LINE1 Methylation is Associated With Lead Exposure and Certain Job-Tasks Performed by Electronic Waste Workers

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Abstract

Background: Electronic waste recycling processes such as dismantling with rudimentary tools and open-air burning result in the release of several toxic chemicals into the environment. Exposure to these toxic chemical mixtures has been associated with many adverse health outcomes affecting respiratory, cardiovascular, neurological, and reproductive systems. DNA methylation has been associated with exposure to toxic chemicals, including heavy metals in several epidemiological studies. DNA methylation profile due to exposure to toxic chemicals among e-waste recyclers has not been studied.

Objective: This study assessed the associations between blood and urine levels of heavy metals; cadmium (Cd), lead (Pb), and arsenic (As) and methylation levels of the LINE1 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. Body burden of Cd, Pb and As was 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.

Results: There was no significant difference in LINE1 methylation between the e-waste and the non e-waste workers (85.16% ±1.32 vs 85.17% ±1.11, $p = 0.950$). However, CpG1 showed significantly lower mean methylation among controls, compared to e-waste workers (81.70% ±1.86 vs 82.48% ±2.20, $p = 0.034$). In linear regression models, blood lead (B-Pb) level was significantly inversely associated with overall LINE1 methylation (β = -0.004; 95%CI: -0.008, -0.0003; $p = 0.034$). Among e-waste recyclers, collectors showed significantly reduced LINE1 methylation levels (β = -0.889; 95%CI: -1.757, -0.021; $p = 0.045$).

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

1. Introduction

The public health concerns due to the high production volumes of electrical and electronic waste (e-waste) especially in low and middle-income countries are well-documented (Alabi et al., 2012; Baldé, Forti, Gray, Kuehr, & Stegmann, 2017; Orlins & Guan, 2016; Robinson, 2009; Song & Li, 2014). The composition of electrical and electronic equipment presents a challenge in the management of e-waste because it is simultaneously a source of recoverable precious materials (especially metals) as well as a myriad of toxic chemicals (Alabi & Bakare, 2017; Amankwaa, Adovor Tsikudo, & Bowman, 2017; Bakhiyi, Gravel, Ceballos, Flynn, & Zayed, 2018; Dias, Bernardes, & Huda, 2019; Fowler, 2017). Effective and adequate recycling processes are, therefore required to recover valuable materials while protecting human and environmental health (Ikhlayel, 2017). This presents serious challenges for informal sector e-waste recycling facilities, especially in developing countries without appropriate recycling infrastructure (Ikhlayel, 2018).

The high influx 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 which has offered employment opportunities to hundreds of young men in the capital Accra (Amankwaa, Bowman, & Tsikudo, 2016). The Agbogbloshie e-waste processing and recycling site (a.k.a., scrapyard) is the main centre in Ghana for recovery of re-usable materials from e-waste. The Agbogbloshie e-waste recyclers are a particularly vulnerable group because it is one of the largest and busiest informal recycling sites worldwide (Srigboh et al., 2016). The workers, often young men, are involved in multiple tasks, work 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 re-usable 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 plastics, foam recovered from old discarded fridges. This singular activity results in the release of a mixture of toxic chemicals such as PAHs, PCBs and heavy metals into the ambient environment. Several studies have documented high concentration of PAHs, chlorinated and brominated dioxin-related compounds (DRCs) and dioxin-like polychlorinated biphenyls (DLPCBs), polybrominated diphenyl ethers (PBDEs) and heavy metals in surface soil samples from the Agbogbloshie e-waste recycling site in Ghana (Akortia, Olukunle, Daso, & Okonkwo, 2017; Daso, Akortia, & Okonkwo, 2016; Tue et al., 2016; Tue et al., 2017). Other studies have found high levels of PAHs-derived metabolites (Feldt et al., 2014) and heavy metals (Srigboh et al., 2016; Wittsiepe et al., 2017) in workers blood and urine.

Heavy metals have been implicated in numerous adverse health outcomes including cancers, cardiovascular diseases, neurological diseases, reproductive toxicity, renal dysfunction and autoimmune diseases (Hu, 2002; Rzymski, Tomczyk, Poniedzialek, Opala, & Wilczak, 2015; Shi, Jing, & Xi, 2019). Bal and colleagues extensively studied the genetic toxicity aspect of metals and reported that heavy metals largely inflicts 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. Epigenetic mechanisms such as DNA methylation - the most widely studied epigenetic marker, may in part mediate the health effects of occupational/environmental exposure to metals (Arita & Costa, 2009).

Long interspersed nucleotide elements (LINE-1) are repetitive elements or transposons, constitute about 18% of the human genome and usually are heavily methylated to ensure genomic stability and integrity (Kahl, Cappetta, & Da Silva, 2019). The alteration of LINE1 is used as a proxy for global methylation changes (Ghosh et al., 2017; Sharma et al., 2019). Decreased levels of LINE1 methylation may result in increased mitotic recombination, and overall genome instability (Brennan & Flanagan, 2012; Duan, He, Ma, Zhang, Sheng, Bin, Cheng, Niu, Dong, & Lin, 2013; Kahl et al., 2019; S. Li et al., 2018), and this has been regarded as a biomarker of exposure to different classes of xenobiotics in occupational settings (Kahl et al., 2019). A study among urban pesticide sprayers in Mexico showed decreased levels of LINE1 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, LINE1 was inversely associated with Pb levels (C. Li, Yang, Xu, Zhang, & Sun, 2013). Duan and co-workers also reported hypomethylation of LINE1 among coke-oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) (Duan, He, Ma, Zhang, Sheng, Bin, Cheng, Niu, Dong, Lin, et al., 2013).

