety of materials such as glass, rubber, printed circuit board, plastics, wood, ceramics, ferrous and non-ferrous metals, and other materials (Alabi & Bakare, 2017; Tsydenova & Bengtsson, 2011). E-waste is a mix of bio-accumulative, non-degradable and carcinogenic toxins such as cadmium, lead, mercury, and brominated flame retardants (Alabi & Bakare, 2017; Bakhiyi et al., 2018; Fowler, 2017), as well as precious materials such as gold, copper, platinum, palladium, silver, and aluminium (Bakhiyi et al., 2018; Ikhlayel, 2017). E-waste, therefore, cannot be treated like any other type of solid waste (Zeng et al., 2017), because of its inherent capacity to be both a source of precious materials and a toxic contaminant (Amankwaa et al., 2017; Dias et al., 2019; Olds, 2012). E-waste requires adequate recycling to recover valuable and precious materials as well as protect human health and the environment (Ikhlayel, 2017). Many developing countries are faced with the problem of processing their own generated e-waste, or e-waste imported illegally as used products from developed countries resulting in a large proportion of the components been disposed of in uncontrolled landfill sites (Ikhlayel, 2018), with little or no regulation for its recycling or disposal (Alabi et al., 2012).

2.3.1 E-waste recycling and heavy metals exposure

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Tchounwou et al., 2012). Heavy metals are ubiquitous environmental and occupational pollutants and have been implicated in numerous adverse health outcomes, including cancers,

cardiovascular diseases, neurological diseases, reproductive toxicity, renal dysfunction and autoimmune diseases (Hu, 2002a; Rzymski et al., 2015; Shi et al., 2019). The recycling of e-waste in both formal and informal settings expose workers to a plethora of hazardous chemicals such as heavy metals (Song & Li, 2014b).

This section of the literature review covers studies across the globe that evaluated heavy metals exposure among e-waste workers. Eighteen peer-reviewed original articles were retrieved from two databases (Scopus and MEDLINE) using the following search strategy: ("e-waste" OR "electronic waste" OR "WEEE") AND ("heavy metals" OR "arsenic" OR "cadmium" OR "lead" OR "chromium" OR "nickel" OR "manganese") AND ("workers" OR "recyclers" OR "occupation"). Of the 18 studies, six were performed in Ghana (Amankwaa et al., 2017; Asante et al., 2012; Srigboh et al., 2016; Takyi et al., 2021; Wittsiepe et al., 2017b; Yang et al., 2020b), three in Thailand (Kiddee & Decharat, 2018; Neitzel et al., 2020; Sirichai, Prueksasit, & Sangsuthum, 2020), two in China (Wang et al., 2011; Wang et al., 2018), one in the Philippines (Alam, Ang, & Bondoc, 2018), one in Sweden (Julander et al., 2014), three in India (Ha et al., 2009; Singh et al., 2018; Upadhyay et al., 2020), and two in Nigeria (Alabi et al., 2020; Igharo et al., 2020). Almost all the studies except two (Julander et al., 2014; Upadhyay et al., 2020), were conducted in the informal sector. Table 3 gives a summary of the studies included in this review.

Various biological matrices - blood, plasma, serum, urine, hair, and skin were used to estimate the body burden of heavy metals (essential and toxic metals) in e-waste workers. Among the studies conducted in Ghana, the majority of the studies reported elevated levels of heavy metals in e-waste workers compared to controls. For example, Wittsiepe et al. (2017a), examined the body burden of heavy metals in e-waste workers at the Agbogbloshie e-waste recycling site in Ghana. The results showed that the median concentration of blood Pb (88.5 vs 41.0 µg/l, p <

0.001) was significantly higher in e-waste workers than in controls. In the same vain, urine Cd, Cr, and Ni were higher in the e-waste worker group than in controls. In contrast, hair Hg levels were higher among the controls than the e-waste worker group (0.43 vs 0.72, $p < 0.001$) (Wittsiepe et al., 2017b). Other studies evaluated heavy metals in individuals who directly recycle e-waste and those not involved in e-waste work at the Agbogbloshie recycling site (Amankwaa et al., 2017; Srigboh et al., 2016). Amankwaa et al. (2017) found that blood Pb levels in the non-e-waste workers at the recycling site were higher (3.54 µg/dL) than those that were directly recycling e-waste (3.49 µg/dL), though not significant. This highlights the fact that the hazardous impacts of e-waste could be extended to other workers and residents around the recycling site. Similarly, Srigboh et al. (2016) found elevated levels of blood Cd and Pb as well as urine As in e-waste workers compared to the general population. In addition, e-waste burners had the highest levels of heavy metals within the worker's group. Recently, Yang et al. (2020b) reported that the median concentrations of inorganic As (As(III), As(V), MMA, DMA) were higher in e-waste workers compared to controls. In addition, Takyi et al. (2021) further reported higher body burden of 'technology-critical elements' such as Strontium (Sr), Thallium (Tl), Europium (Eu), Lanthanum (La), and Terbium (Tb) in e-waste workers than reference population in Ghana.

Three studies were conducted in Thailand to evaluate the levels of heavy metals in the body of e-waste workers. Two of the studies did not include a comparator group and reported lower levels of blood Pb in e-waste workers compared to the Thai occupational exposure limit of 60 µg/dL (Kiddee & Decharat, 2018; Neitzel et al., 2020). The remaining study found significantly higher mean blood Pb level in e-waste workers (6.61±3.07 µg/dl) than that of non-e-waste workers (2.73±0.49 µg/dl), but not blood Cd (Sirichai et al., 2020). Another study conducted in the Philippines using hair matrix to establish the toxicity of e-waste in informal recyclers

reported that Cu, Pb, and K in the hair samples of e-waste workers were significantly higher than those of controls (Alam et al., 2018).

In the Chinese studies, Wang et al. (2011) estimated the concentration of Pb, Cd, and Cu in blood and urine of e-waste workers (n=48), and 56-age and sex-matched controls. The results showed that blood lead levels were significantly higher in the e-waste worker group (median: 11.449 µg/dL, 1st/3rd quartiles: 9.351–14.410 µg/dL) than in the control group (median: 9.104 µg/dL, 1st/3rd quartiles: 7.275–11.389 µg/dL). In contrast, urine Cd was significantly higher in controls (median: 0.002 µg/dL, 1st/3rd quartiles: 0.001–0.004 µg/dL) than in e-waste workers (median: 0.001µg/dL, 1st/3rd quartiles: 0.001–0.002 µg/dL). Similarly, Wang et al. (2018) measured heavy metals in the serum of e-waste workers and controls and reported higher serum Pb in the e-waste workers than in controls. However, the concentrations of essential trace elements (Ca and Mg) were significantly higher in the control group than in the e-waste worker group, while no significant differences in the concentrations of Cu, Zn, Fe or Se in the serum between the two groups was observed (Wang et al., 2018).

The study conducted in Sweden examined the concentration of 20 heavy metals in whole blood, plasma and urine of formal e-waste recyclers (n=55) who have access to proper recycling infrastructure and worker protection compared to the concentrations in office workers (n=10) with no e-waste exposure (Julander et al., 2014). The study found significantly higher concentrations of chromium, cobalt, indium, lead, and mercury in blood, urine, and/or plasma of the recycling workers, compared with the office workers. Another study conducted among formal e-waste recyclers was done in India (Upadhyay et al., 2020). Upadhyay et al. (2020) in their study did not include a comparator group. The concentration of Pb in the blood of e-waste recyclers in this study were all within the acceptable exposure limits. However, environmental Pb exposure correlated significantly with blood Pb levels. Other studies from India also

reported higher levels of heavy metals measured in the skin (Singh et al., 2018) and hair (Ha et al., 2009) of e-waste workers compared to controls.

Alabi et al. (2020) assessed the concentrations of heavy metals in e-waste scavengers in Nigeria compared to a control group with no e-waste exposure. The results showed that the median concentrations of blood Pb (19.55 vs 1.50 µg/L, p=0.01), Cd (1.43 vs 0.18 µg/L, p=0.05), Cr (2.31 vs 0.02, p=0.01), and Ni (1.05 vs 0.01 µg/L, p=0.01) were significantly higher in the exposed scavengers than in the controls. In the same vain, other investigators estimated Pb and Cd in whole blood of occupationally exposed e-waste workers in Nigeria. The mean blood Pb and Cd were significantly higher in the e-waste workers than in the controls (0.05 µmol/L vs 0. 03 µmol/L, p=0.000 and 103.20 nmol/L vs 54.65 nmol/L, p=0.000, respectively).

Table 3: Previous epidemiological studies of exposure to heavy metals associated with e-waste work

Author (year)	Country	Industry	Exposed group	Sample(s)	Metal(s)	Main findings
Alabi et al. (2020)	Nigeria	Informal	E-waste scavengers (n=95) vs controls (n=104)	Serum	Pb, Ni, Cd, and Cr	The median concentrations of blood Pb (19.55 vs 1.50 µg/L, p=0.01), Cd (1.43 vs 0.18 µg/L, p=0.05), Cr (2.31 vs 0.02, p=0.01), and Ni (1.05 vs 0.01 µg/L, p=0.01) were significantly higher in the e-waste scavenger group compared to the control group
Alam et al. (2018)	Philippines	Informal	Recyclers (n=) vs controls (n=50)	Hair	Essential (Zn, Mg, K, Fe, Mn), toxic (Cd, Cu, Cr, Pb and Ni)	Cu, Pb, and K were significantly higher in the e-waste recyclers than the controls. Pb, Cu, Ni, and Cd in recyclers were higher than the permissible limits.
Amankwaa et al. (2017)	Ghana	Informal	e-waste workers (n=81), non-e-waste workers (n=33) vs controls (n=14)	Blood	Pb	Workers who engaged in e-waste burning had high blood Pb levels. No difference between the BLLs of e-waste workers and workers not in e-waste, indicating high environmental exposure.
Asante et al. (2012)	Ghana	Informal	Recyclers (n=20) vs reference-1 (gold mining town) (n=25) and reference-2 (n=3)	Urine	Multiple trace elements (TEs) and As	Concentrations of Fe, Sb, and Pb in the urine of e-waste recycling workers were significantly higher than those of reference sites after consideration of interaction by age, indicating that the recycling workers are exposed to these TEs through the recycling activity.

Table 3: continued

Author (year)	Country	Industry	Exposed group	Sample(s)	Metal(s)	Main findings
Ha et al. (2009)	India	Informal	Recyclers (n=11) vs controls (n=8)	Hair	Trace elements (TEs)	High levels of Cu, Mo, Ag, Cd, In, Sb, Tl, and Pb were observed in the hair of male workers from e-waste recycling sites, indicating the influence of recycling activities.
Igharo et al. (2020)	Nigeria	Informal	e-waste workers (n=63) vs controls (n=41)	Blood	Cd and Pb	E-waste workers had higher blood Cd and Pb levels than non-e-waste workers
Julander et al. (2014)	Sweden	Formal	Recyclers (n=55) vs office workers (n=10)	Blood, plasma, and urine	Sb, As, Be, Cd, Cr, Co, Cu, Ga, In, Fe, Pb, Mn, Hg, Mo, Ni, Pt, Tl, W, V and Zn	Recycling workers showed higher concentration of Cr, Co, In, Pb, and Hg, in blood, urine, and/or plasma compared with the office workers
Kiddee and Decharat (2018)	Thailand	Informal	E-waste workers (n=71)	Blood	Pb and Cd	Blood Pb levels in e-waste workers were lower (median: 4µg/dL) than the Thai acceptable biological exposure index of 30 µg/dL.
Neitzel et al. (2020)	Thailand	Informal	E-waste workers (n=120)	Blood and urine	Cd, Pb, and Mn	significantly higher levels of Cd and Pb were observed in the blood of men compared with those in women
Singh et al. (2018)	India	Informal	e-waste workers (n=20) vs reference group (n=	Skin	As, Cu, Co, Cd, Cr, Ni, Fe, Zn, Pb, Ba	Mean concentrations of Cr, Cu, Pb and Zn in the dermal samples of the e-waste workers were 192.59, 78.08, 30.87 and 37.3 times higher than the reference sample

Table 3 continued

Author (year)	Country	Industry	Exposure group	Sample(s)	Metal(s)	Main findings
Sirichai et al. (2020)	Thailand	Informal	e-waste workers (n=30) vs non-e-waste workers (n=30)	Blood	Pb and Cd	Mean Pb levels of the e-waste workers (6.61±3.07 µg/dl) were significantly higher than those of the non-e-waste workers (2.73±0.49 µg/dl). No difference in Cd between groups.
Srigboh et al. (2016)	Ghana	Informal	e-waste workers (n=58) vs non-e-waste workers (n=11)	Blood and urine	essential (Cu, Fe, Mn, Se, Zn) and toxic (As, Cd, Co, Cr, Hg, Ni, Pb)	Many blood and urinary elements) were within reference ranges. Blood Cd, Pb and urine As were higher in e-waste workers than the general population. E-waste burners had the highest body burden of metals,
Takyi et al. (2021)	Ghana	Informal	e-waste workers (n=100) vs non-e-waste workers (n=51)	Blood and urine	17 elements (Ag, As, Ba, Cd, Ce, Cr, Eu, La, Mn, Nd, Ni, Pb, Rb, Sr, Tb, Tl, Y)	Mean levels of blood Pb, Sr, Tl, and urinary Pb, Eu, La, Tb, and Tl were significantly higher in recyclers than controls. In general, the collectors and sorters tended to have higher elemental levels than other work groups.
Upadhyay et al. (2020)	India	Formal	e-waste workers (n=64)	Blood	Pb	BLLs were within permissible limits. Workers exposed to higher environmental lead levels had significantly higher BLL
Wang et al. (2011)	China	Informal	e-waste workers (n=48) vs controls (n=56)	Bold and urine	Pb, Cu, and Cd	BLLs of the e-waste workers were significantly higher (median: 11.5 µg/dL) than those in the control group (9.1 µg/dL). In contrast, urine Cd was significantly higher in controls than in e-waste workers.
Wang et al. (2018)	China	Informal	e-waste workers (n= 146) vs controls (n=121)	Serum	Pb, Cu, Zn, Ca, Mg, Fe and Se	Serum Pb was significantly higher in e-waste workers than in controls. However, Ca and Mg were significantly lower e-waste workers compared to the control group
Wittsiepe et al. (2017b)	Ghana	Informal	E-waste workers (n=75) vs controls (n=40)	Blood and hair	Cd, Ni, Cr, Hg, Pb	The blood lead levels were higher in the exposed group (median: 88.5 vs 41 µg/ l). Similarly, urine Cd, Cr, and Ni were higher in e-waste workers. In contrast, hair Hg was

						higher in the controls than in e-waste workers.
Yang et al. (2020b)	Ghana	Informal	E-waste workers (n= 84) vs controls (n= 94)	Urine	Inorganic As	E-waste workers showed higher levels of inorganic As compared to controls. Burners had the highest levels of inorganic arsenic in the e-waste group.

2.3.2 E-waste recycling and particulate matter (PM) exposure

Particulate matter (PM) is a heterogeneous mixture of chemicals suspended in the air that originates from multiple sources, such as combustion products from biomass burning for household use, vehicular emissions, and suspension of soil particles through road dust (Pipal, Kulshrestha, & Taneja, 2011). In addition, occupational activities such as agriculture, construction, and mining present another essential source of PM exposure (Garcia et al., 2013; Gautam, Kumar, & Patra, 2016; Kim, Kabir, & Kabir, 2015).

Particulate matter varies significantly in composition and particle size. The major components of PM include nitrates; sulfates; elemental and organic carbon; organic compounds (e.g., PAHs); biological compounds (e.g., endotoxin, cell fragments); and metals (e.g., iron, copper, nickel, zinc, and vanadium) (Gao et al., 2018; Kim et al., 2015). Based on particle size, PM is categorized into three major fractions: (i) coarse PM with aerodynamic diameter ≤ 10 μm (PM_{10}), defined as inhalable particles that can penetrate the lungs, (ii) fine PM with aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$), defined as respirable and can enter the alveoli, and (iii) ultrafine PM with aerodynamic diameter ≤ 0.1 μm ($PM_{0.1}$) (Pipal et al., 2011; Sun et al., 2018). The toxicity of PM is highly correlated with particle size, where smaller particles are associated with increased toxicity (Stanek et al., 2011).

The informal electronic waste (e-waste) recycling industry where proper regulation and controls are lax or absent, and worker protection is often inadequate, has emerged as a significant source of occupational particulate matter (PM) air pollution, especially in developing countries (Bi et al., 2010; Zhang et al., 2019a). Combustion products from burning e-waste generate fine PM, which is linked to pulmonary and cardiovascular diseases (Franchini & Mannucci, 2012; Jin et al., 2015; Kuang et al., 2019). A recent study revealed elevated ambient PM levels at the Agbogbloshie e-waste site over background levels (Kwarteng et al.,

2020). Another study by Amoabeng et al. (2020) , showed an association between PM and adverse respiratory effects in e-waste workers compared to controls.

Previous studies have reported elevated PM exposure in e-waste workers based on personal sampling. At the Agbogbloshie e-waste recycling site, Laskaris et al. (2019) reported that individuals who work on site had significantly higher mean $PM_{2.5}$ compared to those who work off-site (85 vs 71 µg/m^3, p = 0.014). In addition, workers who burned e-waste had the highest mean personal $PM_{2.5}$ exposure (Laskaris et al., 2019). Similarly in Thailand, other researchers found that open burning of e-waste in the Buriram Province resulted in elevated levels of the average concentrations of $PM_{2.5}$ and PM_{10} (2774 µg/m^3 and 3215 µg/m^3, respectively), in e-waste workers (N=33) (Bungadaeng, Prueksasit, & Siriwong, 2019). These high levels of PM among e-waste workers, particularly e-waste burners, might be attributed to the release of fumes and smoke from e-waste combustion, and other mechanical processes (dismantling, sorting, shredding and transportation) which also releases dust into the ambient air (Kwarteng et al., 2020).

2.4 Overview of DNA methylation

The most stable and widely studied epigenetic modification is DNA methylation. It is the addition of a methyl group (CH_3) from the universal methyl donor S-adenosyl-methionine (SAM) to the 5-carbon of the pyrimidine cytosine to form 5-methylcytosine (Laird, 2003; Pogribny, 2019) (Figure 6). DNA methyltransferases are the enzymes that catalyze the methylation reaction utilizing a family of *de novo* methyltransferases (DNMT3A and DNMT3B) to primarily methylate new sites and a maintenance methyltransferase (DNMT1) to restore hemimethylated sites to full methylation (Koturbash, Beland, & Pogribny, 2011; Laird, 2003). 5-methylcytosines are mostly observed within CpG dinucleotides, and approximately 70-90% of them are methylated (Koturbash et al., 2011; Pogribny, 2019).

Majority of methylation reaction occurs at the repeated sequence of the DNA and at the CpG depleted regions of the genome (exonic, intronic, and intergenic regions), whereas high-density CpG sites (CpG islands) around promoter regions of housekeeping genes are demethylated (Kulis & Esteller, 2010; Pogribny, 2019). This makes the overall methylation pattern of the DNA in normal cells bimodal (Bergman & Cedar, 2013) (Figure 7).

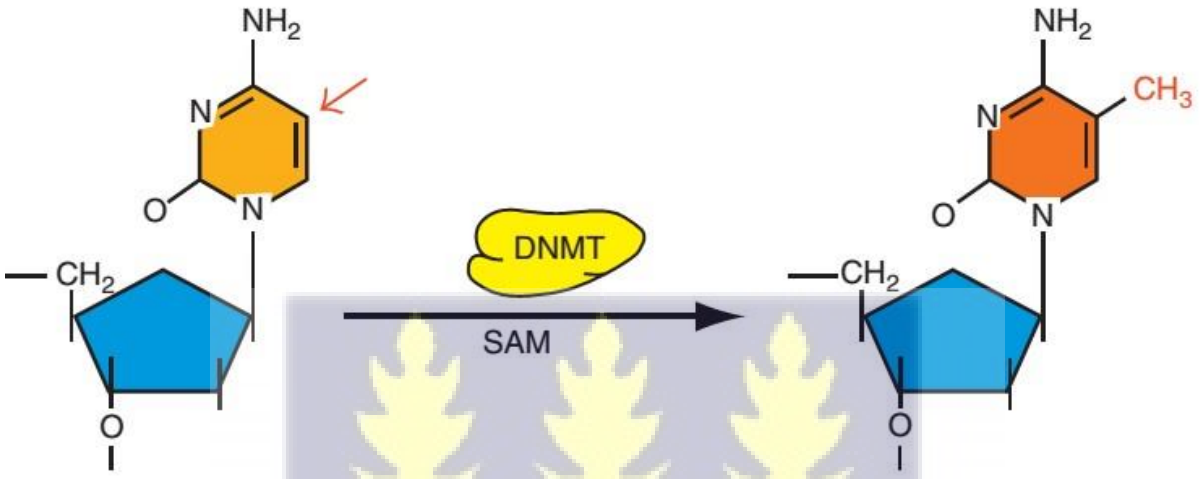


Figure 6: Methylation at the 5' position of the cytosine moiety is catalyzed by DNMT in the presence of S-adenosyl-methionine (SAM)

Source: (Kulis & Esteller, 2010)

Figure 7: Bimodal pattern of DNA methylation. Unmethylated promotor CpG Island and methylated intronic, intergenic, and repetitive sequences.

Unlike germline DNA sequences, DNA methylation patterns are not inherited from parent to child; rather, the methylation patterns acquired from the gametes are rapidly erased in the zygote, giving way for the bimodal methylation pattern to be reset during implantation to

facilitate cell lineage differentiation (Bergman & Cedar, 2013; Cedar & Bergman, 2012; Dor & Cedar, 2018). The resetting of the DNA patterns during embryogenesis is presumed to maintain the stability of the genome expression patterns by repressing genes in other to avoid ectopic gene expression while allowing genes such as housekeeping genes to be expressed in various cell types (Dor & Cedar, 2018). Studies have demonstrated that the relationship between DNA methylation and gene control is largely influenced by the location in which methylation occurs (Jones, 2012; Pisanic II, Athamanolap, & Wang, 2017). For example, methylation within promoter or 5' CpG islands regulates local gene expression by silencing gene transcription (Pisanic II et al., 2017; Wright et al., 2010), while methylation within repetitive sequences is vital for chromosomal stability by suppressing their expression (Ma et al., 2013).

Although DNA methylation is considered a relatively stable heritable event with well-established enzymes mediating the process, recent discoveries have identified mechanisms involved in demethylation. These mechanisms are replication-dependent "passive" and replication-independent "active" demethylation (Biswas & Rao, 2017). The discovery of ten-eleven translocation (TET) proteins has elucidated the mechanisms involved in both passive and active demethylation processes. In the passive demethylation process, TET proteins modify 5-methylcytosine (5-mC) via oxidation into 5-hydroxymethylcytosine (5-hmC). Maintenance methyltransferase (DNMT1) poorly recognizes 5-hmC, leading to the lack of maintenance methylation during DNA replication (Biswas & Rao, 2017; Hassler & Egger, 2012; Scourzic, Mouly, & Bernard, 2015). During active demethylation, TET proteins further oxidize 5-hmC to 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC), which are later removed by thymine DNA glycosylase (TDG) and replaced by unmodified cytosine via a base excision repair (BER) mechanism (Chappell et al., 2016; Maiti & Drohat, 2011).

2.4.1 Repetitive Elements

Repetitive elements are DNA sequences that occur multiple times in the human genome (Padeken, Zeller, & Gasser, 2015). Approximately 50% of the human genome is derived from repetitive elements and is normally heavily methylated (Mallona, Jordà, & Peinado, 2016). Tandem repeats (satellite elements) and interspersed elements constitute the repetitive elements of the human genome. Satellite elements are generally located within the centromere or centromere-adjacent (juxtacentromeric) heterochromatin, whereas interspersed elements are spread throughout the genome (Haider et al., 2012).

The most abundant repetitive elements are interspersed elements (LINE-1 and Alu), also known as retrotransposons (Kazazian Jr & Goodier, 2002; Lambrou et al., 2012). These elements together constitute approximately 30% of the human DNA sequence and are normally silenced in normal cells through DNA methylation (Kazazian Jr & Goodier, 2002). Hypomethylation of these otherwise heavy methylated elements increases their activities as retrotransposable elements, which can in turn adversely affect the normal function of cells by inserting mutations or inducing genomic instability (Baccarelli et al., 2009; Cho et al., 2019; Goetz, Morgan, & Baulch, 2011; Kitkumthorn & Mutirangura, 2011; Wilson, Power, & Molloy, 2007). There are approximately 1.4 million Alu and approximately half a million LINE-1 repetitive elements distributed across the genome, and their methylation levels are used as a surrogate marker for global DNA methylation (Yang et al., 2004). Significant loss of methylation in repetitive elements such as LINE-1 has been suggested to play a vital role in carcinogenesis and other pathological conditions due to exposure to exogenous toxicants (Cao, 2015). Global hypomethylation has been identified in the blood of cancer patients (Sun et al., 2018), and several other studies have also reported global methylation patterns associated with exposure to environmental and occupational toxicants such as benzene (Bollati et al., 2007),

cadmium, lead, mercury, persistent organic pollutants, and PAHs (Ruiz-Hernandez et al., 2015).

2.4.2 Factors influencing global DNA methylation

DNA methylation is a stable epigenetic mark that is laid down by *de novo* methyltransferases (DNMT3a and DNMT3b) and continually maintained by a maintenance methyltransferase (DNMT1) (Faulk, 2019). However, factors found to be influencing DNA methylation in humans have been identified in several studies (Terry et al., 2011). These factors are broadly categorized into demographic, environmental exposures, and behavioural factors.

2.4.2.1 Demographic factors

Age: There is evidence to suggest that DNA methylation is an age-dependent process (Jarmalaite et al., 2003). Studies over the past two decades have provided important information on the effect of age on both global DNA hypomethylation (Fuke et al., 2004; Wilson et al., 2007; Wilson et al., 1987), and gene-specific hypermethylation (Issa, 2000). Several studies have reported inverse relationships between age and total 5-mC content (global DNA methylation) in the blood of healthy individuals (Fuke et al., 2004; Shimabukuro et al., 2007). For example, a study by Bollati et al. (2009), found an inverse correlation between age and LINE-1 and *Alu* in a cohort of elderly subjects. In another study, global DNA methylation measured by using repetitive elements such as LINE-1 and *Alu* among healthy Koreans revealed a significant inverse correlation between age and *Alu* but not LINE-1 (Kim et al., 2010). In contrast, multiple studies did not find any significant relationship between age and blood LINE-1 methylation levels (Benitez-Trinidad et al., 2018; Rusiecki et al., 2008; Zhang et al., 2011a; Zhu et al., 2012).

