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Levels of heavy and essential trace metals and their correlation with antioxidant and health status in individuals occupationally exposed to municipal solid wastes

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Abstract

Recent studies have indicated an increased incidence of toxic neuropathies among waste management workers (WMW) possibly linked to increased detection of heavy metals in municipal solid wastes. The present study evaluated serum levels of some heavy and essential trace metals in relation to oxidant/antioxidant status of WMW. One hundred and twenty-six WMW and 84 non-WMW (control) were recruited. Metal/element concentration was measured by atomic absorption spectrophotometry and oxidant/antioxidant markers were determined using standard procedures. The WMW exhibited significantly ($p < 0.001$) decreased ferric reducing ability of plasma (FRAP) and higher levels of ceruloplasmin (Cp) and malondialdehyde. Iron (Fe) and copper (Cu) levels were significantly lower ($p < 0.05$) and higher ($p < 0.001$), respectively in WMW when compared with control while levels of other trace elements were not significantly different between these groups. Lead (Pb) and chromium levels were significantly higher ($p < 0.001$ and $p < 0.05$, respectively) in WMW while mercury levels were comparable with those of control subjects. In WMW, Cp ($r = -0.182$; $p > 0.05$) and FRAP ($r = 0.277$; $p < 0.05$) negatively and positively correlated with Pb, respectively, while a positive correlation was observed between zinc ($r = 0.230$; $p < 0.05$) and Pb and between Cu ($r = 0.541$; $p > 0.001$) and Fe. Overall, the decreased antioxidant capacity and increased oxidative stress observed in WMW in this study may be related to their blood levels of heavy and essential trace metals. Conscious efforts are required, therefore, to reduce risk and protect WMW from toxic neuropathies and other adverse health consequences of occupational exposure.

Keywords

Municipal solid waste, occupational exposure, trace and heavy metals, oxidative stress

Introduction

Solid waste generation in Nigeria has increased substantially in recent times with a rise in the annual figure from 4.5 million tons in 1999 to 25 million tons in 2009 (Odewabi, 2014; Ogwueleka, 2009; Oyeniyi, 2011). The risk and public health impact of environmental pollution attributable to waste dumps is of considerable interest and has become an issue of global concern (Abah and Ohimain, 2010). Although, significant progress has been made with regards to putting measures in place to minimize work-related risks in the last two decades, the challenges associated with waste generation and accumulation continue to constitute a major public health concern, especially in developing countries (Ekor and Odewabi, 2014; Odewabi et al., 2013a). There is good evidence that work-related adverse health effects are common in waste management workers (WMW), and the risk of

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developing toxic neuropathies is becoming increasingly apparent. This may be associated with the presence of large amount of heavy metals and other hazardous industrial materials in municipal solid waste (MSW) in most parts of the world (Ekor and Odewabi, 2014).

Analysis of some MSW reveals high concentration of heavy metals, polyaromatic hydrocarbons, dioxins, volatile organic compounds, and sharp objects that have serious consequences on waste handlers, public health, and the environment in general (Domingo and Nadal, 2009). Metals appear in MSW from a variety of sources including used and disposed batteries, consumer electronics, ceramics, light bulbs, house dust and paint chips, lead (Pb) foils such as wine bottle closures, used motor oil, and plastics. Also, some inks and glass can all introduce metal contaminants into the solid waste stream. Smith (2009) reported that all types of MSW compost contain more heavy metals than the background concentrations present in soil as there are many heavy metal-containing materials in household municipal waste. In recent times, electronic-waste (e-waste) is one of the fastest growing waste streams (Widmeier et al., 2007). E-waste constitutes 8% of municipal waste generated by the European Union and the disposal amount was expected to double in 2015 (Widmeier et al., 2007). The presence of heavy metals and exposure to flame retardants are some of the hazards associated with e-waste recycling (Tsydenova and Bengtsson, 2011). In Nigeria, several thousands of slightly used computers are imported into the country through the Lagos seaport every month. Many of these computers lose their value within a short time and contribute to the electrical/electronic equipment (or e-waste) pile in Nigeria (Ukem, 2008).

Metals are known to play important roles in a wide variety of biological processes, and deficiency of any of these elements affects normal functions in the human body (Evan and Vousden, 2001; Valko et al., 2006). It has also been reported that overload of micromineral or trace elements produces immunotoxicity (Arinola and Akiibinu, 2006; Dardenne, 2002). Homeostasis of metal ions, which is tightly regulated via mechanisms of uptake, storage, and secretion, is critical for life and is maintained within strict limits (Bertini and Cavallaro, 2008; Jomova and Valko, 2011). Breakdown of metal ion homeostasis can lead to metal binding to uncommon protein sites or displacement of other metals from their natural binding sites (Nelson, 1999). Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protective capacity and subsequently induces DNA damage, lipid peroxidation, protein modification, and other effects, which are associated with numerous diseases like cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders, and chronic inflammatory conditions (Jomova and Valko, 2011).