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 it has been regarded as a hallmark of cancer development (Buj et al., 2016; Das & Singal, 2004). In a meta-analysis (Barchitta, Quattrocchi, Maugeri, Vinciguerra, & Agodi, 2014), LINE-1 methylation level was significantly lower in cancer patients than in control samples ($p < 0.001$). The difference, however, was confined to tissue samples ($p < 0.001$) and not blood ($p = 0.23$).

Global DNA methylation changes associated with heavy metal exposure have been less clear and consistent, as demonstrated by studies showing both hypermethylation and hypomethylation. These studies were limited by differences in tissues examined, populations studied, methods for measuring methylation status, and singular analysis of effect one metal at a time (Bandyopadhyay, Paul, Adak, & Giri, 2016; Goodrich, Basu, Franzblau, & Dolinoy, 2013; Hanna et al., 2012; K. Hossain et al., 2017; M. B. Hossain, Vahter, Concha, & Broberg, 2012; Lambrou et al., 2012; C. Li et al., 2013; Majumdar et al., 2010; Phetliap et al., 2018; Pilsner et al., 2009; Tajuddin et al., 2013; Tellez-Plaza et al., 2014; Wright et al., 2010). Besides, the informal e-waste recycling industry has largely been understudied despite evidence that activities result in release and exposure to several heavy metals (Awasthi, Zeng, & Li, 2016). There is little published data on the association between human metal exposure and DNA damage in the e-waste recycling industry (Alabi, Adeoluwa, & Bakare, 2019; Q. Wang et al., 2011; Y. Wang et al., 2018). To the best of our knowledge, no previous study has investigated the association between metal exposure to the e-waste workers themselves and DNA methylation changes; rather these studies were carried out among non-worker populations residing near informal sector e-waste recycling (Z. Li et al., 2020).

The objectives of this study were to (1) quantify the concentration of heavy metals in blood (Cd and Pb) and urine (Cd, Pb, and As) (2) evaluate the LINE1 DNA methylation, and (3) elucidate the association between concentration of blood and urine heavy metals and LINE1 DNA methylation among e-waste workers and a control group in Ghana. These metals were considered because of their wide-spread use in electrical and electronic products, their toxicity and public health significance (Tchounwou, Yedjou, Patlolla, & Sutton, 2012), and the risk of co-exposure (Ni, Huang, Wang, Zhang, & Wu, 2014).

2. Methods

2.1 Study area and population

The study was conducted in two locations: the e-waste recycling site in Agbogbloshie, Accra, Ghana, and Madina Zongo, a part of greater Accra located approximately 10 km from the e-waste site. The Agbogbloshie e-waste site is the largest and busiest e-waste dump in Africa (Srigboh et al., 2016). The site is situated on the banks of the Korle Lagoon on the western side of the Odaw River in central Accra. To the east are various businesses, including banks, pharmaceutical companies, breweries, shops, and

various manufacturing companies. To the south 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 Agbogbloshie scrapyard is the main centre for the recovery of valuable materials from e-waste, with an estimated population of 80,000 (United Nations Population Fund, 2018). The residents at the site are predominantly migrants from the northern parts of Ghana (Wittsiepe et al., 2017). The recycling process involves manual dismantling, collection, and sorting of electrical equipment, and the burning of electrical and electronic items, including plastic materials such as wire insulation, which emits considerable smoke and pollutes the local enaqa vironment. The recyclers are mostly young men who use rudimentary tools such as a chisel, hammer, pliers etc. or sometimes their bare hands with little or no protective equipment (Acquah et al., 2019).

The controls are resident at Madina Zongo in Accra, which is about 10 km North of Agbogbloshie. There are no e-waste recycling activities in the area, and individuals recruited are not involved in any e-waste work. The residents of Madina Zongo are known to be quite similar to the e-waste workers with respect to the length of time residing in Accra and region of the country from where they moved, socioeconomic background, religion, and culture.

2.2 Study design and participants recruitment

This study utilized existing data and specimens from the parent study (GeoHealth-II), a longitudinal repeated measures study with four rounds of data collection from both Agbogbloshie workers and the comparison group in Madina Zongo. After completing a community entry process which included durbar which brought together researchers, and leaders of the study sites, and the e-waste workers, participants were recruited into the study. Each potential participant was given a detailed explanation of the study procedures and objectives, the benefits and possible risks of participating in the study, and if willing to be enrolled, was asked to provide written consent. The study was conducted after receiving ethical clearance from the College of Health Sciences Ethical and Protocol Review Committee, University of Ghana (protocol identification number CHS-ET/M.4-P 3.9/2015-2016). Briefly, there were 151 participants recruited from the first round of data collection from both Agbogbloshie (100) and Madina Zongo (51). Due to higher than expected loss to follow up rates in the second round of data collection, 56 new participants (Agbogbloshie = 42 and Madina Zongo = 14) were recruited to address this challenge. Some of the participants provided multiple blood and urine samples during the four sampling periods from March 2017 to August 2018, bringing the total number of samples to 598. This study utilized data from the first sampling event (Agbogbloshie = 100 and Madina Zongo =51) to investigate associations between heavy metals (Cd, Pb, and As) and global (LINE1) methylation levels at baseline.

