



Chemical characteristics of bottom ash from biomedical waste incinerators in Ghana

Justice Kofi Debrah ·
Maria Alzira Pimenta Dinis

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Abstract Biomedical waste (BMW) incineration is the most used alternative disposal method in developing countries, such as Ghana. The improper disposal of incinerator-generated bottom ash (BA) is a significant concern due to the hazardous nature of waste. A study was conducted at Tema Hospital (TGH) and Asuogyaman Hospital (VRAH) incinerator sites. The BA samples were sent to the Council for Scientific and Industrial Research, Institute of Industrial Research, Ghana. The BA samples were weighed with fisher analytical balance, ground, and sieved with standard grade meshes of 120, 100, and 80 to determine the BA particle size distribution. The chemical composition and heavy metals were analysed using X-ray fluorescence spectrometry (XRF) and atomic absorption spectroscopy (AAS) techniques. The results indicated the chemical composition of the analysed BA samples was CaCO_3 (49.90%), CaO (27.96%) and MgCO_3 (6.02%) for TGH and CaCO_3 (48.30%), CaO (27.07%), and SiO_2 (6.10%) for VRAH, respectively. The mean concentration (M) (kg m^{-3}) and standard deviation (SD) for

TGH in the BA were 7.082 ± 0.478 (Ti), 4.657 ± 0.127 (Zn) and 4.271 ± 1.263 (Fe), while that of VRAH consisted of 10.469 ± 1.588 (Ti), 7.896 ± 2.154 (Fe) and 4.389 ± 0.371 (Zn). Therefore, the heavy metals' mean concentration at the BA is above the WHO permissible limits of soil, i.e., 0.056 kg m^{-3} (Ti), 0.085 kg m^{-3} (Pb), 0.100 kg m^{-3} (Cr) and 0.036 kg m^{-3} (Cu). Furthermore, the heavy metal mean concentrations of TGH and VRAH present in the BA analysed samples were ranked in descending order: $\text{Ti} > \text{Zn} > \text{Fe}$ and $\text{Ti} > \text{Fe} > \text{Zn}$, respectively. It is therefore recommended that BA must be properly disposed of because of the hazardous nature of heavy metals present in the analysed samples, which are able to cause environmental and public health issues.

Keywords Biomedical waste (BMW) · Incinerator · Bottom ash (BA) · Heavy metal · Ghana

Introduction

The recent population growth and the outbreak of diseases such as the Ebola virus, severe acute respiratory syndrome (SARS), coronavirus disease of 2019 (COVID-19) and other illnesses (Bucătaru et al., 2021) have significantly increased medical activities globally. Unfortunately, medical activities have also contributed to the rising generation of biomedical waste (BMW), making it difficult to be managed (Olaniy et al., 2018; Chisholm et al., 2021), especially

J. K. Debrah (✉)
Faculty of Science and Technology, University Fernando Pessoa (UFP), Praça 9 de Abril 349, 4249-004 Porto, Portugal
e-mail: 40511@ufp.edu.pt

M. A. P. Dinis (✉)
UFP Energy, Environment and Health Research Unit (FP-ENAS), University Fernando Pessoa (UFP), Praça 9 de Abril 349, 4249-004 Porto, Portugal
e-mail: madinis@ufp.edu.pt

in developing countries such as Ghana (Debrah et al., 2022b, d, e). Improper handling of BMW contributes to the rising volume of infectious BMW. In sub-Saharan Africa (SSA), for instance, in Addis Ababa, Ethiopia, health facilities generate about 41% of infectious BMW (Debere et al., 2013). A study by Oli et al. (2016) also revealed that more than 40% of infectious BMW is generated in Southeast Nigeria health facilities. Infectious BMWs (49.1%) are generated in six selected healthcare facilities in Eastern and Greater Accra regions (Debrah et al., 2021a), and 49.6% infectious BMW is generated in health facilities in Kumasi (Oduro-Kwarteng et al., 2021), in Ghana. These values are higher than the recommended 15% limit by the World Health Organization (WHO, 2018), to 35–50% (Debere et al., 2013; Debrah et al., 2021a; Oduro-Kwarteng et al., 2021; Oli et al., 2016).