Hazardous effects of occupational exposure to waste-associated chemicals are still poorly characterized based on limited information in most parts of the world (Tsydenova and Bengtsson, 2011). Recently, we observed elevated levels of markers of systemic inflammation and oxidative stress in WMW of Ogun State in southwest Nigeria (Odewabi et al., 2013a). So far, the biochemical mechanisms responsible for the observed oxidative stress are yet to be fully elucidated. In the present study, we determined and correlated the serum concentration of some essential trace elements and heavy metals with levels of some markers of oxidant/antioxidant status in WMW.

Materials and methods

Subjects and work condition

Study participants (age, 34.45 $\pm$ 8.81 years) included healthy male and female (34/50) non-waste workers ($n = 84$) and male and female (51/75) WMW ($n = 126$) recruited from four private waste management companies in Ijebu-Ode and Sagamu of Ogun State, Southwest Nigeria. Subjects were selected by purposive sampling in line with our previous studies (Odewabi et al., 2013a). The duration of employment of the WMW who enrolled for study ranged between 0.5 and 6.75 years and worked 8 h daily for 6 days every week. Individuals with visible wound/lesion, infections, inflammatory diseases, or those who were taking drugs that could interfere with inflammatory mechanisms were excluded. The medical ethics committee of the Olabisi Onabanjo University Teaching Hospital (OOUTH) and the Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, approved the study (ethical approval number: OOUTH/DA.226/T/2). The participants gave informed written consent in accordance with the amended Helsinki Declaration of 1983 (World Medical Organization, 1996).

Assessment of health status of subjects

Self-reported work-related health complaints were used to evaluate health status of participants and data were obtained as described in our previous study (Odewabi et al., 2013b). Signs and symptoms presented by individuals at least once in the last week and twice in the previous 3 months were recorded.

Collection and processing of blood samples for analysis

Five milliliters of blood was collected from the antecubital vein of waste workers and controls. Three milliliters of blood was separated into sterile universal plastic bottles and allowed to clot and retract at room temperature, and serum was obtained by centrifugation. Hemolyzed samples were excluded from the study. Serum was separated into two sets of sterile Eppendoff plastic bottles and immediately frozen at −20°C until analysis. One set was used for metal analysis while the other set was used for lipid peroxidation and ceruloplasmin (Cp) analysis. The remaining 2 ml of blood was immediately transferred into sterile lithium heparin bottles, and plasma was obtained by centrifugation, which was separated into sterile Eppendoff plastic bottles and immediately frozen at −20°C until analysis of ferric-reducing ability of plasma. Analysis of samples for both control and WMW was done simultaneously to eliminate the confounding effect of environmental variation on health response.

Determination of lipid peroxidation

Lipid peroxidation was estimated by the thiobarbituric acid reactive substance method as described by Varshney and Kale (1990) and malondialdehyde (MDA) was quantified using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Buege and Aust, 1978). Briefly, 0.4 ml of plasma was mixed with 1.6 ml of Tris-potassium chloride buffer to which 0.5 ml of 30% trichloroacetic acid was added. Then 0.5 ml of 0.75% thiobarbituric acid was added and placed in a water bath for 45 min at 80°C. The mixture was then cooled in ice and centrifuged at 3000 × *g*. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm.

Measurement of Cp

Cp was estimated from its oxidase activity using *o*-dianisidine dihydrochloride as previously described by Schoslnsky et al. (1974). This method involves enzymatic oxidation of *o*-dianisidine dihydrochloride (4,4′-diamino-3,3′-dimethoxy-biphenyl) by ceruloplamin oxidase in the presence of oxygen at 30°C resulting in the formation of a yellowish-brown product. Briefly, equal volumes of sodium acetate buffer and serum sample in two test tubes were incubated at 30°C in water bath for 5 and 15 min, respectively, with *o*-dianisidine dihydrochloride reagent followed by sulfuric acid (9.0 M) to form the purplish-red solution. The absorbance of the purple-red solution was measured at 540 nm in a cuvet having a 1-cm light path against distilled water as blank. Cp oxidase activity was quantified using the product of difference in absorbance of the purplish-red solution and $\sum = 9.6$ ml μmol^{-1} cm^{-1} absorptivity of colored solution in terms of substrate consumed (milliliter per micromole per centimeter) (Lehmann et al., 1974).