2.3 Data collection

2.3.1 Questionnaire survey

All participants answered a questionnaire which was administered by trained staff. Interviews were conducted in English, Dagbani, Twi and Hausa. The interview included demographics (age, gender,

religion/ethnicity, education, measures of socioeconomic position, location of birth and childhood and location of all residences), information to assess past and current potential exposures to air pollutants (use of tobacco/exposure to environmental tobacco smoke, exposure to indoor cooking using biomass fuels, type of housing, detailed job history), personal and family medical history (diagnosed illnesses, reported symptoms), and other health-related measurements such as weight, height, and blood pressure.

2.3.2 Blood and urine samples collection

A temporary structure (clinic) was erected and arranged to allow for the various data collection for each sampling round in both Agbogbloshie and Madina Zongo. Each participant at enrollment and in subsequent rounds provided 20 ml of venous whole blood. The blood was collected by an experienced phlebotomist following sterile procedures via venepuncture of the antecubital fossa into EDTA tubes. In addition, each participant was given a 100 ml, sterile plastic container to provide a urine sample. The urine was then aliquoted into three 10 ml sterile tubes for storage. The blood and urine samples were placed in a cooler with ice blocks and then transported to the University of Ghana after each sampling day, stored at -80 C, and later transported on dry ice to the University of Michigan, USA and McGill University, Canada for DNA extraction and metal analysis, respectively.

2.4 Elemental Analysis

Inductively Coupled Plasma mass spectrometer (ICPMS Varian; 820MS) was used to detect the levels of blood and urine Cd, Pb and As. The blood and urine samples were digested with nitric acid as detailed by Basu, Nam, Kwansaa-Ansah, Renne, and Nriagu (2011).

All the analytical quality control measures previously described by Srigboh et al. (2016), were used. In summary, all laboratory glassware and plastic were acid-washed (cleaned, soaked 24 h in 20% nitric acid, and rinse 3 times in Milli-Q water) before use. Accuracy and precision were measured by use of certified reference materials (INSPQ; QM-U-Q1109 [urine]; and then QM-B-Q1506 and QM-B-Q1314 [blood]) obtained from the Institut National de Sante Publique du Quebec. Also, each batch run contained procedural blanks and replicate runs. For each element analyzed, the theoretical detection limit was calculated as three times the standard deviation of the mean blank value (Supplemental Table 1).

2.5 Extraction of DNA from whole blood for LINE-1 methylation

DNA was extracted in the laboratory at Michigan Public Health using the Qiagen DNA Blood Mini Kit, following manufacturer's recommendations. Purity and quantity of DNA samples were assessed with the Qubit Broad Range Double-stranded DNA assay and Nanodrop Spectrophotometer through the University of Michigan DNA Sequencing Core. The extracted DNA was then stored at -20 ºC until LINE-1 methylation analysis was conducted.

2.5.1 LINE-1 methylation analysis

Sodium bisulfite conversion was performed on 300 ng of extracted genomic DNA using the Qiagen EpiTect Bisulfite Kit per the manufacturer's protocols. PCR amplification was performed for the promoter region of LINE-1 using a previously published assay (Yang et al. 2004). In summary, HotStarTaq Master Mix (Qiagen, Valencia, CA), water, and desalted FWD/RVS primers were combined to create a PCR master mix. Lastly, 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 product quality was confirmed using 2 % agarose gels and gel red stain. 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 re-run if any of the controls failed. Samples were measured in duplicate.

2.6 Statistical analysis

Statistical analysis was done with Stata v15.1 (STATA Corp LLC. Texas, USA) and GraphPad Prism v8.3.1 was used to generate graphs. Shapiro-Wilk test was used to assess the normality of continuous variables. The data were presented as mean (standard deviation) for normally distributed data or median (interquartile range) for data that were not normally distributed. The non-parametric Mann-Whitney U test was used to compare differences across study sites for continuous abnormally distributed data while t-test compared normally distributed data. In the case of categorical variables, Chi-squared test of association was used for the comparison while one-way analysis of variance (ANOVA) compared continuous variables (e.g. LINE1) and categorical variables (e.g. job categories). Since Cd, Pb, and As were not normally distributed, they were log-transformed for the bivariate analysis. Pearson correlation was used to assess the correlation between the heavy metals and the average methylation of the four CpG sites (LINE1) and methylation of each CpG sites. Multivariable linear regression was used to explore relationships between DNA methylation and heavy metal biomarker levels while adjusting for confounders. Statistical significance was set at $p<0.05$.