This could adversely affect the environment and public health through uncontrollable disposals, like dumping in the open space and pit burning (Debrah et al., 2021a, b, c; Debrah et al., 2022a, b, c, d, e, f; Leal Filho et al., 2022a), so common in Africa. The improper handling of BMW has hindered achieving of some of the sustainable development goals (SDGs) in most developing countries, specifically good health and well-being (SDG3), clean water and sanitation (SDG6) and climate action (SDG13) (Leal Filho et al., 2022b, c). It is the case in Ghana.

Considering the significant volume of infectious BMW produced by health facilities in SSA countries, the incineration method is currently used to minimize generated BMW and reduce the problems posed by BMW infectious waste (Awodele et al., 2016), negatively impacting the environment (Debrah et al., 2021a). Studies in developing countries have shown that incineration reduces the weight of BMW by more than 70% (Xiao et al., 2018; Zhao et al., 2010) and the volume by 90% (Debrah et al., 2021a; Xiao et al., 2018). After the incineration, significant combustion residues remain in the form of bottom ash (BA). Although BMW incineration disposal is widely used in SSA, its environmental impact has resulted in public health problems caused by gases being released (Rahman & Singh, 2019). A few epidemiological studies in developed countries have shown that incinerator workers/operators and residents closer to incinerators (< 10 km) present diseases related to their work environment. These include laryngeal cancer (Michelozza et al., 1998), gastric cancer (Dockery and Pope III 1994; Rapiti et al., 1997), liver cancers (Elliott

et al., 1996), and urinary mutagen (Landrigan et al., 1987). Other research also indicate that incinerator operators can present a significant level of mercury in their hair (Kurttio et al., 1998), lead and cadmium in blood (Wrbitzky et al., 1995) and hexachlorobenzene in blood/urine (Angerer et al., 1992). These effects show why some industrialized nations, such as the United States of America (USA), Germany and the Netherlands, have prevented the usage of incinerators in the management of BMW (Abor & Bouwer, 2008). Instead, most countries have opted for the most acceptable alternative available technology to handle BMW, such as microwave, plasma pyrolysis and ionized autoclave, with minimum environmental and fewer health issues (Dharmaraj et al., 2021; Xu et al., 2020; Zhao et al., 2021; Zimmermann, 2018), representing less threat to the environment and human health (Zhao et al., 2021). In developing countries, especially in SSA, these alternative technologies to handle BMW are rare due to financial implications associated with its purchasing and management (Leal Filho et al. 2022d; Dinis et al., 2022; Debrah et al., 2022a). Although current modern incinerators operate within a temperature range of 850–1200 °C, BA containing metals that are non-biodegradable affects the entire ecosystem and human health (Dwivedi et al., 2019; Premkumar et al., 2018; Wei et al., 2021). Recent studies by Agnihotri and Kesari (2019), Tait et al. (2020), and Wallace et al. (2020) revealed that heavy metals such as Cd, Hg and Pb could cause chronic diseases like cancer and long-term neurological conditions, leading to possible morbidity (Bennett et al., 2001; Yang et al., 2020). Also, these and other heavy metals can leach through the soil and contaminate drinking water (Kapoor & Singh, 2021; Mukherjee et al., 2021), which is then absorbed by plants, animals, and other organisms in the food chain (Feng et al., 2020; Manzoor & Sharma, 2019; Sonone et al., 2020). Meanwhile, the accumulated heavy metals cause chronic and acute toxic effects in the various living beings in nature.

In Ghana, BMW disposal equipment has changed from the traditionally built container through open brick (De Montfort) to the current covered brick 6-regulator cylinder gas incinerator. Only a limited number of medical centres have this new 6-regulator cylinder gas incinerator equipment. However, few studies referring to incinerators have addressed the BMW BA in Ghana. For instance, a study by Amfo-Otu et al. (2015) showed that heavy metals such as Pb, Cd, Hg and Cr were found in the BA of a De Montfort-type incinerator, with a concentration of Pb

equal to 147.50 mg/kg. Also, a similar study by Adama et al. (2016) reported the release of Hg, Pb, Zn, Ag, Cr and Cd in the De Montfort incinerator. However, to this study authors' best knowledge, there is not yet a study in Ghana on particle size distribution, the main chemical composition and the concentrations of heavy metals residuals present in the 6-regulator cylinder gas incinerator involving BA.