Gender: Previous studies that have compared global DNA methylation measured through LINE-1 repetitive elements among males and females found significantly increased

methylation in males than in females (El-Maarri et al., 2007; Zhang et al., 2011a; Zhu et al., 2012). In the Zhu et al. (2012) study, a combined analysis of five studies was done by pooling data from 1465 participants in Italy, Poland and the United States (U.S.), reported and found that LINE-1 methylation was significantly higher in men (β = 0.796, 95% CI: 0.261 to 1.330) than women. Recent studies in Mexico found lower methylation levels of LINE-1 in females compared to males (Benitez-Trinidad et al., 2018; Paredes-Céspedes et al., 2020). Similarly, Marques-Rocha et al. (2016) found approximately 7.3% decline in LINE-1 methylation levels among females ($P < 0.01$). Some researchers suggest that the consistent decrease in global methylation observed in women compared to men could be attributed to decreased availability of dietary folate and other one-carbon metabolism nutrients including vitamins vitamin B2 and B6 due to increased utilization in women (El-Maarri et al., 2007).

Race/ethnicity: There is evidence that global DNA methylation differs among racial groups (Xia et al., 2014). Recently, a considerable literature has grown around the theme of race/ethnicity and global DNA methylation (Dong et al., 2017; Subramanyam et al., 2013; Terry et al., 2008; Zhang et al., 2011a; Zhu et al., 2016). Zhang et al., (2011) studied 161 cancer-free subjects and found a decrease of 2.2% of LINE-1 methylation in non-Hispanic blacks and 1.3% in Hispanics compared to non-Hispanic whites. Similarly, another study reported higher LINE-1 methylation in African-Americans and Hispanics than whites (Subramanyam et al., 2013). Contrary to the previous results, Hsiung et al. (2007) in an earlier study found about 1.26% increase in LINE-1 DNA methylation in non-Caucasians than in Caucasians. Among fire fightehrs in USA, an epigenome-wide analysis showed differences in methylation at 76 CpG sites when Hispanic and non-Hispanic whites were compared with decreased methylation in Hispanic than non-Hispanic firefighters (Goodrich et al., 2021). The

differences in DNA methylation by race/ethnicity maybe explained by differences in genetics and other environmentals exposures (Bell et al., 2011; Galanter et al., 2017).

2.4.2.2 Environmental factors

Exposure to exogenous factors including heavy metals, air pollution, benzene, pesticides and persistent organic pollutants (POPs) have associated with DNA methylation in humans. For example, alterations to DNA methylation have been observed following occupational and environmental exposure to metals such as Cd, Pb, and As (Ameer et al., 2017; Devóz et al., 2017; Hossain et al., 2012). In a cross-sectional study in Bangladesh, Hossain and colleagues demonstrated that chronic exposure to As was inversely associated with LINE-1 DNA methylation (Hossain et al., 2017). Similarly, Phetliap et al. (2018), showed that As contaminated environment in Thailand was associated with global DNA hypomethylation. Bollati et al. (2007) conducted a study among gasoline filling attendants (n = 78) and traffic police officers (n = 77) exposed to low-dose benzene and found that exposure to airborne benzene was associated with a significant reduction in LINE-1 DNA methylation. Similarly, a study among urban pesticide sprayers in Mexico showed decreased levels of LINE-1 methylation among the pesticide-exposed group (Benitez-Trinidad et al., 2018). In another study in China where workers in a battery plant were occupationally exposed to Pb, LINE-1 methylation was inversely associated with Pb levels (Li et al., 2013). Duan and co-workers also reported hypomethylation of LINE-1 among coke-oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) (Duan et al., 2013).

The possible mechanisms through which environmental exposures alter DNA methylation patterns include 1) inhibition of DNA methylation pathways such as the depletion of SAM by As, reduction of DNMTs activity by Cd, and Pb; 2) promotion of DNA demethylation pathways such as increasing TET activities by metals, POPs, and PAHs; and 3) promotion of

DNA methylation pathway such as the overexpression of DNMTs by PM, and chronic Cd exposure (Bhargava et al., 2019; Ruiz-Hernandez et al., 2015).

2.4.2.3 Behavioural factors

Smoking: DNA methylation is considered as a possible pathway through which cigarette smoke exerts its deleterious health effects, including cancer, and chronic cardio-pulmonary effects (Breitling et al., 2011). Recent evidence suggests that smoking is associated with a reduction in global DNA methylation levels (Avram et al., 2020; Caliri et al., 2020). For example, Avram et al. (2020), measured global DNA methylation in buccal cells of 47 individuals, including smokers, former smokers, and comparison group who had never smoked using MethylFlash Methylated DNA Fluorometric Quantification Kit. The results showed a reduction in the methylation levels in smokers (3.1%) and former smokers (2.16%) compared with never smokers (4.16%). Similarly, Caliri et al. (2020), found a significant reduction of methylation of the LINE-1 gene in both vapers and smokers. However, several other studies failed to find an association between smoking and LINE-1 DNA methylation (Fa et al., 2016; Figueiredo et al., 2009; Kim et al., 2009; Paredes-Céspedes et al., 2020). Multiple pathways are linked to smoking-related alteration of DNA methylation patterns. Some researchers suggest that smokers have reduced levels of blood folate compared to non-smokers (Piyathilake et al., 1994). In addition, tobacco smoke leads to DNA damage such as strand breaks and DNA adducts (Alsaad et al., 2019), and subsequent recruitment of DNMTs from lesion-free DNA to the site of damage which results in altered methylation patterns in these regions (Terry et al., 2011).

Alcohol: Chronic alcohol use has been associated with altered DNA methylation patterns in humans (Zhang & Gelernter, 2017; Zhang et al., 2019b). Alcohol consumption may alter DNA methylation pattern by inhibiting the pathways that regulate the availability of the universal methyl donor, S-adenosylmethionine (SAM) (Varela-Rey et al., 2013). For example, chronic

alcoholism is associated with low plasma level of folate due to decreased folate absorption (Halsted et al., 2002). Evidence exists of the association between alcohol consumption and increased LINE-1 methylation in sperm of 143 male residents in southwest China (Zhang et al., 2019b). In addition, Benitez-Trinidad et al. (2018), found a marginal decrease in LINE-1 methylation levels among ever-drinkers compared to never-drinkers in a cohort of urban sprayers in Mexico. However, other studies did not find an association between alcohol consumption and LINE-1 DNA methylation (Shigaki et al., 2012; Zhang et al., 2011a; Zhu et al., 2012).

Body mass index: The association between body mass index (BMI) and global DNA methylation have shown inconsistent results (Lopes et al., 2019). Zhu et al. (2012) performed a combined analysis of five studies and found no association between BMI and LINE-1 or *Alu* repetitive elements. Similarly, among urban sprayers in Mexico, LINE-1 methylation was not correlated with the BMI of the participants (Benitez-Trinidad et al., 2018). In contrast, other studies have found an inverse association between BMI and LINE-1 DNA methylation (Castellano-Castillo et al., 2019; Perng et al., 2013; Piyathilake et al., 2011).

Physical activity: There is evidence that long term physical activity or exercise is associated with global DNA methylation (Boyne et al., 2018; Kawi et al., 2018). Boyne et al. (2018), in a meta-analysis of 14 studies, found an overall trend towards higher levels of physical activities over long periods and higher levels of global DNA methylation. Kawi et al. (2018) also found a positive association between exercise and global DNA methylation in females with chronic low-back pain. Similarly, Zhang et al. (2011b) in an earlier study, measured the methylation level of LINE-1 using real-time PCR (MethyLight) in 161 participants enrolled in the North Texas Healthy Heart Study. The results showed that participants with physical activity of about

30 minutes per day had higher LINE-1 methylation levels than their counterparts with ≤ 10 minutes of physical activity per day.

Dietary folate: It is established that one-carbon metabolism pathway plays an important role in DNA methylation by regulating the availability of methyl groups (Brennan & Flanagan, 2012; Cancarini et al., 2015; Friso et al., 2002). Folate, a major dietary source of methyl groups, converts homocysteine to methionine and the production of S-adenosyl methionine (SAM) – a universal methyl donor essential for DNA methylation. Riboflavin (vitamin B2) and vitamin B6 are one-carbon metabolism pathway-dependent micro-nutrients that may influence methyl group bioavailability. A deficiency in dietary folate, and these micro-nutrients (vitamins B2 and B6), are therefore implicated in global DNA hypomethylation (Cancarini et al., 2015; Cho et al., 2010; Friso et al., 2002; Nelson, Marsit, & Kelsey, 2011; Schernhammer et al., 2010; Vineis et al., 2011).

2.4.3 Global DNA methylation and disease

Several studies (Heyn & Esteller, 2012; Pacchierotti & Spanò, 2015; Portela & Esteller, 2010; Santos-Reboucas & Pimentel, 2007), have reported on the role of altered epigenetic marks such as DNA methylation in the aetiology of disease, including cancer, infertility, cardiovascular, respiratory, metabolic, immunologic, and neurodegenerative pathologies. Human tumours often display changes in DNA methylation, including global hypomethylation and gene-specific hypermethylation (Eden et al., 2003). Methylation at promoter regions of tumour suppressor genes along with global hypomethylation is thought to contribute to increased disease risk, including tumorigenesis (Feinberg & Tycko, 2004). Promotor hypermethylation contributes to tumorigenesis by silencing tumour suppressor genes (Figure 8). This result in loss of function that increases cell proliferation leading to the initiation and progression of cancer (Cho et al., 2010; Ha & Califano, 2006), while global hypomethylation, on the other hand, induces genome instability and chromosomal aberrations, and increase cell proliferation

through the activation of pro-oncogenes (Hsiung et al., 2007). The methylation pattern in tissues in the build-up to disease especially cancer is therefore observed to be reversed from the global (intergenic regions/repeats) hypermethylation and CpG islands promoter regions hypomethylation to global hypomethylation and promoter hypermethylation (Figure 9).

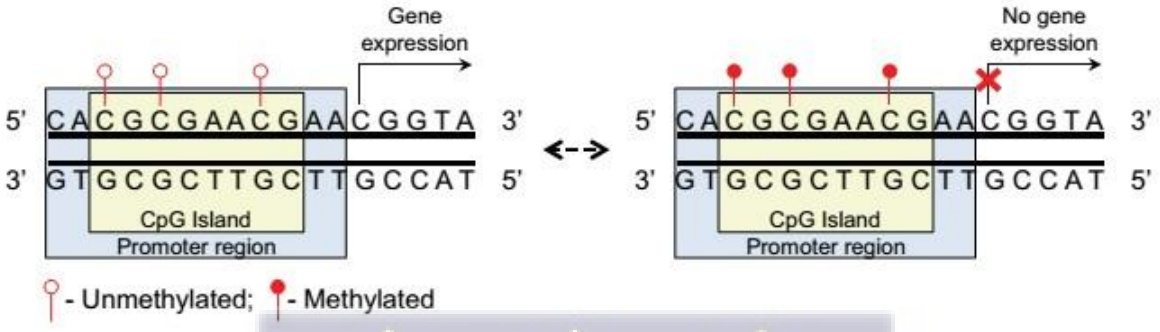


Figure 8: Association between DNA methylation and gene transcription

Source: Dreval and Pogribny (2018).

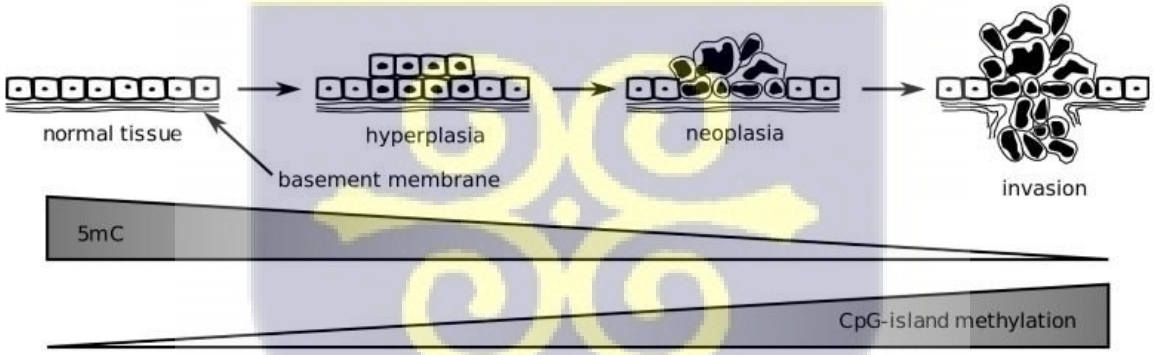


Figure 9: Epigenetic alterations over time

Source: (Philippe Hupé [CC-BY-SA-3.0], via Wikimedia Commons)

Global genomic hypomethylation is a common epigenetic event and was the first epigenetic alteration to be identified in cancers (Feinberg & Tycko, 2004; Feinberg & Vogelstein, 1983; Herceg & Vaissière, 2011; Wilson et al., 2007). Even though global DNA hypomethylation is

virtually found in all human cancers, there is still debate in the scientific literature on its role in tumour development. The bone of contention remains whether DNA global hypomethylation is an early cancer-causing event or a consequence of cancer (Cao, 2015; Herceg & Vaissière, 2011). However, hypomethylation has been detected in some normal colonic and urothelial bladder mucosae, suggesting hypomethylation as an early event in the development of colorectal and urothelial bladder cancers (Hoffmann & Schulz, 2005). In addition, global hypomethylation is observed to be an early event for breast cancer, and chronic lymphocytic leukaemia (Wilson et al., 2007). LINE-1 hypomethylation has been observed in several types of cancers (Ehrlich, 2002; Hsiung et al., 2007; Woo & Kim, 2012; Zhu et al., 2011), and has been regarded as a hallmark of cancer development (Buj et al., 2016; Das & Singal, 2004). In a meta-analysis (Barchitta et al., 2014), LINE-1 methylation level was significantly lower in cancer patients than in control samples ($P<0.001$). The difference, however, was confirmed in tissue samples ($P<0.001$) and not blood ($P=0.23$). In a previous meta-analysis by Woo and Kim (2012), global DNA hypomethylation in peripheral blood leukocytes was associated with increased cancer risk. In addition, an inverse relationship was observed between LINE-1 methylation and bladder cancer risk in a Chinese population (Cash et al., 2012). Zhu et al. (2011), reported a 3.2 fold (95% CI 1.4-7.5) prevalence of lung cancer among persons in the Normative Aging Study with reduced LINE-1 methylation. However, recent evidence suggests that global DNA hypermethylation is associated with disease, including myopia (Hsi et al., 2019) and Head and Neck Squamous Carcinoma (Akinmoladun et al., 2020).

2.4.4 Methods for measuring global DNA methylation

Global DNA methylation assays are used to estimate the total 5-mC content of the genome (Sant & Goodrich, 2019). Various assays are developed to reliably measure global DNA methylation in humans including LUminometric methylation assay (LUMA), bisulfite

pyrosequencing of repetitive elements, mass spectrometry, enzyme-linked immunosorbent assay (ELISA), and [^{3}H]-methyl acceptance assay (Lisanti et al., 2013).

2.4.4.1 LUminometric methylation assay (LUMA)

The Luminometric methylation (LUMA) is used to measure genome-wide DNA methylation (Karimi et al., 2006). A number of researchers have utilized LUMA to estimate the changes in total percent methylation associated with chemical exposures in humans (Cho et al., 2019; Pilsner et al., 2012). LUMA relies on genomic cleavage by methylation-sensitive and -insensitive restriction enzymes; difference in the amount of cleavage between these two types of enzymes is a readout for global methylation (Lee & Pausova, 2013). LUMA is a high-throughput assay, require only 200-500 ng of unmodified DNA, highly reproducible, and have a very short run time (Karimi et al., 2006). However, the reproducibility of LUMA assay may be affected by the DNA isolation method (Soriano-Tárraga et al., 2013). In addition, LUMA assay is not able to discriminate between methylated and hydroxymethylated cytosines, hence the global methylation estimated is in reality, a combination of both 5-mC and 5-hmC (Sant & Goodrich, 2019).

2.4.4.2 Bisulfite pyrosequencing of repetitive elements

The measurement of CpG within repetitive DNA sequences, such as long interspersed element-1 (LINE-1), and *Alu*, is one of the most widely used assays in estimating global DNA methylation as these elements exhibit high copy number and are widespread throughout the human genome (Buj et al., 2016; Caliri et al., 2020; Cho et al., 2019; Toraño et al., 2012). To estimate the methylation level of the CpG sites within the repeat elements, DNA first undergoes bisulfite conversion which converts unmethylated cytosines to uracils and leaves methylated cytosines unchanged (Goodrich et al., 2013; Toraño et al., 2012). Each repetitive element is then amplified by PCR and sequenced in order to determine the percent methylation at each CpG site (Yang et al., 2004). Sequencing of bisulfite converted DNA has revolutionized the

study of DNA methylation, and it is regarded as the "gold standard" method for methylation analysis (Beck & Rakyan, 2008; Docherty et al., 2009; Jordà & Peinado, 2010; Rakyan et al., 2011).

2.4.4.3 Mass spectrometry

Another method currently available to provide reliable, accurate, cost-effective, and also address the issue of precision and regarded as the "gold standard" in the quantification of global genomic methylation is mass spectrometry. This method has the ability to distinguish 5-mC from other DNA modification and requires only 10 ng of genomic DNA (Sant & Goodrich, 2019). However, expensive instrumentation and technical expertise are required to perform these methods.

2.4.4.4 Enzyme-linked immunosorbent assay (ELISA)

ELISA, a high-throughput assay, is easy to perform and uses methylcytosine-specific antibodies to quantify the relative DNA methylation between samples. ELISA assay is capable of differentiating 5-mC from other DNA modifications. Several kits such as MethylFlash ELISA Easy Kit (Colorimetric) (EpiGentek) are available for estimating Global DNA Methylation (5-mC).

Table 4: Characteristics of methods used to measure global DNA methylation

Method	Genomic Coverage (%), Humans	Input DNA required	Bisulfite conversion required?	Can distinguish between 5-mC and other modifications?
LUMA	~8%	>250 ng	No	No
Repetitive elements (Alu/LINE-1)	~ 10% - 15% each	>250 ng	Yes	No
Mass spectrometry	100%	>10 ng	No	Yes
ELIZA	100%	>20 ng	No	Yes

Source: (Sant & Goodrich, 2019)

2.4.5 Occupational metals exposure and global DNA methylation

Bal and colleagues extensively studied the genomic toxicity aspect of metals and reported that heavy metals largely influence their toxicity either through direct interaction with nuclear DNA or indirectly through generated reactive intermediates reacting with other cellular pathways such as inhibition of DNA repair mechanisms or both (Bal, Protas, & Kasprzak, 2011). Recent developments in the field of metal toxicity and carcinogenicity suggest that genetics alone cannot fully explain metal-induced chronic diseases, especially cancer, since most of the metals are weak mutagens (Arita & Costa, 2009). Growing data have linked metal-induced nongenotoxic carcinogenesis with epigenetic modifications, such as DNA methylation. Although there is growing interest in the association between heavy metal exposure and DNA methylation, most epidemiological studies have mainly focused on the general population with little attention to the occupationally exposed population, especially workers within the informal sector with uncontrolled exposure sources.

To date, there are few studies that have investigated the association between heavy metals and global DNA methylation profile of workers exposed to these metals. A search of the literature revealed only four studies that examined the relationship between occupational exposure to heavy metals and global DNA methylation (Devóz et al., 2017; Goodrich et al., 2013; Li et al., 2013; Wang et al., 2012). Two of the studies were performed in China (Li et al., 2013; Wang et al., 2012), one in Brazil (Devóz et al., 2017), and one in the US (Goodrich et al., 2013). Table 5 provides a summary of studies of included in this review.

Among chromate manufacturing workers in China, Wang et al. (2012) evaluated the levels total 5-mC in chromate manufacturing workers and a control group. The results showed a significant decline in in global 5-mC in the Cr exposed workers (1.48%) as compared to 2.10% in the control group. The global hypomethylation observed in the chromate workers was attributed to the significant depletion of folate associated with Cr exposure (Wang et al., 2012).

Another study examined global DNA methylation in 53 workers from a battery plant exposed to elevated levels of Pb and age and gender-matched 57 healthy volunteers (Li et al., 2013). Global DNA methylation was measured using LINE-1 repetitive elements as a proxy. The study found a significant reduction in LINE-1 methylation in the Pb exposed workers compared to the control group. Additionally, a negative correlation was observed between LINE-1 and blood Pb levels in the exposed workers (Li et al., 2013).

Similarly, a Brazilian study reported an inverse association between Pb and global DNA methylation in a group of automotive battery factory workers (Devóz et al., 2017). Dentists were studied in USA and their occupation was predictor of higher Hg blood levels (Goodrich et al., 2013). Although no correlation was observed between Hg and global DNA methylation, LINE-1 showed a positive correlation with age (Goodrich et al., 2013).

Up to date, far too little attention have been paid to workers involved in the informal processing of e-waste with regards to heavy metals exposure and worker's global DNA methylation profiles. A recent study in China found an association between e-waste exposure and decreased global methylation levels (Li et al., 2020). However, the study was done among residents living near e-waste dismantling factories and not the e-waste workers themselves (Li et al., 2020).

2.4.6 Occupational particulate matter exposure and global DNA methylation

Particulate matter (PM) air pollution is associated with adverse health outcomes especially to the respiratory and cardiovascular systems (Anderson, Thundiyil, & Stolbach, 2012; Brook et al., 2010; Cavallari et al., 2016; Panis et al., 2017; Riediker et al., 2018). It has been suggested that DNA methylation may be a potential pathway by which PM is linked to adverse health effects (Kile et al., 2013; Madrigano et al., 2012; Sayols-Baixeras et al., 2019). Available data suggest that LINE-1 hypomethylation is associated with various pathological conditions, including cardiovascular diseases and cancer (Hossain et al., 2017; Kemp & Longworth, 2015).

However, a recent evidence suggest that global DNA hypermethylation is associated with myopia (Hsi et al., 2019) and Head and Neck Squamous Carcinoma (Akinmoladun et al., 2020).

There is little published data on the relationship between occupational PM exposure and global DNA methylation (Sun et al., 2018). However, whether occupational PM exposure is associated with hyper- or hypomethylation throughout the genome is yet to be settled. For example, Tarantini et al. (2009) examined the relationship between PM_{10} and global DNA methylation in 63 male workers in a steel production plant in northern Italy. LINE-1 and Alu repetitive elements were used to estimate the total 5-mC content of the genome. The results showed that PM_{10} was negatively associated with both LINE-1 ($\beta = -0.34$, $p = 0.04$) and Alu ($\beta = -0.19$, $p = 0.04$) (Tarantini et al., 2009). Similarly, Wang et al. (2020) found a significant decreased in total 5-mC in relation to increasing $PM_{2.5}$ in Coke oven workers in China. In contrast, Fan et al. (2014) reported a positive association between $PM_{2.5}$ and LINE-1 methylation among occupational welders in the U.S, while Kile et al. (2013) found a null association between $PM_{2.5}$ and both Alu and LINE-1 among boilermaker welders.

The inconsistent findings observed in the previous occupational PM exposure studies could be attributed to the differences in PM components originating from different sources. Further research is warranted to confirm the direction of occupational PM exposure on global DNA methylation. Table 5 presents with summary of studies between occupational PM exposure and global DNA methylation.

Table 5: Previous epidemiological studies on the association between exposure to occupational metals and PM and global DNA methylation

Author (year)	Country	Population	Pollutant	Outcome measure	Results
Wang et al. (2012)	China	Chromate production plant workers (n=115) vs controls (n=60)	Cr	Global DNA methylation	Significant decrease in global DNA methylation in chromate workers compared to controls (1.48% vs 2.10%, $p<0.001$)
Li et al. (2013)	China	Battery plant workers (n=53) vs controls (57)	Pb	LINE-1	LINE-1 significantly reduced in the exposed group compared to the control group (76.6% vs 86.3%, $p<0.001$. An inverse correlation between LINE-1 and blood Pb in exposed group was observed.
Devóz et al. (2017)	Brazil	Automotive battery factory workers (n=100)	Blood and plasma Pb	Global DNA methylation	Negative correlations were found between B-Pb, P-Pb and % global DNA methylation.
Goodrich et al. (2013)	USA	Dentists	Hg	LINE-1	No significant correlation between Hg and LINE-1
Tarantini et al. (2009)	Italy	Steel production plant workers (n=63)	PM_{10}	Alu and LINE-1	PM_{10} concentration was negatively associated with both Alu and LINE-1
Wang et al. (2020)	China	Coke oven workers (n=558) vs controls (n=210)	$PM_{2.5}$	Global DNA methylation (%5-mC)	There was observed decreasing trend in %5-mC levels with increasing $PM_{2.5}$ concentration
Fan et al. (2014)	USA	Boilermaker welders (n=66)	$PM_{2.5}$	Alu and LINE-1	$PM_{2.5}$ showed a positive association with LINE-1 methylation ($\beta = 0.79\%$, $p = 0.013$)
Kile et al. (2013)	USA	Boilermaker welders (n=38)	$PM_{2.5}$	Alu and LINE-1	No significant association was observed between $PM_{2.5}$ and Alu or LINE-1

2.5 Conclusion

The literature reviewed revealed that informal e-waste recycling exposes workers to elevated concentrations of particulate matter and heavy metals. Previous studies have found a consistent inverse association between heavy metals and global DNA methylation in occupationally exposed populations. Particulate matter on the other hand showed both positive and negative associations with global DNA methylation. Most of the studies estimated global DNA methylation levels using repetitive elements methylations such as LINE-1 and Alu. Some limitations of the studies reviewed include, single metal exposure assessment which does not reflect the reality of metals exposure in occupational settings, and most of the studies did not include a reference population, and therefore do not allow for comparison of exposure and outcome relationships between the exposed and reference groups. I addition, there is little published data on the global DNA methylation profile of individuals involved in the recycling of e-waste. Despite these limitations, these studies have provided some elucidation on the effects of occupational toxic metals on DNA methylation and/or indicate toxic levels of metals in occupational environments. In Ghana, no previous study has investigated the genetic or epigenetic effect of pollutants associated with e-waste recycling among e-waste workers. In addition, previous studies that examined the effect of PM and heavy metals on global DNA methylation in other occupational fields used traditional statistical methods to estimate the effect of one chemical at a time, however, occupational exposures is to a multiple of chemicals simultaneously. There is therefore the need to employ a novel and robust statistical method to estimate the mixture effect of chemicals among informal e-waste workers and individuals engaged in other similar unregulated type of work, especially, in developing countries.

CHAPTER THREE

3.0 METHODS

3.1 Introduction

This chapter describes the materials and methods used to answer the questions related to specific objectives 1, 2, 3 and 4. The study sites and population recruitment procedures as well as the study design, sample size calculation and statistical analyses used to answer the research questions are described. The chapter concludes with ethical considerations for this study.

3.2 Study sites

This study was conducted in two locations: the e-waste site in Agbogbloshie (Figure 10) and a reference site in Madina Zongo (Figure 11). The Agbogbloshie e-waste recycling site is one of the busiest sites of its kind in the world and has become a hub for informal e-waste processing in Ghana (Simon, 2018; Srigboh et al., 2016). Agbogbloshie is located in central Accra and rated as one of the most contaminated site on earth (Amoyaw-Osei et al., 2011; Blacksmith Institute, 2013). The site is situated on the banks of Korle Lagoon on the western side of the Odaw River, about 1 km from central Accra. To the north-east are various businesses, including banks, pharmaceutical company, brewery, shops, and various manufacturing companies. To the south-east is a densely populated, 'resource-poor' community with the majority of residents lacking access to essential services such as clean water and sanitation (Amankwaa et al., 2016). The e-waste workers are mostly young men who migrated from the northern part of Ghana in search of employment opportunities. These workers' main jobs include collection, dismantling, and open burning of the e-waste (Acquah et al., 2019). These activities are carried out with little or no use of personal protective equipment, thus, exposing workers to multiple pollutants, including metals, and particulate matter of varying sizes.