Measurement of ferric-reducing ability of plasma

Ferric-reducing ability of plasma (FRAP) was determined according to the method described by Benzie and Strain (1993). Assay was based on the ability of plasma to reduce ferric tripyridyltriazine (Fe III–TPTZ) complex at low pH to an intense blue-colored ferrous (Fe II) form. The latter has an absorption maximum at 593 nm and the intensity of the blue color is proportional to the antioxidant capacity of the sample. Briefly, 300 µl of plasma was added to 3 ml of FRAP reagent. The absorbance was read at 593 nm after incubating for 4 min at ambient temperature against a distilled water blank. The FRAP values expressed in micromoles per liter were read from a prepared standard curve relating millimole Fe^{2+} l^{-1} to absorbance.

Estimation of metal concentrations

All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). Nitric acid (HNO_3) approximately 16 M and 30% hydrogen peroxide (H_2O_2) were used for wet-acid digestions. Ultrapure water was prepared by passing deionized water through a Milli-Q system (Bedford, Massachusetts, USA) and was used throughout the study. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg l^{-1} of the given element supplied by Sigma-Aldrich (St. Louis, Missouri, USA). The analysis was carried out according to the direct method

described by Kaneko (1999) using a Buck 205 atomic absorption spectrophotometer (AAS; Buck Scientific, England). The method is based on the principle that atoms of the element when aspirated into the AAS vaporized and absorbed light of the same wavelength as that emitted by the element when in the excited state.

A deuterium background corrector was used for background corrections. The operating parameters for the elements were set as recommended by the manufacturer (Table A1). For flame measurements, a 10-cm long slot burner head, a lamp, and an air–acetylene flame were used.

For microwave digestion, the protocol of Yahaya et al. (2013) was employed. Serum (0.5 ml) was pipetted into a pyrex flask and 3 ml freshly prepared mixture of concentrated HNO_3 and 30% H_2O_2 in a 2:1 v/v ratio was added to each sample and allowed to stand for 10 min. The mixture was then microwave digested following a one-stage process programmed at 80% of the total power in a SharpLight – up (Dial) domestic microwave oven (Canada) with maximum heating power of 800 W for 3 min. After digestion, the solution was diluted with 10 ml deionized water. A blank digest (without the sample) was carried out in the same way.

In order to establish the efficiency, accuracy, the validity of results, as well as reliability of the analytical procedures used in this study, a recovery experiment was conducted. Briefly, 1.5 ml of blood serum samples were spiked with 10 ppm of magnesium (Mg), manganese (Mn), selenium (Se), zinc (Zn), Cu, chromium (Cr), Pb, mercury (Hg), and Fe in separate Teflon beakers and digested. The digested spiked samples were diluted with 10 ml deionized water. The worked-up samples were stored in polyethylene containers at 4°C prior to flame atomic absorption spectrophotometric (FAAS) analysis. Also 10 ml each of 10 ppm standards were taken for FAAS analysis so that the results obtained could be compared with those of the spiked blood serum samples to obtain the recovery. The recovery was done in triplicate and the percentage recovery was calculated using the equation (AOAC, 1995):

$$R = \left[\frac{(\text{Amount after spike} - \text{amount before spike})}{\text{Amount added}}\right] \times 100\%.$$

The percentage recoveries of metals in the spiked blood serum samples were 82–99% as shown in Table B1. Generally, good recoveries were obtained for all metals. Each determination was carried out at least three times in order to ensure precision and reproducibility. The relative standard deviations were less than 10% for all measurements. In this study, the detection limit of each element was calculated as three times the standard deviation of the blank (3σ blank, $n = 5$) (Christian, 2004; Miller and Miller, 2000).

Quality control of trace elements determination

Because methods for trace elements analysis are affected by both matrix and contamination problems, effective measures of quality assurance were incorporated into the trace element analysis including reagent blanks, replicate analyses to assess precision, use of calibrators of the trace elements in the expected concentration range of the specimen analyzed, and a control prepared in-house with concentrations of the trace elements determined to assess accuracy and batch-to-batch precision. The control material used was of the same matrix type and contained approximately the same amounts of analyte as the specimens. If the coefficient of variation for control samples run in triplicate for each batch of analysis exceeded 10%, the results were considered unreliable and the samples were re-assayed.

Correction of the matrix difference between samples and standards

Matrix interference was corrected and controlled by the use of the *method of addition.* Three aliquots (0.5 ml) of each sample were taken into three Teflon beakers. Two milliliters each of diluents, standard containing 5 μg ml^{-1} of the metal, and standard containing 10 μg ml^{-1} was added to the first, second, and third aliquots, respectively. The percent absorption on each of the three aliquots was determined. The values were converted to absorbance and plotted against concentration on linear graph paper (White et al., 1976):

$$A = -\log\left(1 - \frac{R}{100}\right),$$

where A is absorbance and R is % absorption.