Therefore, this study aims to assess and determine the significant elements in terms of chemical components found in the BA in two cases: the Tema Hospital (TGH) and the Asuogyaman Hospital (VRAH) in Ghana. The particle size distribution and the concentration of heavy metals present in the BMW BA were obtained from the 6-regulator cylinder gas incinerator in TGH in Greater Accra Region and VRAH in Easter Region, Ghana.

Materials and methods

The BA samples used in this study were collected from TGH and VRAH facilities, with bed capacities of 409 and 55 patients, respectively. TGH and VRAH generate an estimated amount of 1.10 kg/day and 2.30 kg/day of BMW infectious waste, representing an accumulated value of 3.20 tons and 0.90 tons per week, respectively. BAs (0.27 tons and 0.07 tons) were produced every week after the incineration of BMW from TGH and VRAH incinerators. The incinerators used in TGH and VRAH were constructed with bricks and covered with corrugated iron sheets. The walls of the incinerators are composed of three layers to reduce the heat transmissions

during the combustion process. In addition, fibres were placed between the walls and the covered corrugated iron sheets to prevent heat transfer. The incinerators have 2 chambers, a blower with six outlets and a front-loading window with 7 BA outlets. The two chambers operate within a temperature range of 800 ± 50 °C and 1100 ± 50 °C respectively, which is powered by gas from two gas cylinders with six regulators. Infectious BMWs collected from the various wards of TGH and VRAH are loaded directly into the incinerator by the operators and combusted continuously within 2–3 h with a maximum temperature of 800 °C. The BA generated from VRAH is hauled to the landfills, while the TGH is dumped close to the incinerator. Figure 1 shows the incinerators of TGH, VRAH.

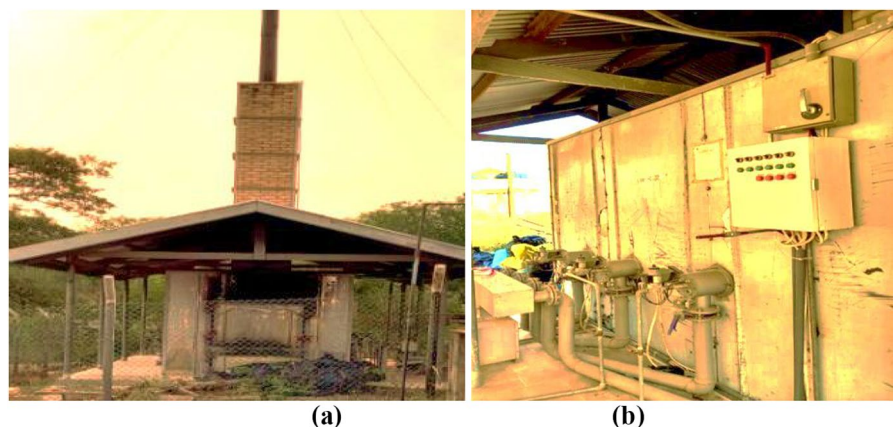
Data sources and study period

Qualitative data on the incineration process of BMW in TGH and VRAH were obtained through critical observation and BA analyses. The time frame for the data collection was a month, i.e. March 2021. There was a daily check and observation of the waste type fed into the incinerators and how the BA was disposed of after incineration. In addition, the incinerated BAs were collected and sent to the Council for Scientific and Industrial Research-Institute of Industrial Research (CSIR-IIR), Ghana laboratory, for analysis.

