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Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity

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Abstract

Heavy metals in municipal solid waste incineration bottom ash (MSWIBA) may leach into soil and groundwater and pose long-term risks to the environment. In this study, toxicity characteristic leaching procedure (TCLP) was carried out on the MSWIBA from Macao. Heavy metals in leachates were determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES), and genotoxicity of leachates was also evaluated by micronucleus (MN) assay with *Vicia faba* root tip cells. The results showed that the concentrations of aluminium (Al), manganese (Mn), cobalt (Co), cadmium (Cd) and mercury (Hg) in the leachates were less than 0.01 mg l^{-1}, and those of iron (Fe), copper (Cu) and molybdenum (Mo) were less than 0.1 mg l^{-1}. The concentrations of chromium (Cr), zinc (Zn), selemium (Se), strontium (Sr), barium (Ba) and caesium (Cs) were between 0.11 mg l^{-1} and 2.19 mg l^{-1}. Lead (Pb) concentrations, in particular, reached as high as 19.6 mg l^{-1}, significantly exceeding the maximum concentration limit (5 mg l^{-1} for lead by TCLP). Compared with the negative group, a significant increase of MN frequencies was observed in the leachate-exposed groups ($P < 0.05$). With the increase of heavy metals in the leachates, the toxic effects on the *Vicia faba* root tip cells increased, implying that heavy metals were the main factors causing the genotoxic effects. Our results suggested that apart from chemical analysis, bioassays like the MN assay of *Vicia faba* root tip cells should also be included in a battery of tests to assess the eco-environmental risks of bottom ashes before decisions can be made on the utilization, treatment or disposal.

Keywords: Municipal solid waste incineration bottom ash (MSWIBA); Toxicity characteristic leaching procedure (TCLP); Heavy metal; Genotoxicity; Micronucleus (MN) assay

1. Introduction

Incineration, which aims to reduce the volume, the toxicity and the reactivity of the waste (Klein et al., 2001), is a viable management strategy throughout the world for treating the increasing combustible municipal solid waste (MSW) that cannot be recycled (Eighmy et al., 1995; Ferreira et al., 2003). Although incineration reduces greatly the volume (by about 90%), the mass (by about 75%) of MSW and provides energy (Chimenos et al., 1999), it is not the final solution of managing MSW (Ferreira et al., 2003). Since the solid residues (bottom ashes (BA), fly ashes) it generates still amount to roughly 17 Mt per year world-wide, which must subsequently be disposed of in an environmentally acceptable manner (Chimenos et al., 1999; Klein et al., 2001). This amount is expected to double within the next 10 or 15 years (Chimenos et al., 1999; Klein et al., 2001). Moreover, the advance of air pollution control measures in municipal solid waste incineration (MSWI) has resulted in a shift of constituents of concern from air emissions to the solid

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residues (Sawell et al., 1995; van der Sloot et al., 2001). Heavy metals (after undergoing gasification, oxidation, chlorination, condensation, coagulation, and nucleation), which come from raw wastes, are condensed into incinerated residues and thus may pose a threat to the environment (Gau and Jeng, 1998). During MSWI, lithophilic metals such as Fe, Cu, Cr, and Al remained mainly in the BA while Cd volatilized and condensed to the fly ash. About two thirds of Pb and Zn was found in the BA despite their high volatility (Jung et al., 2004). BA, which is the object of this study, represents about 80% of the residues and contains various substances that may pose a threat to the environment (Klein et al., 2001). The evaluation of the environmental quality of such residues is necessary before decisions can be taken on the utilization, treatment or disposal of them (van der Sloot et al., 2001).

In the last decade, most of studies focus on the chemical composition, mineralogical characteristics, and heavy metals leaching behaviors of MSWIBA (Eighmy et al., 1995; Gau and Jeng, 1998; Chimenos et al., 1999; Chang et al., 2001; van der Sloot et al., 2001; Bruder-Hubscher et al., 2002; Chimenos et al., 2003), fewer studies have addressed the issue of its (eco)toxicological consequences (Schramm et al., 1999; Radetski et al., 2004). Although in the past the analytical techniques improved rapidly, there is a huge lack in the quantitative evaluation of the risk of a mixture of compounds (such as BA) determined by physical-chemical analytical techniques (Schramm et al., 1999). Therefore, a complemental bioassay strategy should be developed with a focus on the most important (eco)toxicological effects. The biological endpoints are chosen according to their importance of known biological targets. Genotoxicity and/or disruption of the genome are ones of the first targets of concern (Schramm et al., 1999). Micronucleus (MN) assay in *Vicia faba* root cells, which were validated and its protocol were standardized through a program under the International Program on Chemical Safety, is highly sensitive and capable of detecting mutagens, clastogens and carcinogens from the environment, and showed excellent correlations with tests in the mammalian systems and human lymphocytes systems (Grant, 1994; Ma, 1999). It has been recommended for use in mutation screening or monitoring by the Royal Swedish Academy of Sciences, Committee 17 of the Environmental Mutagen Society and the World Health Organization (Grant, 1994).