Madina Zongo is an area of greater Accra, about 18 km north-east of the Agbogbloshie e-waste recycling site. Previous e-waste studies have successfully recruited reference participants from Kwabenya North, a suburb of Accra. Residents of Madina Zongo are demographically similar those in Agbogbloshie with respect to age, socioeconomic class, religion (Islam) and culture (Wittsiepe et al., 2017b). There are no e-waste recycling activities in the area, and the individuals recruited were not involved in any e-waste work.

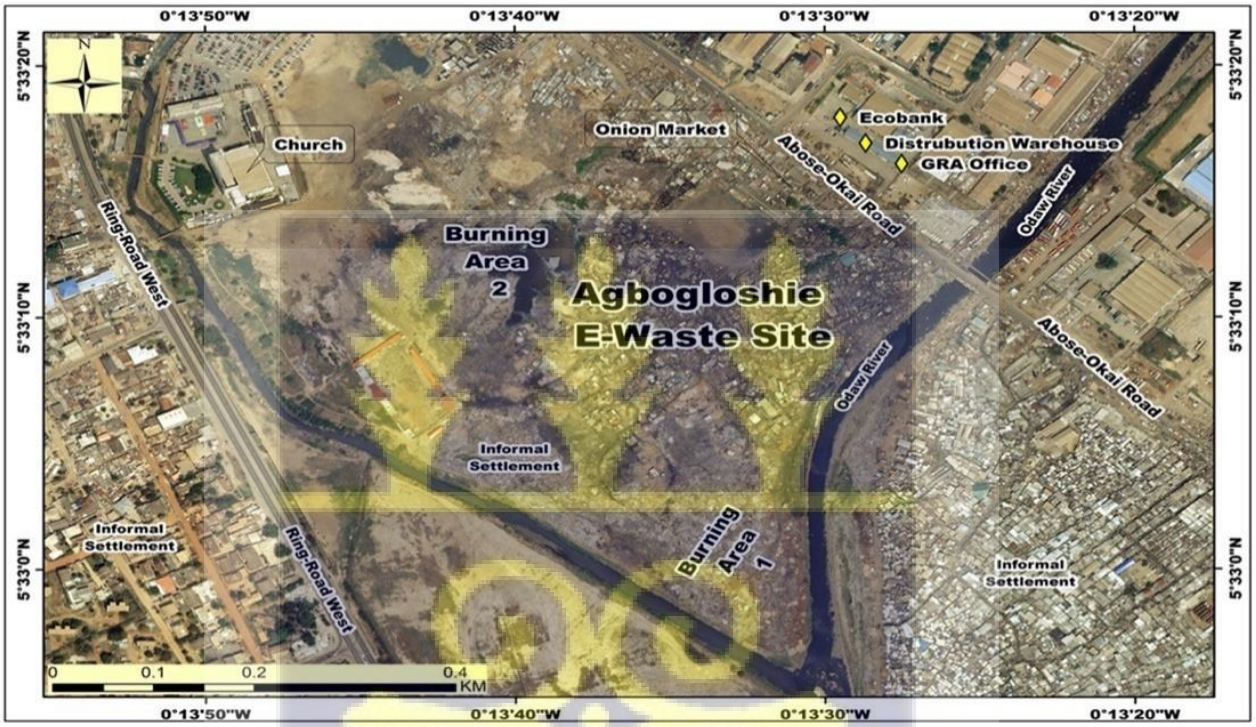


Figure 10: Activity map of the Agbogbloshie e-waste site

Source: (Amoabeng et al., 2020)

Figure 11: Google earth view of the study site at Madina Zongo, Accra

Source: (Takyi et al., 2020)

3.3 Study design

This study made use of biological samples and exposure data collected during the first round of a parent/larger GEOHealth II study - a longitudinal study. Details of the GEOHealth II study design has been described elsewhere (Laskaris et al., 2019).

3.4 Study participants

The samples used for this research work originated from the Global Environmental and Occupational Health II (GEOHealth II) study. The GEOHealth II study was a longitudinal cohort study with the broad aims of increasing multidisciplinary understanding of the environmental and occupational health risks associated with e-waste recycling at the Agbogbloshie e-waste site in central Accra, Ghana, and to use study findings to inform evidence-based implementation activities and policy options at the national, regional, and international levels. The study participants of the GEOHealth II study comprised of e-waste

recyclers at the Agbogbloshie e-waste site, and a reference group from Madina Zongo. E-waste recycling at Agbogbloshie is male dominated; therefore, participants recruited for this study were all males for both e-waste workers and the reference population. The inclusion criteria were adult males aged 18 years and above who had previously worked at the e-waste site for at least six months. Similarly, participants from the control site (Madina Zongo) must have lived in the area for at least six months. Persons with mental or physical disabilities interfering with their ability to understand the informed consent or complete health status measures were excluded.

3.5 Sample size

The formula for calculating sample size for the analysis of longitudinal data by Diggle et al. (2002) was used to calculate the sample size required to achieve the desired power of 80% and α - level of 0.05 for the main GEOHealth II study. The formula is as follows:

$$N = \frac{2(Z_{\alpha} + Z_{\beta})^2 (1+(n-1)\rho)}{n[(\mu_1 - \mu_2)/\delta]^2}$$

Where:

N = sample size

δ^2 is the assumed common variance in the two groups

$\mu 1 - \mu 2$ is the difference in means of the two groups

n is the number of time points

ρ is the assumed correlation of the repeated measures

One-hundred and fifty-one (151) participants, 100 from Agbogbloshie, and 51 from Madina Zongo were recruited at inception or baseline. Data obtained from the baseline (wave 1) of the GEOHealth II study were used to answer the specific research question of this thesis.

3.6 Sampling strategy

In March 2017, a community durbar was organized at study sites to engage and inform community leaders and potential participants about the study. Leaders and potential

participants were given a detailed explanation of the study, its objectives, nature and protocols for sample collection, and ethical issues and possible risks and potential benefits to participants, and hose willing to participate were asked to provide written consent before enrolment. A total of 151 participants were enrolled for the study at baseline, of which 100 were e-waste workers, and the remaining 51 served as the control group. Analyses for this thesis was restricted to the baseline sample (N = 151).

3.6.1 Overview of field data collection for the GEOHealth II study

The diagram (Figure 12) below illustrates the breakdown of tasks (workflow) during field data collection. As shown in Station 1, a participant entered the study after informed consent and registration, which culminated in the creation of a personal biodata file. Upon completion of registration, the participant was handed copies of the complete surveys (including a checklist of tasks to be accomplished at each of the remaining six (6) stations) and proceeded to Station 2. At Station 2, anthropometric/physical measurements were taken and then the participant proceeded to the next stage, until all stations were visited. After Station 6 (the last station), the participant returned to Station 1, with the checklist for an audit of the completed tasks. Once the auditor was satisfied that the participant had been seen at all the work stations and had completed all the tasks required at each station, the auditor scheduled the subject for backpack and personal air monitoring data.

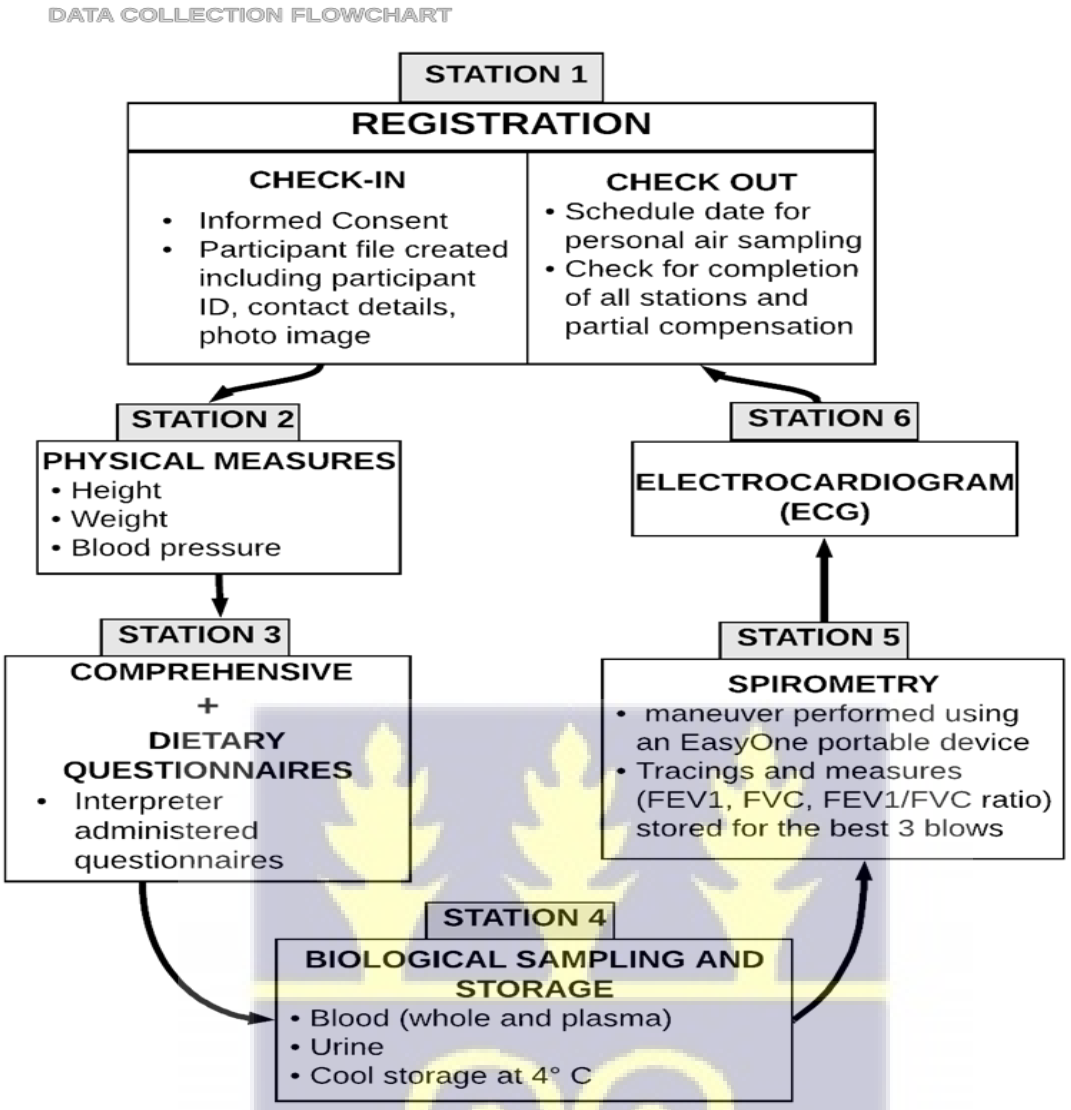


Figure 12: A flowchart showing data collection steps for the GEOHealth II study

3.7 Data collection methods

3.7.1 Sociodemographic data

Each participant answered series of questions using a structured questionnaire that was administered by trained staff. Interviews were conducted in either English or other local languages (Dagbani, Twi and Hausa). Data collected included demographic (age, gender, religion/ethnicity, education, measures of socioeconomic position, location of birth and childhood, and location of all residences), information on job related and past exposures, use

of tobacco, exposure to indoor biomass fuels, type of housing, detailed job history, personal and family medical history (diagnosed illnesses, reported symptoms), and other related anthropometric measurements such as weight, height, and blood pressure.

3.7.2 Blood sample collection

Blood was collected into EDTA tubes by an experienced phlebotomist making sure all sterile procedures were followed for venupuncture of the antecubital fossa. Blood samples were aliquoted into 2.5 mls cryotubes and placed in a cooler with ice packs and later transported to the University of Ghana for storage at -80°C, and later transported on dry ice to the University of Michigan, USA for DNA extraction and methylation analysis, or McGill University in Canada for metals analysis.

3.7.3 Personal particulate matter (PM) exposure assessment

A detailed description of personal PM measurements has previously been described by Laskaris et al. (2019). In summary, personal PM of varying sizes (1, 2.5, 4, and 10) was collected minute-by-minute in real-time from all participants using a battery-operated 5-channel optical particle counter (OPC; Aerocet 831, Met One Instruments, Inc, Oregon, USA) at a flow rate of 2.8 L/min. In addition, $PM_{2.5}$ was collected on a 47 mm Teflon filters with a high flow (SKC, 10 L/min) impact sampler. The sampling pumps were carried in a backpack and worn by participants during the work shift. The inlet of the sampling pump was placed on a shoulder strap of the backpack to collect PM from each participant's breathing zone. Five backpacks were used, allowing only five participants to be studied daily. The sampling time was set to 4 hours during peak working hours (8:00 am - 12:00 pm) to ensure uninterrupted exposure assessment.

PM measurement for the controls was conducted in the same manner as with the e-waste workers. Participants wore the backpacks for 4 hours, usually between 8 am to 12 pm, while

going about their routine activities. The overall sampling period lasted for six weeks (March 2017 to May 2017). Before deployment, OPC batteries (including an auxiliary battery) were charged, flow rates were set and confirmed using a flowmeter (VFB-67, Dwyer Instrument Inc, IN, USA) connected to a HEPA capsule filter (Pall Gelman Science, Ann Arbor, MI, USA), which also confirmed the "zero" test. After sampling, flow rates were rechecked, and OPC data was downloaded to a laptop.

3.8 Laboratory analysis of metals in blood

Metal levels in blood were measured at the Basu laboratory in McGill University, Montreal, Canada, using Inductively Coupled Plasma Mass Spectrometer (ICPMS Varian; 820MS). The metals measured included: cadmium (Cd), arsenic (As); lead (Pb), manganese (Mn), silver (Ag), calcium (Ca), magnesium (Mg), iron (Fe), selenium (Se), copper (Cu), zinc (Zn), strontium (Sr), cerium (Ce), rubidium (Rb), yttrium (Y), europium (Eu), lanthanum (La), neodymium (Nd), thallium (Tl) and terbium (Tb). All tubes and pipette tips used were acid-washed (cleaned, soaked 24 hr in 10% hydrochloric acid and rinsed three times in Milli-Q water) before use. Certified standard reference materials (INSPQ; QM-B-Q1505 blood; QM-B-1506 blood; and QM-B-Q1314 blood) obtained from the Institut National de Santé Publique du Québec were used to measure accuracy and precision (Appendix 4). In addition, each batch run included procedural blanks and replicates. The theoretical detection limit was determined for each element analyzed as three times the standard deviation of the mean blank value.

In this study, five metals (Cd, Pb, Se, Zn, and Mn) were selected for analysis as evidence of their association with DNA methylation has been reported in other populations (Hossain et al., 2012; Montes-Castro et al., 2019; Zhang et al., 2019c), and blood has been reported to be an acceptable matrix for their exposure assessment (Barbosa Jr et al., 2005; Cowan et al., 2009).

3.9 Extraction of DNA from whole blood

DNA was extracted from whole blood in the laboratory at the University of Michigan School of Public Health using Qiagen DNA Mini Kit (Qiagen, Valencia, C.A), following manufacturer's instruction. Specifically, 20µl of Qiagen protease inhibitor provided by the manufacturer was dispensed into 1.5ml microcentrifuge tube. 200 µl of whole blood was pipetted into the tube, followed by 200 µl of lysis buffer AL, and mixed completely by pulse-vortexing for 15s. The sample was then incubated at 56 ^{o}C for 10 min, followed by brief centrifugation to remove drops from the inside of the lid of the tube. Next, 200 µl of ethanol (96 - 100%) was added to the sample, mixed completely again by pulse-vortexing for 15s, and centrifuged briefly to remove drops from the inside of the lid.

The mixture was then transferred to the QIAamp mini spin column in a 2mls collection tube and centrifuged at 6000 x *g* for 1min. The QIAamp mini spin column was placed in a 2ml collection tube provided by the manufacturer and the filtrate was discarded. 500 µl of washing buffer AW1 was added to the mixture and centrifuged at 6000 x *g* for 1 min. The previous step was repeated with 500 µl of washing buffer AW2 and centrifuged at 20,000 x *g* for 3 min. The mini spin column was then placed in a 2 ml collection tube and centrifuged at full speed for 1 min to eliminate the chance of the washing buffer AW2 carryover. The mini spin column was then transferred in to a 1.5 ml microcentrifuge tube. 200 µl of DNA elution buffer AE was added to the column matrix, incubated at room temperature (15 – 25 ^{o}C) for 5 min, and then centrifuged at 6000 x g for 1 min to elute the DNA. The purity and quantity of DNA samples were assessed with Qubit Broad Range Double-staranded DNA Assay and Nanodrop spectrophotometer.

3.10 Bisulphite conversion of DNA

Sodium bisulfite conversion was performed on 300 ng of genomic DNA using Qiagen EpiTect Kit per the manufacturer's protocol. 800 µl RNase-free water was added to each bisulfite mix

aliquot and vortexed for 5 min to completely dissolve the bisulfite mix. For the next step, 300 ng DNA, RNase-free water, 85 µl dissolved bisulfite mix, and 35 µl DNA protect buffer were combined in 200 µl PCR tubes to create a total volume of 140 µl of bisulfite reactions, and mixed thoroughly. The bisulfite DNA conversion was then performed using a thermal recycler according to the cycling program described in table 6.

Table 6: Bisulfite conversion thermal cycler conditions

Step	Time	Temperature
Denaturation	5 min	95ºC
Incubation	25 min	60ºC
Denaturation	5 min	95ºC
Incubation	85 min (1 h 25 min)	60ºC
Denaturation	5 min	95ºC
Incubation	175 min (2 h 55 min)	60ºC
Hold	Indefinite†	20ºC

† Converted DNA can be left in the thermal cycler overnight without any loss of activity

3.10.1 Cleaning up of bisulfite converted DNA

After the bisulfite conversion was completed, the PCR tubes containing the bisulfite reactions were taken out of the thermal recycler and centrifuged briefly. The completed bisulfite reactions were then transferred into 1.5 ml microcentrifuge tubes and 560 µl buffer BL containing 10 µl/ml carrier RNA was added to each sample. The samples were transferred to corresponding EpiTect spin columns, centrifuged at full speed for 1 min and the flow-through discarded. The spin columns were placed back in to the collection tubes. Exactly 500 µl of washing buffer BW was added to the spin columns, centrifuged at full speed for 1 min, and the flow-through discarded. The spin columns were again placed back in to the collection tubes, and 500 µl desulfonation buffer BD was added to each spin column and incubated at room temperature (15-25ºC) for 15 min. After the 15 min incubation period, the spin columns were then centrifuged at full speed for 1 min and the flow-through discarded. The spin columns were

placed back in the collection tubes and DNA washing procedure was repeated 2x with 500 µl each of the washing buffer BW. The spin columns were then placed in new 2 ml collection tubes and centrifuged at full speed for 1 min to remove residual liquid. Finally, the spin columns were placed in new 1.5 ml microcentrifuge tubes. The purified DNA was eluted by adding 20 µl of elution buffer BE and centrifuged for 1 min at 15,000 x *g*.

3.11 PCR amplification of Bisulphite converted DNA and pyrosequencing

PCR amplification was performed for the promoter region of LINE-1 as previously described (Yang et al. 2004). In summary, 15 µL of HotStarTaq Master Mix (Qiagen, Valencia, CA), water, and 15 µL desalted forward and reverse primers were combined to create a PCR master mix. Finally, 3 µL of bisulfite-converted DNA was added to each well to bring the final primer concentration to 0.2 mM and the total reaction volume to 30 µL. PCR cycling conditions were 95°C for 30 s, 50°C for 30 s, and 72°C for 60 s for 35 cycles. PCR product quality was confirmed using 2% agarose gels and gel red staining. Following amplification, 12 µL of PCR product was combined with each sequencing primer and analyzed for CpG-specific methylation using the PyroMark MD System (Qiagen, Valencia, CA). Four bisulfite conversion controls (EpigenDX) and four pyrosequencing controls (Qiagen) were prepared at methylation levels of 0%, 30%, 60% and 100%. CpG site-specific methylation percentages (0 – 100%) were generated for each of the four CpG sites included in the assay. All samples on a plate were rerun if any of the controls failed, and samples were measured in duplicate.

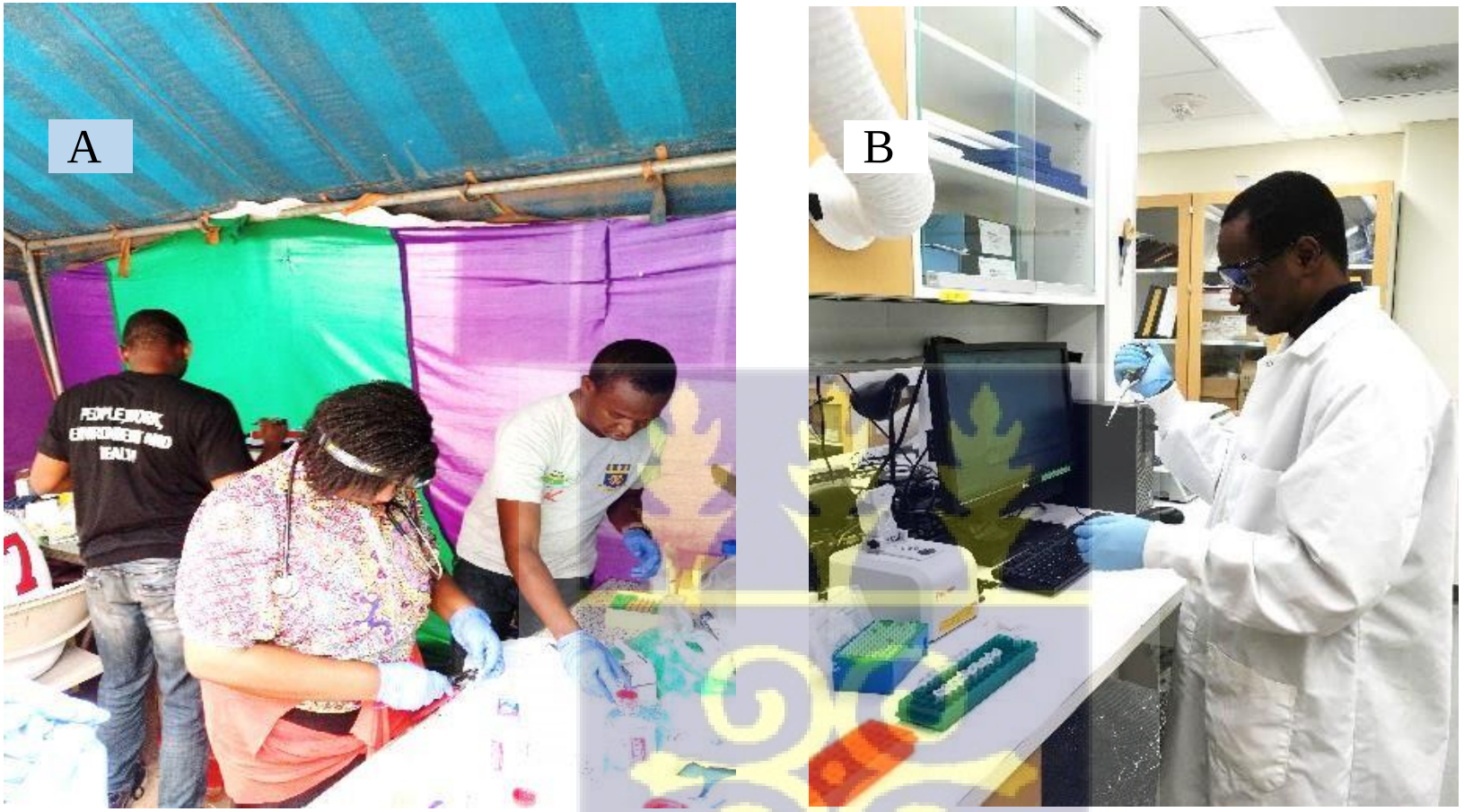


Figure 13: Pictures showing data collection and laboratory analysis; A=biological samples collection at Agbogbloshie, B=DNA extraction at University of Michigan School of Public Health

3.12 Statistical analysis

The normality assumptions of the data were tested using the Shapiro Wilk's test. Demographic and other characteristic differences between e-waste workers and controls were presented as mean ± SD for continuous variables that exhibited normal distribution (age, BMI, hours worked/day, days worked/week) and compared by student t-test. All categorical variables were presented as frequencies (percent frequency) and compared using the chi-squared test.

$PM_{2.5}$ and PM_{10} were not normally distributed and were therefore presented as median (IQR) and compared between e-waste workers and controls by the non-parametric Mann-Whitney U test. To determine whether the specific job-tasks performed by e-waste workers were associated with PM exposure levels, the e-waste workers were categorized into three main groups based on their primary job tasks as burners, dismantlers, and collectors to identify high-risk worker groups. A self-reported primary job tasks was relied upon to categorize the workers since there were no documented job titles or task protocols at the informal e-waste site. They were categorized into the different job categories if they reported having been performing a specific task for approximately 70% of their time for the past month. Kruskal-Wallis test was used to compare the concentrations of PM across the primary job-tasks. The concentrations of $PM_{2.5}$ and PM_{10} were compared to air quality guidelines reference values set by the World Health Organization (WHO) and the Ghanaian reference values set by the Ghana Standards Authority (GSA) (GSA, 2019). Differences between LINE-1 DNA methylation and specific CpG sites methylation of the LINE-1 gene were compared between the e-waste workers and controls using student t-test. A further bivariate analysis was performed to evaluate the relationships between anthropometric and lifestyle factors, and LINE-1 methylation levels.

All metals were transformed using natural logarithm (ln) to approximate normality. Metals were presented as geometric mean (95% CI) and compared between e-waste workers and reference population by t-test. Analysis of variance (ANOVA) was used to compare the

geometric mean concentration of metals across the different job tasks performed by e-waste workers. The levels of metals in blood were compared to background levels of the U.S population using the 95th percentile (P95) values of the U.S National Health and Nutrition Examination Survey (NHANES) (CDC, 2019). The P95 helped determine whether levels observed in other studies were unusual (CDC, 2019).

Linear regression model with robust standard errors (SE) from ordinary least squares (OLS) was used to evaluate the associations between PM and metals exposure and LINE-1 DNA methylation. Both mean percent methylation of four CpG sites of LINE-1 and methylation of specific CpG sites were modelled. Methylation units were in percent (%) change, and models for PM exposure were interpreted as a unit increase in predictor associated with percent change in outcomes, whereas models for metals exposure were interpreted as percent increases in predictor associated with percent changes in outcomes. All models were adjusted for age, BMI, smoking, indoor use of biomass fuel, alcohol intake, and e-waste exposure status (Agbogloshie/Madina Zongo). Covariates included in the models were based on evidence of their association with DNA methylation from previous studies (Alegría-Torres, Baccarelli, & Bollati, 2011).