Sampling preparation

Granulated BA samples (0.300 kg) were collected from the VRAH and TGH 6-regulator cylinder gas

Fig. 1 Six-cylinder gas incinerators: (a) front view and (b) cylinder regulators



incinerators every 9 days within March 2021. The collected BA also contained other coarse materials such as glasses, bricks, concretes and heavy metals (Ti, Fe and Cu), resulting from medical bottles and needles in the BMW or used in the construction of the incinerators. The hot BA samples were cooled at room temperature and then dried at 105 °C for 24 h, similar to Bakkali et al. (2013) procedure. Each dried BA sample was ground gradually using laboratory mortar and pestle into smaller particle sizes to allow the exchange between residue phases and the extraction solutions (Bakkali et al., 2013; Guérin, 2000). The grounded BA was sorted into sizes with shaker-fitted standardized grade meshes of 120, 100, and 80. However, within 20 min, the sampled BAs were sieved to the particle size below 125 µm, 125–150 µm and 150–180 µm and above 180 µm.

BA chemical composition

X-ray fluorescence spectrometry analysis

The elemental composition in the BA was determined using X-ray fluorescence (XRF) spectroscopy. Portions of the 0.300 kg prepared bottom ash sample of TGH and VRAH were pressed into a cake. The chemical components of the BA were qualitatively analysed using S2 Ranger Energy Dispersive equipment to determine the unknown elements.

Heavy metal analysis

Before heavy metals were analysed using the SP-IAA320 Atomic Absorption Spectrophotometer (AAS)

model, with a range of 190–900 nm and an accuracy level of $\leq \pm 0.5$ nm, the laboratory was thoroughly cleaned to eliminate possible contamination affecting the results. Dried sample (0.001 kg) was transferred into a round-bottom flask with 10 cm³ HNO₃, evaporated over 1 h on a hot plate. The residue obtained from the evaporated mixture was digested with concentrated HNO₃ and HClO₄ in the ratio of 3:1 for 10–15 min at 25 °C. The digested mixture was steadily heated at 300 °C for about 120 min on a hot plate until all HClO₄ gases evaporated. The mixture was allowed to cool at 25 °C and then filtered with Whatman No. 42 filter paper. The filtrate was kept in a 0.001 m³ well-labelled polyethylene bottle, ready to be analysed. The same procedure was carried out with estimated trace metals recorded and analysed with the ASS.

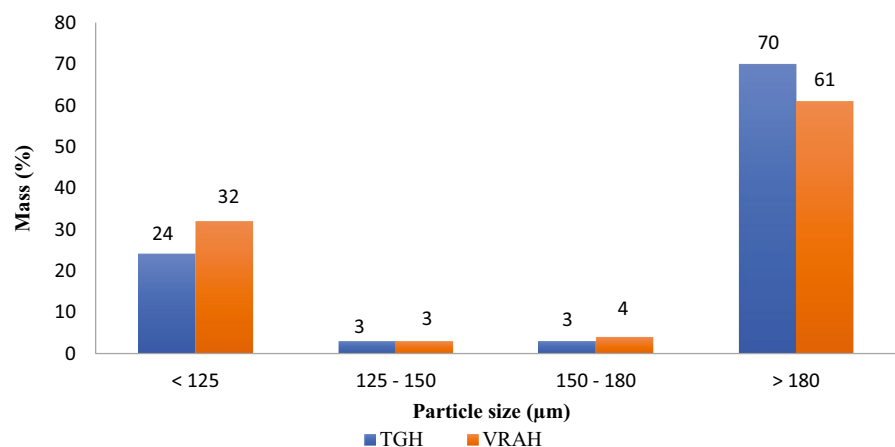
Results and discussion

Particle size distribution

The sieved particle size distribution of BA from TGH and VRAH is shown in Fig. 2. Most BA particles in this study were found above 180 µm and below 125 µm. This could result from the treatment of the sample, such as grinding or quantity of grounded sample on the sieve surface.

Figure 2 shows that the mass percentage of the particle size above 180 µm of VRAH is reduced by 9% of the total 70% TGH BA sample. The difference in the percentage might be due to the high content of the glass, ceramics, and other materials found in TGH BMW incinerated. The mass percentage content of

Fig. 2 BA samples particle size distribution in the VRAH and TGH incinerators



the BA particles decreases with the particle size, from 180 to 125 µm. Below 125 µm, VRAH mass percentage particles increased by 12% (34% of the VRAH mass samples and 24% TGH particle mass sample) due to the high content of the heavy metals present in VRAH BA. The particle size distribution of this study is similar to a study by Yu et al. (2013), which was carried out in China, but opposite to a study conducted by Bakkali et al. (2013) in Rabat, Morocco.