Statistical analysis

Results are presented as mean ± standard deviation. Data were analyzed using Statistical Package for the Social Sciences version 16.0. Comparison between waste workers and control was performed using Student's t test for unpaired data and Pearson's

correlation coefficient. The statistical significance was set at $p < 0.05$.

Results

Physical examination and medical history, risk behavior, and exposure

The WMW were apparently healthy on physical examination and assessment of past medical history. The medical history and clinical profile of the WMW along with those of the control group are presented in Table 1. Seven and 12 percent of the WMW and control subjects are smokers respectively (Table 1). The most frequently occurring self-reported symptoms experienced by subjects over the previous year are those related to the digestive, respiratory, nervous, and musculoskeletal systems (see correlation analysis of analyzed metals with work-related health complaints in Table 7). Close observation of the WMW reveals that most of them did not always use personal protective equipment at workplace. Some of the workers exhibited poor personal hygiene habits, such as eating or smoking cigarettes at the workplace without first washing their hands, living within the dumpsite/workplace, or not taking their bath or changing into clean clothing after the day's work. The frequencies of these risk behaviors and exposure to work-related injuries/risks are presented in Tables 2 and 3. Plasma levels of MDA, ceruloplasmin oxidase (Cp) activity, and FRAP of WMW and control subjects are shown in Table 4. The WMW exhibited significant ($p < 0.001$) increases in Cp activity and MDA level together with marked decrease in FRAP ($p < 0.001$) when compared with control.

Trace elements and heavy metals

Tables 5 and 6 summarize the levels of trace elements and heavy metals in plasma of WMW and control subjects. Cu and Pb levels were significantly ($p < 0.001$) higher and Fe concentration significantly ($p < 0.05$) lower in the WMW group when compared with control. Levels of other trace elements and heavy metals analyzed (Mn, Mg, Cr, Hg, Se, and Zn) were not significantly ($p > 0.05$) different from that of the control.

Correlation analysis between FRAP and trace/heavy metals

Table 7 shows the degree of association between Cp, Pb, Cu, Zn, Fe, and FRAP. The FRAP ($r = 0.277$;

Table 1. Socio- and bio-physical data of WMW and control.

Parameters	WMW ($n = 126$)	Control ($n = 84$)	t Values	p Values
Age (years)[a]	34.45 ± 8.81	34.42 ± 9.91	0.210	0.984
Exposure (years)	6.5 ± 1.5	–	–	–
BMI[a] (kg m^{-2})	22.72 ± 1.96	22.67 ± 3.68	0.304	0.738
WHR[a]	0.85 ± 0.34	0.84 ± 0.05	0.404	0.669
Smokers[b] (males)	9.52 (12)	8.33 (7)	0.029[c]	0.960
Alcohol use[b] (males)	12.69 (16)	13.09 (11)	0.176[c]	0.864
Sex (M:F)	51/75 (0.68:1)	34/50 (0.68:1)	–	–

BMI: body mass index; M: male; F: female; WHR: waist hip ratio; WMW: waste management workers; *n*: number of subjects.
[a]Data expressed as mean ± standard deviation.
[b]Data expressed as percentage (%) of individuals.
[c]χ^2 values.

Table 2. Risk behavior frequency of the waste workers.

Behavior	Risk frequency (%)
Not wearing goggles	97.3
Not wearing mask	77.1
Not wearing gloves	75.3
Not wearing protective clothing	72.5
Not wearing protective shoes	82.3
Smoking without washing hands first	7.0
Eating at work	71.8
Hands not washed with water alone before eating	52.2
Hands not washed with water and soap before eating	35.0

Table 3. Frequency of exposure of subjects to work-related injuries and risks.

Injury/risk	Exposure frequency (%)
Contusion	35.7
Laceration	34.7
Needle injuries	37.3
Abrasion	41.6
Sharp object sighting	75.3
Fecal sighting	81.2
Blood sighting	68.2
Metal object sighting	71.8
Animal products sighting	65.3
Battery products sighting	65.3

Table 4. Levels of oxidant/antioxidant markers of waste workers and control.[a]

Parameters	WMW ($n = 126$)	Control ($n = 84$)	t Values	p Values
Cp ($U\ l^{-1}$)	138.54 ± 45.86^{b} (17.94%)[c]	117.07 ± 38.03	−5.732	0.000
MDA ($nmol\ ml^{-1}$)	3.70 ± 1.33^{b} (28.91%)[c]	2.87 ± 1.02	−2.975	0.004
FRAP ($\mu mol\ Fe^{2+}\ l^{-1}$)	877.83 ± 153.69^{b} (−19.32%)[c]	1088.10 ± 158.66	−7.493	0.000

WMW: waste management workers, Cp: ceruloplasmin oxidase activity, MDA: malondialdehyde, FRAP: ferric reducing ability of plasma; *n*: number of subjects.
[a]Data expressed as mean ± standard deviation.
[b]Significantly different from the control.
[c]Percentage change relative to control.

