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RESEARCH ARTICLE

Association between toxic and essential metals in blood and global DNA methylation among electronic waste workers in Agbogbloshie, Ghana

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Abstract

Aberrant global DNA methylation status is a known biomarker for increased disease risk, especially cancer. There is little published data on the association between toxic and essential metal mixtures and global DNA methylation in electronic waste (e-waste) workers. We aimed to establish the association between toxic and essential metals in blood and the effect of their interactions on global DNA methylation among e-waste recyclers and a reference group in Ghana. We used ICP-MS to measure the level of five metals (Se, Zn, Mn, Cd, and Pb) in the blood of 100 e-waste workers and 51 controls. We quantified blood DNA methylation levels of LINE-1 as an indicator of global DNA methylation. Cd, Mn, and Se levels were significantly higher in the reference group than in e-waste workers. Only Pb was significantly higher in the e-waste workers compared to the controls. Our linear regression analysis results showed a significant inverse association between Zn and LINE-1 DNA methylation ($\beta_{Zn} = -0.912$; 95% CI, −1.512, −0.306; $p = 0.003$) which corresponds to a 0.009 decrease in %LINE-1 methylation (95% CI, −0.015, −0.003; $p = 0.003$) for a 1% increase in Zn concentration. Potential interactions between Cd and Zn on global DNA methylation were observed. In summary, co-exposure to toxic and essential metals is associated with global (LINE-1) DNA methylation.

Keywords DNA methylation · Epigenetics · E-waste recyclers · LINE-1 · Toxic metals · Essential metals

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Introduction

Metals are toxicologically important compounds that are ubiquitous in the environment and are largely used in the manufacture of electrical and electronic equipment (Woo et al. 2016). End-of-life electrical and electronic equipment (EEE or e-waste) are increasingly been recognized as having serious global environmental and public health concern due to the lack of technology for efficient recycling or recovery (Krishnamoorthy et al. 2018; Kumar and Singh 2014). E-waste contains toxic metals such as chromium (Cr), lead (Pb), mercury (Hg), iron cadmium (Cd), arsenic (As), and essential metals such as iron (Fe), zinc (Zn), and copper (Cu) (Fujimori et al. 2012). Recycling e-waste predominantly occurs in the informal sector, where regulations are weak or absent with inadequate worker protection (Forti et al., 2020). Several researchers have reported elevated levels of toxic metals, including Pb, Cd, nickel (Ni), Cr, manganese (Mn), Hg, and As, in e-waste workers' biological samples (urine, blood, serum, and hair) (Alabi et al. 2020; Neitzel et al. 2020). Exposure to these toxic metals could have adverse health implications, especially in the long term.

Evidence suggests that toxic metals are among the most critical risk factors for many chronic diseases such as cardiovascular diseases, neurological diseases, reproductive toxicity, renal dysfunction, autoimmune diseases, and cancers (Hu 2002; Lim et al. 2019; Rzymski et al. 2015; Shi et al. 2019). Due to the elevated levels detected in occupational environments (Baloch et al. 2020; Han et al. 2019) and the potentially deleterious effects of these toxic metals on human health in the long term, there is growing interest to determine the intermediate events, especially at the gene/DNA level, before the onset of clinical occupational disease (Leelapongwattana and Bordeerat, 2020; Salemi et al. 2017). Epigenetic modifications are highly suspected as intermediaries between toxic metal exposure and adverse health outcomes (Chervona et al. 2012; Stein and Davis 2012).

Long interspersed nuclear elements (LINE-1) are a family of repetitive sequences that constitute a significant portion (approximately 17%) of the human genome (Yang et al. 2004). They are most heavily methylated to prevent retrotransposition, and their methylation levels are used as a surrogate marker for global DNA methylation of the human genome (Rodić and Burns, 2013; Yang et al. 2004). Toxic metal exposure can influence LINE-1 DNA methylation and may be a mechanism by which these metals are associated with disease (Lee et al. 2009).