Chemical composition of BA samples analysed by XRF

XRF analysis is the main technique used to determine the status of BA elements of BMW. The composition of determinant elements depends on the toxicity of the waste in the incinerator (Bakalár et al., 2021; Li et al., 2004), furnace type, and gas velocity (Chang et al., 2009). Table 1 presents the chemical composition of the oxides obtained from XRF analysis samples of TGH and VRAH incinerators. The results obtained show that each BA analysed sample contains a higher percentage of CaCO₃ than all the remaining oxides. For TGH, the main BA elements consist of CaCO₃ (49.90%), CaO (27.96%), MgCO₃ (6.02%), MgO (2.87%), SO₃ (2.34%) and SO₄ (2.80%), while the main components of VRAH are constituted by CaCO₃

(48.30%), CaO (27.07%), SiO₂ (6.10%), MgCO₃ (6.12%), MgO (2.93%) and Al₂O₃ (3.74%). The high percentages of the oxides from TGH and VRAH BA samples show that most of the BMW incinerated include domestic waste, which emphasizes the need to proper segregation at the source.

Although BA contains CaCO₃, its high content in this study, with a mean of 49%, may be due to the presence of papers in incinerating BMW (Chang et al., 2017). It could also be due to the presence of the marbles in constructing the incinerator (de Oliveira et al., 2021; Hashimoto et al., 2017; Krajewska, 2018) and possibly through the use of CaCO₃ dietary supplements in the form of medication by patients (Salomão et al., 2017; Zhai et al., 2018). According to Schabel et al. (2014), He and Liu (2017) and Indriati et al. (2020), CaCO₃ is used as a filler in paper production to ensure the brightness of the paper. The analysis of the BA from these researched hospitals indicated a silicon/calcium (Si/Ca) ratio of 8.64:55.03 and <0.3, which is far less than a study conducted by Li et al. (2004) and Bakkali et al. (2013) in China and Morocco, with Si/Ca ratio of 3 and 5, respectively. Therefore, the Si/Ca ratio indicates the content of generated non-infectious waste within a BMW. This study's 0.3 Si/Ca ratio revealed that BMW generated from the TGH and VRAH was not adequately segregated.

The laboratory analysis from the TGH and VRAH incinerators showed mixed results of oxide compositions. This could be attributed to the size of the hospital, the area situated and the nature of activities performed by each hospital (Christiana & Anushree, 2021). In this study, the level of SO₄ and SO₃ in TGH BA was greater than that of VRAH, oppositely for K₂O. The content of SO₃ can be due to the presence of papers, food waste, plastics, leather mix, rubber, textile, wood, glass, ceramics and metals, which remains residual in BA (Kaiser, 1968). Nevertheless, a higher concentration of SO₃ in BA will be problematic since it converts into SO₄ at temperatures below 500 °C, becoming corrosive (Hardman & Stacy, 1998; Jaworowski & Mack, 1979; Marier & Dibbs, 1974; Sarbassov et al., 2017).

BA heavy metal detection in TGH and VRAH incinerators

Heavy metals such as Zn, Pb, Cr, Ni, Cu and Cd are primarily used to manufacture or coat medical tools and photographic (Bakkali et al., 2013) due to their resistance to corrosion and antibacterial surface factor.