3. Results

3.1 Demographic characteristics

Overall, participants in the control group were significantly older than their e-waste counterparts (25.4±6.3 vs 32.5±10.4, Table 1). The BMI of the control group (mean = 23.8±3.5) was significantly higher than that of e-waste workers (mean = 21.6±2.7, Table 1). A majority, of e-waste workers, live within 1km of the e-waste site, worked on the average10 hr per day, for an average of 6 days per week. Regards the level of education, 25.3% of e-waste workers had no formal education compared to 13.0% of the controls. Only 16.2% of e-waste workers had secondary school education or higher, compared to 52.2% of the controls. Majority of the e-waste workers (80%) earned between 20 – 80 Ghanaian Cedi (GHC); the equivalence of

5 – 15 USD. The prevalence of smoking among the e-waste workers was significantly higher (27.8%) than among the non e-waste group (12.2%, Table 1).

Table 1: Demographic and lifestyle characteristics study participants (e-waste workers and controls).

	Total	E-waste workers	Non e-waste workers	p-value
N	151	100	51	
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)		6.0(1.0)		
Hours work/day, mean(±SD)		9.7(4.4)		
Sleep location, n(%)		n=97		
On the site		54(55.7)		
≤1km off-site		35(36.1)		
>1km off-site		8(8.3)		
Education, n(%)	n=145	n=99	n=46	**<0.001[b]**
No formal education	31(21.4)	25(25.3)	6(13.0)	
Primary	30(20.7)	26(26.3)	4(8.7)	
Middle/JHS	44(30.3)	32(32.3)	12(26.1)	
Secondary/SHS+	40(27.6)	16(16.2)	24(52.2)	
Marital status, n(%)	n=150	n=99	n=51	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=149	n=99	n=50	0.072[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 141-200	8(5.4)	5(5.1)	3(6.0)	
> GHC 200	11(7.4)	4(4.0)	7(14.0)	
Indoor cooking, n(%)	n=147	n=98	n=49	**0.009[b]**
Yes	30(20.4)	14(14.3)	16(32.7)	
No	117(79.6)	84(85.7)	33(67.4)	
Alcohol use, n(%)	n=148	n=99	n=49	0.086[b]

Never	125(84.9)	83(83.8)	42(87.7)	
Former	6(4.1)	2(2.0)	4(8.2)	
Occasional/regular	17(11.5)	14(14.1)	3(6.1)	
Smoking, n(%)	n=146	n=97	n=49	**0.033[b]**
Yes	33(22.6)	27(27.8)	6(12.2)	
No	113(77.4)	70(72.2)	43(87.8)	

Abbreviations: SD=standard deviation, N= Total number of participants, n(%)= frequency(percent frequency). [a] p-values obtained by t-test, [b] p-values obtained by chi-square test

3.2 Heavy metals concentration in blood and urine in e-waste workers and controls

As shown in Table 2, E-waste workers had lower median blood cadmium (B-Cd) concentration compared to the control group, (respectively, 0.59µg/L and 0.81µg/L, p=0.003), while concentrations of urine Cd (U-Cd), which is a biomarker of long term exposure did not differ among the two populations. Blood lead (B-Pb) and urine lead (U-Pb) on the other hand showed higher significant median concentration among the e-waste worker group than the control group; (76.82µg/L and 40.25µg/L, p=<0.001; and 6.89µg/L and 3.43µg/L, p=<0.001, respectively). Similar to Cd, arsenic in urine (U-As) was higher among the control group (Table 2).

The levels of heavy metals in blood and urine were compared to background levels in the US population, using the P95 values of the National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control and Prevention, 2019). For blood and urine lead, 99% and 97% of the samples of e-waste workers far exceeded the reference value of 29.3µg/L and 1.26µg/L, respectively. In the controls, 74% of the population had B-Pb higher than 29.3µg/L and 94.1% had U-Pb higher than 1.26µg/L. For U-As, 68.6% of the controls had concentrations higher than 49.9µg/L compared to 41.8% of the e-waste workers (Table 2).

Table 2: Heavy metals concentration in blood (Cd and Pb) and urine (Cd, Pb, and As) in e-waste workers and controls

Heavy metals (µg/L)	Total	E-waste	Controls	P
B-Cd	n=150	n=100	n=50	
Mean ±SD	0.80 ± 0.59	0.73 ± 0.55	0.93 ± 0.64	0.003
Median (IQR)	0.66 (0.44)	0.59 (0.43)	0.81 (0.55)	
B-Cd > 1.17, n (%)	21 (14)	11 (11)	10 (20)	
B-Pb	n=150	n=100	n=50	
Mean ±SD	75.12 ± 58.42	92.35 ± 63.69	40.67 ± 19.12	<0.001
Median (IQR)	518.95 (44.01)	76.82 (49.37)	40.25 (17.45)	
B-Pb > 29.3, n (%)	135 (90.67)	99 (99)	37 (74)	
U-Cd	n=149	n=98	n=51	
Mean ±SD	0.62 ± 0.98	0.68 ± 1.18	0.50 ± 0.35	0.878
Median (IQR)	0.40 (0.45)	0.38 (0.56)	0.42 (0.44)	
U-Cd > 0.77, n(%)	31 (20.8)	22 (22.4)	9 (17.6)	
U-Pb	n=149	n=98	n=51	
Mean ±SD	6.49 ± 5.03	7.76 ± 4.87	4.06 ± 4.45	<0.001
Median (IQR)	5.05 (4.98)	6.89 (5.22)	3.43 (2.38)	
U-Pb > 1.26, n (%)	145 (97.3)	97 (99)	48 (94.1)	
U-As (µg/L)	n=149	n=98	n=51	
Mean ±SD	69.63 ± 63.39	58.75 ± 59.45	90.55 ± 66.02	0.001
Median (IQR)	51.59 (71.35)	42.90 (570.7)	69.53 (101.08)	
U-As > 49.9, n (%)	76 (51.0)	41 (41.8)	35 (68.6)	