Several researchers have reported that occupational exposure to toxic metals 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), and lead in automotive battery factory workers (Devóz et al., 2017). However, there are still data gaps addressing the issue of DNA 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 interaction affect DNA methylation (Vidal et al. 2015). A recent study indicated that higher concentrations of Zn, As, and Hg were associated with decreased LINE-1 methylation in neonates (Montes-Castro et al. 2019). We, therefore, aimed to examine the effect of Cd, Pb, Mn, selenium (Se), and Zn or their interaction on global DNA methylation (LINE-1) among e-waste workers in Ghana.

Materials and methods

Study area

The study was conducted in two locations: the e-waste site in Agbogbloshie and Madina Zongo, a control site. These study sites were previously described (Amoabeng et al. 2020; Laskaris et al. 2019b). Briefly, the Agbogbloshie e-waste recycling site is one of the busiest sites of its kind globally and has become a hub for the informal e-waste sector in Ghana and many other formal and informal businesses (Simon 2018; Srigboh et al. 2016). It is located in central Accra and rated as one of the most contaminated sites on earth (Amoyaw-Osei et al., 2011; Blacksmith Institute 2013). The e-waste workers are mostly young men who migrated from the northern part of Ghana, in search of employment opportunities. E-waste recycling activities include collection, dismantling, and open air burning to retrieve reusable parts (Acquah et al., 2019). These activities are carried out in the open, with little or no use of personal protective equipment (PPE) for worker protection, thereby exposing workers to multiple pollutants, including particulate matter (PM) of varying sizes and chemicals such as polychlorinated biphenyls (PCB) and dioxins (Dai et al. 2020; Laskaris et al. 2019a).

The control site, Madina Zongo, is in another metropolitan area of Accra, more than 10 km northeast of the Agbogbloshie e-waste recycling site. A previous study has successfully recruited control participants from Kwabenya North (Wittsiepe et al., 2017), a suburb of Accra not far from Madina Zongo. Population in Madina Zongo are similar to those in Agbogbloshie with respect to age, length of time residing in the greater Accra area (and region of the country from where they moved), socioeconomic position, religion, and culture. There are no e-waste recycling activities in the area, and the individuals recruited were not involved in any e-waste work.

Study population

The study population is from the GeoHealth II study described in Takyi et al. (2020), a longitudinal cohort study, with broad aims of increasing multidisciplinary understanding of the health risks at the Agbogbloshie e-waste site in central Accra, Ghana, and to use study findings to inform evidence-based policy development and implementation at the national, regional, and international level. 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. For this current study, we restricted our analyses to the baseline sample. 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 GeoHealth II study protocol.

Blood sample collection

Ten ml of whole blood (venous) was drawn from each participant and used for metal analysis and DNA extraction for methylation analysis. Blood was collected by an experienced phlebotomist following sterile procedures via venepuncture of the antecubital fossa into EDTA tubes. Blood samples were aliquoted into 2.5-ml cryotubes and placed in a cooler with ice blocks and later transported to the University of Ghana for storage at − 80 °C. Stored whole blood aliquots were later shipped on dry ice to the University of Michigan, Ann Arbor, USA, or McGill University, Montreal, Canada, for DNA extraction and methylation analysis or for metals analysis, respectively.

Measurement of metals in whole blood

An inductively coupled plasma mass spectrometer (ICPMS Varian; 820MS) was used to measure levels of blood Cd, Pb, Mn, Se, and Zn. Blood samples were digested with nitric acid as detailed by Basu et al. (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 for 24 h in 20% nitric acid, and rinsed 3 times in Milli-Q water) before use. Accuracy and precision were measured using certified reference material (QM-B-Q1506 and QM-B-Q1314 [blood]) obtained from the Institut National de Sante Publique du Quebec. Additionally, 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 S1).

Extraction of DNA from whole blood for LINE-1 methylation

DNA was extracted in the laboratory at the University of Michigan School of Public Health using the Qiagen DNA Blood Mini Kit (Qiagen, Valencia, C.A), following the manufacturer's instructions. The 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.