Table 1 BA content obtained by the X-ray fluorescence spectrometry

Incinerator chemical composition		
Oxides	TGH sample (%)	VRAH sample (%)
MgO	2.87	2.93
Al ₂ O ₃	2.04	3.74
SiO ₂	2.54	6.10
P ₂ O ₅	1.14	1.01
SO ₄	2.80	0.98
SO ₃	2.34	0.81
K ₂ O	1.01	0.74
CaO	27.96	27.07
TiO ₂	0.82	1.28
Fe ₂ O ₃	0.42	0.83
MgCO ₃	6.02	6.12
CaCO ₃	49.90	48.30
Others	0.14	0.09

TGH Tema hospital, VRAH Asuogyaman hospital, % percentage

The results in Table 2 indicate the heavy metal concentration of the BA samples in TGH and VRAH. Even though TGH and VRAH BA samples showed different mean (*M*) and standard deviation (*SD*) concentrations, both had an amount of Ti, Cr, Mn, Fe, Ni, Cu, Zn and Pb. The mean concentrations of Ti, Fe and Cu heavy metals in VRAH BA were higher than in TGH BA, while Cr, Mn and Ni were opposite. The difference in the mean concentrations of the BAs from TGH and VRAH could be attributed to improper waste segregation, since coloured plastic materials in BMW contain heavy metals (Tufail, 2008). The results from the analysis for TGH and VRAH indicate that Ti has the highest value, $7.082 \pm 0.478 \text{ kg m}^{-3}$ and $10.469 \pm 1.588 \text{ kg m}^{-3}$. Ni has the least $0.030 \pm 0.021 \text{ kg m}^{-3}$ and $0.025 \pm 0.011 \text{ kg m}^{-3}$ for TGH and VRAH, respectively. The mean concentrations of TGH and VRAH heavy metals as ranked in order of descending are as follows: $\text{Ti} > \text{Zn} > \text{Fe} > \text{Cr} > \text{Pb} > \text{Mn} > \text{Cu} > \text{Ni}$ and $\text{Ti} > \text{Fe} > \text{Zn} > \text{Cu} > \text{Mn} > \text{Pb} > \text{Cr} > \text{Ni}$. Heavy metals such as Fe, Zn and Cu are essential to humans but become poisonous in high concentrations. Pb and Cr heavy metals are hazardous and have a high negative effect on humans (Tchounwou et al., 2012). The order raking of the heavy metals in TGH and

VRAH BA indicates that TGH BA is more toxic than VRAH.

This study allowed to obtain higher mean concentrations of Ti, Fe and Zn in TGH and VRAH, similar to a study conducted in China on pollutants of BMW BA (Zhao et al., 2010). The higher concentration of Ti, Fe and Zn in BA is because they are widely used as metal alloys in medical equipment (Dehghan-Manshadi et al., 2020; Hernández-Escobar et al., 2019; Kazemi et al., 2020). However, due to their high melting point, above the temperature used in incinerators, Ti and Fe tend to be present in BA.

Some metals found in BA could contain toxic contaminants that can be persistent in the environment (such as Pb, Ni, Cu and Cr) (Kumar et al., 2021; Thuy et al., 2021), negatively impacting it (Andreola et al., 2019; Lemly, 2018; Manzoor & Sharma, 2019; Yin et al., 2020). The mean concentration of Ti, Cr, Cu and Mn and Pb released from TGH BA and VRAH BA exceeded the WHO permissible limits of heavy metals in soil, including Fe from VRAH BA, as shown in Table 2. Due to its high content of heavy metals, the improper disposal of this BA could negatively impact the environment and public health. Each heavy metal has different properties and impacts, in terms of human health effects. As example, the exposure to high concentrations of Ti contained in BA samples could cause coughing, tightness and chest pains (Lenntech, 2022). Zn and Fe present in the BA may cause nausea, vomiting and anaemia (Kim et al., 2019; Njoku et al., 2020; Saria, 2016).

As seen by the results being presented, the disposal of BA resulting from BMW incineration needs to be properly addressed since the resulting BA can threaten ecosystems and humans. The heavy metal component in the BA can leach out into water bodies, groundwater and soil, polluting the environment and possibly causing cancer respiratory and other issues severely affecting human health (Mozhi et al., 2022; Munawer, 2018).