p-value estimate from the Wilcoxon rank-sum (Mann-Whitney) test

p-values < 0.05 were considered significant

IQR=interquartile range

source of reference values: USA, NHANES, Survey 2011–2016 (U.S. Department of Health and Human Services - Centers for Disease Control and Prevention, 2019)

3.3 LINE1 DNA methylation in e-waste workers and controls

Methylation of four CpG sites of LINE1 was quantified from whole blood samples (N=149). As expected, all CpG sites were heavily methylated (Table 3). There was no significant difference in LINE1 methylation

among the e-waste workers and the non e-waste workers (85.16 ± 1.32% vs 85.17 ± 1.11%, p=0.950). 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%, p=0.034) and CpG4 had the highest (91.28%) mean methylation level (Table 3).

Table 3: LINE1 methylation levels (%) in e-waste workers and controls

Methylation (%), mean ± SD	**Total** (n=149)	**E-waste** (n=99)	**Controls** (n=50)	**p**
CpG1	82.23 ± 2.12	82.48 ± 2.20	81.70 ± 1.86	0.034
CpG2	83.91 ± 1.21	83.93 ± 1.33	83.87 ± 0.95	0.789
CpG3	83.25 ± 2.18	83.07 ± 2.34	83.60 ± 1.82	0.162
CpG4	91.28 ± 2.46	91.16 ± 2.18	91.52 ± 2.93	0.403
LINE1	85.16 ± 1.25	85.16 ± 1.30	85.17 ± 1.10	0.950
*The mean methylation of all CpG sites is reported along with methylation at specific CpG sites Abbreviations: CpG=cytosine-guanine dinucleotide, LINE1=mean methylation of all CpG sites, p-values obtained by t-test				

3.2.1 LINE1 methylation levels of e-waste workers by primary job-tasks performed.

The main e-wate recycling activities at the Agbogbloshie site include sorting and transport of e-waste materials, manual dismantling of larger waste types, and open burning of smaller insulated wires to recover copper and other valuables (Kwarteng et al., 2020). Therefore, the e-waste workers at the Agbogbloshie site in Ghana are categorised into 3 main groups; collectors/sorters, dismantlers, and burners based on their most recent job tasks (Acquah et al., 2019).

LINE1 methylation level was compared among the different categories of e-waste workers defined by primary job tasks. Overall, e-waste collectors had the lowest mean methylation level compared to burners and dismantlers (Figure 1). However, the difference in methylation was not significant (ANOVA, p=0.104)

(figure 1 here)

3.3 Relationship between LINE1 methylation and anthropometric and lifestyle factors

The mean methylation of LINE1 was assessed based on anthropometric and lifestyle factors such as age, BMI, smoking, alcohol consumption, and indoor via one-way analysis of variance (ANOVA) (Table 4). LINE1 methylation was not related with age, BMI, smoking or alcohol consumption ($p_{all} > 0.05$). However, the proximity of e-waste workers residence in relation to the e-waste site was associated with LINE1

methylation (p=0.019). A Tukey post-hoc test revealed that LINE1 methylation was statistically lower in workers who lived within 1km of the e-waste site compared to those who lived on-site (p=0.034).

Table 4: Relationship between LINE1 methylation and anthropometric and lifestyle factors

	LINE1 methylation			
Variable	Total (n=151)	E-waste workers (n=100)	Non e-waste workers (n=51)	p-value
Age (years)				
≤20	85.3(1.1)	85.2(1.2)	85.4(0.6)	0.817
21-30	85.1(1.4)	85.1(1.4)	85.3(1.3)	0.520
31-40	85.1(1.2)	85.3(1.3)	84.8(1.2)	0.3995
>40	85.3(1.2)	85.73(2.0)	85.2(1.0)	0.541
	P=0.885	P= 0.803	P=0.697	
Smoking				
Yes	85.2(1.2)	85.2(1.3)	85.1(0.7)	0.805
No	85.2(1.3)	85.2(1.3)	85.2(1.2)	0.892
	P=0.914	P=0.830	P= 0.832	
Alcohol intake				
occasional/regular	85.4(1.4)	85.4(1.5)	85.6(1.3)	0.797
former	85.2(1.6)	84.2(2.5)	85.7(1.2)	0.339
never	85.2(1.2)	85.2(1.3)	85.1(1.1)	0.744
	P=0.675	P=0.471	P=0.481	
BMI (kg/m^2)				
Low weight	84.8(1.1)	84.6(1.2)	85.2(0.5)	0.492
Normal weight	85.2(1.3)	85.2(1.3)	85.2(1.1)	0.796
Overweight	85.3(1.2)	84.9(1.3)	85.5(1.1)	0.307
Obesity	84.5(1.3)	85.7(0.0)	84.2(1.3)	0.364
	P=0.363	P= 0.529	P=0.244	
Sleep location				
On-site	-	85.4(1.4)	-	-
≤1km off-site	-	84.7(1.1)	-	-
≥1km off-site	-	85.8(1.4)	-	-
	-	P=**0.019**	-	-