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). 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. Samples were measured in duplicate.

Assessment of covariates

Trained staff administered interview questionnaires face to face to collect information including socio-demographic and lifestyle characteristics including age, smoking status (yes, no), indoor use of biomass fuel for cooking (yes, no), alcohol consumption (occasional/regular, former, never), and educational attainment (primary, junior high, senior high +). The interview was conducted in English and local languages (Dagbani, Housa, and Twi). The body mass index (BMI) of the participants was calculated by dividing their weight in kilograms (kg) by height in meters squared (m^2).

Statistical analysis

All metals were transformed using natural logarithm (ln) to approximate normality before proceeding with any analysis. Descriptive statistics of participant's characteristics are presented for e-waste workers and controls as mean ± SD for continuous variables that exhibited normal distribution (age, BMI, and LINE-1) and compared by student *t* test. Metals were presented as geometric mean (95% CI) and compared by *t* test. All categorical variables were presented as frequencies (percent frequency) and compared by chi-squared test.

We used linear regression models with robust standard errors (SEs) from ordinary least squares (OLS) to assess the associations between single and multiple metals exposures, with LINE-1 DNA methylation (the average of all four CpG site methylation of LINE-1) and at each CpG site. E-waste workers and non-e-waste workers were analyzed together in the model. The model is of the form

$$Y_i = \beta_o + \beta_1 Se_i + \beta_2 Zn_i + \beta_3 Mn_i + \beta_4 Cd_i + \beta_5 Pb_i + B^T Zi + e_i$$

where *Y* is the LINE-1 DNA methylation levels. Se, Zn, Mn, Cd, and Pb are the natural log-transformed concentrations of selenium, zinc, manganese, cadmium, and lead. *Z* represents additional potential confounders, and β represent the coefficients of the exposure variables. 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 were interpreted as percent increases in predictor associated with percent changes in outcomes. We further fitted OLS models stratified by study sites due to differences in exposure profiles between e-waste workers and controls. 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 et al., 2011). We explored the possible modification effect of the selected toxic metals (Cd and Pb) on DNA methylation caused by essential metals (Mn, Se, and Zn) concentrations by incorporating the corresponding interaction terms into the regression model. We further performed sensitivity analyses using different variants of the outcome model (robust and cross-fit partialling-out least absolute shrinkage and selection operator (LASSO) linear regression models) to compare with the OLS results with robust SEs. All statistical analyses were performed using Stata v16.1 (STATA Corp LLC. Texas, USA). The results were considered statistically significant if *p* values were < 0.05.

Results

Characteristics of study participants

The e-waste workers were significantly younger (mean age = 25.4 years) than the controls (mean = 32.5 years) (Table 1). Even though the BMI of both the e-waste and the controls were within normal weight according to the World Health Organization (WHO) parameters, BMI of controls (mean = 23.8) was significantly higher than that of e-waste workers (mean = 21.6) (Table 1). Significant differences were observed in educational level between the e-waste workers and controls: 25.0% of e-waste workers had no formal education at all (vs 13.7% of the controls), 32.0% had up to middle/junior high school (vs 25.5% of the controls), and only 16.0% had secondary school education or higher (vs 51.0% of the controls). No differences in smoking and alcohol intake were observed between the two groups. The indoor use of biomass fuel was significantly higher in the controls (31.4%) than e-waste workers (14.0%).