The associations between PM and metals exposure and DNA methylation of LINE-1 (average of all 4 CpG sites) and at each specific CpG site were estimated using independent linear regression models. In addition to analyzing each metal separately, multiple linear regression model was used to simultaneously evaluate all the five metals as a mixture by mutually adjusting for all metals and the same covariates as in the single metal analysis. To explore the possible modification effect of the selected toxic metals (Cd and Pb) on DNA methylation caused by essential metals (Mn Se and Zn) concentrations, the corresponding interaction terms were incorporated into the regression model. A further sensitivity analysis using different variants of the outcome model (robust and cross-fit partialling-out least absolute shrinkage and

selection operator (LASSO) linear regression models) was performed to compare with the results of the OLS with robust SEs. All statistical analyses were performed using Stata v16.1 (STATA Corp LLC. Texas, USA) and GraphPad Prism v8.3.1 was used to generate some graphs. The results were considered statistically significant if p-values were < 0.05.

3.13 Data storage and sharing

Data entered in to excel, and processed in STATA and GraphPad Prism were stored in a computer and protected with a password. In addition, data were backed up in the cloud with sufficient encryption to ensure maximum security.

3.14 Ethical consideration

The Ethical and Protocol Review Committee at the College of Health Sciences, University of Ghana (protocol identification number CHS-ET/M.4-P 3.9/2015-2016) approved the study protocol. In addition, the local chiefs of Agbogbloshie and Madina-Zongo permitted the study research team to enter the community to conduct the study. Leaders and study participants were given a detailed explanation of the study, its objectives and procedures, and the benefits and possible risks of participating in the study. Written and verbal consent was obtained from all participants before participating in the study.

CHAPTER FOUR

4.0 RESULTS

4.1 Introduction

This chapter is divided into four main sections, each of which presents the results relating to the three major themes of the thesis.

4.2 Sociodemographic characteristics of e-waste workers and reference population

E-waste workers were significantly younger (mean age = 25.4±6.3 years) compared to the reference population (mean = 32.5±10.4 years). Even though the BMI of both e-waste and the reference population were within normal weight according to the World Health Organization (WHO) parameters, the BMI of the controls (mean = 23.8) was significantly higher than that of the e-waste workers (mean = 21.6, Table 7). E-waste workers worked for an average of 9 hours per day, 6 days per week, with 59.8% of them living and working on the e-waste site while the rest lived off-site, but within 1 km of Agbogbloshie (40.2%). Significant differences were observed in educational level between e-waste workers and the reference population, i.e., 25.0% of e-waste workers had no formal education at all (vs 13.7% of the reference population), 33.0% had up to middle/junior high school (vs 25.5% of the reference population), and only 16.0% had secondary school education or higher (vs 51.0% of the reference population). The majority of the participants were Muslims, and more than 80% earned 20 – 80 Ghanaian Cedi (GHS); the equivalence of 5 – 15 USD per day. The prevalence of smoking was not statistically different between the e-waste workers (28.0%) and non-e-waste workers (15.7%, Table 7). The indoor use of biomass for fuel was significantly higher in the controls (31.4%) than the e-waste workers (14.0%).

Table 7: Characteristics of e-waste workers (n=100) and controls (n=51) enrolled for the study, March 2017-May 2017 at Agbogbloshie and Madina Zongo, Accra, Ghana

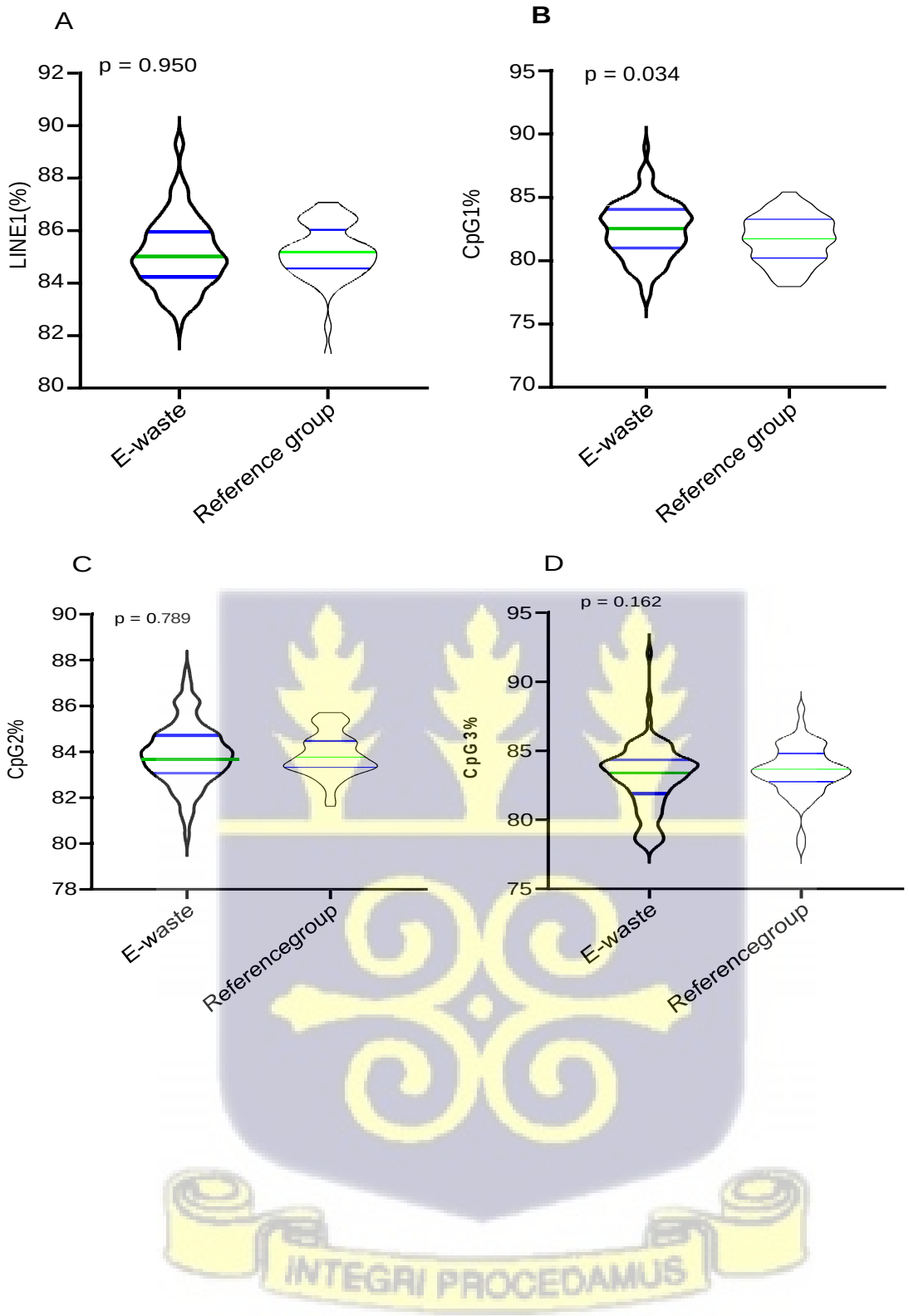
	Total	E-waste workers	Controls	p-value
Characteristics	n=151	n=100[c]	n=51[c]	
BMI (kg/m²), mean(±SD)	22.4(3.2)	21.6(2.7)	23.8(3.5)	**<0.001[a]**
Age (years), mean(±SD)	27.8(8.6)	25.4(6.3)	32.5(10.4)	**<0.001[a]**
Workdays/week, mean(±SD)	NA	6.0(1.0)	NA	
Hours work/day, mean(±SD)	NA	9.3(2.5)	NA	
Sleep location, n(%)				
On the site	NA	58(59.8)	NA	
≤1km off-site	NA	39(40.2)	NA	
Education, n(%)				**<0.001[b]**
No formal education	32(21.2)	25(25.0)	7(13.7)	
Primary Middle/JHS	31(20.5)	26(26.0)	5(9.8)	
Secondary/SHS+	46(30.6)	33(33.0)	13(25.5)	
	42(27.8)	16(16.0)	26(51.0)	
Marital status, n(%)				0.074[b]
Single	73(48.7)	43(43.4)	30(58.8)	
Married	77(51.3)	56(56.6)	21(41.2)	
Income, n(%)			n=50	0.059[b]
GHC 20-80	120(80.5)	81(81.8)	39(78.0)	
GHC 81-140	10(6.7)	9(9.1)	1(2.0)	
> GHC 140	19(12.8)	9(9.1)	10(20.0)	
Indoor use of biomass				**0.011[b]**
Yes	30(19.9)	14(14.0)	16(31.4)	
No	121(80.1)	86(86.0)	35(68.6)	
Alcohol use, n(%)				0.183[b]
Regular	18(1.9)	15(15.0)	3(87.7)	
Former	12(8.0)	9(9.0)	3(8.2)	
Never	121(80.1)	76(76.0)	45(6.1)	
Smoking, n(%)				0.093[b]
Yes	36(23.8)	28(28.0)	8(15.7)	
No	115(76.2)	72(72.0)	43(84.3)	
E-waste job category, n(%)				
burners	NA	32(32.0)	NA	
dismantlers	NA	49(49.0)	NA	
collectors/sorters	NA	19(19.0)	NA	

Abbreviations: SD=standard deviation, N= Total number of participants, n(%)= frequency(percent frequency), JHS = junior high school, SHS = senior high school. [a] p-values obtained by t-test, [b] p-values obtained by chi-square test, [c]some figures may not add up to the total numbers because of missing values, **bold** p-values are statistically significant.

4.3 Results for objective 1

4.3.1 Global (LINE-1) DNA methylation in e-waste workers and reference population

Methylation of four CpG sites of LINE-1 (CpG1, CpG2, CpG3, and CpG4) were quantified from whole blood samples (N=151). As expected, all CpG sites were heavily methylated. There was no significant difference in mean LINE-1 DNA methylation among the e-waste workers and the non-e-waste workers (85.16 ± 1.32% vs 85.17 ± 1.11%, t(147) = 0.063, $p=0.950$) (Fig 14A). CpG1 showed significantly lower mean methylation among the non-e-waste workers compared to the e-waste workers (81.70 ± 1.86% vs 82.48 ± 2.20%, t(147) = 2.144, $p = 0.034$) (Fig 14B), and CpG4 had the highest (91.28%) mean methylation level among the e-waste workers (Fig 14D).



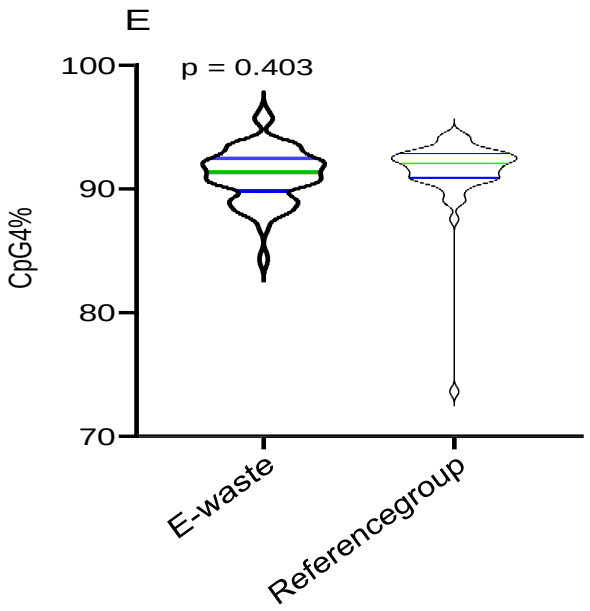


Figure 14: Violin plots. The violin plots [A-E] present the distribution of individual methylation of LINE-1 and site-specific CpG methylation of LINE-1 in e-waste workers and controls. P-values were calculated by t-test. The green line represents median values, and blue lines represent interquartile ranges.

4.3.2 Global (LINE-1) DNA methylation levels of e-waste workers by primary job tasks performed

LINE-1 methylation level was compared among the different categories of e-waste workers defined by their primary job tasks i.e., burners, dismantlers, and collectors. Overall, e-waste collectors had the lowest mean methylation level than burners and dismantlers (Figure 15). However, no significant differences were observed between group means as determined by one-way ANOVA ($F(2, 96) = 2.316$), $p = 0.104$).

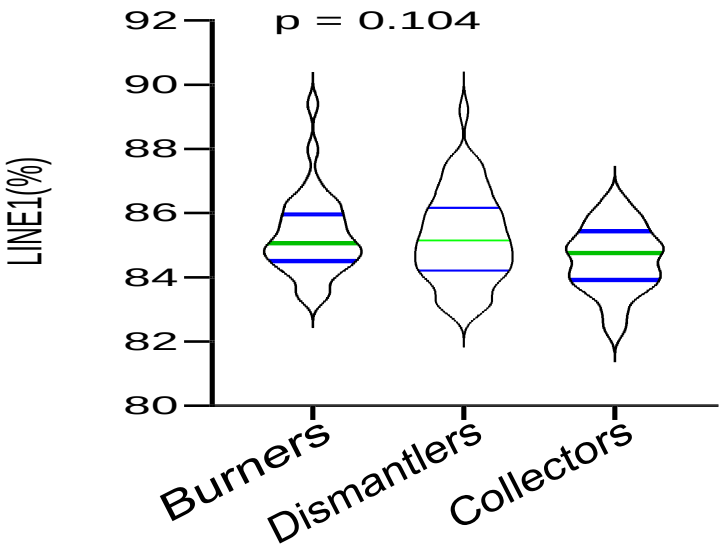


Figure 15: LINE-1 methylation across primary job-tasks performed by e-waste workers; collectors had the lowest mean methylation levels than burners and dismantlers. The green line indicates median methylation, and the blue lines represent interquartile ranges.

4.4 Relationship between LINE-1 methylation and anthropometric and lifestyle factors

Methylation of LINE-1 between e-waste workers and controls was assessed based on anthropometric and lifestyle factors such as age, BMI, smoking, alcohol consumption, and indoor use of biomass fuel for cooking via one-way analysis of variance (ANOVA) (Table 8). In this study, LINE-1 methylation was not related to age, BMI, smoking, alcohol consumption, or indoor use of biomass fuel for cooking amongst either group ($p_{all} > 0.05$).

Table 8: Relationship between LINE-1 methylation and anthropometric and lifestyle factors

	LINE-1 methylation		
Variable	E-waste workers (n=100)	Controls (n=51)	p-value
Age (years)			
≤20	85.2(1.2)	85.4(0.6)	0.817
21-30	85.1(1.4)	85.3(1.3)	0.520
31-40	85.3(1.3)	84.8(1.2)	0.399
>40	85.73(2.0)	85.2(1.0)	0.541
Smoking			
Yes	85.2(1.3)	85.1(0.7)	0.805
No	85.2(1.3)	85.2(1.2)	0.892
Alcohol intake			
occasional/regular	85.4(1.5)	85.6(1.3)	0.797
former	84.2(2.5)	85.7(1.2)	0.339
never	85.2(1.3)	85.1(1.1)	0.744
BMI (kg/m^2)			
Low weight	84.6(1.2)	85.2(0.5)	0.492
Normal weight	85.2(1.3)	85.2(1.1)	0.796
Overweight	84.9(1.3)	85.5(1.1)	0.307
Obesity	85.7(0.0)	84.2(1.3)	0.364
Indoor use of biomass			
Yes	85.3(1.5)	84.9(1.1)	0.370
No	85.2(1.3)	85.3(1.1)	0.574
Job category			
Burners	85.3(1.3)	NA	
Dismantlers	85.2(1.4)	NA	
Collectors/sorters	84.6(1.0)	NA	

Body Mass Index (BMI) categorization according to World Health Organization (WHO) parameters: low weight (≤18.5 kg/m^2); normal weight (> 18.5 kg/m^2 and ≤24.9 kg/m^2); overweight (> 24.9 kg/m^2, and ≤29.9 kg/m^2), and obesity (≥30 kg/m^2), P-values were obtained by the ANOVA test, NA: Not applicable. LINE-1 methylation presented as mean (±SD)

4.5 Results for objective 2

4.5.1 Particulate matter (PM) exposure in personal air among e-waste workers and reference population

A Mann-Whitney U test indicated that the median concentration of personal air $PM_{2.5}$ was significantly higher among e-waste workers (median = 77.32 µg/m^3) than the reference population (median = 34.88 µg/m^3, U = 320.5, $p < 0.001$. Similarly PM_{10} concentration was significantly higher among the e-waste workers (median = 210.21 µg/m^3) than the reference population (median = 121.92 µg/m^3, U = 1032, $p < 0.001$) (Figures 16A and B). The median

concentrations of PM obtained for both e-waste workers and reference population exceeded the World Health Organization (WHO) Air Quality Guideline (AQG) for short-term exposure of 25 µg/m^3 and 50 µg/m^3 for $PM_{2.5}$ and PM_{10}, respectively (Appendix 5.5). Furthermore, the concentrations of $PM_{2.5}$ and PM_{10} among e-waste workers far exceeded the reference values for the Ghanaian Ambient Air Quality Standards for short-term exposure, i.e. 35 µg/m^3 and 70 µg/m^3, respectively. However, the median concentrations of $PM_{2.5}$ (34.88 µg/m^3) in the reference population was lower than the Ghanaian standards 35 µg/m^3, whereas PM_{10} (121.92 µg/m^3) concentration was higher than the Ghanaian standards of 70 µg/m^3 (Appendix 5.5).

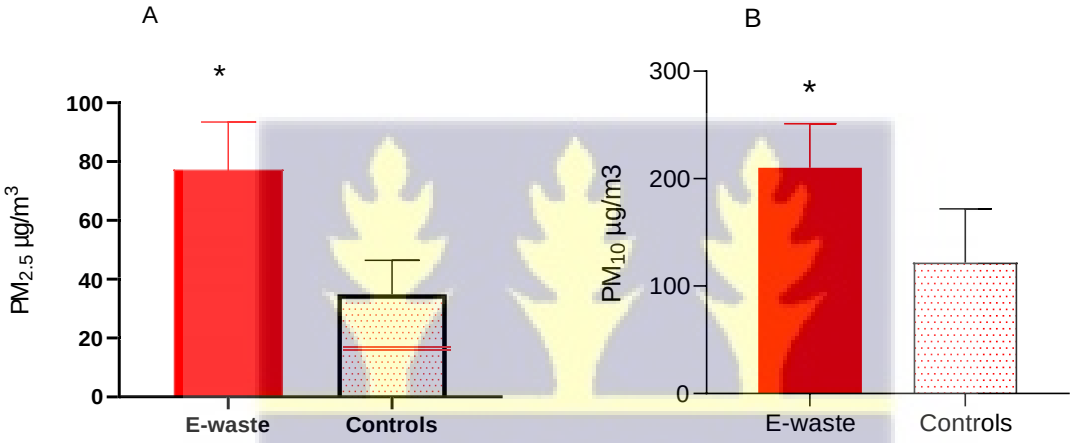


Figure 16: Measurement of personal air particulate matter among e-waste workers and reference group. Data are presented as the median (interquartile range). A: PM2.5, and B: PM10, *= p ≤ 0.05, p-values are obtained by Mann-Whitney-U test

4.5.2 Personal particulate matter exposure across primary job-tasks

Personal air particulate matter concentrations were measured among e-waste workers based on their primary job-tasks, i.e. collectors, dismantlers and burners. There was a statistically significant difference in the median $PM_{2.5}$ and PM_{10} levels between the groups as determined by the Kruskal-Wallis test (p = 0.013 and p = 0.027, respectively). Dunn's post hoc test revealed that the medians of both $PM_{2.5}$ and PM_{10} were significantly higher among burners than among collectors (p = 0.009 and p = 0.029, respectively). No significant differences were

observed when burners were compared to dismantlers for $PM_{2.5}$ and PM_{10} exposure (Figures 17A and B).

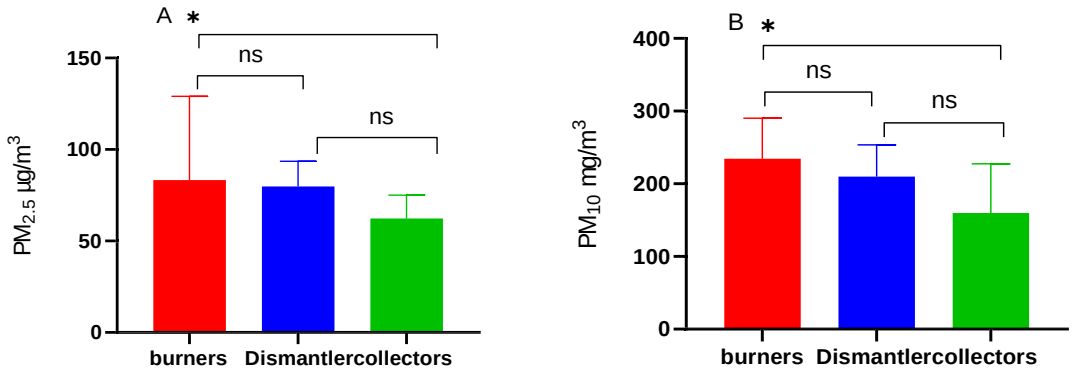


Figure 17: Personal air particulate matter concentrations among e-waste workers by primary job tasks performed shows high exposure among burners, A: PM2.5, B: PM10. Data are presented as the median (interquartile range), *= $p < 0.05$ and ns = non-significant. P- values obtained by Kruskal-Wallis and Dunn's post-hoc tests

4.5.3 Metal concentrations in blood of e-waste workers and reference population

As shown in table 10, the essential metals Se and Mn were significantly higher among the control group than the e-waste worker's group (geometric mean: Se = 190.6 vs 147.7 µg/L, Mn = 14.7 vs 11.4 µg/L, $p_{all} < 0.05$). In addition, except for blood Pb concentration which was significantly higher among e-waste workers (geometric mean: Pb = 79.6 vs 37.7 µg/L, $p < 0.001$), Cd, which is also a toxic metal like Pb was also rather higher in reference population than e-waste workers (Table 9).

Levels of metals in blood were compared to background population levels among Americans using the 95th percentile (P95) values of the U.S National Health and Nutrition Examination Survey (NHANES) (CDC, 2019). The P95 is helpful when determining whether levels observed in other studies are unusual (CDC, 2019). Among the e-waste workers, only Zn and Pb showed higher blood levels than the U.S general population according to the NHANES (Table 9).

Similarly, among reference population, three metals (Se, Zn, and Pb) were higher than the P95 values according to the NHANES (Table 9).

An evaluation of metals concentrations across job categories of e-waste workers showed that the levels of Se, and Zn were highest in e-waste collectors, followed by the dismantlers and then the burners (Table 10).

Table 9: Metal concentrations in blood of e-waste workers and reference population

Metals, µg/L	Reference (P95) GM(95% CI)	E-waste workers, GM(95% CI)	Controls, GM(95% CI)	P value
Se	161 (157-162)	147.7 (140.1, 155.8)	190.6 (179.4, 200.7)	**<0.001**
Zn	113 (107-119)	7417 (6910.9, 7954.3)	7960 (7301.5, 8677.8)	0.226
Mn	15.1 (14.5-15.5)	11.4 (10.2, 12.7)	14.7 (13.4, 16.1)	**0.003**
Cd	1.17 (.990-1.37)	0.6 (0.5, 0.7)	0.8 (0.7, 0.9)	**0.003**
Pb	2.93 (2.75-3.26)	79.6 (71.9, 88.1)	37.7 (33.8, 42.0)	**<0.001**

Abbreviations: Se; blood selenium, Zn; blood zinc, Mn; blood manganese, Cd; blood cadmium, Pb; blood lead, GM (95% CI); geometric mean (95% confidence interval), P95; 95th percentile. **Bold** p-values are statistically significant. P-values obtained using t-test.
source of reference values: USA, NHANES, Survey 2011–2016 (U.S. Department of Health and Human Services - Centers for Disease Control and Prevention, 2019)

Table 10: Metals concentrations in blood across job tasks

Metals, µg/L	Burners GM(95% CI)	Dismantlers GM(95% CI)	Collectors GM(95% CI)	P values
Se	131.1(119.7, 143.5)	152.9(141.2, 165.5)	165.5(150.4, 182.0)	**0.004**
Zn	6156.9(5443.6, 6963.7)	7736.6(7087.5, 8445.3)	9085.1(7628.8, 10819.2)	**<0.001**
Mn	9.2(7.1, 12.0)	12.9(11.6, 14.4)	12.0(9.6, 15.0)	**0.023**
Cd	0.6(0.4, 0.7)	0.6(0.5, 0.7)	0.6(0.4, 0.9)	0.817
Pb	76.2(66.1, 87.7)	83.4(71.5, 97.4)	75.8(56.0, 102.6)	0.665

Abbreviations: Se; selenium, Zn; zinc, Mn; manganese, Cd; cadmium, Pb; lead, GM (95% CI); geometric mean (95% confidence interval). **Bold** p-values are statistically significant. P-values obtained using ANOVA.

4.6 Results for objective 3

4.6.1 Associations between global DNA methylation and PM exposure

Using linear regression models and controlling for relevant confounders (indoor use of biomass fuel for cooking, age, cigarette smoking, alcohol consumption, smoking status, location of study (Agbogloshie or Madina Zongo), and BMI), no significant associations were observed between exposure to $PM_{2.5}$ and $PM_{10,}$ and LINE-1 DNA methylation ($\beta_{PM2.5}$ = 0.003; 95% CI; -0.001, 0.009, p = 0.159), and (β_{PM10} = 0.002; 95% CI; -0.001, 0.004, p = 0.121), respectively (Table 12) when the e-waste workers and reference population were analyzed together. However, a significant positive association was observed between $PM_{2.5}$ and LINE-1 CpG2 methylation when specific CpG sites were assessed (β = 0.003; 95% CI; 0.001, 0.006; p = 0.022). This estimate suggests that a 1 µg/m^3 increase in $PM_{2.5}$ levels resulted in a 0.003 increase in LINE-1 CpG2 DNA methylation percent (Table 11). Sensitivity analyses using different variants of the outcome model (robust and cross-fit partialling-out LASSO regressions) all showed similar results in the associations between DNA methylation and PM exposure (Table 11). In addition, linear regression models stratified by e-waste exposure status (e-waste workers/controls) showed similar estimates of the associations between $PM_{2.5}$ and PM_{10} exposure and LINE-1 DNA methylation among only the e-waste workers (Appendix 5.6)

Table 11: Associations between PM exposure and total vs CpG site specific DNA methylation of LINE-1 in total sample (n=151)

		Linear regression with robust standard errors from OLS	**Sensitivity analysis**	
			Robust regression	**Cross-fit partialling-out LASSO regression**
PM exposure	Outcomes (LINE-1) DNA methylation	β(95% CI)	β(95% CI)	β(95% CI)
$PM_{2.5}$	Average of 4 CpGs	0.003(-0.001, 0.009)	0.003(-0.001, 0.006)	0.004(-0.001, 0.009)
	CpG1	0.001(-0.008, 0.009)	-0.001(-0.007, 0.005)	0.002(-0.007, 0.010)
	CpG2	0.003(0.001, 0.006)*	0.004(0.001, 0.007)*	0.003(0.001, 0.006)*
	CpG3	0.009(-0.001, 0.018)	0.004(-0.002, 0.010)	0.008(-0.001, 0.017)
	CpG4	0.002(-0.002, 0.007)	0.001(-0.004, 0.007)	0.001(-0.002, 0.005)
PM_{10}	Average of 4 CpGs	0.002(-0.001, 0.004)	0.002(-0.000, 0.003)	0.002(-0.001, 0.004)
	CpG1	0.002(-0.002, 0.005)	0.001(-0.002, 0.005)	0.001(-0.003, 0.005)
	CpG2	0.002(-0.000, 0.003)	0.002(0.000, 0.004)*	0.001(-0.000, 0.003)
	CpG3	0.003(-0.002, 0.008)	0.000(-0.002, 0.003)	0.004(-0.001, 0.008)
	CpG4	0.001(-0.001, 0.004)	0.001(-0.002, 0.004)	0.001(-0.001, 0.003)

P-value notation: *p<0.05, β: average DNA methylation change, CI: Confidence Interval, **$PM_{2.5}$** = particulate matter ≤ 2.5μm in aerodynamic diameter, **PM_{10}** = particulate matter ≤ 10μm in aerodynamic diameter; LINE-1: long interspersed nucleotide element; CpG: cytosine guanine dinucleotide. All models adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking, study location and BMI.