Table 5. Essential trace metals levels in WMW and control.[a]

Parameters	WMW ($n = 126$)	Control ($n = 84$)
Fe ($\mu g\ dl^{-1}$)	77.46 ± 26.87^{b} (−9.55%)[c]	84.86 ± 26.04
Mg ($mg\ dl^{-1}$)	15.60 ± 8.20 (−1.51%)[c]	16.29 ± 7.82
Cu ($\mu g\ dl^{-1}$)	45.96 ± 14.74^{d} (23.58%)[c]	37.19 ± 8.07
Mn ($ng\ dl^{-1}$)	40.36 ± 15.03 (0.001%)[c]	42.21 ± 15.30
Zn ($\mu g\ dl^{-1}$)	63.41 ± 11.62 (−6.57%)	67.87 ± 13.93
Se ($\mu g\ dl^{-1}$)	25.30 ± 8.49 (−2.42%)[c]	26.15 ± 8.76

Cu: copper; Fe: iron; Mg: magnesium; Mn: manganese; Se: selenium; Zn: zinc; WMW: waste management workers; *n*: number of subjects.
[a]Data expressed as mean ± standard deviation.
[b]$p < 0.05$ when compared with control.
[c]Percentage change relative to control.
[d]$p < 0.001$ when compared with control.

$p < 0.05$) and Zn ($r = 0.230$; $p < 0.05$) exhibited significant positive correlation respectively with Pb, while Cp activity ($r = -0.182$; $p > 0.05$) exhibited significant negative correlation with Pb. Furthermore, Cu ($r = 0.541$; $p > 0.001$) significantly correlated with Fe, whereas this correlation did not exist in control group.

Correlation analysis between analyzed metals and work-related health complaints

The correlation coefficient between some of the analyzed metals with work-related health complaints is presented in Table 8. A significant positive correlation was observed between diarrhea ($r = +0.205$; $p < 0.01$), tiredness ($r = +0.227$; $p < 0.01$), shoulder pain ($r = +0.178$; $p < 0.05$), and Pb level in the waste workers. Negative correlation was observed between phlegm production ($r = -0.238$; $p < 0.01$), chest pain ($r = -0.186$; $p < 0.05$), and Cu level as well as between tiredness ($r = -0.179$; $p < 0.05$), chest pain ($r = -0216$; $p > 0.01$), and Fe level. A significant correlation also existed between tiredness ($r = +0.263$, $p < 0.01$), chest pain ($r = +0.202$, $p < 0.01$), shoulder pain ($r = +0.204$, $p < 0.01$), and Zn level. Similarly, there was a significant correlation between diarrhea ($r = +0.245$; $p < 0.01$), phlegm production ($r = +0.208$; $p < 0.01$), chest pain ($r = +315$; $p > 0.001$), shoulder pain ($r = +0.218$, $p < 0.01$) and tiredness as well as between chest pain ($r = +0.320$, $p < 0.001$) and shoulder pain.

Discussion

We reported the presence of systemic inflammation, oxidative stress, and elevated levels of immunoglobulin G and immunoglobulin A and adenosine deaminase activity in WMW in our earlier studies (Odewabi et al., 2013a, 2013b). Several studies have suggested a possible link between oxidative stress and disruption of metal ion homeostasis (Halliwell and Gutteridge, 1990; Jomova and Valko, 2011; Mates, 2000; Nelson, 1999; Valko et al., 2005). In the present study, therefore, we investigated the relationship between exposure to MSW and the levels of some essential trace elements, toxic metals, and oxidant/antioxidant markers among WMW of Ogun State, southwest, Nigeria.

The major frequently occurring symptoms of health problems in the past year as reported by the WMW investigated in this study are those relating to the digestive, respiratory, nervous, and musculoskeletal systems. These symptoms are similar to those reported in the study carried out by Ray et al. (2005) and Rouse (2006). Our result further demonstrated significantly higher plasma level of Cp in the WMW when compared with control, thus, corroborating our previous findings of WMW's predisposition to systemic inflammation (Odewabi et al., 2013a). Cp is an acute phase protein with ferroxidase activity and

Table 6. Levels of some heavy metals in WMW and control.