Table 1 Characteristics of e-waste workers ($n = 100$) and controls ($n = 51$) enrolled for the study, March 2017 to May 2017

Variables	e-waste workers	Controls	*p* value
Age (years) (mean ± SD)	25.4 ± 6.3	32.5 ± 10.4	**< 0.001**.[a]
BMI (kg/m.^{2}) (mean ± SD)	21.6 ± 2.7	23.8 ± 3.5	**< 0.001**.[a]
Education, ***n*** (%)	25 (25.0)	7 (13.7)	**< 0.001**.[b]
No formal	27 (27.0)	5 (9.8)	
Primary	32 (32.0)	13 (25.5)	
Middle/JHS	16 (16.0)	26 (51.0)	
Secondary/JHS			
Indoor biomass use, ***n*** (%)	14 (14.0)	16 (31.4)	**0.011**.[b]
Yes	86 (86.0)	35 (68.6)	
No			
Alcohol intake, ***n*** (%)	15 (15.0)	3 (5.9)	0.183.[b]
Occasional/regular	9 (9.0)	3 (5.9)	
Former	76 (76.0)	45 (88.2)	
Never			
Smoking status, ***n*** (%)	28 (28.0)	8 (15.7)	0.093.[b]
Yes	72 (72.0)	43 (84.3)	
No			
LINE-1 (mean ± SD)	85.1 ± 1.3	85.2 ± 1.1	0.785.[a]

SD, standard deviation; *BMI*, body mass index; *n* (%), frequency(percent frequency); *JHS*, junior high school; *SHS*, senior high school; *LINE-1*, long interspersed nuclear elements l; [a]*p* values obtained by *t* test; [b]*p* values obtained by chi-square test; bold *p* values are statistically significant

Overall, there were no significant differences ($p = 0.785$) in LINE-1 methylation (%) between e-waste workers and controls (Table 1).

Concentrations of metals in blood of e-waste workers and controls

Blood concentrations of essential metals Se and Mn were significantly higher in 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, $p_{all} < 0.05$) (Table 2). In addition, blood Cd was higher in controls than e-waste workers (Table 2). In contrast, blood Pb concentration was significantly higher among e-waste workers than the controls (geometric mean, Pb = 79.6 vs 37.7 µg/L, $p < 0.001$).

The levels of metals in blood were compared to background levels of the US population using the 95th percentile (P95) values of the US National Health and Nutrition Examination Survey (NHANES) (CDC 2019). The P95

Table 2 Metal concentrations in blood of e-waste workers and controls

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**

Se, selenium; *Zn*, zinc; *Mn*, manganese; *Cd*, cadmium; *Pb*, 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 (US Department of Health and Human Services – Centers for Disease Control and Prevention, 2019)

helps determine 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 US general population, according to the NHANES. However, except for Cd and Mn concentrations, the remaining three metals (Se, Zn, Pb) were higher in the control population than the P95 values according to the NHANES (Table 2).

To determine whether metals concentration differ based on the specific tasks performed by e-waste workers, we compared the levels of metals across job categories or tasks of e-waste workers. The results showed that Se and Zn's levels were the in e-waste collectors, followed by the dismantlers and lowest in burners (Table S2).

Effect of single and multiple metals on LINE-1 methylation using linear regression analysis

First, we evaluated each metal exposure effect on global DNA methylation using single-metal linear regression models controlling for age, BMI, smoking, alcohol intake, indoor use of biomass fuel for cooking, and exposure status (location). The results showed a significant inverse association between Zn and LINE-1 DNA methylation (i.e., the average of all 4 CpG sites of LINE-1) ($\beta_{Zn} = -0.912$; 95% CI, −1.512, −0.306; $p = 0.003$) which corresponds to ($\beta_{Zn} = -0.009$; 95% CI, −0.015, −0.003; $p = 0.003$) (Table 3). This estimate suggests that a 1% increase in Zn was associated with a 0.009 decrease in %LINE-1 methylation. Similarly, significant inverse associations were observed between CpG2 methylation and Pb concentration ($p = 0.048$) and 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 3).

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

To further evaluate the associations between metals and LINE-1 methylation based on e-waste exposure status, two separate linear regression models were fitted to examine and compare the associations between metals and DNA methylation in e-waste workers and the control group. Both single-metal and multiple-metal regression models showed a significant inverse relationship between Zn concentration and LINE-1 DNA methylation only in the e-waste workers (Table S3).