4.6.2 Global DNA methylation across job tasks

The relationship between e-waste workers by tasks performed (burners, dismantlers, and collectors) and LINE-1 methylation were further assessed using linear regression with robust standard errors from OLS and cross-fit partialling-out linear regression from LASSO. The reference group was used as a reference, while controlling for age, BMI, smoking, alcohol use, indoor biomass fuel use, and PM exposure (Table 12). Results from the analyses showed that, compared to the controls, e-waste collectors had significantly decreased LINE-1 methylation (β = -0.667; 95% CI; -1.266, -0.069; p = 0.029). This estimate suggests that, working as an e-waste collector results in 0.67 decrease in LINE-1 methylation (percent) compared to the reference population.

Table 12: Electronic waste recycling activities and LINE-1 DNA methylation using non-e-waste workers as reference.

Covariates	LINE-1 DNA methylation			
	Linear regression with robust standard errors from OLS		Cross-fit partialling-out Lasso regression	
	β(95% CI)	P value	β(95% CI)	P value
Job category				
Ref=control group				
Burners	-0.165(-0.865, 0.534)	0.641	0.114(-0.492, 0.721)	0.712
Dismantlers	-0.153(-0.740, 0.433)	0.606	0.026(-0.498, 0.550)	0.923
Collectors	-0.668(-1.266, 0.069)	0.029	-0.651(-1.200, -0.102)	0.020

*p<0.05, β: average DNA methylation change, CI: Confidence Interval. Note: models adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking, $PM_{2.5}$, PM_{10} and BMI

4.7 Results for objective 4

4.7.1 Effect of single and multiple metals exposure on DNA methylation

The effect of individual metal exposure on global DNA methylation was determined, using single metal linear regression models while controlling for age, BMI, smoking, alcohol intake, indoor use of biomass fuel for cooking, and exposure status (location) was evaluated. The results showed a significant inverse association between Zn and LINE-1 DNA methylation (average of 4 CpG sites) (β_{Zn} = -0.912, 95% CI: -1.512, -0.306, *p = 0.003*) which corresponds to (β_{Zn} = -0.009, 95% CI: -0.015, -0.003, *p = 0.003*) (Table 13). This estimate suggests that a

1% increase in Zn was associated with a 0.009 decrease in percent LINE-1 DNA methylation. Similarly, significant inverse associations were observed between CpG2 methylation and Pb concentration (*p = 0.048*), CpG3 methylation and Se concentration (*p = 0.048*), as well as CpG4 and Zn concentration (*p = 0.030*). Sensitivity analysis using robust and LASSO regression models showed similar results as linear regression with robust SEs from OLS (Table 13).

The results of analysis; using mutual regression model by mutually adjusting for all metals without accounting for potential collinearity or interactions, showed that a percent increase in Zn concentration remained significantly associated with global (LINE-1) DNA methylation (average of 4 CpG sites) Zn (β_{Zn} = -0.767, 95% CI: -1.447, -0.087, *p = 0.027*) which corresponds to (β_{Zn} = -0.008, 95% CI: -0.014, -0.001, p = 0.027), and with CpG1 methylation (*p = 0.039*) (Table 14).

To further delineate and establish associations between metals exposure due to e-waste processing and LINE-1 methylation, two separate linear regression models were fitted to examine and compare the associations between metals and DNA methylation in e-waste workers compared to reference population. Both single metal and multiple metals regression models showed that increased blood Zn concentration was associated with a significant decrease in LINE-1 DNA methylation only in the e-waste workers (Appendix 5.7).

4.7.2 Potential interaction effects of toxic and essential metals on global DNA methylation

The effects of interaction between toxic and essential metals on LINE-1 methylation were evaluated. The results showed modifications of global (LINE-1) DNA methylation (average of 4 CpG sites) and specific CpG sites of LINE-1 due to potential interaction between toxic and essential metals (Figure 18). The results showed that the negative effect of Cd on LINE-1 methylation was reversed in the presence of increased concentrations of Zn (p = 0.001); (Figure

18A). Regarding the specific CpG sites, increased levels of Zn potentially antagonizes the toxic effect of Cd on CpG1 methylation. In addition, potential greater than additive effect (synergism) was observed in CpG1 due to Pb concentration been modified by Zn (Figure 18C), and CpG2 due to Pb concentration been modified by Mn concentration (Figure 18D).

Table 13: Single metal linear regression models of Global Repetitive (LINE-1) Methylation. Mean methylation is modelled for average CpG sites (all) and specific CpG sites (n=151)

Outcomes (LINE-1 methylation	**Metals (µg/L)**	Linear regression with robust standard errors from OLS	Sensitivity analyses	
			Robust regression	**Cross-fit partialling-out LASSO linear regression**
		β(95% CI)	**β(95% CI)**	**β(95% CI)**
Average of 4 CpG sites	Se	- 0.774(-1.775, 0.226)	-0.554(-1.416, 0.308)	-0.727(-1.598, 0.145)
	Zn	**- 0.912(-1.517, -0.306)***	-0.717(-1.320, -0.114)*	-0.912(-1.496, -0.329)*
	Mn	- 0.297(-0.695, 0.102)	-0.206(-0.634, 0.222)	-0.289(-0.657, 0.080)
	Cd	- 0.107(-0.509, 0.296)	-0.003(-0.386, 0.380)	-0.088(0-.434, 0.257)
	Pb	-0.425 (-0.897, 0.047)	-0.295(-0.742, 0.153)	-0.428(-0.872, 0.016)
CpG1	Se	0.239(-1.115, 1.592)	0.530(-0.958, 2.017)	0.092(-1.163, 1.348)
	Zn	-0.933(-1.988, 0.122)	-0.766(-1.805, 0.273)	-1.192(-2.262, -0.123)*
	Mn	0.053(-0.723, 0.830)	-0.012(-0.752, 0.728)	-0.157(-0.927, 0.613)
	Cd	0.165(-0.428, 0.759)	0.242(-0.415, 0.898)	-0.092(-0.641, 0.456)
	Pb	-0.338(-1.084, 0.409)	-0.283(-1.049, 0.484)	-0.330(-1.031, 0.371)
CpG2	Se	-0.487(-1.306, 0.331)	-0.398(-1.249, 0.454)	-0.488(-1.239, 0.263)
	Zn	-0.387(-0.938, 0.165)	-0.491(-1.080, 0.099)	-0.426(-0.971, 0.119)
	Mn	-0.256(-0.583, 0.071)	-0.276(-0.692, 0.139)	-0.238(-0.537, 0.061)
	Cd	-0.188(-0.547, 0.170)	-0.192(-0.566, 0.182)	-0.209(-0.529, 0.112)
	Pb	**-0.412(-0.820, -0.004)***	-0.464(-0.891, -0.036)*	-0.413(-0.786, -0.040)*
CpG3	Se	**-1.604(-3.190, -0.018)***	-1.240(-2.665, 0.186)	-1.369(-2.838, 0.099)
	Zn	-1.313(-2.645, 0.020)	-0.874(-1.864, 0.116)	-1.056(-2.405, 0.293)
	Mn	-0.440(-1.146, 0.266)	-0.097(-0.804, 0.611)	-0.310(-1.039, 0.419)
	Cd	-0.474(-1.304, 0.357)	-0.198(-0.832, 0.435)	-0.392(-1.135, 0.350)
	Pb	-0.409(-1.078, 0.260)	-0.524(-1.245, 0.197)	-0.411(-1.009, 0.187)
CpG4	Se	-1.615(-4.517, 1.287)	-0.377(-1.733, 0.979)	-1.656(-4.161, 0.849)
	Zn	**-0.984(-1.874, -0.095)***	-0.896(-1.825, 0.033)	-0.890(-1.714, -0.067)*
	Mn	-0.440(-0.996, 0.116)	-0.365(-1.030, 0.300)	-0.328(-0.850, 0.194)
	Cd	-0.079(-0.664, 0.506)	-0.291(-0.884, 0.302)	0.029(-0.453, 0.511)
	Pb	-0.652(-2.289, 0.984)	0.293(-0.401, 0.987)	-0.599(-2.115, 0.918)

P-value notation: *p<0.05, β: average LINE-1 methylation change, CI: Confidence Interval; All models are adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking status, BMI, and e-waste exposure status. All Heavy metals biomarkers are natural log-transformed.

Table 14: Multiple metals linear regression models of Global Repetitive (LINE-1) Methylation. Mean methylation is modelled for average CpG sites (all) and specific CpG sites (n=151)

Outcomes (LINE-1 methylation)	Metals (µg/L)	Linear regression with robust standard errors from OLS	Sensitivity analyses	
			Robust regression	Cross-fit partialling-out LASSO linear regression
Average of 4 CpG sites	Se	-0.251(-1.251, 0.749)	-0.135(-1.099, 0.829)	-0.289(-1.139, 0.561)
	Zn	**-0.767(-1.447, -0.087)***	-0.661(-1.386, 0.064)	-0.774(-1.404, -0.145)*
	Mn	-0.041(-0.425, 0.342)	-0.017(-0.485, 0.452)	-0.019(-0.363, 0.324)
	Cd	0.079(-0.282, 0.441)	0.112(-0.291, 0.515)	0.050(-0.276, 0.375)
	Pb	-0.226(-0.69, 0.242)	-0.148(-0.630, 0.333)	-0.193(-0.624, 0.239)
CpG1	Se	0.868(-0.607, 2.342)	1.097(-0.574, 2.769)	0.679(-0.683, 2.042)
	Zn	**-1.356(-2.641, -0.071)***	-1.172(-2.418, 0.075)	-1.442(-2.767, -0.116)*
	Mn	0.299(-0.504, 1.102)	0.188(-0.621, 0.997)	0.147(-0.626, 0.919)
	Cd	0.323(-0.260, 0.906)	0.309(-0.388, 1.006)	0.219(-0.379, 0.819)
	Pb	-0.281(-1.066, 0.504)	-0.275(-1.103, 0.552)	-0.208(-0.921, 0.504)
CpG2	Se	-0.150(-0.991, 0.691)	0.016(-0.920, 0.952)	-0.129(-0.847, 0.589)
	Zn	-0.100(-0.683, 0.482)	-0.259(-0.957, 0.439)	-0.159(-0.734, 0.415)
	Mn	-0.127(-0.463, 0.209)	-0.137(-0.589, 0.316)	-0.104(-0.435, 0.227)
	Cd	-0.103(-0.443, 0.236)	-0.117(-0.507, 0.273)	-0.139(-0.439, 0.159)
	Pb	-0.326(-0.740, 0.088)	-0.387(-0.851, 0.076)	-0.293(-0.668, 0.081)
CpG3	Se	-0.980(-2.488, 0.528)	-0.817(-2.404, 0.769)	-0.884(-2.201, 0.432)
	Zn	-0.917(-2.200, 0.366)	-0.612(-1.795, 0.571)	-0.691(-2.041, 0.659)
	Mn	-0.038(-0.666, 0.590)	0.131(-0.637, 0.899)	-0.076(-0.688, 0.536)
	Cd	-0.225(-0.982, 0.532)	-0.072(-0.733, 0.589)	-0.111(-0.806, 0.585)
	Pb	-0.026(-0.762, 0.710)	-0.274(-1.060, 0.512)	-0.075(-0.690, 0.540)
CpG4	Se	-1.119(-4.037, 1.799)	0.174(-1.315, 1.662)	-1.084(-3.473, 1.305)
	Zn	-0.541(-1.785, 0.703)	-0.899(-2.009, 0.210)	-0.455(-1.739, 0.828)
	Mn	-0.130(-0.849, 0.589)	-0.222(-0.942, 0.498)	-0.079(-0.681, 0.524)
	Cd	0.164(-0.457, 0.785)	-0.145(-0.765, 0.476)	0.289(-0.185, 0.763)
	Pb	-0.367(-1.925, 1.191)	0.510(-0.227, 1.247)	-0.488(-1.922, 0.946)

P-value notation: *p<0.05, β: average LINE-1 methylation change, CI: Confidence Interval; Model is adjusted for all metals, indoor use of biomass fuel for cooking, alcohol consumption, age, smoking status, BMI, and e-waste exposure status. All Heavy metals biomarkers are natural log-transformed.

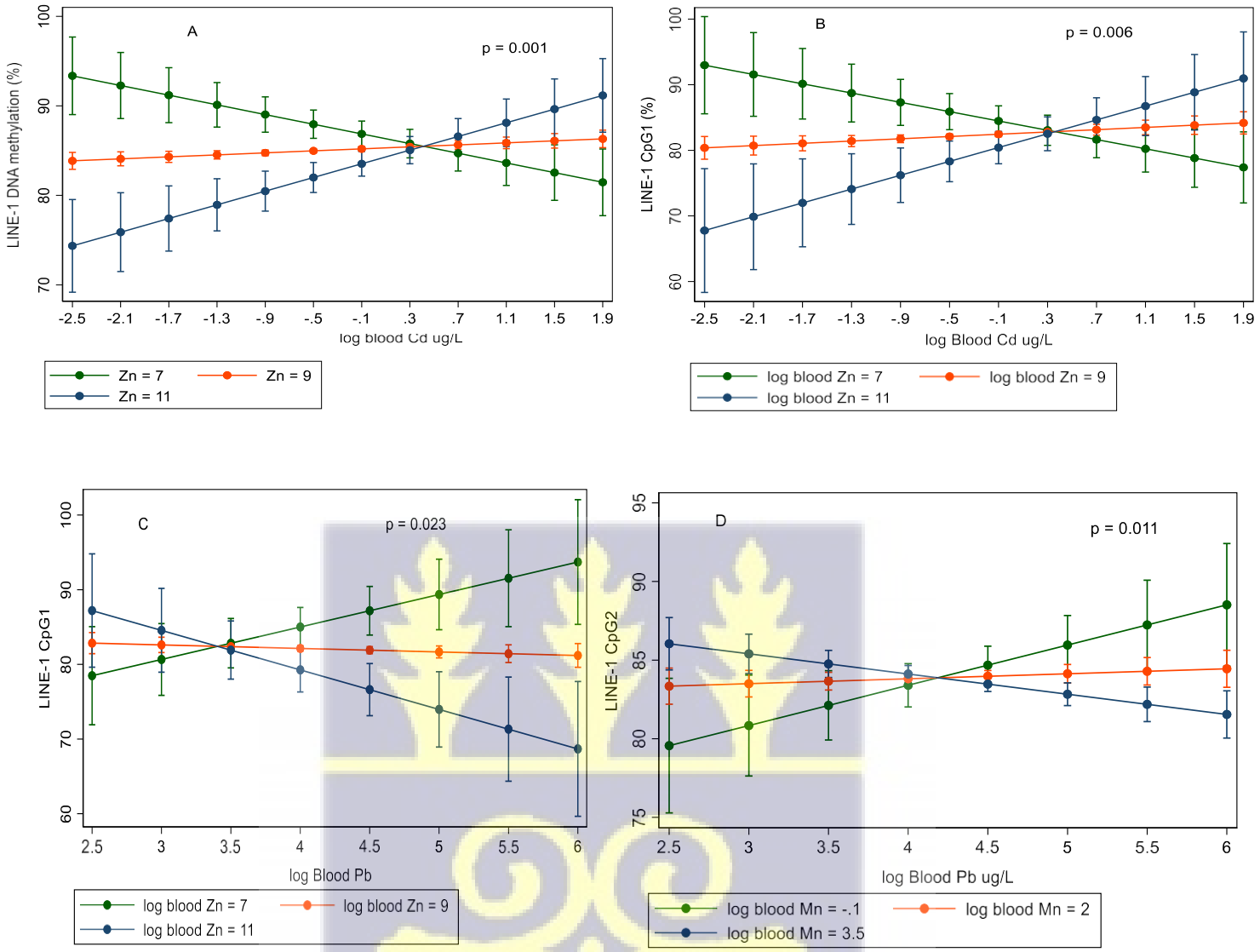


Figure 18: Interaction effects of toxic and essential metals on DNA methylation of LINE-1 CpG sites. A=interaction between Cd and Zn on all CpG sites of LINE-1, B=interaction between Cd and Zn on CpG1 of LINE-1, C= interaction between Pb and Zn on CpG1 of LINE-1, D= interaction between Pb and Mn on Cp2 of LINE-1

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

In this chapter, a discussion of the key findings of exposure assessment of breathing zone PM and blood metals among the e-waste workers and reference population is presented. Finally, a more detailed discussion of the results of multiple linear regression analyses to assess the effects of PM and metals exposure on LINE-1 DNA methylation is highlighted.

5.2 Discussion of results for specific objective 1

5.2.1 Global (LINE-1) DNA methylation among e-waste workers and reference population

Informal e-waste recycling activities generate a myriad of pollutants, including metals (essential and toxic), particulate matter, organics (e.g. PCBs) exposure of which could have deleterious health effects (Alabi & Bakare, 2017). Several studies have reported associations between occupational exposures and DNA methylation; however, there are few studies, if any, which have examined exposure levels and DNA methylation among informal e-waste workers (Goodrich et al., 2013; Li et al., 2011; Li et al., 2013; Wen et al., 2016).

The findings of this study show that LINE-1 was heavily methylated in both e-waste workers and reference population as demonstrated with analysis with whole blood. Overall, there was no significant difference in LINE-1 methylation between e-waste workers and reference population in this study. However, when individual CpG sites were analyzed, CpG1 showed a significant decrease in methylation among reference population compared to e-waste workers even though this decrement in the specific CpG site was not sufficient to influence the overall difference in LINE-1 methylation between the groups. Although, these results differ from some published studies (Benitez-Trinidad et al., 2018; Cho et al., 2019; Yang et al., 2018) where

global DNA methylation significantly decreased among occupationally exposed population compared to reference populations, they are consistent with those of (Chen et al., 2019; Ghosh et al., 2017). For example, in their study to establish whether or not workers exposed to multi-wall carbon nanotubes had different LINE-1 methylation, Ghosh et al. (2017) found no significant differences between occupationally exposed individuals and unexposed population. Similarly, Chen et al. (2019) reported no differences in global DNA methylation among interventional physicians and controls, results consistent with current findings. The non-significant difference in global (LINE-1) methylation between this study's populations may be attributable to the choice of the control group in this study since the categorization of hyper- or hypomethylation is dependent on the methylation levels of the comparator group (Phetliap et al., 2018). For example, this study's reference group was composed of population-based subjects, most of whom live and work near a busy highway with frequent vehicular traffic and may be exposed to traffic-related air pollutants including higher concentrations of metals such as Cd. The limited variability in pollutants exposure between the e-waste workers and reference population may partly explain the lack of difference in global DNA methylation status between the two groups. This phenomenon is succinctly stated by Wynder and Stellman as follows: "If cases and controls are drawn from a population in which the range of exposures is narrow, then a study may yield little information about potential health effects" (Wynder & Stellman, 1992).

5.2.2 LINE-1 methylation levels of e-waste workers by primary job tasks performed

Although different e-waste workers seem to perform particular tasks more than others, say burners often keep to burning, they can at the same time be involved in multiple tasks, such as burners being engaged in dismantling, since there no formal protocols prescribing specific job tasks to specific individuals. Job is based on self-reporting of present or recent task by e-waste worker (Feldt et al., 2014; Wittsiepe et al., 2015). Based on this categorization, e-waste

collectors had reduced LINE-1 methylation, compared to dismantlers or burners, but difference was not statistically significant. Job misclassification has been reported to have the ability to conceal the true effect of exposure on health outcome (Laskaris et al., 2019). The use of wearable cameras proposed by Laskaris et al. (2019) can significantly minimize task misclassification and improve occupational exposure assessment among informal sector workers. E-waste collectors often travel long distances away from e-waste site into different parts of the city (primarily by foot, bicycle or tricycle) to purchase or scavenge e-waste materials (Acquah et al., 2019; Laskaris et al., 2019). This predisposes them to other ambient air pollutants such as that from vehicular emissions, roadside burning, PM from construction sites etc. In the end, collectors likely are exposed to varied air pollutants, and may explain the decline in their LINE-1 DNA methylation.

5.3 Discussion of results for specific objective 2

5.3.1 Exposure of e-waste workers to PM at recycling site

Processes used in informal e-waste recycling rely on rudimentary tools and crude methods, often resulting in release of PM and toxic chemicals into the ambient environment (Bi et al., 2010; Zhang et al., 2019)(Acquah et al., 2019; Amoabeng et al., 2020; Kwarteng et al., 2020; Laskaris et al., 2021; Laskaris et al., 2019; Lin et al., 2021). As expected, $PM_{2.5}$ and PM_{10} were significantly higher among the e-waste workers compared to the reference population and in agreement with the findings of previous studies by Zhang (2017) and Zheng et al. (2015). Regards to specific tasks, significantly higher PM (2.5 and 10) levels were measured in personal air of burners, compared to those engaged in other recycling activities such as collection. Practically, burning sites always had this dark plume of smoke hanging in the air. It is therefore not surprising that higher PM levels were measured among burners, result of which is consistent with the finding by Bungadaeng et al. (2019), where open burning of e-waste in the Buriram Province of Thailand resulted in elevated levels of the average concentrations of

$PM_{2.5}$ and PM_{10}, in e-waste workers. Similarly, in accordance with this study's results, previous studies from Agbogbloshie have demonstrated that burning e-waste generates $PM_{2.5}$ and PM_{10} concentration over and above acceptable limits (Amoabeng et al., 2020; Kwarteng et al., 2020; Laskaris et al., 2021; Laskaris et al., 2019). These high levels of PM among e-waste workers, particularly e-waste burners, might be attributed to the release of fumes and smoke from e-waste combustion as well as other mechanical processes (dismantling, sorting, shredding and transportation), which also released dust into the ambient air (Kwarteng et al., 2020). In addition, there are other sources of PM such as the often seen biomass burning of refuse pile near the e-waste site, cooking of food with firewood by several local food sellers near the e-waste site, vehicular exhaust emissions all likely contribute to the higher levels of PM at the Agbogbloshie e-waste recycling site (Kwarteng et al., 2020). However, the fractions contributed by each activity at the site were not evaluated.

Notably, almost all the PM measured in the reference population exceeded the WHO Ambient Air Quality Guidelines (WHO, 2006). The high concentration of PM measured in the reference population suggests a high background concentration of PM in urban Ghana. This can be attributed to the over-reliance on solid fuels for cooking, biomass burning, vehicular emissions, and road dust (Health Effects Institute, 2019; Ofosu et al., 2012).

5.3.2 Measurement of metals in blood of e-waste workers and reference population

In this study, concentration of blood Pb was significantly higher in e-waste workers than in reference population, a finding consistent with data from previous biomonitoring studies among e-waste workers at Agbogbloshie (Srigboh et al., 2016; Takyi et al., 2021; Wittsiepe et al., 2017b). Higher levels of Pb was also measured in blood of teenage e-waste scavengers in Nigeria than unexposed reference group (Alabi et al., 2019), suggesting e-waste recycling activities (exposure) as a critical contributor to increased Pb levels. The high concentration of Pb observed in the blood of e-waste workers could be attributed to the increased use of Pb for

soldering purposes in electronic equipment (Mohammadyan et al., 2019), which is directly released during e-waste dismantling. In addition, burning solid waste near the e-waste recycling site and vehicular exhausts could also contribute to higher levels of Pb among e-waste workers.

Cadmium levels in blood was however significantly higher among the reference population than in e-waste workers even though levels detected in both populations were lower than background levels established among the US general population NHANES (CDC, 2019). Levels of Cd in e-waste and reference population in this current study are similar to those previously reported by Wittsiepe et al. (2017b) at the same site, when e-waste workers were compared to a reference population in Kwabenya North, a suburb of Accra. Alabi et al. (2019) reported similar body burden of Cd in e-waste scavenging workers and controls in Nigeria. It is possible that higher body burden of Cd in the reference group is attributable to ingestion of contaminated food or water other than from occupational exposure. For instance, tubers of yam and potatoes, rice, and vegetables are staple foodstuffs for this study's population and are mostly farmed in contaminated soil (Bortey-Sam et al., 2015); therefore, exposure to Cd could be via the ingestion of these contaminated foodstuffs.

For the essential metals, both Se and Mn were significantly higher in the reference population than e-waste workers, but similar to concentrations in US general population. Selenium and Mn, unlike Pb and Cd are essential dietary micronutrients, and differences could be explained by dietary habits and socioeconomic status (SES). Higher levels of blood Zn and Mn were measured in e-waste workers than previously recorded (Srigboh et al., 2016). Further, the high body burden of essential elements (Zn, Se, and Mn) in this study population could be attributed to exposure from food such as red meat, fish, poultry, whole grains and beans other than from occupational exposure.

Generally, in addition to occupational exposure of metals in e-waste workers, the high concentration of metals in both e-waste workers and reference group could be explained by the possible reliance on solid fuels for cooking, which is a significant source of ambient or outdoor air pollution. The Health Effects Institute (HEI) – Ghana working group estimated that residential sources (including household cooking, lighting and heating) contributed approximately 65% of the total national primary air pollution in Ghana, followed by transport and road dust (13.9%) (Health Effects Institute, 2019). In addition, the use of metal (galvanized iron) pipes for water distribution in Ghana and corroded household plumbing systems could be possible source of metals exposure through tap water (Asante et al., 2012).

5.4 Discussion of results for specific objective 3

5.4.1 Associations between global DNA methylation and PM exposure

The relationship between exposure to PM (2.5 and 10) and LINE-1 methylation was not statistically significant. These findings are consistent with a previous study where the investigators examined a cohort of male welders in the USA, and found that occupational exposure to $PM_{2.5}$ was not significantly associated with LINE-1 and Alu methylations (Kile et al., 2013.). A possible explanation for these results might be that PM concentrations in these studies were not high enough to induce epigenetic alterations. However, the findings do not support many previous occupational and environmental studies (Fan et al., 2014; Mishra et al., 2021; Tarantini et al., 2009; Wu et al., 2021). For instance, a study by Fan et al. (2014) among welders found a significant positive association between $PM_{2.5}$ and LINE-1 methylation measured in blood. In addition, Mishra et al. (2021) in their pilot study found an increase in LINE-1 DNA methylation among individuals exposed to high levels of air pollution.