Parameters	WMW ($n = 126$)	Control ($n = 84$)	t Values	p Values
Cr ($\mu g\ l^{-1}$)	56.87 ± 12.36[a] (7.30%)[b]	53.00 ± 12.32	2.280	0.023
Hg ($\mu g\ dl^{-1}$)	8.21 ± 2.72 (−0.48%)[b]	8.25 ± 2.93	−0.119	0.905
Pb ($\mu g\ dl^{-1}$)	50.13 ± 13.40[c] (46.28%)[b]	34.27 ± 14.27	11.802	0.000

WMW: waste management workers; n: number of subjects; Cr: chromium; Pb: lead; Hg: mercury.
[a]$p < 0.05$ when compared with control.
[b]Percentage change relative to control.
[c]$p < 0.001$ when compared with control.

Table 7. Coefficient of correlation between parameters in WMW ($n = 126$).

Parameters	Correlation coefficient (r)						
	Cp	Pb	Cu	Zn	Fe	FRAP	MDA
Cp	1.00	−0.182[a]	0.147	−0.076	−0.039	0.113	0.196[a]
Pb	−0.182[a]	1.00	0.067	0.230[b]	0.132	−0.277[a]	0.065
Cu	0.147	0.067	1.00	−0.023	0.541[c]	−0.148	−0.031
Zn	−0.076	0.230[b]	−0.023	1.00	−0.044	−0.140	−0.058
Fe	−0.039	0.132	0.541[c]	−0.044	1.00	0.017	0.024
FRAP	0.113	−0.277[a]	−0.148	−0.140	0.017	1.00	0.011
MDA	0.196[a]	0.065	−0.031	−0.058	0.024	0.011	1.00

Cu: copper; Fe: iron; Pb: lead; Zn: zinc; FRAP: ferric reducing ability of plasma; MDA: malondialdehyde; WMW: waste management workers; Cp: ceruloplasmin activity.
[a]Coefficient of correlation (r): $p < 0.05$.
[b]Coefficient of correlation (r): $p < 0.01$.
[c]Coefficient of correlation (r): $p < 0.001$.

Table 8. Correlation analysis of some of the analyzed metals with work-related health complaints ($n = 126$).

	Pb	Cu	Zn	Fe	Diarrhea	Tiredness	Nose irritation	Phlegm production	Chest pain	Shoulder pain
Pb	1	0.067	0.230[a]	0.132	0.205[b]	0.227	−0.123[b]	0.145	−0.114	0.178[b]
Cu	0.067	1	−0.023	0.541[c]	−0.149	−0.152	−0.076	−0.238[a]	−0.186[b]	−0.168
Zn	0.230[a]	−0.023	1	−0.044	0.131	0.263[a]	−0.014	0.110	0.202[a]	0.205[a]
Fe	0.132	0.541[c]	−0.044	1	−0.148	−0.179[b]	0.088	−0.193[b]	−0.216[a]	−0.063
Diarrhea	0.205[a]	−0.149	0.131	−0.148	1	0.245[a]	0.066	0.242[a]	0.228[a]	0.235[a]
Tiredness	0.227[a]	−0.152	0.263[a]	−0.179[b]	0.245[a]	1	0.097	0.207[a]	0.315[c]	0.228[a]
Nose irritation	0.123	−0.076	−0.014	−0.088	0.066	0097	1	0.120	0.146	0.133
Phlegm production	0.145	−0.238[a]	0.110	−0.193	0.242[a]	0.208[a]	0.120	1	0.010	0.156
Chest pain	−0.114	−0.186[b]	0.202[a]	−0.216[a]	0.228[a]	0.315[c]	0.146	0.010	1	0.320[a]
Shoulder pain	0.178[b]	−0.168	0.204[a]	−0.063	0.235[a]	0.228[a]	0.137	0.156	0.320[a]	1

Fe: iron; Cu: copper; Hg: mercury; Pb: lead; Se: selenium; Zn: zinc.
[a]$p < 0.01$.
[b]$p < 0.05$.
[c]$p < 0.001$.

an important antioxidant not only in plasma but also in the bronchoalveolar lining (Leelakunakorn et al., 2005; Mongiat et al., 1992). This protein is, therefore, considered to be a significant pulmonary antioxidant and its presence in plasma may reflect the condition of the lungs (Mongiat et al., 1992). Cp, as ferroxidase, converts the ferrous iron to oxidized ferric iron and facilitates the movement of Fe from the cells to the

blood (Freiden and Hsich, 1976). The redox state of the cell is predominantly dependent on a Fe and Cu redox couple and it is maintained within strict physiological limits (Park et al., 2009). The WMW in this study exhibited significantly decreased plasma Fe concentration when compared with the control subjects. Our result is in consonance with the findings of Ray et al. (2005) who reported hypochromic anemia alongside anisocytosis and poikilocytosis in landfill workers in India.