Potential interaction effects of toxic and essential metals on global methylation

We evaluated the effects of interaction between toxic and essential metals on LINE-1 methylation. The results showed modifications of DNA methylation of LINE-1 due to potential interaction between toxic and essential metals (Fig. 1). We observed that at a lower concentration of Zn, Cd significantly decreased LINE-1 methylation ($p = 0.001$); however, when Zn concentration increased, LINE-1 methylation was increased (Fig. 1).

Discussion

In the present study, blood concentration of Cd, Mn, and Se was higher in the control group than in the e-waste worker group, whereas Pb concentration was higher in the e-waste workers. However, we did not observe any significant differences in LINE-1 methylation between e-waste workers and the control group; hence, we combined them in the linear regression analysis and controlled for the study site. The linear regression results from OLS showed that increased Zn concentrations significantly reduced LINE-1 methylation by either single or multiple metals analyses. In addition, an

Table 3 Single metal linear regression models of global repetitive (LINE-1) methylation. Mean methylation is modelled for average CpG sites (all) and specific CpG sites ($n = 151$)

		Linear regression with robust standard errors from OLS	Sensitivity analyses Robust regression	Cross-fit partialling-out LASSO linear regression
Outcomes (LINE-1 methylation)	Metals (μg/L)	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 metal biomarkers are natural log-transformed

interaction effect between Cd and Zn on LINE-1 methylation was observed.

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); therefore, our results may provide a potential explanation for occupational metal mixture exposure and DNA methylation mediated diseases. Our results contribute to the recently 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 concern. 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).

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. 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) and 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 reference group in this study since the

Table 4 Multiple 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
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 metal biomarkers are natural log-transformed

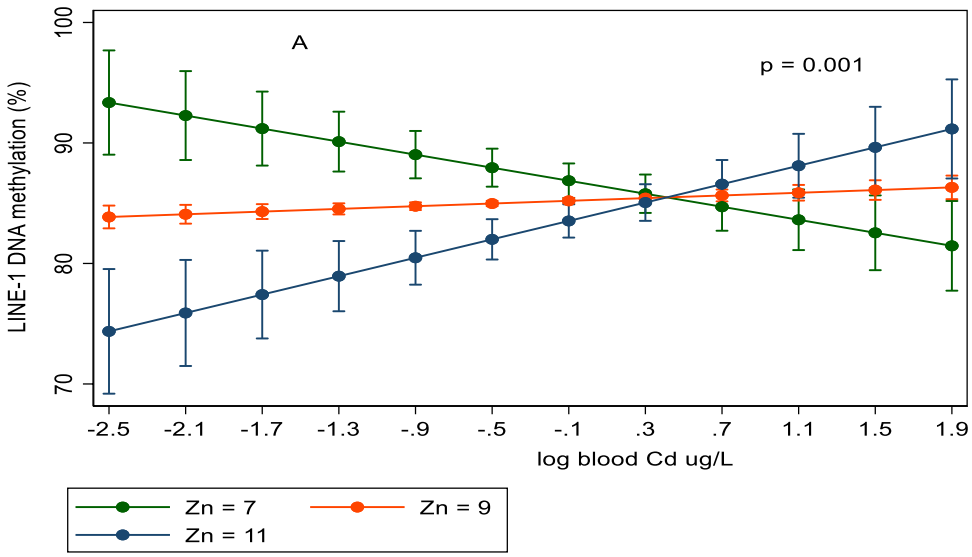

Fig. 1 Interaction effects of Cd and Zn on global DNA methylation

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 pollutant 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 and Stellman, 1992).