Several other studies have reported inverse relationships between PM and DNA methylation in both occupational and environmental settings. For instance, Tarantini et al. (2009) found a decrease in LINE-1 methylation with high PM_{10} exposure in peripheral blood samples of steel

production plant workers in Italy. A similar association has been reported in Thailand, where decreased LINE-1 methylation was associated with working in an industrial estate with elevated levels of air pollution (Peluso et al., 2012). Additionally, a decrement in LINE-1 methylation was reported in a cohort of 718 elderly participants exposed to $PM_{2.5}$ in the Boston area Normative Aging Study (Baccarelli et al., 2009). Furthermore, a recent systematic review and meta-analysis by Wu et al. (2021) found an inverse association between $PM_{2.5}$ and global DNA methylation in adults. The variability of results seen in studies where PM alter DNA methylation could be attributed to the source and composition of PM. For example, Tarantini and co-workers measured PM in steelworkers (Tarantini et al., 2009), Fan et al. (2014) measured PM concentrations in welders, while this present study measured PM from e-waste recyclers. Different sources of pollution may result in different complex mixtures of toxic organic chemicals which in turn might have different biological effects (Peluso et al., 2012). For example, welders may be exposed to high levels of metals (Ding et al., 2016), while PM generated through e-waste recycling comprised of a relatively lower concentration of metals (Bi et al., 2010). The discrepancies in findings may also reflect the variability of epigenetic responses to environmental exposure since factors such as ethnicity, occupation, or tissue type may influence the effect of the same pollutant on the epigenome (Jamebozorgi et al., 2018). Also worthy of note is the fact that, DNA methylation may be dependent on other factors such as timing and length of exposure, routes of exposure, and host genetics (Galanter et al., 2017; Ji & Hershey, 2012), and therefore could contribute to the variability observed from different studies. The mechanism of these contributory factors therefore require further studies and clarification.

Previous studies have shown that LINE-1 specific CpG sites by default differ in methylation levels (Sharma et al., 2019b) and respond differentially to environmental factors (Goodrich et al., 2015). This suggests that a site-specific approach to the assessment of environmental

pollutants and epigenetic modifications is critical in understanding the biological mechanism of exposure-outcome relationships (Goodrich et al., 2015; Hanna et al., 2012). This study found a significant positive association between $PM_{2.5}$ and LINE-1 CpG2 site methylation. The results suggest that a 1 $\mu g/m^3$ resulted in a 0.003 increase in %LINE-1 CpG2 DNA methylation. This finding should be taken with caution because, even though specific CpG sites methylation levels provide a limited assessment of the region of interest (Hanna et al., 2012), the evidence available suggests that alteration of one of the four CpG sites may alter the overall LINE-1 DNA methylation (Hata & Sakaki, 1997).

The relationship between specific e-waste job tasks performed by e-waste workers and LINE-1 methylation was also assessed. The results showed a significant decline in LINE-1 methylation among e-waste collectors compared to those in the reference group. This results suggests that e-waste collectors maybe susceptible to adverse health outcomes due to genome instability associated with significant decrease in global (LINE-1) DNA methylation (Hypomethylation). However, this difference could be due to the relatively small sample size in each of the job categories which may increase the likelihood of chance finding due to the effect of multiple comparison analysis.

The mechanism linking PM exposure to DNA methylation is not fully understood. Exposure to high PM concentrations increases reactive oxygen species (ROS) generation (Gurgueira et al., 2002), and subsequent DNA damage, such as strand brakes (James et al., 2003). DNA damage increases DNA methyltransferase (DNMT) activities with high affinity to DNA repair sites that methylate adjacent CpG nucleotides (Cuozzo et al., 2007; James et al., 2003). The increased DNMT activity secondary to DNA damage may increase LINE-1 CpG methylation levels (Bollati et al., 2011), as observed in this study. Recent reports showed that cells exposed to PM resulted in the upregulation of all three DNA methyltransferases (DNMT1, 3a 3b),

positively corresponding with a considerable increase in global DNA methylation (Bhargava et al., 2019; Sunil et al., 2017).

5.5 Discussion of results for specific objective 4

5.5.1 Association between a mixture of toxic and essential metals and global DNA methylation

The association between both toxic and essential metals and global DNA methylation was evaluated using linear regression models and other variants of the outcome model (robust and LASSO regressions) as sensitivity analyses was examined. The study aimed to examine the overall effect of both toxic and essential metals blood levels on global (LINE-1) DNA methylation and the effects of their interactions on LINE-1 DNA methylation. The results showed that blood Zn levels significantly reduced LINE-1 methylation in both the single and multiple metals regression analyses. In addition, the interaction between toxic and essential elements on LINE-1 methylation was observed.

Results across single metal linear regression, mutual regression, and sensitivity analyses consistently indicate that Zn is inversely associated with LINE-1 DNA methylation, at least in the populations studied. This inverse association between Zn and LINE-1 DNA methylation is consistent with Zn's role as a micronutrient essential for the epigenome stability (Maret & Sandstead, 2008; Wallwork & Duerre, 1985) which directly contributes to the methyl pool, i.e. the amount of methyl (CH_3) group available for DNA methylation purposes. Therefore, deficiency or excess of this essential element may contribute to epigenetic alteration such as DNA hypomethylation. The concentration of Zn in this study is extremely high and far exceeds that levels measured by NHANES among American populations. The high concentration of blood Zn observed in this study could be evidence of non-homeostatic Zn retention in the body due to excess exposure from several sources, including diet. Evidence suggests that either Zn deficiency or excess causes cellular oxidative stress (Lee, 2018) which could alter DNA

methylation (Liou et al., 2017). Therefore, the high Zn concentrations observed in this study population could be a source of DNA oxidative stress, implicated in a decrease in global DNA methylation (LINE-1). In their paper, Valinluck et al. (2004) showed that oxidative stress markers (8-OHdG) could alter the ability of human DNMTs to methylate the adjacent cytosine. Consistent with findings by Valinluck and colleagues, Liou et al. (2017), demonstrated a significant negative correlation between 8-OHdG and global DNA methylation in peripheral blood lymphocytes (PBL) of healthy nanomaterial handling workers. The oxidative demethylation reaction could be attributed to the accumulation of α-ketoglutarate (α-KG) (a co-factor of ten-eleven translocation (TET) protein) arising from metals-induced oxidative stress (Sen et al., 2015). The increase in the amount of α-KG might increase the activities of TET proteins which oxidizes 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) (a key intermediate in active demethylation pathways) in the DNA (Chia et al., 2011; Kohli & Zhang, 2013). Furthermore, oxidative stress DNA damages induced by metals can induce DNA hypomethylation by blocking DNA to function as a substrate for DNMTs (Valinluck et al., 2004).

Although ample scientific evidence exists to suggest that Cd, Pb, and Mn may be associated with DNA methylation (Hou et al., 2012), this study did not observe any significant association of these metals with LINE-1 DNA methylation (the average of all 4 CpGs of LINE-1) in the linear regression analyses. Due to the small sample size, there may be insufficient power to detect any significant association or the confounding effects of unmeasured exposures from other sources such as diet. In addition, there is evidence of weak reproducibility between studies when using LINE-1 as a biomarker of global DNA methylation (Choi et al., 2009; Ohka et al., 2011) due to the use of different assays targeting different CpG sites within LINE-1 (Sharma et al., 2019a).

Based on Goodrich et al. (2015)'s recommendation to evaluate site-specific effects of exposures, each of the four specific CpG sites of LINE-1 using both single and multiple metals models were further analyzed. Pb and Se concentrations showed a significant inverse association with CpG2 ($p = 0.048$) and CpG3 ($p = 0.048$), respectively, only in the single metal analysis. These findings are consistent with a recent study that reported an association between Se and DNA methylation at specific CpG sites (Tian et al., 2020). Similarly, occupational Pb exposure was associated with specific CpG sites methylation measured using the Illumina Infinium Methylation EPIC BeadChip (850 K) (Zhang et al., 2019c). The consistencies observed in our study and those of previous researchers could be attributed to the fact that individual CpG sites of LINE-1 may exhibit different methylation levels in response to the same exposure (Sharma et al., 2019a).

The changes in DNA methylation levels by toxic metals such as Pb and Cd are well documented in the literature (Devóz et al., 2017; Hossain et al., 2012; Tellez-Plaza et al., 2014; Wright et al., 2010), whereas less is known concerning their interaction with essential metals on DNA methylation. The results showed that Cd significantly reduced LINE-1 methylation at low Zn levels; however, LINE-1 methylation significantly increased at a higher concentration of Zn. This finding is consistent with findings from both human studies and animal models where Zn has been reported to mitigate epigenetic effects (Reeves & Chaney, 2004; Vidal et al., 2015). Several studies have reported that increased Zn concentration may reduce Cd absorption and accumulation and mitigate Cd's adverse effects (Brzóska & Moniuszko-Jakoniuk, 2001). The mechanism by which Zn antagonizes Cd is not fully known; however, it has been partly attributed to the competition between Cd and Zn at co-factor sites requiring Zn such as methionine synthase, which may decrease the activity of these enzymes (Elinder & Piscator, 1978; Maret & Sandstead, 2008).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

The final chapter of this thesis is divided into two parts. The first part draws conclusions based on the results of the thesis. The second part presents recommendations to all stakeholders involved in the management of e-waste, followed by the limitations and strengths of the study as well as the contribution of the study to scientific knowledge.

6.2 Conclusions

Personal PM (2.5 and 10) and blood concentration of toxic (Cd and Pb) and essential (Se, Zn, and Mn) metals and their associations with global (LINE-1) DNA methylation were assessed among informal e-waste workers at Agbogbloshie and a reference population at Madina Zongo.

Personal PM (2.5 and 10) concentrations were higher among e-waste workers, especially workers who burned e-waste compared to the reference population. PM concentrations were over and above the WHO recommended exposure limits for short-term exposure. However, no significant association was observed between PM (2.5 and 10) and global (LINE-1) DNA methylation in both e-waste workers and reference population. This could indicate an overall high level of exposures in the general population.

Regards toxic metals exposure, Pb concentration was higher among e-waste workers than reference population whilst Cd was rather higher among the reference population. Majority of the essential metals (Se and Mn) were also higher among the reference population compared to the e-waste workers. The high concentration of metals in both the e-waste workers and reference population may be a consequence of defused or generalized pollution sources rather than from a point source such as e-waste recycling activities.

High Zn concentration was associated with a significant reduction in global (LINE-1) DNA methylation among e-waste workers and a potential antagonism effect may exist between Zn and Cd on global (LINE-1) DNA methylation. The alteration of LINE-1 DNA methylation by metals could serve as an early epigenetic marker of effect that could mediate metals-induced related adverse health outcomes. This could also suggests the utility of epigenetic markers such as global DNA methylation for indicating toxic exposures among people who directly recycle e-waste.

6.3 Recommendations

The need to manage e-waste, especially processing in a manner that will improve public and environmental health in Ghana cannot be over emphasized. The key stakeholders who are responsible for the management of e-waste in Ghana include among others; government agencies such as the Ministry of Health and Ghana Health Service (MOH/GHS), the Environmental Protection Agency (EPA), and the Ministry of Environment Science Technology and Innovation (MESTI), and the e-waste recyclers and their associations. These stakeholders need to collaborate in order to win the fight of indiscriminate dumping and crude recycling of e-waste in Ghana. The following are some recommendations to the various stakeholders to help improve the management of e-waste in Ghana.

6.3.1 Recommendations e-waste workers

- Educate e-waste workers and their unions in an understandable fashion on the long-term health effects of pollutants exposure associated with their work. When e-waste workers understand and believe that they are at risk for developing a disease, and the benefits of taking an action to avoid it, they may be motivated to take responsibility and act on behalf of their own health to the greatest extent possible.
- Provide and educate e-waste workers on the need to wear appropriate personal protective equipment (PPEs) such as gloves, well-fitting mask, boots, and coveralls.

PPEs will help reduce inhalation, ingestion and dermal exposures while performing recycling activities

- Efforts should be made to keep homes of e-waste workers separate from their work area. This will help minimise exposure to the rest of the family.

6.3.2 Recommendations to policy makers

- The already existing hazardous and electrical waste control and management act, 2016 (Act 197), which aims to provide for the control, management, and disposal of electrical and electronic waste in Ghana should be enforced by the EPA.
- The EPA should derive locally acceptable exposure limits for hazardous chemicals that will serve as a guide in managing the control of occupational and environmental exposures in Ghana.
- Government through the MESTI should develop a technically advanced recycling facility to help formalize the recycling process and reduce the levels of chemicals exposure from open-pit burning.

6.4 Future research direction

- Researchers should scale-up molecular epidemiology studies at the Agbogbloshie e-waste recycling site to aid in the early detection of adverse effects of e-waste exposure before the onset of clinical symptoms. Researchers should investigate gene-specific methylation alterations (e.g. tumour suppressor genes, oncogenes, or DNA repair genes) to help identify specific potential downstream health consequences in this vulnerable population. Findings of such studies will further provide elucidation of the pathways by which e-waste related chemical will induce adverse health effects, and help to guide in the design and implementation of prevention strategies.
- Even though the e-waste recycling industry is male dominated, vulnerable populations such as children and pregnant women live and work at the recycling site or nearby.

These populations are continually exposed to the high levels of chemicals released during the recycling process. There is therefore the need to scale-up research in these categories of people since some of the chemicals are developmental neurotoxicants that can cross the placenta and affect birth outcomes.

- Future research should again consider mixture analysis that will reflect the reality of e-waste exposure since workers and residents are exposed to a myriad of chemicals released during recycling and not one chemical at a time.

6.5 Strengths and Limitations of the study

Limitations

- The cross-sectional nature of this study limits its ability to draw conclusions about the causality of PM or metals on global DNA methylation in this population
- This study examined the associations between PM and metals on one hand and global DNA methylation on the other hand, however, workers involved in informal e-waste recycling are exposed to a myriad of chemicals including PAHs, VOCs, BFRs, and PCBs; which could also influence LINE-1 DNA methylation. This study, therefore, is unable to rule out that other pollutants may have effects on DNA methylation profiles in blood of the population studied.
- Other domains of environmental exposure, such as nutrition (including dietary intake data on folic acid, choline, vitamin B12, and betaine), and psychosocial stress, which could affect DNA methylation were not measured in this study.
- PM was measured for 4 hours of the worker's work-shift, which may not be representative of the overall 8-hour occupational exposure. However, since there were no regulations and documented work schedules in place, the 4-hours monitoring in the

informal e-waste site was sufficient to monitor participants during their active work hours.

Strengths

- To the best of my knowledge, this is the first study to examine DNA methylation among informal e-waste recyclers with rich characterization of exposures
- As part of a bigger study (GEOHealth II study), this study benefitted from high-quality protocols for recruiting participants, conducting interviews, collecting biological samples, and laboratory analyses
- This study assessed the interaction effects of toxic metals and essential elements on global DNA methylation, which has not been previously reported in occupationally exposed populations.
- The Agbogbloshie e-waste recycling site is arguably one of the most contaminated, best researched, and most easily accessible site worldwide and therefore represents a good place to investigate pollution exposure to workers and associated adverse health outcomes.

6.5 Contribution to knowledge

To the best of my knowledge, this is one of the first studies to examine this population in an epigenetic context. Since the loss of methylation in LINE-1 repetitive elements is associated with genomic instability and plays a vital role in carcinogenesis and other pathological conditions (Cao, 2015), the results of this study may provide a potential explanation for occupational metal mixture exposure and DNA methylation mediated diseases associated with occupational exposures. The results of this study will further contribute to the rather slowly growing interest in evaluating the health effects of multiple metal exposure from e-waste recycling activities, especially in developing countries (Oguri et al., 2018), which is an

understudied global health issue. The present study further adds to the growing body of research that indicates that the adverse effects of metal mixtures are different from that of a single metal, and the effects of toxic metals can be modified by the concentration of essential metals (Wu et al., 2016).

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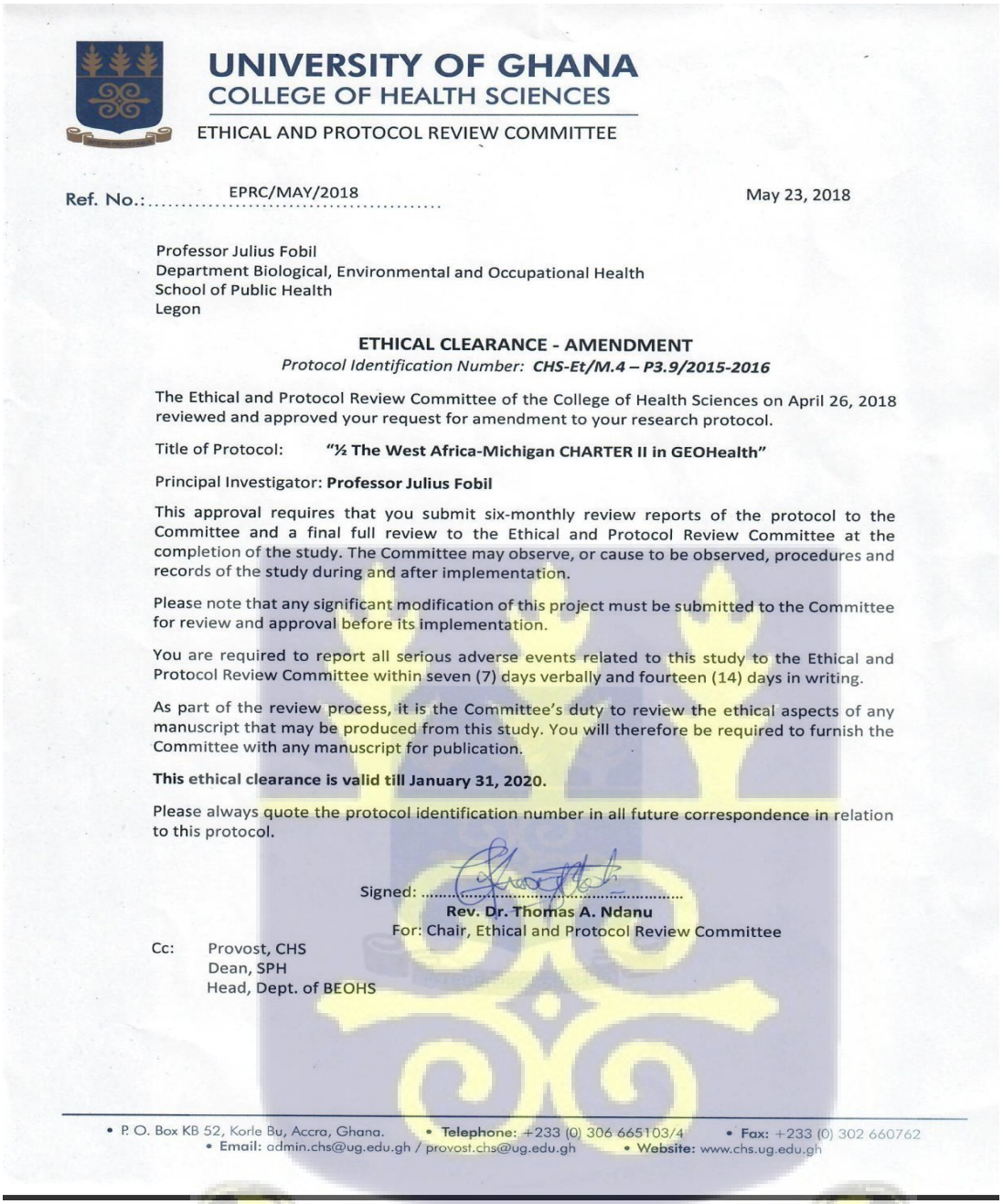
APPENDICES

Appendix 1: Ethical clearance



UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/MAY/2018 May 23, 2018

Professor Julius Fobil
Department Biological, Environmental and Occupational Health
School of Public Health
Legon

ETHICAL CLEARANCE - AMENDMENT
Protocol Identification Number: ***CHS-Et/M.4 – P3.9/2015-2016***

The Ethical and Protocol Review Committee of the College of Health Sciences on April 26, 2018 reviewed and approved your request for amendment to your research protocol.

Title of Protocol: **"½ The West Africa-Michigan CHARTER II in GEOHealth"**

Principal Investigator: **Professor Julius Fobil**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till January 31, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Rev. Dr. Thomas A. Ndanu
For: Chair, Ethical and Protocol Review Committee

Cc: Provost, CHS
Dean, SPH
Head, Dept. of BEOHS

• P. O. Box KB 52, Korle Bu, Accra, Ghana. • Telephone: +233 (0) 306 665103/4 • Fax: +233 (0) 302 660762
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UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

My Ref. No.....

14th January, 2016.

Prof. Julius Fobil
Department of Biological,
Environmental and Occupational Health,
School of Public Health,
University of Ghana
Legon, Accra

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.4 – P 3.9/2015-2016**

The Ethical and Protocol Review Committee of the College of Health Sciences unanimously approved your research proposal.

TITLE OF PROTOCOL: **The West Africa-Michigan collaborative Health Alliance for Reshaping Training, Education and Research for Global Environmental and Occupationa Health – Investing in Innovation (Short Title: 1/2 The West Africa-Michigan CHARTER II for GEOHealth)**

PRINCIPAL INVESTIGATORS: **Prof. Julius Fobil**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 31st January, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed:.....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SMD
Head of Department

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Appendix 2: Consent Form

U01 Research Study: Integrated assessment of exposures due to informal level recycling of electronic wastes at Agbogbloshie

Participant Information Leaflet and Consent Form
What every prospective participant should know before deciding to or not to participate

Title of Research: "Integrated assessment of exposures due to informal level recycling of electronic wastes at Agbogbloshie"

Name(s) and affiliation(s) of researcher(s):
This study is being conducted by Prof. Julius N. Fobil, Dr. John Arko-Mensah, Dr. Judith Stephens, Sylvia A. Takyi, Lawrencia Kwarteng, Afua Amoabeng, & Augustine Acquah of the University of Ghana, School of Public Health, Legon-Accra, and Zoey Laskaris, Prof. Thomas G. Robins, and Prof. Stuart Batterman of the Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, USA, and Prof. Niladri Basu of the Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Canada

About the informed consent to participate in the study:
We are inviting you to take part in a research study. Before you decide whether to take part, it is important that you to understand why the research is being done and what it will involve. Ask us if there is anything, which is not perfectly clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Background of the study:
Large amounts of electronic waste (e-waste) are discarded and processed at recycling sites in developing countries. E-waste includes for example old televisions, monitors, computers, and cell phones. The e-waste dump at Agbogbloshie is one of the largest worldwide. During the work in the recycling process, for example by burning plastic material, high doses of toxic substances are released (for example lead) and may be taken up by exposed persons. The amount of poisonous chemicals present in the human body in persons exposed is not well investigated. Those poisonous chemicals can have serious effects on your health, including lung problems affecting breathing, but the effects of a combination of poisonous chemicals are not well known to date.

Purpose(s) of research:
The purposes of this research are to systematically investigate the levels of those environmental poisons and the health status in workers at the e-waste dump at Agbogbloshie, and compare them to exposures and health problems in persons not exposed to e-waste processing activities. The results of the study will demonstrate the risk of health damage for persons involved in the recycling process. The results can guide actions against pollutions, will point out the need for further investigations of the effects of the poisons on your health, and may lead to changes in work methods to better protect the health of workers.

Procedure of the research, what shall be asked of each participant and approximate total number of participants that would be involved in the research:
If you agree to participate in this study, you will be asked on three occasions (baseline, 12 months later, 24 months later) to complete questionnaires, blow into a hand-held portable device used to measure your lung function (how well you are breathing), provide blood and urine samples to detect possible exposures to harmful chemicals, and wear a vest during one of your workdays containing instruments to measure levels of chemicals in the air you are breathing, as well as providing a buccal swab for possible future genetic studies.

Questionnaire. Answer questions on your life situation, health problems you may have experienced, your daily routine and your work on the recycling site. One of our field workers will ask you these questions in your preferred language (English, Dagbani, or Hausa). The questionnaire will require approximately 90 minutes for completion. Also, a doctor will perform a short medical examination (about 5 minutes).

Lung function test. You will be asked to blow into a hand-held portable device used to measure your lung function (how well you are breathing). The information retrieved from the device will be sent directly to your medical doctor if you ask us to do so.

Blood and urine samples. Then, you will be asked to produce a urine sample to help detect potentially hazardous chemicals which may have entered your body. In addition, we will insert a needle into a vein in your arm to withdraw 20 to 30 mL of blood (approximately a teaspoonful of blood) also for the detection of potentially hazardous chemicals in your body. The samples obtained will be shipped to USA and Canada and tested in a specialized laboratories.

Buccal swab for possible future genetic analyses. Only the first time you participate in the study you will be asked to provide a buccal swab, obtained by gentle rubbing of the inside of the cheek inside your mouth. The sample will be used only to look at genes affecting how likely it is that potentially harmful exposures will cause breathing problems.

Potential Risk(s): There is no significant risks for your health from participating in the study.

From obtaining blood samples. You may experience modest discomfort from needle sticks to obtain the blood sample. Rarely, a small hematoma (blood clot) may form at the site. **Protective measures**: Blood samples will be obtained only by study staff well-trained in phlebotomy (drawing blood) using standard sterile equipment and procedures, to minimize discomfort and prevent any risk of infection.

From the air sampling procedures. The pump (mounted at the waist on a belt) may cause slight discomfort because of its weight and because it needs to be worn for the full shift. **Protective measures**: The lightest available pumps will be used, and level of comfort assured by the study staff placing the pump on individual.

From lung function tests. Some persons may experience transient lightheadedness from performance of the lung function test. Rarely persons with pulmonary conditions such as asthma, may experience increased obstruction to airflow with wheezing and/ or shortness of breath in response to repeated testing. **Protective measures**: Prior to the administration of spirometry, the study staff will ask you about any underlying lung conditions as well as your state of health at the current time. Study staff will be trained to monitor both the appearance of the participant and the real-time outcome of maneuvers to detect evidence of any increasing airway obstruction. If detected, study staff will end the test without obtaining further maneuvers.

From interviews and questionnaires: Rarely some individuals may experience unease or discomfort at answering questions about family socioeconomic position, use of tobacco products, or previous or current illnesses. **Protective measures**: your answers will be completely confidential and seen only by study staff carrying out required aspects of the study. Also, you are free to decline to answer any question you wish without penalty.

From providing buccal swab samples for genetic analysis: Some individuals may experience unease at providing samples for genetic information. **Protective measures**: We will check the sample only for genes that may put an individual at risk for more severe effects on respiratory health owing to e-waste associated exposures. Also, you are free to decline participation in this aspect but still participate in all other aspects of the study.

Benefit(s): You will be seen and examined by a doctor, who will provide some basic medical treatment on site. If he finds a health problem, which requires further medical treatment, he will write a short report and make a recommendation where to present. The costs of the further medical treatment cannot be covered. Information documenting and interpreting your levels of their exposure to potentially adverse agents, and measures of your current health status will be provided to you. More generally, results of the study should provide information that can help guide effective interventions and solutions to health risks for those working at the Agbogbloshie site.