During infection or inflammatory stress, there is a rise in the production of pro-inflammatory cytokines like interleukin (IL)-1b, IL-6, IL-8, and tumor necrosis factor (Michel, 1997; Ulmer, 1997) with a corresponding increase in serum Cu concentration because of the acute phase action of IL-1. The action of IL-1 may cause an elevation in serum Cu and a decrease in Zn concentrations (Milne, 2003). The significant elevation in Cu concentration observed in the WMW in this study may be due to an increase in the level of IL-1 arising from an underlying inflammatory process in these subjects. Cu is capable of inducing oxidative stress via two mechanisms. It can either directly catalyze the formation of ROS through a Fenton-like reaction (Liochev and Fridovich, 2002; Prousek, 2007) or significantly decrease glutathione levels (Speisky et al., 2009). Decrease in glutathione concentration was associated with occupational exposure to MSW as reported previously in our study (Odewabi et al., 2013a). Glutathione depletion is capable of enhancing the cytotoxic effect of ROS and allows Cu to be more catalytically active to produce higher levels of ROS. The increase in Cu toxicity resulting from GSH depletion demonstrates clearly that GSH is an important cellular antioxidant that acts against Cu toxicity (Steinebach and Wolterbeek, 1994).

We also observed in this study that the WMW's serum Zn concentration was slightly lower than that of the control subjects. Studies have associated Zn deficiency with increased oxidative modification of lipid, protein, and DNA oxidation (Prasad, 2009; Valko et al., 2005). Chronic or long-term absence of Zn in experimental models has also been shown to predispose to oxidative stress-mediated injuries. Furthermore, previous studies have reported greater prevalence of work-related respiratory symptoms in waste workers (Krajewski et al., 2001; Odewabi et al., 2013a; Ray et al., 2005). Zinc, as an antioxidant, has been shown to reduce formation of free radicals via several mechanisms: it acts as an inhibitor of nicotinamide adenine dinucleotide phosphate oxidase, inducer of metallothionein (effective scavenger of radicals) and it is an integral metal of Cu, Zn-superoxide dismutase (Bray and Bettger, 1990; Prasad, 2009). ROS are known to activate nuclear factor κB (NF-κB) which in turn activates growth factors, antiapoptotic molecules, inflammatory cytokines, and adhesion molecules (Prasad, 2009). Zinc reduces inflammatory cytokine production by upregulation of a Zn-finger protein, A20, which inhibits NF-κB activation via TRAF pathway (Prasad, 2008). Thus Zn functions not only as an antioxidant but also as an anti-inflammatory agent. The observed decrease in the serum concentration of this essential metal in the WMW in our study may be related to its continuous utilization due to its involvement in protection against inflammation and oxidative stress associated with occupational exposure to MSW.

Our study also demonstrated that the WMW blood Pb concentration was significantly greater than that of the control subjects. This suggests that there are high levels of this heavy metal in the WMW's work environment and a possible risk of Pb toxicity with continuous exposure. High blood Pb levels (mean of 28 $\mu g\ dl^{-1}$) were reported for child waste pickers in Metro Manila. More than 70% of children working at Metro Manila's largest dumpsite had blood Pb levels that exceeded WHO guideline of 20 $\mu g\ dl^{-1}$ (Carotti and Smith, 1974; Cointreau, 2006). Pb is capable of causing free radical-mediated organ or tissue damage and this is usually accomplished by two independent but related mechanisms (Ercal et al., 2001). The first involves the direct formation of ROS including singlet oxygen, hydrogen peroxides, and hydroperoxides. The second mechanism is achieved through depletion of cellular antioxidant pools. A previous study demonstrated that blood Pb levels in workers cleaning electrostatic precipitators were greater than those in the group not working in a municipal incinerator (Malkin et al., 1992). Pb exposure has been reported to deplete antioxidants *in vivo* (Hermes-Lima et al., 1991; Yoshida et al., 2003). Because MSW generally are exposed to heavy metals such as cadmium, Cr, cobalt, nickel, Mg, Fe, and Zn (Hong et al., 2000), other heavy metals in the waste disposal environment could also be systemically absorbed. High concentrations of these heavy metals in the body is thought to enhance redox cycling and consequently induce ROS, especially hydroxyl radicals (Halliwell, 1988). Furthermore, Pb has been reported to affect Cp level in children living near a secondary Pb

smelting plant. Beside the inhibition of heme-synthesizing enzymes, Pb may also bind to transferrin and Cp, which may induce anemia, since Cp catalyzes the transport of Fe to transferrin, the essential step in heme synthesis. This probably provides reason for the elevated Cp observed in the present study.