Table 2 Heavy metal concentration ($M \pm SD$) in BA samples from TGH and VRAH incinerator

Heavy metal	TGH $M \pm SD$ (kg m^{-3})	VRAH $M \pm SD$ (kg m^{-3})	WHO permissible value soil content ^b (kg m^{-3})
Ti	7.082 ± 0.478	10.469 ± 1.588	0.056
Cr	0.676 ± 0.162^a	0.169 ± 0.028	0.100
Mn	0.321 ± 0.033	0.265 ± 0.015	0.012
Fe	4.271 ± 1.263	7.896 ± 2.154	5.000
Ni	0.030 ± 0.021	0.025 ± 0.011	0.035
Cu	0.083 ± 0.005	0.292 ± 0.057^a	0.036
Zn	4.657 ± 0.127	4.389 ± 0.371	0.050
Pb	0.477 ± 0.020	0.243 ± 0.033	0.085

1Kg m^{-3} = 1000 ppm

BA bottom ash, TGH Tema hospital, VRAH Asuogyaman hospital, *M* mean concentration, *SD* standard deviation

^aValue: middle data

^bWHO permissible limit (International Programme on Chemical Safety 1982; Anjali et al., 2018; Osobamiro et al., 2019; Ullah et al., 2022)

Conclusions

When improperly handled and inadequately disposed, BMW generated from diagnostics centres, healthcare centres, blood banks, research institutions and

laboratories are hazardous and negatively impact the environment and public health. In SSA countries in particular, and also in Ghana, BMW is primarily incinerated. Although this incineration process reduces the volume and weight of BMW, it also produces BA that contains heavy metals and oxides, toxic to human health and the environment. Departing from the analysis of the results from this study, involving XRF and ASS techniques, the results from this study using BA from two healthcare facilities in Ghana, i.e. TGH and VRAH, revealed that the BA generated by the BMW incinerators from both healthcare facilities contains high concentration of significant non-hazardous waste containing CaCO_3 , CaO , SiO_2 , MgCO_3 , MgO , SO_3 and Al_2O_3 in the incinerated BMW, resulting from materials used in the construction and domestic waste such as food waste, ceramics, plastics and papers. These values can be reduced if proper segregation is addressed prior to incineration. High concentrations of heavy metals, specifically Ti, Fe, Zn, Pb, Cu, Mn and Cr, were found in both incinerators, above WHO permissible soil limits, except Ni. In both TGH and VRAH incinerators, Ti heavy metal was the highest by-product in the BA, corresponding to $7.082 \pm 0.478 \text{ kg m}^{-3}$ and $10.469 \pm 1.588 \text{ kg m}^{-3}$, respectively, in TGH and VRAH. Ti is one of the most hazardous heavy metals, negatively impacting the environment and human health.

From the results obtained in this study, it is concluded that improper disposal of BA from incinerators may pollute the environment and water bodies through leachate, leading to possible health implications such as respiratory diseases and cancers, while also negatively impacting the environment. Ensuring proper disposal of BA through sanitary landfills may contribute to minimize the heavy metals and hazardous components in the environment, also protecting the human health. The advance of specific SDGs, such as SDG3, 6, and 13, strongly depend on actions aimed at contributing to better handling of waste in Africa. Accordingly, this study is of fundamental importance in Ghana, a developing country that needs to focus on environmental issues that also benefit human health. It represents a contribution to fulfil the gap of knowledge at this respect and the scarcity of studies in the SSA region.

Limitations and future studies

This study was conducted at a time when COVID-19 pandemic in Ghana was rising, and the selected hospitals were assigned as COVID-19 centres. The authors were unable to sort and classify BMW waste before being incinerated. Therefore, future studies must attempt to classify the types of BMW waste incinerated, as well as to analyse the percentage of limestone present in the BA of the incinerators, possibly used for binding materials.

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Author contribution Conceptualization, Justice Kofi Debrah; data curation, Justice Kofi Debrah; methodology, Justice Kofi Debrah; supervision, Maria Alzira Pimenta Dinis; validation, Maria Alzira Pimenta Dinis; visualization, Justice Kofi Debrah and Maria Alzira Pimenta Dinis; writing-original draft, Justice Kofi Debrah and editing, Justice Kofi Debrah and Maria Alzira Pimenta Dinis. All the authors have read and accepted to the published version of the manuscript.

Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval All the authors have read, understood, and have complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors and are aware that with minor exceptions, no changes can be made to authorship once the article is submitted.

Competing interests The authors declare no competing interests.

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