Confidentiality: All data collected within this study will be treated confidentially. Your records are confidential and will be available only to study staff as needed only for purposes of carrying out the study. Overall results for groups of individuals participating in the study may be

published, but will be presented in such a way that the identities of participating individuals are never revealed.

Voluntariness: Whether to agree to participate in this study it is entirely up to you. You are not under any obligation to participate. There are no penalties for declining to participate in the study.

Withdrawal from the research:
You may choose to withdraw from the research at anytime without having to explain yourself. Also, you may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:
There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study at any time. Please note however, that some of the information that may have been obtained from you without identifiers (name etc), before you chose to withdraw, may have been modified or used, without any personal identifiers (name, address, etc.), in analysis, reports and publications.

Compensation:
You will receive a consolidated compensation in two parts as follows: **1)** *upon completion of questionnaire, spirometry as well as donation of blood and urine samples, you receive - a bottle of water, milo, a tin of milk and one meat pie*; **2)** *on a separate day to be agreed between you and research team, upon completion of 8hrs of wearing backpack which contains personal air samplers - you receive 20 GHc cash and a T-Shirt*.

Contacts: One of the study coordinators will be present during the study recruitment. If you still have any question concerning this study, please do not hesitate to contact Prof. Julius N. Fobil at the University of Ghana, School of Public Health, Legon-Accra, Telephone: 0243 462514).

Your rights as a Participant
This research has been reviewed and approved by the Institutional Review Board of the College of Health Sciences (CHS-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0244 061 270 or email addresses: feyanumu@chs.edu.gh or feyanumu@ug.edu.gh.

U01 Research Study: Integrated assessment of exposures due to informal level recycling of electronic wastes at Agbogbloshie

CONSENT FORM

Title of Research:

"Integrated assessment of exposures due to informal level recycling of electronic wastes at Agbogbloshie"

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision on whether to participate or not participate.

NAME: _____

DATE: _____ SIGNATURE: _____

Statement of person giving consent:

I have read and understood the background information on this study or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time without penalty. I have received a copy of this information leaflet and consent form to keep for myself.

NAME_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

WITNESS' NAME: _____

U01 Research Study: Integrated assessment of exposures due to informal level recycling of electronic wastes at Agbogbloshie

WITNESS' SIGNATURE:

_____

Appendix 3: Questionnaire

ID number: •☐☐☐☐

7. To which of the following religious groups do you belong?

☐0 No religion ☐1 Catholic Christian ☐2 Protestant Christian
☐3 Muslim ☐4 Traditional ☐5 Other (specify) _____

8. What is your usual daily income?

☐1 ≤GHS 20 ☐2 GHS 21-40 ☐3 GHS 41-60 ☐4 GHS 61-80
☐5 GHS 81-100 ☐6 GHS 101-120 ☐7 GHS 121-140 ☐8 GHS 141-200
☐9 GHS > 200

9. What is your marital status?

☐1 Single ☐2 Married ☐3 Not married, living with partner
☐4 Divorced ☐5 Widowed ☐6 Separated

10. What is the highest level of school you attended: primary, middle/JSS, secondary/SSS, or higher?

☐0 None ☐1 Primary ☐2 Middle/JSS ☐3 Secondary/SSS ☐4 Higher

Part 3. OCCUPATIONAL - ELECTRONIC-WASTE TASKS

Now I will ask you about tasks related to electronic waste.

11. Have you worked at this electronic-waste site in Agbogbloshie for more than ONE YEAR?

☐1 Yes ☐2 No [GO TO 14]

12. How long have you worked at this electronic-waste site in Agbogbloshie?

_____years _____months _____days

13. Have you EVER worked ____[fill in blank with job types listed]____ since arriving at Agbogbloshie?

[ASK QUESTION AGAIN FOR EACH JOB ACTIVITY LISTED]

a. Repairing electronics → Year started:_____, Year Ended:_____ or ☐(check) if current
b. collecting or off-loading e-waste → Year started:_____, Year Ended:_____ or ☐(check) if current
c. Dismantling electronic waste → Year started:_____, Year Ended:_____ or ☐(check) if current
d. Removing covering off of wires → Year started:_____, Year Ended:_____ or ☐(check) if current
e. Sorting electronic waste → Year started:_____, Year Ended:_____ or ☐(check) if current
f. Burning electronic waste → Year started:_____, Year Ended:_____ or ☐(check) if current
g. Burning wires only → Year started:_____, Year Ended:_____ or ☐(check) if current
h. Collecting wires after burning → Year started:_____, Year Ended:_____ or ☐(check) if current
i. Trading or selling e-waste → Year started:_____, Year Ended:_____ or ☐(check) if current
j. Smelting lead batteries → Year started:_____, Year Ended:_____ or ☐(check) if current
k. OTHER:_____ → Year started:_____, Year Ended:_____ or ☐(check) if current

[When finished with 13, GO TO 16]

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ID number: •☐☐☐☐

14. How long have you worked at this electronic-waste site in Agbogbloshie?

_____months _____days

15. Have you EVER worked _____ since arriving at Agbogbloshie?
[ASK QUESTION AGAIN FOR EACH JOB ACTIVITY LISTED]

a. Repairing electronics → Month started:_____, Month Ended:_____ or ☐(check) if current
b. Collecting or off-loading e-waste → Month started:_____, Month Ended:_____ or ☐(check) if current
c. Dismantling electronic waste → Month started:_____, Month Ended:_____ or ☐(check) if current
d. Removing covering off of wires → Month started:_____, Month Ended:_____ or ☐(check) if current
e. Sorting electronic waste → Month started:_____, Month Ended:_____ or ☐(check) if current
f. Burning electronic waste → Month started:_____, Month Ended:_____ or ☐(check) if current
g. Burning wires only → Month started:_____, Month Ended:_____ or ☐(check) if current
h. Collecting wires after burning → Month started:_____, Month Ended:_____ or ☐(check) if current
i. Trading or selling e-waste → Month started:_____, Month Ended:_____ or ☐(check) if current
j. Smelting lead batteries → Month started:_____, Month Ended:_____ or ☐(check) if current
k. OTHER:_____ → Month started:_____, Month Ended:_____ or ☐(check) if current

16. In the past THREE MONTHS which job did you do the MOST [Rank =1]? [CHECK ONE ONLY]

☐1 Repairing electronic waste
☐2 Collecting electronic waste
☐3 Sorting electronic waste
☐4 Removing covering of wires
☐5 Dismantling Electronic Equipment
☐6 Burning Electronic waste
☐7 Burning wires only
☐8 Ash/wire collection after burning
☐9 Lead Smelting
☐10 Buying or trading e-waste
☐777 Other → Please specify: _____
☐789 I haven't worked 3 months [GO TO 20]

17. In the past THREE MONTHS, which job did you do the SECOND MOST [Rank =2]? [CHECK ONE ONLY]

☐1 Repairing electronic waste
☐2 Collecting electronic waste
☐3 Sorting electronic waste
☐4 Removing covering of wires
☐5 Dismantling Electronic Equipment
☐6 Burning Electronic waste
☐7 Burning wires only
☐8 Ash/wire collection after burning
☐9 Lead Smelting
☐10 Buying or trading e-waste
☐777 Other → Please specify: _____
☐789 I never worked more than 1 job [GO TO 20]

ID number: •☐☐☐☐

18. In the past THREE MONTHS, which job did you do the THIRD MOST [Rank =3]? [CHECK ONE ONLY]

☐1 Repairing electronic waste
☐2 Collecting electronic waste
☐3 Sorting electronic waste
☐4 Removing covering of wires
☐5 Dismantling Electronic Equipment
☐6 Burning Electronic waste
☐7 Burning wires only
☐8 Ash/wire collection after burning
☐9 Lead Smelting
☐10 Buying or trading e-waste
☐777 Other → Please specify: _____
☐789 I never worked more than 2 jobs [GO TO 20]

19. In the past THREE MONTHS, which job did you do the FOURTH MOST [Rank =4]? [CHECK ONE ONLY]

☐1 Repairing electronic waste
☐2 Collecting electronic waste
☐3 Sorting electronic waste
☐4 Removing covering of wires
☐5 Dismantling Electronic Equipment
☐6 Burning Electronic waste
☐7 Burning wires only
☐8 Ash/wire collection after burning
☐9 Lead Smelting
☐10 Buying or trading e-waste
☐777 Other → Please specify:_____
☐789 I never worked more than 3 jobs [GO TO 20]

20. Over the past WEEK, how many days did you work at this electronic-waste site in Agbogbloshie?
_____# of days

21. Over the past WEEK did you _____ for at least ONE HOUR?
[ASK QUESTION AGAIN FOR EACH JOB ACTIVITY LISTED]

a. Repair electronics → How many days did you do this:_____ # of Days
b. collect or off-loading e-waste → How many days did you do this:_____ # of Days
c. Dismantle electronic waste → How many days did you do this:_____ # of Days
d. Remove covering off of wires → How many days did you do this:_____ # of Days
e. Sort electronic waste → How many days did you do this:_____ # of Days
f. Burn electronic waste → How many days did you do this:_____ # of Days
g. Burn wires only → How many days did you do this:_____ # of Days
h. Collect wires after burning → How many days did you do this:_____ # of Days
i. Trade or selling e-waste → How many days did you do this:_____ # of Days
j. Smelt lead batteries → How many days did you do this:_____ # of Days
k. OTHER:_____ → How many days did you do this:_____ # of Days

22. On average, how many HOURS PER DAY did you work last week?
_____# of Hours per day

ID number: •☐☐☐☐

23. On the LAST DAY you worked, did you _____ for at least 30 MINUTES?
[ASK QUESTION AGAIN FOR EACH JOB ACTIVITY LISTED and ROUND TO THE NEAREST HOUR]

a. Repair electronics → How long did you do this:_____ # of Hours
b. collect or off-loading e-waste → How long did you do this:_____ # of Hours
c. Dismantle electronic waste → How long did you do this:_____ # of Hours
d. Remove covering off of wires → How long did you do this:_____ # of Hours
e. Sort electronic waste → How long did you do this:_____ # of Hours
f. Burn electronic waste → How long did you do this:_____ # of Hours
g. Burn wires only → How long did you do this:_____ # of Hours
h. Collect wires after burning → How long did you do this:_____ # of Hours
i. Trade or selling e-waste → How long did you do this:_____ # of Hours
j. Smelt lead batteries → How long did you do this:_____ # of Hours
k. OTHER:_____ → How long did you do this:_____ # of Hours

24. In the past ONE MONTH, which job did you do the MOST? [CHECK ONE ONLY]

☐1 Repairing electronic waste
☐2 Collecting electronic waste
☐3 Sorting electronic waste
☐4 Removing covering of wires
☐5 Dismantling Electronic Equipment
☐6 Burning Electronic waste
☐7 Burning wires only
☐8 Ash/wire collection after burning
☐9 Lead Smelting
☐10 Buying or trading e-waste
☐777 Other → Please specify: _____
☐789 Did not work a whole month

25. In your lifetime, have you worked at other electronic waste or scrap metal sites?

☐1 Yes ☐2 No

→ 25A. If yes, for how long? _____(# of years, or months if less than 1 year)

[GO TO NEXT PAGE]

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ID number: •☐☐☐☐

26. Please tell me if you think any of the jobs you have EVER done since working with electronic waste in Agbogbloshie have caused you to have the following problems.

Symptoms	Irritation or burning of the eyes, nose, or throat?	Wheezing or whistling sound in your chest apart from colds?	Shortness of breath, difficulty catching your breath, or a smothering feeling?	Chest tightness or a sensation of a band around the chest?	Other problem: Please describe
Repair electronics	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Collect or off-load e-waste	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Dismantle electronic waste	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Remove covering of wires	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Sort electronic waste	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Burn electronic waste	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Burn wires only	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Ash/wire collection after burning	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Smelt lead batteries	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Trade e-waste	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	
Other Job (from above)	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	☐1 Yes ☐2 No	

☐789 Check here if participant never worked jobs that involved burning and GO TO 29

27. Think about the last day you worked burning wires, burning electronic-waste, ash/wire collection after burning, or smelting. What was used to light the fire?

[Check ALL that apply]

☐1 Styrofoam ☐2 Petrol/ kerosene ☐3 Tires ☐4 Only lighters and matches
☐5 Saw dust ☐6 Coconut shell
☐777 Other → Please describe:_____ ☐789 I don't know [GO TO 29]

ID number: •☐☐☐☐

28. Is this the usual way fires are started?

☐$_1$ Yes [GO TO 29]
☐$_2$ No ⟶ 28A. If No, what is usually done?_____
☐$_{789}$ I don't know.

29. In your job, do you regularly wear safety clothing or equipment like gloves, boots, ear plugs or dust masks?

☐$_1$ Yes ☐$_2$ No [GO TO 30]

29A. If Yes, which clothing or equipment do you regularly wear?

Safety glasses, goggles, or other eye protection such as face shields?	☐$_1$ Yes	☐$_2$ No
Rubber-soled boots or shoes?	☐$_1$ Yes	☐$_2$ No
Gloves?	☐$_1$ Yes	☐$_2$ No
Dust mask or respirator?	☐$_1$ Yes	☐$_2$ No
Long trousers?	☐$_1$ Yes	☐$_2$ No
Ear plugs or earmuffs to block sound?	☐$_1$ Yes	☐$_2$ No
Helmet?	☐$_1$ Yes	☐$_2$ No

EXAMPLES OF SAFETY EQUIPMENT

7

ID number: •☐☐☐☐

Part 4. JOB HISTORY

Now I will ask you some questions about all the jobs you have had for at least 6 months in your lifetime.

30. At the same time as working in Agbogbloshie with electronic waste, have you done any other jobs in the LAST MONTH?

☐$_1$ YES ☐$_2$ NO [GO TO 31] ☐$_{789}$ Did not work in Agbogbloshie for a total of one month [GO TO 31]

→ 30A. If yes, please describe your other current jobs.

JOB TITLE	JOB DESCRIPTION	EMPLOYER NAME / [If family, write "Family"]	Average HOURS/ WEEK	START (MM/YYYY)
1.				
2.				

31. Other than e-waste work at Agbobgloshie, have you ever held <u>any</u> kind of job for at least 6 months?

☐$_1$ YES ☐$_2$ NO [GO TO 35]

→ **31A. Let's think about those past jobs that you have had for <u>SIX (6) months or more</u>. Start with the most recent and work backwards from there. For your most recent job before Agbogbloshie, what was your job title? Etc.**

JOB HISTORY

JOB TITLE	JOB DESCRIPTION	Employer Name / [If Family, write "Family"]	Average HOURS per WEEK	START (MM/YYYY)	END (MM/YYYY)
1.					
2.					
3.					
4.					
5.					
6.					

8

ID number: •☐☐☐☐

32. In your previous jobs, do you think you were exposed to any of the following?

	Exposure List	Which past job?
32A)	Smoke from burning fields or from scrapyards	_____ (job title)
32B)	Lead, mercury, arsenic, cobalt, chromium, "hard metal" tungsten carbide, other metals	_____ (job title)
32C)	Mining	_____ (job title)
32D)	Asbestos, silica, coal dust, wood dust, grain dust or other dust	_____ (job title)
32E)	Solvents/Degreasers	_____ (job title)
32F)	Animal/Plant products (cotton, animal hides, infections materials, etc.)	_____ (job title)
32G)	Foundry Work / Blacksmith	_____ (job title)
32H)	Fumes or smoke from welding	_____ (job title)
32I)	Formaldehyde	_____ (job title)
32J)	Pesticides ("Poison")	_____ (job title)
32K)	Any other chemical or other type of exposure you feel we should know about?	_____ (job title)

33. Do you believe any of your previous or second jobs caused you any health problems?

☐$_1$ Yes ☐$_2$ No [GO TO 34] ☐$_{888}$ Don't Know [GO TO 34]

→ 33A. If yes, complete the following:

Which job?	What problem(s)?
_____ (job title)	What problem(s):_____
_____ (job title)	What problem(s):_____
_____ (job title)	What problem(s):_____

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ID number: •☐☐☐☐

34. Do you believe any of your previous or second jobs have caused you to have any of the following specific problems?

34A. ☐1 Irritation or burning of the eyes, nose, or throat?	_____ (job title)
34B. ☐2 Cough?	_____ (job title)
34C. ☐3 Wheezing or whistling sound in your chest apart from colds?	_____ (job title)
34D. ☐4 Shortness of breath, difficulty catching your breath, or a smothering feeling?	_____ (job title)
34E. ☐5 Chest tightness or a sensation of a band around the chest?	_____ (job title)

Part 5: LIVING ARRANGEMENTS

35. When you are working, after work where do you usually sleep?
☐1 On the site ☐2 Off site, but within 1km of Agbogbloshie ☐3 More than 1 km away

36. The usual place you sleep is?
☐1 Rented Room/ Block ☐2 Rented Kiosk/Shop ☐3 Open space (no walls and/or no ceiling)
☐4 Mosque ☐5 Own Home ☐6 Family Home ☐7 Mud House
☐777 Other ⟶ Please describe:_____

37. How long have you been sleeping there?
_____ years, _____months, _____days

[GO TO NEXT PAGE]

ID number: •☐☐☐☐

38. At the place you currently sleep, do you own any of the following items?

A. Smart Phone "Whatsapp phone"?	☐1Yes ☐2 No	B. Washing machine?	☐1Yes ☐2 No
C. Any other type of mobile phone?	☐1Yes ☐2 No	D. Computer?	☐1Yes ☐2 No
E. Bed?	☐1Yes ☐2 No	F. Digital photo-camera?	☐1Yes ☐2 No
G. A wall clock?	☐1Yes ☐2 No	H. Non-digital photo-camera?	☐1Yes ☐2 No
I. A radio?	☐1Yes ☐2 No	J. Video deck?	☐1Yes ☐2 No
K. Table?	☐1Yes ☐2 No	L. DVD/VCR?	☐1Yes ☐2 No
M. Cabinet/Cupboard?	☐1Yes ☐2 No	N. Sewing machine?	☐1Yes ☐2 No
O. A land-line telephone?	☐1Yes ☐2 No	P. A black/white television?	☐1Yes ☐2 No
Q. Electricity?	☐1Yes ☐2 No	R. A color television?	☐1Yes ☐2 No
S. Refrigerator?	☐1Yes ☐2 No	T. Car or truck?	☐1Yes ☐2 No
U. Ceiling Fan?	☐1Yes ☐2 No	V. Motor cycle or Motor scooter?	☐1Yes ☐2 No
W. Air conditioner?	☐1Yes ☐2 No	X. A freezer?	☐1Yes ☐2 No
Y. Bicycle?	☐1Yes ☐2 No	Z. Electric generator/Invertor(s)?	☐1Yes ☐2 No
AA. Hand Truck?	☐1Yes ☐2 No		

39. Where you currently usually live, does anyone do cooking indoors?

☐1 Yes ☐2 No [GO TO 40]

→ 39A. Do you sleep in the same room as where the cooking is done?

☐1 Yes [GO TO 41] ☐2 No

40. Where is the cooking usually done?

☐1 In a separate room from where I sleep, but in the same compound.
☐2 Outdoors
☐3 No cooking is done, I only buy food [GO TO 45]
☐777 Other → Please describe:_____
☐888 Don't Know

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ID number: •☐☐☐☐

41. What types of fuel are used for cooking? Check ALL that apply

☐1 Electricity ☐2 Kerosene ☐3 Charcoal
☐4 Wood/ firewood ☐5 LPG / Gas ☐6 Natural Gas
☐7 Biogas ☐8 Straw/shrub/grass ☐9 Agricultural crop residue
☐8 Animal dung ☐10 No food cooked in house
☐777 Other ⟶ Please describe: _____
☐888 Don't Know

42. Which of the fuel sources that you just described is used MOST often? Check ONE only

☐1 Electricity ☐2 Kerosene ☐3 Charcoal
☐4 Wood/ firewood ☐5 LPG ☐6 Natural Gas
☐7 Biogas ☐8 Straw/shrub/grass ☐9 Agricultural crop residue
☐8 Animal dung ☐10 No food cooked in house
☐777 Other ⟶ Please specify type: _____
☐888 Don't Know

43. Is the food cooked on a/an: Check ALL that apply

☐1 Open fire (with firewood) ☐2 Stove/ coal pot with ventilation method (e.g., chimney)
☐3 Stove/ coal pot without ventilation method
☐4 LPG cook stove
☐777 Other ⟶ Please specify type: _____
☐888 Don't Know

44. Which cooking method is used MOST often? Check ONE only

☐1 Open fire (with firewood) ☐2 Stove/ coal pot with ventilation method (e.g., chimney)
☐3 Stove/ coal pot without ventilation method
☐4 LPG cook stove
☐777 Other ⟶ Please specify type: _____
☐888 Don't Know

45. Where did you sleep just before the place you currently sleep?

☐1 Rented Room/ Block ☐2 Rented Kiosk/Shop ☐3 Open space (no walls and/or no ceiling)
☐4 Mosque ☐5 Own Home ☐6 Family Home ☐7 Mud House
☐777 Other ⟶ Please describe: _____

46. Where was this place located?

☐1 In Accra
☐2 Outside of Accra ⟶ Specify location (Country, Region): _____

ID number: •☐☐☐☐

47. Have you ever lived, including when you were a child, other places for 6 months or more where someone did cooking indoors?

☐1 Yes ☐2 No [GO TO 48] ☐888 Don't Know [GO TO 48]

→47A. If yes, please list the location of where this was and fill in the rest of the table.

Country or Ghana Region	Did you sleep in the same room as the cooking?	Start Date (MM/YYYY)	End Date (MM/YYYY)
1.	☐1 Yes ☐2 No ☐888 Don't Know		
2.	☐1 Yes ☐2 No ☐888 Don't Know		
3.	☐1 Yes ☐2 No ☐888 Don't Know		

Part 6: HEALTH STATUS

Now I will ask you some questions about your health.

48. Would you say your health in general is:

☐1 Excellent ☐2 Very good ☐3 Good ☐4 Fair ☐5 Poor

49. Are you limited in the kind or amount of work you can do because of any impairment or health problem?

☐1 Yes ☐2 No [GO TO 50]

→ 49A. If yes, what is that impairment or health problem:

_____

→ 49B. If yes, how does it limit your work?

_____

50. How often have you had skin rashes over the past two weeks?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

51. How often have you had itching around your anus over the past two weeks?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

52. How often have you had a headache over the past two weeks?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

53. How often have you had an episode of stomach ache over the past two weeks?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

54. How often have you seen blood in your urine over the past two weeks?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

55. How often have you seen blood in your stool over the past two weeks?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

13

ID number: •☐☐☐☐

56. How often have you had a cough over the last two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

57. How often have you experienced shortness of breath or difficulty in breathing over the last two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

58. How often have you experienced dizziness over the last two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

59. How often have you felt your heart beating abnormally over the last two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

60. How often have you had diarrhea over past two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

61. How often in the last two weeks did you have fever?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

62. How often did you experience nausea over the last two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

63. How often have you vomited in the last two weeks?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

64. Have you taken any treatment for worms over the last three months?
☐$_1$ Yes ☐$_2$ No ☐$_{888}$ Don't know

65. Have you experienced tremors (shakiness in your fingers or hands) making handwriting (or any other activity) difficult in the past year?
☐$_1$ Never ☐$_2$Rarely ☐$_3$ Frequently ☐$_4$ Always

66. Do you think you've lost weight recently (when not trying to lose weight; i.e., not dieting)? [Do you think that your clothes are too large for you due to a loss of weight?]
☐$_1$ Yes ☐$_2$ No ☐$_{888}$ Don't know

☐$_{789}$ If "NEVER" to ALL of the above health problems, skip to question 69.

67. Did you seek treatment for any of these problems or conditions you had?
☐$_1$ Yes ☐$_2$ No [Go To 69] ☐$_{888}$ Don't know [Go To 69]

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68. If yes where did you receive treatment for the most severe condition?
[Check ALL that apply]

☐1 Self Medication ☐2 Traditional Healer ☐3 Drug Store/Pharmacy
☐4 Clinic/Hospital ☐777 Other → Please specify: _____

69. Have you ever been told by a doctor or health professional that you have any of the following medical conditions?

→ 69A. High Blood Pressure
☐1 Yes → Are you taking medication for this? ☐1 Yes ☐2 No
☐2 No ☐888 Don't Know

→ 69B. Sugar disease/ Diabetes Mellitus
☐1 Yes → Are you taking medication for this? ☐1 Yes ☐2 No
☐2 No ☐888 Don't Know

→ 69C. High fat content in blood/ High Cholesterol
☐1 Yes → Are you taking medication for this? ☐1 Yes ☐2 No
☐2 No ☐888 Don't Know

70. During the last 12 months, how often did you usually have any kind of drink containing alcohol?
One drink is the equivalent to 1 can or glass of beer, 1 shot of liquor, 1 glass of wine (150ml), 2 glasses of palm wine, or 0.5 calabash of pito.
[Choose only one]

☐1 Every day ☐2 5 to 6 times a week ☐3 3 to 4 times a week
☐4 Twice a week ☐5 Once a week ☐6 2 to 3 times a month
☐7 Once a month ☐8 3 to 11 times in the past year ☐9 1 or 2 times in the past year
☐10 I never drank any alcohol in my life [GO TO 71]
☐11 I did not drink any alcohol in the past year, but I did drink in the past

→ 70A. During your lifetime, what is the maximum number of drinks containing alcohol that you drank within a 24-hour period?

☐1 36 drinks or more ☐2 24 to 35 drinks ☐3 18 to 23 drinks ☐4 12 to 17 drinks ☐5 8 to 11 drinks
☐6 5 to 7 drinks ☐7 4 drinks ☐8 3 drinks ☐9 2 drinks ☐10 1 drink

71. How often do you wash your hands before eating?
☐1 Never ☐2 Rarely ☐3 Frequently ☐4 Always

→ 71A. How often do you wash your hands with soap?
☐1 Never ☐2 Rarely ☐3 Frequently ☐4 Always

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72. How often do you wash your hands after defecation?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

72A. How often do you wash your hands with soap?

☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

Part 7: RESPIRATORY SURVEY

I am going to ask you some additional questions about your health. Most of these will be about your breathing. Wherever possible, I would like you to answer 'YES' or 'NO'.