Our results across the single-metal linear regression, mutual regression, and sensitivity analyses consistently indicate that Zn is inversely associated with LINE-1 methylation, at least in the populations studied. This inverse association between Zn and LINE-1 methylation is consistent with Zn's role as a micronutrient essential for the epigenome (Maret and Sandstead, 2008; Wallwork and Duerre 1985), which directly contributes to 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 our study is extremely high and far exceeds that detected in the NHANES study in American populations. The high concentration of Zn in our participants' blood could be evidence of non-homeostatic Zn retention in the body due to excess exposure from several sources, including diet (King et al. 2000). Available scientific evidence suggests that both Zn deficiency and excess cause cellular oxidative stress (Lee 2018). 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 this study. 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 metal-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 and 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 evidence exists to suggest that Cd, Pb, and Mn may be associated with DNA methylation (Hou et al. 2012), we did not observe any significant association of these metals with LINE-1 DNA methylation (the average of all CpGs of LINE-1) in our linear regression analyses. Due to our 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. 2019). However, based on the recommendation of Goodrich et al. (2015) to evaluate site-specific effects of exposures, we further modelled each of the four specific CpG sites of LINE-1 using both single- and multiple-metal models. We observed that 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. Our 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 site methylation measured using the Illumina Infinium Methylation EPIC BeadChip (850 K) (Zhang et al. 2019). 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. 2019).

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. Our results showed that Cd significantly reduced LINE-1 methylation at low Zn levels; however, LINE-1 methylation significantly increased at a high Zn concentration. This observation is consistent with findings from both human studies and animal models where Zn has been reported to mitigate epigenetic effects (Reeves and 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 and 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 and Piscator 1978; Maret and Sandstead 2008).

This current study is not without limitations. For instance, the current study has only examined metals; however, the e-waste recycling process, especially, open-pit burning, generates other chemicals such as polycyclic aromatic hydrocarbons (PAHs) and other persistent organic pollutants (POPs) that could also alter DNA methylation. In addition, we used a cross-sectional design, which makes it impossible to infer a

causal relationship between the observed DNA methylation and metal exposure. Our small sample size did not allow us to evaluate the metals' overall mixture effect on DNA methylation using other novel statistical methods.

Despite the limitations mentioned above, a key strength of our study is that we could assess the interaction effects of toxic metals by essential elements on global DNA methylation, which has not been previously reported in occupationally exposed populations. In addition, our study is the first that examined the association of metals and epigenetic alterations (global DNA methylation) in individuals involved in informal e-waste recycling.

Conclusion

In conclusion, we observed that environmental and occupational metal exposure showed an inverse relationship with global DNA methylation when e-waste workers and controls were analyzed together. Within the mixture, Zn was the most important metal that drove the association. Our findings suggest that DNA methylation could be altered due to interactions between toxic and essential elements. Future studies should consider mixture analysis using longitudinal study designs with a large sample size and further include the wide range of pollutants associated with e-waste recycling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-20954-z.

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Author contribution Conceptualization, Ibrahim Issah, Thomas G. Robins, Julius N Fobil, Laura S Rozek, and John Arko-Mensah. Methodology, Ibrahim Issah., Laura S Rozek, Katie R Zarins, Julius N. Fobil, and John Arko-Mensah. Formal analysis and investigation, Ibrahim Issah, Duah Dwomoh, Thomas G. Robins, Julius N Fobil, John Arko-Mensah, Niladri Basu, Thomas P. Agyekum, Laura S Rozek, and Katie R Zarins. Writing — original draft preparation, Ibrahim Issah. Writing — review and editing, Ibrahim Issah, Laura S Rozek, Katie R Zarins, Julius N Fobil, John Arko-Mensah, and Thomas P. Agyekum. Funding acquisition, Thomas G. Robins, Stuart Batterman, Niladri Basu, and Julius N Fobil. Resources, Thomas G. Robins and Julius N. Fobil. Supervision, Julius N Fobil, Thomas G Robins, John Arko-Mensah, and Laura S Rozek. All authors read and approved the final manuscript.

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Data Availability The datasets generated and/or analyzed during the current study are not publicly available due to privacy reasons but are available from the corresponding author on reasonable request.

Declarations

Ethics approval 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).

Consent to participate 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 was asked to provide written consent if willing to be enrolled.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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