Although a study by Cointreau (2006) reported a significant increase in blood levels of Hg among garbage handlers and paper sorting workers, the blood levels of Hg in the municipal WMW investigated in the present study did not differ significantly from those of the control subjects. We also observed that Cp exhibited significant positive correlation with MDA and significant negative correlation with Pb. Significant negative correlation between FRAP and Pb and between Fe and Cu was also observed in MSW management workers, but such correlation was not seen in the control subjects. Furthermore, a significant positive correlation was observed between diarrhea ($r = +0.205$; $p < 0.01$), tiredness ($r = +0.227$; $p < 0.01$), shoulder pain ($r = +0.178$; $p < 0.05$), and Pb level in the waste workers. Also, a negative correlation was observed between phlegm production ($r = -0.238$; $p < 0.01$), chest pain ($r = -0.186$; $p < 0.05$), and Cu level as well as between tiredness ($r = -0.179$; $p < 0.05$), chest pain ($r = -0216$; $p > 0.01$), and Fe level. A significant correlation also existed between tiredness ($r = +0.263, p < 0.01$), chest pain ($r = +0.202, p < 0.01$), shoulder pain ($r = +0.204$, $p < 0.01$), and Zn level. These suggest that the levels of these metals may be contributing to self-reported symptoms and general health status of the waste workers.

In conclusion, our data suggest that the WMW investigated in this study may be exposed to high levels of toxic metals in the workplace. The blood levels of these heavy metals in addition to altered levels of essential trace elements may contribute to the presence of some of the frequently reported symptoms by these individuals. However, since the levels of these toxic metals were not analyzed in the solid wastes, it is not clear if the source of contamination or exposure is only limited to the work environment of the WMW. We believe this is one of the limitations of our study. Considering the significant level of exposure at the workplace, we recommend that government at all levels in conjunction with other relevant stakeholders review existing policies and put in place measures that will promote risk reduction. We also believe that continuous education of the WMW on the need for compliance to safety regulations and practice is very crucial in all of these.

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Appendix 1

Table A1. Working conditions of atomic absorption spectroscopy.

Element	Wavelength (nm)	Slit width (nm)	Flame type	Sensitivity	Linearity ranges
Cr	357.9	0.7	Rich, yellow	0.1 $\mu g\ ml^{-1}$ for 1% absorption	Up to 5 $\mu g\ ml^{-1}$
Cu	324.8	0.7	Lean, blue	0.09 $\mu g\ ml^{-1}$ for 1% absorption	Up to 5 $\mu g\ ml^{-1}$
Fe	248.3	0.2	Lean, blue	0.12 $\mu g\ ml^{-1}$ for 1% absorption	Up to 5 $\mu g\ ml^{-1}$
Mg	285.2	0.7	Lean, blue	0.007 $\mu g\ ml^{-1}$ for 1% absorption	Up to 0.5 $\mu g\ dl^{-1}$
Mn	275.5	0.2	Lean, blue	0.55 $\mu g\ ml^{-1}$ for 1% absorption	Up to 3 $\mu g\ ml^{-1}$
Pb	283.3	0.7	Lean, blue	0.5 $\mu g\ ml^{-1}$ for 1% absorption	Up to 20 $\mu g\ ml^{-1}$
Se	196.0	2.0	Lean, blue	0.5 $\mu g\ ml^{-1}$ for 1% absorption	Up to 50 $\mu g\ ml^{-1}$
Zn	213.9	0.7	Lean, blue	0.018 $\mu g\ ml^{-1}$ for 1% absorption	Up to 1 $\mu g\ ml^{-1}$
Hg	253.6	0.7	Lean, blue	0.5 $\mu g\ ml^{-1}$ for 1% absorption	Up to 10 $\mu g\ ml^{-1}$

Cr: chromium; Cu: copper; Fe: iron; Mg: magnesium; Mn: manganese; Pb: lead; Se: selenium; Zn: zinc; Hg: mercury.

Table B1. Percentage recoveries of trace metals in blood serum after digestion and detection limits for the FAAS determination of the elements.

Sample Elements	Amount Spiked ($\mu g\ ml^{-1}$)	Amount Recovered ($\mu g\ ml^{-1}$)	Percentage Recovery (%)	Detection Limit ($\mu g\ ml^{-1}$)
Fe	10	9.3	93	0.005
Mg	10	9.8	98	0.0001
Cu	10	8.9	89	0.001
Pb	10	9.1	91	0.01
Mn	10	8.9	89	0.002
Zn	10	9.8	98	0.001
Se	10	9.9	99	0.05
Cr	10	9.7	97	0.003
Hg	10	8.2	82	0.25

Cr: chromium; Cu: copper; Fe: iron; Mg: magnesium; Mn: manganese; Pb: lead; Se: selenium; Zn: zinc; Hg: mercury; FAAS: flame atomic absorption spectrophotometric analysis.