Indoor cooking				
Yes	85.1(1.3)	85.3(1.5)	84.9(1.1)	0.370
No	85.2(1.2)	85.2(1.3)	85.3(1.1)	0.574
	0.733	P=0.635	P=0.252	

Body Mass Index (BMI) 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.

3.4 Relationship between LINE1 methylation and blood and urine levels of Cd, Pb, and As

Bivariate analyses (Pearson correlation) showed a trend toward negative correlations between the log-transformed heavy metals and LINE1 methylation, even though the correlations were not significant (Table 5). No correlation was observed between LINE1 and the specific CpG sites.

Table 5: Pearson correlation coefficients between LINE1 and specific CpG sites methylation and log-transformed blood and urine Cd, Pb, and As

Heavy metals	LINE1		CpG site1		CpG site2		CpG site3		CpG site4	
	r	p	r	P	r	p	r	p	r	P
B-Cd	-0.06	0.476	-0.00	0.996	-0.11	0.176	-0.11	0.171	0.04	0.668
B-Pb	-0.14	0.085	0.04	0.599	-0.13	0.128	-0.15	0.076	-0.13	0.104
U-Cd	-0.14	0.089	-0.14	0.088	-0.06	0.498	-0.06	0.445	-0.08	0.333
U-Pb	-0.03	0.729	0.11	0.200	0.02	0.833	-0.01	0.940	-0.13	0.117
U-As	-0.07	0.395	-0.12	0.149	-0.05	0.573	0.02	0.776	-0.04	0.647

3.5 LINE1 methylation and exposure to heavy metals: Multiple Linear Regression

Associations between LINE1 methylation and body burden of heavy metals were assessed using multiple linear regression model, adjusting for confounding factors that may influence methylation status (age, BMI, smoking, alcohol consumption). A statistically significant inverse relationship was observed between blood Pb and LINE1 methylation (β = -0.004; 95%CI: -0.008, -0.0003; p = 0.034) (Table 6). This association persisted only among the non e-waste workers. Further adjusting for primary job taks performed by e-waste workers, we observed a modest significant association in LINE1 methylation levels between e-waste collectors compared to those who work as burners (β = -0.889; 95%CI: -1.757, - 0.021; p = 0.045) (Table 6).

Table 6: Association between LINE1 methylation and levels of Cd, Pb, and As in blood and urine.

LINE1 methylation						
Predictors	**Total**		**e-waste workers**		**Controls**	
	β(95%CI)	p-value	β(95%CI)	p-value	β(95%CI)	p-value
B-Pb	-0.004(-0.008, -0.0003)	0.034	-0.003(-0.008, 0.001)	0.191	-0.025(-0.04, -0.008)	0.004
Collectors[a]	-	-	-0.889(-1.757, -0.021)	0.045	-	-
All models are adjusted for age, BMI, smoking status, and alcohol intake, (in total population and stratified by e-waste exposure), [a] = further adjusted for primary job tasks. p-values < 0.05 are significant						

4. Discussion

Informal e-waste recycling activities generate a considerable amount of air pollutants, including heavy metals, some of which are carcinogenic (Alabi & Bakare, 2017). Several studies (Goodrich et al., 2013; C. Li et al., 2011; C. Li et al., 2013; Wen et al., 2016) have reported associations between occupational heavy metals exposure and DNA methylation; however, there are few studies, if any, that have looked at exposure levels and DNA methylation among informal e-waste workers.

Our study shows that lead levels (B-Pb and U-Pb) were significantly higher in e-waste workers compared to the controls. Our finding is consistent with other biomonitoring studies among e-waste workers at Agbogbloshie (Wittsiepe et al., 2017) and teenage e-waste scavengers in Nigeria (Alabi et al., 2019), where B-Pb and serum Pb levels were consistently higher in the exposed group than controls suggesting e-waste recycling activities as a critical contributor to the elevated Pb levels. Contrary to our expectation, B-Cd and U-As were significantly higher among the controls. In this study, the control population had a generally high levels of Cd, Pb, and As in blood and urine, when compared with the P95 values of the NHANES survey (Centers for Disease Control and Prevention, 2019). The high concentration of heavy metals in the unexposed group could be attributed to the 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 total national primary $PM_{2.5}$ in Ghana, followed by transport and road dust (13.9%) (Health Effects Institute, 2019). Also, the control group for this study 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. In addition, drinking water could be a major source of heavy metals exposure in Ghana. For example, Asante et al. (2012) estimated the levels of trace elements (T.E.s) concentration in tap water in Accra, Ghana and found significant variations in heavy metals concentration even though the samples were taken from the same water treatment source. The use of

metal (galvanized iron) pipes for water distribution in Ghana and corroded household plumbing systems were reported as possible sources of heavy metals found in tap water (Asante et al., 2012).