Macao is a small island less than 27.3 km^2, yet has more than 0.46 million inhabitants. In additional to this high population density, accelerated economic development and enhanced living standards in the last decade have led to the generation of more MSW with a more complex composition. Today, incineration is a major approach in Macao for managing the increasing production of MSW. Landfilling is the main management option for the MSWIBA in Macao, as in many countries and territories (Ibanez et al., 2000; Klein et al., 2001; Ferreira et al., 2003). The potential pollution risks of the BA are of wide concern.

At present, the available (eco)toxicological data on the MSWIBA are relatively scarce. Moreover, the mineralogical characteristics and the chemical composition of MSWIBA varied either in different areas or during different periods. Thus, putting the extrapolation of published results to the MSWIBA from Macao is under question.

In the present study, toxicity characteristic leaching procedure (TCLP) suggested by the USA Environmental Pollution Agency (EPA) has been carried out on the MSWIBA from Macao. The Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) were employed to determine the contents of heavy metals in the leachates. The genotoxicity of the leachates was screened by MN assay in *Vicia faba* root cells. The aim is to provide data on its potential risks on the environment, and to provide policy maker with better information for planning of sound environmental actions and control measures.

2. Materials and methods

2.1. Sampling of bottom ash

Samples of MSWIBA were provided by Macao EPA. The sampling of MSWIBA was carried out in accordance with the recommendations of the International Ash Working Group (IAWG, 1997). Homogeneous steps were applied to the materials in order to derive representative laboratory samples to be used for the following experiments.

2.2. Leaching extraction procedure

The leaching extraction procedure followed the USA EPA Method # of 1311 with minor modifications (EPA., 1990). Triplicate BA samples were leached by three different extraction fluids respectively, which were deionized water (A) and two acetic extractants (B: pH 4.9, and C: pH 2.9). Since the average precipitation of a year was about 1869.4 mm in Macao in last century (SMG, 2000), the BA in the landfills will be leached by relatively more rain. The liquid-to-solid ratio was enlarged to 40:1, following an agitation extraction with a speed of 30 rpm for 20 h. Leachates were collected and filtered through a 0.45-μm membrane filter (Millipore). Each leachate was immediately prepared for metal elements analysis and MN assay in *Vicia faba* root cells, respectively.

2.3. Analysis of metal elements

The metal elements leached were determined by Perkin Elmer Elan 6000 ICP-MS and Varian Vista ICP-AES in the Laboratory of Isotope Geochronology and Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, with external standard calibrations (Wei et al., 2003a; Wei et al., 2003b). Analytical precision was better than 5% for the metal elements except Cd and Mo (better than 10%). Several Chinese rock standards (GSR-1, GSR-2, GSR-3, GSR-4, GSR-5, GSR-6), which have different mineralogy and a fairly large span of element concentrations (Wei et al., 2003b), were repeatedly measured along with the samples for quality control. Accuracy was within ± 5% for the metal elements except Cd and Mo (± 15%).

2.4. Micronucleus assay in Vicia faba root cells

The protocol published by Ma et al. (1995), with minor modifications, was adopted for this study and only brief description present here. *Vicia faba* seeds (provided by the School of Life Sciences, Huazhong Normal University, PR China) were stored at 4 °C in dry conditions until use. Before soaking, seeds were disinfected by a short immersion (3 min) in a 5% calcium hypochlorite solution and then thoroughly rinsed five times with distilled water. Seeds soaked in 48-h aerated tap water (ATW) for 20 h were allowed to germinate between two moist filter papers for 4 d at 22 °C. After removing the tips of the primary roots, the seedlings were transferred to ATW maintained at 22 °C for 4 d with daily renewal of water. Secondary roots of every six seedlings were treated with the leachates abovementioned for 24 h in darkness at 22 °C. ATW was used as the negative control and potassium dichromate solution ($K_2Cr_2O_7$, 0.01 g l^{-1}) as the positive. After a post-exposure recovery period of 48 h, roots were isolated and fixed (at 4 °C during 16 h) in freshly prepared 3:1 ethanol/glacial acetic acid mixture. Then, root tips were immersed in distilled water for 5 min and hydrolyzed in 5 M HCl at 22 °C for 1 h. After subsequent staining in orcein (1%) for 5 min, root tips were prepared for enumerating micronucleated cells. Micronucleated cells were scored on at least 1000 cells/root and for 1 root/seedling under a microscope (Eclipse 80i, Nikon, Japan; ×400). Only micronuclei distinctly separated from the main nucleus were scored (Fig. 1). MN frequency (MN‰) was calculated as follows:

$$\text{MN‰} = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} \times 1000 \quad (1)$$