Section I. Wheezing and tightness in the chest

73. Have you had wheezing or whistling (noise in your chest when you breathe) in your chest at any time in the last *12 months*?	☐1Yes ☐2 No [GO TO 74]
73A. Have you been short of breath when the wheezing noise was present?	☐1Yes ☐2 No
73B. Have you had this wheezing or whistling when you *did not* have a cold or flu?	☐1Yes ☐2 No
74. Have you been woken up with a feeling of tightness in your chest at any time in the last *12 months?*	☐1Yes ☐2 No

Section II. Shortness of breath

75. Have you had an attack of shortness of breath that came on during the daytime when you were at rest at any time in the last *12 months?*	☐1Yes ☐2 No
76. Have you had an attack of shortness of breath that came on following running or exercise at any time in the last *12 months?*	☐1Yes ☐2 No
77. Have you been woken by an attack of shortness of breath at any time in the last *12 months?*	☐1Yes ☐2 No

Section III. Cough and phlegm from the chest

78. Have you been woken by an attack of coughing at any time in the last *12 months?*	☐1Yes ☐2 No [GO TO 79]
78A. Do you *usually* cough first thing in the morning?	☐1Yes ☐2 No

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79. Do you *usually* cough during the rest of the day, or at night?	☐1Yes ☐2 No [GO TO 80]
79A. Do you cough like this on most days/nights for as much as three or more months in each of the last two years?	☐1Yes ☐2 No
80. Do you usually bring up any phlegm from your chest first thing in the morning?	☐1Yes ☐2 No
81. Do you usually bring up any phlegm from your chest during the day, or at night?	☐1Yes ☐2 No [GO TO 82]
81A. Do you bring up phlegm like this on most days/nights for as much as three or more months in each of the last two years?	☐1Yes ☐2 No

Section IV. Breathing

82. Do you ever have trouble with your breathing?	☐1Yes ☐2 No [GO TO 83]
82A. Do you have this trouble: Give all options at once, and insert cross (X) next to ONE answer only	☐1 Continuously so that your breathing is never quite right? ☐2 Repeatedly, but it goes away completely between the times when it troubles you? ☐3 Only rarely?
83. Do you have problems walking with a condition *other than* heart or lung disease?	☐1Yes, please state condition: _____ ☐2 No
84. Are you troubled by shortness of breath when walking fast on level ground?	☐1Yes ☐2 No [Go TO 85]
84A. Do you get short of breath walking with other people of your own age on level ground?	☐1Yes ☐2 No
84B. Do you have to stop for breath when walking at your own pace on level ground?	☐1Yes ☐2 No

Section V. Asthma

85. Have you ever had asthma?	☐1Yes ☐2 No [GO TO 92]
85A. *If yes*, was this confirmed by a doctor?	☐1Yes ☐2 No
85B. How old were you when you were first told you have asthma?	_____ years old

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The following references to "attack" of asthma refers to episodes of wheezing, shortness of breath, chest tightness or cough attributed to asthma	
86. How old were you when you had your first attack of asthma?	_____ years old
87. How old were you when you had your most recent attack of asthma?	_____years old
88. Have you had an attack of asthma in the last *12 months?*	☐1Yes ☐2 No [GO TO 89]
88A. How often have you had an attack of asthma in the last *12 months?* [Check ONE answer only]	☐1 Every day ☐2 More than 2 times a week ☐3 More than 1 time per month ☐4 3 to 12 times in the whole year ☐5 1 to 2 times in the whole year
89. Are your chest symptoms caused by, or made worse by any of the following: [ANSWER ALL QUESTIONS]	
89A. Contact with animals/pets	☐1Yes ☐2 No
89B. Grass or flowers	☐1Yes ☐2 No
89C. Heavy exercise	☐1Yes ☐2 No
89D. Breathing cold air	☐1Yes ☐2 No
89E. Dusts or sprays at work	☐1Yes ☐2 No
89F. Exposure to open fires at work?	☐1Yes ☐2 No
89G. Tobacco smoke	☐1Yes ☐2 No
89H. Change in the weather	☐1Yes ☐2 No
90. Do your chest symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and holidays)?	☐1 Stay the same ☐2 Get better ☐3 Get worse ☐4 Never off work
91. Does being at work ever make your chest tight or wheezy?	☐1Yes ☐2 No [GO TO 92]
91A. When did you first notice having problems with chest tightness or wheeze at work?	Date: Month _____Year _____
91B. Is there anything that you work with that causes you to have these chest symptoms?	☐1 Yes ☐2 No
91C. What do you think is causing these symptoms?	WRITE ANSWER:

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Section VI. TB Questions

92. . Have you ever been told by a doctor that you had chest tuberculosis or TB?	☐1Yes ☐2 No [GO TO 99]
93. How old were you when you were first told that you had TB?	_____ years ☐888 Don't know
94. . How long did you take medication for TB? (First) episode of TB?	_____months ☐1 Did not take any medication
95. . Have you ever been told by a doctor that you had a second episode of TB?	☐1Yes ☐2 No [GO TO 99]

96. . How old were you when you were told that you had a second episode?	_____ years ☐888 Don't know
97. . For how long did you take medication for this second episode?	_____ months ☐1 Did not take any medication
98. . Do you still have TB?	☐1Yes ☐2 No ☐888 Don't know

Section VI. Smoking

99. Have you smoked at least 100 cigarettes during your entire life (equivalent to about 5 packs)?
☐1 Yes ☐2 No [GO TO 105]

100. Do you smoke cigarettes now?
☐1 Yes [Go To 93] ☐2 No

101. How old were you when you stopped for good?
_____years old

102. How old were you when you started?
_____years old

103. Since you started smoking, were there periods of time that you did not smoke?
☐1 Yes ☐2 No [GO TO 104]

→ 103A. If yes, how many years did you not smoke? _____years

104. During all the periods that you were smoking, on average, how many cigarettes did you smoke per day?
_____cigarettes per day

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Part 8: STRESSORS

Now I will ask you about stress in your life.

105. In the last month, how often have you been upset because of something that happened unexpectedly?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

106. In the last month, how often have you felt that you were unable to control the important things in your life?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

107. In the last month, how often have you felt nervous and "stressed"?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

108. In the last month, how often have you felt confident about your ability to handle your personal problems?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

109. In the last month, how often have you felt that things were going your way?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

110. In the last month, how often have you found that you could not cope with all the things that you had to do?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

111. In the last month, how often have you been able to control irritations in your life?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

112. In the last month, how often have you felt that you were on top of things?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

113. In the last month, how often have you been angered because of things that happened that were outside of your control?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

114. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

[GO TO NEXT PAGE]

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Part 9: RISK FACTORS OF WORK-RELATED STRESS

Now I will ask you about Job demands and working conditions

115. How often do you feel exhausted after work?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

116. How often are you exposed to unfavourable physical conditions in your work (for example, unfavourable climate, noise, chemicals, sharp or moving objects, slippery surfaces, constant repetitive work, heavy lifting or strenuous work)
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

117. Do you have a supervisor that you report to at work?
☐1 Yes ☐2 No

118. How often does someone else decide your work methods, pace, and/or order?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

119. How often do you feel you are not receiving support from your supervisor and/or fellow workers?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

120. How often do you experience violence or harassment at work?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

121. How many hours do you work on a typical day? _____

122. How often do you work evenings/ nights?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

123. Do you ever work on weekends?
☐1 Saturday only ☐2 Sunday only ☐3 Saturday and Sundays ☐4 No [GO TO 124]

→ **123A.** How often do you work on this day or both days?
☐1 Rarely ☐2 Frequently ☐3 Always

124. How often does your work interfere with your family responsibilities or leisure time activities?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

125. How often do you feel your income is not sufficient to support yourself and your family?
☐1 Never ☐2Rarely ☐3 Frequently ☐4 Always

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Part 10. OTHER INFORMATION

Is there anything else you would like to say, perhaps related to this survey, your life, work or health?

If you answered Yes, please explain:

_____
_____
_____

We thank you very much for your cooperation and patience. You have reached the end of the questionnaire.

[END OF QUESTIONNAIRE]

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APPENDIX 4: Summary of elemental biomarker quality control measures

Summary of whole blood elemental biomarker quality control measures. The limit of detection (values reported as µg/L) was calculated as the mean value of several blank samples plus 3x the standard deviation of the mean. Accuracy (closeness to actual value) and precision (reproducibility) of each element was determined by use of certified blood reference materials (numbers 1314, 1505, 1506) obtained from the Institut National de Santé Publique du Québec (INSPQ).

Table S1: Summary of elemental biomarker quality control measures

Biomarker	Element	Detection Limit (ug/L)	Precision	Accuracy
Blood	Se	9.1	8%	86%
	Zn	11,169.4	11%	94%
	Mn	12.9	13%	124%
	Cd	0.3	21%	119%
	Pb	7.1	6%	103%

Appendix 5: Supplementary Table of Results

Appendix 5.1: Search strategies and results from different electronic databases

Databases	Keywords	Results
MEDLINE with Full Text, Academic Search Complete, CINAHL Complete, Education Research Complete, GreenFILE, Health Source: Nursing/Academic Edition, Library, Information Science & Technology Abstracts	("electronic waste" OR "e-waste" OR "WEEE") AND ("DNA damage" OR "chromosomal aberration" OR "DNA strand breaks" OR "micronucl*" OR "Sister chromatid exchanges" OR "oxidative DNA damage" OR "genotox*" OR "oxidative stress")	83
ProQuest central…1998-aug 2020	(("electronic waste" OR "e-waste" OR "WEEE") AND ("DNA damage" OR "chromosomal aberration" OR "DNA strand breaks" OR "micronucl*" OR "Sister chromatid exchanges" OR "oxidative DNA damage" OR "genotox*" OR "oxidative stress")) AND PEER(yes)	654
SCOPUS..2008- Aug. 2020	TITLE-ABS-KEY (("electronic waste" OR "e-waste" OR "WEEE") AND ("DNA damage" OR "chromosomal aberration" OR "DNA strand breaks" OR "micronucl*" OR "Sister chromatid exchanges" OR "oxidative DNA damage" OR "genotox*" OR "oxidative stress"))	85
Hand search		1
Total		823
Duplicates		106
Total after duplicates		717

Appendix 5.2: Excluded articles and the reasons for their exclusion

Author(s)/year	Resason for exclusion
Alabi and Bakare (2017)	1
Alabi et al. (2012)	3
Alam et al. (2019)	3
Awasthi (2016)	1
Du et al. (2018b)	1
Du et al. (2018a)	4
Grant (2013)	1
Khlaif and Qumsiyeh (2018)	2
Lu et al. (2017)	2
Song (2015)	1
Zhang et al. (2012)	3
Zhang et al. (2016)	2
1) Not original study (e.g. reviews); 2) shared the same population and outcome variable with another article reported in this review; 3) objective did not meet inclusion criteria (e,g. not human study or did not measure DNA damage; 4) retracted article	

Appendix 5.3: Quality assessment of included studies based on the modified Newcastle–Ottawa Scale for cross-sectional studies

	Sample selection Criteria (4 stars)			Comparability (4 stars)		Exposure (1 star)	Outcome (1 star)	Total (10 stars)
Publication	1.Representativeness of sample: a ** Random; b * Non-Random; c Selected Groups; d No Description	2. Sample Size: a * Justified and Satisfactory; b Not Justified	3. Non-Respondents: a * Comparability and Response Rate Satisfactory; b Comparability and/or Response Rate Unsatisfactory; c No Description	4. Comparison Group: a * Described by Authors as Geographically Distinct; b * Same Community; c No Comparison Group	5. Subjects in Outcome Groups Comparable: a * Study Controls for Most Important Confounder; b * Study Controls for Any Additional Confounder; c Study Did not Control for Any Confounder.	6. Exposure Measurements a * Exposure Chemical(s) Quantified; b Exposure Chemicals not Reported	1) Outcome Measurements a * Validated Methods Described; b No Description of methods.	
Alabi et al. (2020)	b *	b	c	a * b *	a * b *	a *	a *	7/10 (high)
Chen et al. (2010)	b *	b	c	a *	a * b *	B	a *	5/10 (low)
He et al. (2015)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Khlaif and Qumsiyeh (2017)	b *	b	c	a *	c	B	a *	3/10 (low)
Li et al. (2014)	b *	b	c	a * b *	c	a *	a *	5/10 (low)
Li et al. (2008)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Lin (2013)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Liu et al. (2009)	a **	b	c	a *	a * b *	B	a *	6/10 (high)
Lu et al. (2016)	b *	b	c	a *	c	a *	a *	4/10 (low)
Neitzel et al. (2020)	b *	b	c	c	a * b *	a *	a *	5/10 (low)
Ngo et al. (2020)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Ni et al. (2014)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Sheng et al. (2008)	a **	b	c	c	c	a *	a *	4/10 (low)
Wang et al. (2010)	a **	b	c	a *	a * b *	a *	a *	7/10 (high)
Wang et al. (2011)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Wang et al. (2018)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Xu et al. (2018)	b *	b	c	c	a * b *	a *	a *	5/10 (low)
Yu et al. (2018)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Yuan et al. (2008)	b *	b	c	a *	a * b *	a *	a *	6/10 (high)
Berame et al. (2020)	b*	b	c	a*	a * b *	B	a *	5/10 (low)

Appendix 5.4: Summary of DNA damage biomarkers and samples used. Blue cells indicate biomarkers assessed in the study, and gray cells indicate the type of sample used.

	Sample						DNA damage biomarkers					
Publications	EFBC	Blood	UCB	Placenta	Urine	Semen	MN	CA	Comets*	8-OHdG	TL	A.R.
Alabi et al. (2020)												
Chen et al. (2010)												
He et al. (2015)												
Khlaif and Qumsiyeh (2017)												
Li et al. (2014)												
Li et al. (2008)												
Lin (2013)												
Liu et al. (2009)												
Lu et al. (2016)												
Neitzel et al. (2020)												
Ngo et al. (2020)												
Ni et al. (2014)												
Sheng et al. (2008)												
Wang et al. (2010)												
Wang et al. (2011)												
Wang et al. (2018)												
Xu et al. (2018)												
Yu et al. (2018)												
Yuan et al. (2008)												
Berame et al. (2020)												
Total	**2**	**9**	**2**	**1**	**6**	**2**	**9**	**3**	**6**	**7**	**1**	**1**

Abbreviations: EFBC=exfoliated buccal cells, UCB=umbilical cord blood, MN=micronuclei, CA=chromosomal aberrations, 8-OHdG= 8-hydroxy-2′-deoxyguanosine, TL=telomere length, AR=apoptosis rate. * =Comet assay (%TDNA, TM, and OTM)

Appendix 5.5: Comparison of the levels of $PM_{2.5}$ and PM_{10} exposure with WHO and NAAQS reference values

			Median (IQR) µg/m^3			
Reference	**Reference values**		**e-waste**		**Controls**	
	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}	$PM_{2.5}$	PM_{10}
AQG(WHO)	25	50	77.32(34.08)	210.21 (93.32)	34.88 (16.55)	121.92 (82.93)
Ghana AQS	35	70	77.32(34.08)	210.21 (93.32)	34.88 (16.55)	121.92 (82.93)

AQG: Air Quality Guidelines, **WHO**: World Health Organization, References: (WHO, 2006); (GSA, 2019)

Appendix 5.6: Associations between total and specific CpG sites DNA methylation of LINE-1 and levels of PM exposure in e-waste exposure workers (n=100) and controls (n=51)

		Linear regression with robust standard errors from OLS	Sensitivity analysis	
			Robust regression	Cross-fit partialling-out LASSO regression
PM exposure	Outcomes (LINE-1)	β(95% CI)	β(95% CI)	β(95% CI)
			E-waste workers	
$PM_{2.5}$	(average of 4 CpGs)	0.004(-0.001, 0.010)	0.004(-0.000, 0.008)	0.004(-0.001, 0.009)
	CpG1	0.002(-0.007, 0.011)	0.001(-0.006, 0.008)	0.001(-0.007, 0.009)
	CpG2	0.004(0.001, 0.007)*	0.004(0.001, 0.008)*	0.003(0.001, 0.006)*
	CpG3	0.010(-0.000, 0.021)	0.007(0.000, 0.014)	0.009(-0.001, 0.018)
	CpG4	0.002(-0.003, 0.006)	0.002(-0.005, 0.009)	0.002(-0.002, 0.006)
PM_{10}	(average of 4 CpGs)	0.002(-0.000, 0.005)	0.002(0.000, 0.004)	0.002(-0.001, 0.004)
	CpG1	0.002(-0.002, 0.007)	0.002(-0.001, 0.006)	0.001(-0.003, 0.005)
	CpG2	0.002(0.000, 0.004)*	0.002(0.000, 0.004)*	0.002(-0.000, 0.003)*
	CpG3	0.004(-0.001, 0.010)	0.001(-0.002, 0.005)	0.004(-0.000, 0.009)
	CpG4	0.001(-0.001, 0.003)	0.001(-0.002, 0.005)	0.000(-0.001, 0.003)
			Controls	
PM	(average of 4 CpGs)	-0.009(-0.028, 0.010)	-0.011(-0.036, 0.014)	-0.007(-0.026, 0.012)
	CpG1	0.004(-0.050, 0.058)	0.033(-0.011, 0.076)	0.006(-0.041, 0.052)
	CpG2	-0.011(-0.033, 0.012)	-0.013(-0.037, 0.011)	-0.007(-0.025, 0.012)
	CpG3	-0.023(-0.063, 0.016)	-0.012(-0.058, 0.033)	-0.022(-0.058, 0.014)
	CpG4	-0.004(-0.047, 0.038)	-0.014(-0.047, 0.020)	0.005(-0.023, 0.033)
PM_{10}	(average of 4 CpGs)	0.000(-0.005, 0.005)	-0.001(-0.006, 0.004)	0.000(-0.004, 0.005)
	CpG1	-0.001(-0.010, 0.007)	-0.000(-0.010, 0.009)	-0.001(-0.009, 0.007)
	CpG2	-0.000(-0.005, 0.004)	-0.002(-0.007, 0.003)	0.001(-0.003, 0.004)
	CpG3	-0.003(-0.011, 0.005)	-0.002(-0.011, 0.007)	-0.002(-0.009, 0.004)
	CpG4	0.000(-0.012, 0.013)	-0.003(-0.010, 0.004)	0.001(-0.006, 0.009)

P-value notation: *p<0.05, β: average DNA methylation change, CI: Confidence Interval, **$PM_{2.5}$** = particulate matter ≤ 2.5µm in aerodynamic diameter, **PM_{10}** = particulate matter ≤ 10µm in aerodynamic diameter; LINE-1: long interspersed nucleotide element; CpG: cytosine guanine dinucleotide. All models adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking, and BMI.

Appendix 5.7: Associations between total and specific CpG sites DNA methylation of LINE-1 and levels of metals exposure in e-waste exposure workers (n=100) and controls (n=51)

	LINE-1 methylation		
	Linear regression with robust standard errors from OLS	Sensitivity analysis	
		Robust regression	Cross-fit partialling-out LASSO linear regression
Single metal model	**β(95% CI)**	**β(95% CI)**	**β(95% CI)**
Metals (µg/L)		**E-waste workers**	
Se	-1.087(-2.167, -0.007)*	-1.008(-2.097, 0.080)	-0.995(-1.956, -0.034)*
Zn	-1.240(-2.079, -0.401)**	-1.030(-1.828, -0.231)	-1.242(-2.007, -0.477)**
Mn	-0.364(-0.816, 0.087)	-0.274(-0.780, 0.232)	-0.374(-0.835, 0.088)
Cd	-0.232(-0.784, 0.319)	-0.099(-0.609, 0.412)	-0.271(-0.759, 0.217)
Pb	-0.274(-0.726, 0.178)	-0.232(-0.785, 0.320)	-0.367(-0.814, 0.081)
		Controls	
Se	-0.334(-2.909, 2.242)	0.799(-0.651, 2.250)	-0.191(-2.185, 1.802)
Zn	-0.212(-1.149, 0.725)	-0.366(-1.369, 0.637)	-0.188(-0.907, 0.532)
Mn	-0.146(-1.046, 0.754)	-0.154(-1.151, 0.842)	0.159(-0.694, 1.011)
Cd	0.251(-0.319, 0.821)	0.075(-0.590, 0.740)	0.155(-0.307, 0.618)
Pb	-0.897(-2.263, 0.469)	-0.376(-1.204, 0.451)	-0.656(-1.825, 0.514)
Multiple metals model		**E-waste workers**	
Se	-0.334(-1.559, 0.891)	-0.410(-1.740, 0.920)	-0.005(-1.066, 1.057)
Zn	-1.180(-2.199, -0.161)*	-0.988(-2.014, 0.038)	-1.226(-2.152, -0.301)*
Mn	-0.138(-0.553, 0.277)	-0.093(-0.643, 0.458)	-0.139(-0.520, 0.243)
Cd	0.150(-0.339, 0.638)	0.209(-0.360, 0.777)	0.125(-0.320, 0.569)
Pb	0.054(-0.408, 0.515)	0.048(-0.556, 0.653)	-0.078(-0.516, 0.361)
		Controls	
Se	-0.231(-2.332, 1.869)	1.046(-0.538, 2.629)	-0.188(-1.854, 1.478)
Zn	0.189(-1.462, 1.840)	-0.813(-2.123, 0.497)	-0.093(-1.306, 1.119)
Mn	-0.077(-1.710, 1.555)	0.674(-0.624, 1.971)	0.353(-1.063, 1.769)
Cd	0.313(-0.334, 0.960)	0.052(-0.628, 0.732)	0.263(-0.201, 0.728)
Pb	-0.948(-2.319, 0.424)	-0.274(-1.139, 0.591)	-0.803(-1.891, 0.286)

P-value notation: *p<0.05, **p<0.005, β: average LINE-1 methylation change, CI: Confidence Interval; All models are adjusted for indoor use of biomass fuel for cooking, alcohol consumption, age, smoking status, and BMI. All Heavy metals biomarkers are natural log-transformed.

Appendix 6: Abstract of manuscript published related to this work

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ORIGINAL ARTICLE

Global DNA (LINE-1) methylation is associated with lead exposure and certain job tasks performed by electronic waste workers

Ibrahim Issah[1] · John Arko-Mensah[1] · Laura S. Rozek[2] · Katie R. Zarins[2] · Thomas P. Agyekum[1] · Duah Dwomoh[3] · Niladri Basu[4] · Stuart Batterman[2] · Thomas G. Robins[2] · Julius N. Fobil[1]

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Abstract

Objective This study assessed the associations between blood and urine levels of toxic metals; cadmium (Cd) and lead (Pb), and methylation levels of the LINE-1 gene among e-waste and control populations in Ghana.

Methods The study enrolled 100 male e-waste workers and 51 all-male non-e-waste workers or controls. The concentrations of Cd and Pb were measured in blood and urine using inductively coupled plasma mass spectrometry, while LINE1 methylation levels were assessed by pyrosequencing of bisulfite-converted DNA extracted from whole blood. Single and multiple metals linear regression models were used to determine the associations between metals and LINE1 DNA methylation.

Results Blood lead (BPb) and urine lead (UPb) showed higher median concentrations among the e-waste workers than the controls (76.82 µg/L vs 40.25 µg/L, $p \leq 0.001$; and 6.89 µg/L vs 3.43 µg/L, $p \leq 0.001$, respectively), whereas blood cadmium (BCd) concentration was lower in the e-waste workers compared to the controls (0.59 µg/L vs 0.81 µg/L, respectively, $p = 0.003$). There was no significant difference in LINE1 methylation between the e-waste and controls (85.16 ± 1.32% vs 85.17 ± 1.11%, $p = 0.950$). In our single metal linear regression models, BPb was significantly inversely associated with LINE1 methylation in the control group ($\beta_{BPb} = -0.027$, 95% CI − 0.045, − 0.010, $p = 0.003$). In addition, a weak association between BPb and LINE1 was observed in the multiple metals analysis in the e-waste worker group ($\beta_{BPb} = -0.005$, 95% CI − 0.011, 0.000, $p = 0.058$).

Conclusion Continuous Pb exposure may interfere with LINE1 methylation, leading to epigenetic alterations, thus serving as an early epigenetic marker for future adverse health outcomes.

Keywords Electronic waste · Toxic metals · DNA methylation · LINE-1

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Appendix 7: Abstract of manuscript published related to this work

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Association between global DNA methylation (LINE-1) and occupational particulate matter exposure among informal electronic-waste recyclers in Ghana

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ABSTRACT

This study examined the associations between PM (2.5 and 10) and global DNA methylation among 100 e-waste workers and 51 non-e-waste workers serving as controls. Long interspersed nucleotide repetitive elements-1 (LINE-1) was measured by pyrosequencing. Personal $PM_{2.5}$ and PM_{10} were measured over a 4-hour work-shift using real-time particulate monitors incorporated into a backpack . Linear regression models were used to assess the association between PM and LINE-1 DNA methylation. The concentrations of $PM_{2.5}$ and PM_{10} were significantly higher among the e-waste workers than the controls (77.32 vs 34.88, $p < 0.001$ and 210.21 vs 121.92, $p < 0.001$, respectively). $PM_{2.5}$ exposure was associated with increased LINE-1 CpG2 DNA methylation ($\beta = 0.003$; 95% CI; 0.001, 0.006; $p = 0.022$) but not with the average of all 4 CpG sites of LINE-1. In summary, high levels of $PM_{2.5}$ exposure was associated with increased levels of global DNA methylation in a site-specific manner.

ARTICLE HISTORY
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KEYWORDS
DNA methylation; LINE-1; $PM_{2.5}$; PM_{10}; e-waste; e-waste workers

Appendix 8: Conference abstract publication and poster presentation related to this work (ISEE 2020). https://ehp.niehs.nih.gov/doi/abs/10.1289/isee.2020.virtual.P-0459

LINE1 methylation is differentially associated with lead exposure and job-tasks performed by electronic waste workers

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Background

- Informal e-waste recycling generate and release several toxic chemicals into the environment including heavy metals
- Heavy metals exposure has been associated with many adverse health outcomes
- DNA methylation may in part mediate the health effects of heavy metals
- This study assessed the associations between heavy metals and LINE1 methylation among e-waste workers and controls in Ghana.

Methods

- The study enrolled 100 male e-waste workers and 51 all-male controls
- Cadmium (Cd), lead (Pb) and arsenic (As) were measured in blood and urine using Inductively Coupled Plasma Mass Spectrometry
- LINE1 methylation was assessed by pyrosequencing of bisulfite-converted DNA extracted from whole blood.

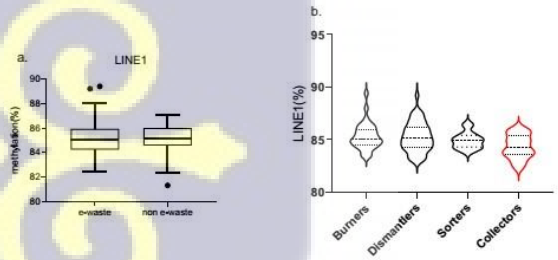
E-waste workers had a higher concentration of **blood Pb**, which was associated with global DNA **hypomethylation** measured through **LINE1**

Results

Table 1: Association between LINE1 methylation and level of Pb in blood all participants and across primary job-tasks performed by e-waste workers

	LINE1 methylation	
Predictor	**β(95% CI)**	**P-value**
Model 1		
Blood Pb	-0.004(-0.008- - 0.0002)	0.038*
Model 2		
Job category		
Burners	1.00(Ref)	
Dismantlers	-0.140(-0.783 - 0.504)	0.667
Sorters	-0.721(-1.890 - 0.449)	0.224
Collectors	-1.068(-2.040 - - 0.095)	0.032*

Model1= adjusted for age, smoking status, BMI, and alcohol consumption; model 2= adjusted as for model 1 and also adjusted for primary job categories of e-waste workers



LINE1 methylation a. in e-waste and non e-waste workers; b. across primary job-tasks performed by e-waste workers; collectors had the lowest median methylation levels.

Discussion

- Continuous exposure to Pb may interfere with LINE1 methylation
- E-waste collectors are at an increased risk
- LINE1 methylation status can be used as a biosensor in risk assessment of workers with uncontrolled exposures

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