Our findings show that LINE1 was heavily methylated in whole blood of e-waste workers (mean ± SD: 85.2 ± 1.3%). Overall, there was no significant difference in LINE1 methylation between the e-waste workers and controls in this study. This is consistent with the findings of Ghosh et al. (2017), where no significant difference in LINE1 methylation was observed between workers exposed to multi-wall carbon nanotubes and controls. The non-significant difference in LINE1 methylation between our study populations may be attributable to the choice of the control group in this study since the categorization of hyper- or hypo-methylation is dependent on the methylation levels of the comparator group (Phetliap et al., 2018). Indoor and outdoor urban air pollution especially in the form of particulate matter (PM) generated from the wide-spread use of biomass fuelwood as energy sources for cooking is a major public health concern in Ghana (Cobbinah, Poku-Boansi, & Peprah, 2017). This and other sources such as vehicular emissions exposes a large proportion of the urban population to PM from where our control group for this study was recruited. Our control group, therefore, are substantially exposed to a high concentration of ambient air pollution which could explain the lack of difference in LINE1 methylation in our study since traffic-related particles ($PM_{2.5}$) altered the level of LINE-1 methylation in blood cells (Baccarelli et al., 2009).

Workers at the e-waste recycling site in Ghana are involved in multiple tasks since job titles and task protocols are not present, and previous studies (Feldt et al., 2014; Wittsiepe et al., 2015), as well as this study, derived job categories based on workers self-report. Regarding the job-tasks performed by the e-waste workers, collectors had lower LINE1 methylation, even though the difference was not statistically significant. This could be attributable to job misclassification, which may conceal a significant difference in health effects (Laskaris et al., 2019). The use of wearable cameras proposed by Laskaris et al. (2019) can significantly minimize the task misclassification and improve occupational exposure assessment among informal sector workers. Adjusting for confounders, our multivariable linear regression analysis showed a significant decline in LINE1 methylation among collectors. E-waste collectors often travel off-site, within the communities (primarily by foot, bicycle or tricycle) to purchase or scavenge e-waste materials (Acquah et al., 2019; Laskaris et al., 2019) and may, therefore, be exposed to other sources of pollutants (including vehicular emissions and road dust) outside the e-waste recycling site. The significant decline in LINE1 methylation among collectors could, therefore, be attributed to the higher exposures due to multiple exposure sources.

Our multivariable regression showed that blood Pb was associated with decreased LINE1 methylation. Previous studies found an association between LINE1 hypomethylation and exposure to lead (Wright et al., 2010). Our finding is consistent with previous occupational exposure studies in China (C. Li et al., 2013), and Brazil (Devóz et al., 2017). In the Chinese study, methylation levels of LINE1 were assessed among battery workers exposed to Pb (n = 53) and a healthy control group (n = 57), and they reported a significant decrease in LINE1 methylation among the Pb exposed group (C. Li et al., 2013). Among workers in an automotive factory in Brazil, there was a negative association between Pb exposure and

global DNA methylation (Devóz et al., 2017). Previous experimental study suggests that Pb decreases the activities of DNA methyltransferase (DNMT); the enzyme that regulates DNA methylation reaction by catalyzing methyl groups (CH_3) through SAM to DNA, which initiates DNA hypomethylation (Sanchez et al., 2017). Contrary, recent epigenome-wide study did not show any significant methylation changes in DNMTs among workers occupationally exposed to Pb, suggesting that other genes may mediate Pb-induced DNA methylation changes (Zhang, He, Feng, & Shao, 2019).

There are some limitations to this study. First, exposure in the informal e-waste recycling sector is to a complex mixture of chemicals including PAHs and other persistent organic pollutants (POPs) which may also alter DNA methylation, data of which were not included in this study. Second, other domains of environmental exposures such as nutrition and psychosocial stress may result in epigenetic modifications that may contribute to an increased risk of disease (Thayer & Kuzawa, 2011), data of which were not considered for this study.

The strength of the present study was that we examined three heavy metals concurrently using two types of biological media (blood and urine) for the exposure assessment. Also, this present study benefitted from the high-quality protocols for the recruitment of participants, conduction of interviews, collection of biological samples, and laboratory analyses.

In conclusion, the high internal concentration of heavy metals in the control group in this study suggests that heavy metals exposure is a nationwide problem in Ghana. However, we found that e-waste workers tend to have a higher concentration of Pb in particular, which was associated with global DNA hypomethylation, as shown in LINE1 methylation. This may serve as an early epigenetic marker which mediates the adverse effects of Pb exposure. In addition, e-waste collectors had decreased LINE1 methylation levels compared to the other categories of workers. To the best of our knowledge, this is the first study that looked at this population in an epigenetic context. Since global methylation provides important preliminary information about the genome stability, further epigenetics epidemiologic research is needed using candidate genes and other epigenetic markers such as histone modifications to provide a comprehensive understanding of specific pathways through which heavy metals exert their toxic effects, especially among the unprotected informal sector workers.

Abbreviations

As Arsenic

Cd Cadmium

CpG Cytosine-guanine dinucleotide

DNMTs DNA methyltransferases

E-waste Electronic waste

GeoHealth Global Environmental and Occupational Health

HEI Health Effect Institute

LINE1 Long Interspersed Nucleotide Element-1

NHANES National Health and Nutrition Examination Survey

PAHs Polycyclic Aromatic Hydrocarbons

Pb Lead

POPs Persistent Organic Pollutants

SAM S-adenosyl methionine

Declarations

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' Contributions

Conceptualization, I.I., T.G.R., J.N.F. L.S.R and J.A.-M.; **Methodology**, I.I., L.S.R., K.R.Z., J.N.F. and J.A.M.; **Software**, L.S.R., and K.R.Z; **Investigation**, I.I., T.G.R., J.N.F., J.A.-M., N.B., T.P.A., L.S.R, and K.Z;

Validationandformalanalysis, I.I., and D.D.; **Resources**, J.N.F., T.G.R., S.B., L.S.R. **Datacuration**, I.I., T.P.A., and D.D.; **Writing—original draft preparation**, I.I. **Writing—review and editing**, I.I., L.S.R., K.R.Z., T.G.R., J.N.F., J.A.-M., S.B., and T.P.A.; **Visualization**, I.I., L.S.R and D.D.; **Supervision**, T.G.R., J.N.F., J.A.-M., and L.S.R.; **Project administration**, T.G.R., J.N.F.; **Funding acquisition,** T.G.R., S.B, L.S.R and J.N.F. All authors read and approved the final manuscript.

Consent for publication

Not applicable

Competing interests

None declared

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Figures

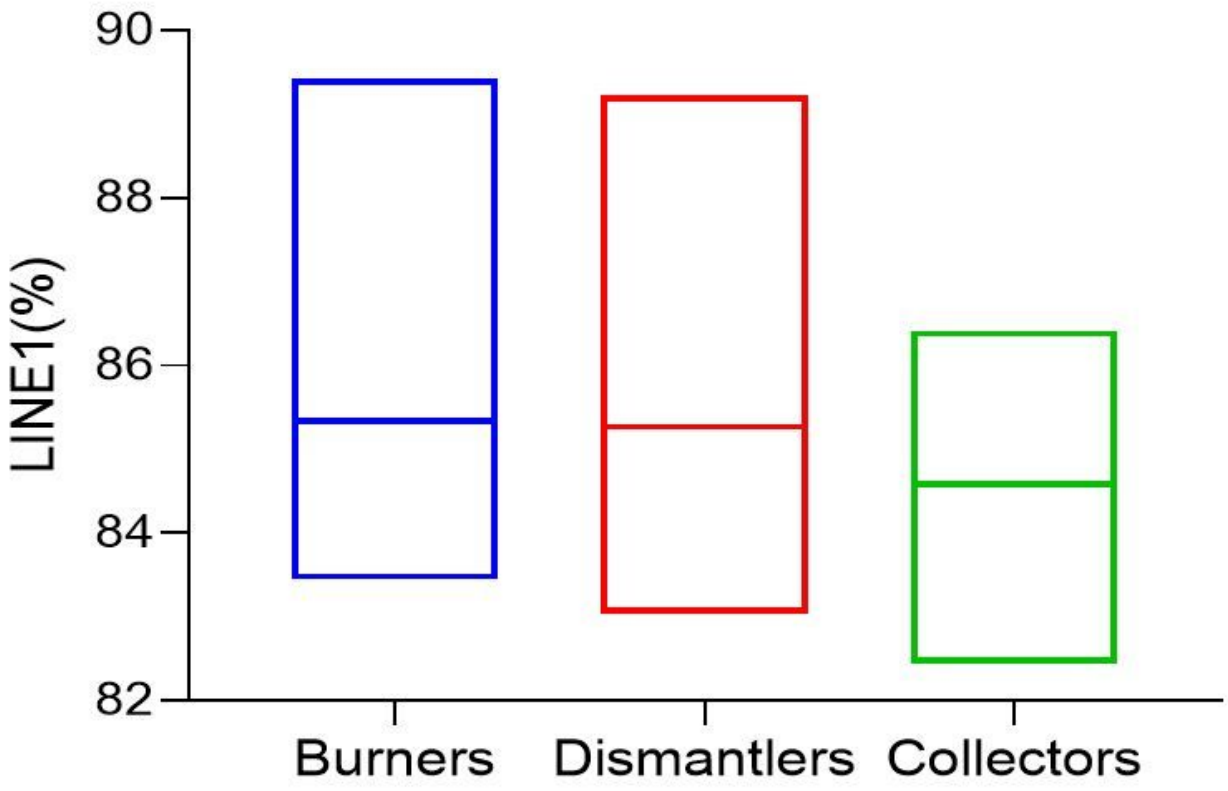

Figure 1

Floating bars reporting (min to max) LINE1 methylation across primary job-tasks performed by e-waste workers; collectors had the lowest mean methylation levels.

Supplementary Files

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