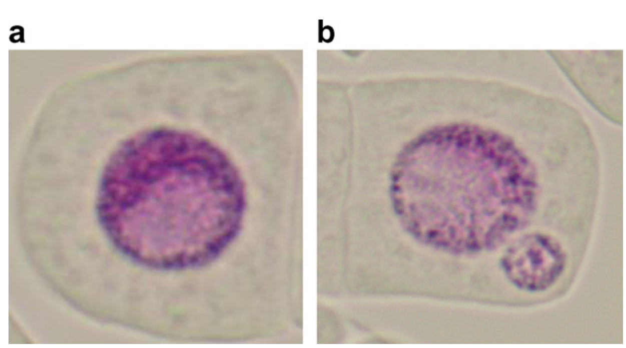

Fig. 1. The natural cell and the micronucleated cell in *Vivia. faba* root tip (a. natural cell; b. micronucleated cell: the MN was under the main nucleus in this cell; ×400).

Statistical differences between control and exposed groups were determined with Dunnett's *t*-test.

3. Results and discussion

The environmental compatibility of the waste incineration process mainly depends on the characteristics and possible applications of the incineration residues (Bipp et al., 1998). At present, the landfilling is still the main management option for BA in many countries and territories, including Macao (Ibanez et al., 2000; Klein et al., 2001; Ferreira et al., 2003). After disposal, the BA may constitute a long-term risk for the environment. The highest attention is directed to the soluble heavy metal compounds which may migrate into soil and groundwater, and pose a health risk to humans via food chain contamination (Bipp et al., 1998; Bruder-Hubscher et al., 2002).

In this study, the concentrations of metal elements in the leachates were listed in Table 1. The results showed that different metal elements in the BA exhibited different leaching characteristics. The concentrations of Al, Mn, Co, Cd and Hg in the leachates were less than 0.01 mg l^{-1}, and that of Fe, Cu and Mo were less than 0.1 mg l^{-1}. The concentrations of Cr, Zn, Se, Sr, Ba and Cs were between 0.11 mg l^{-1} and 2.19 mg l^{-1}, beyond the limits of Level III water quality standards of China (SEPAC, 2002). Particularly, Pb concentrations were very high with a maximum of 19.6 mg l^{-1}, exceeding significantly the maximum concentration limit, which is 5 mg l^{-1} for Pb (by TCLP), and, moreover, the European Environmental Legislation intends to lower this limit (Ioannidis and Zouboulis, 2003).

It was found that many factors, such as the amounts and speciation of metal elements in the BA, the extraction fluids, buffering capacities of the BA, and some of physical characteristics of the BA including specific surface area,

Table 1
The metal elements leached by the three different extraction fluids

Metal elements	Concentrations of metal elements (mg l^{-1})		
	A leachate	B leachate	C leachate
Al	<0.01	<0.01	<0.01
Cr	0.11	0.11	0.13
Fe	0.04	0.04	0.04
Mn	<0.01	<0.01	<0.01
Co	<0.01	<0.01	<0.01
Cu	0.06	0.09	0.10
Zn	0.49	0.89	1.71
As	0.10	0.11	0.12
Se	0.12	0.22	0.24
Sr	1.54	1.94	2.19
Mo	0.06	0.08	0.07
Cd	<0.01	<0.01	<0.01
Cs	0.10	0.13	0.14
Ba	0.33	0.37	0.41
Hg	<0.01	<0.01	<0.01
Pb	9.14	16.52	19.06

A: deionized water; B: acetic extractant (pH 4.9); C: acetic extractant (pH 2.9).

porous size, particle sizes and amount of carbon, have great effects on the leaching process of metal elements in the BA (Wei et al., 1998). In this study, it was very difficult to disentangle which factor is mainly responsible for the leaching behaviors of the metal elements in the BA. But higher Pb concentrations in the leachates may be due to that Pb had higher leaching rate than the other metals by TCLP, since lead acetate is one of the most soluble lead compounds (44.2 g/100 ml H_2O) (Wei et al., 1998; Ioannidis and Zouboulis, 2003). Moreover, Pb solubility increases at lower (acidic) pH values (Ioannidis and Zouboulis, 2003).

The amounts of metal elements leached by the different extraction fluids were compared in the Fig. 2. With the decreasing pH values of the extraction fluids, the amounts of metal elements in the leachates increased, especially significant for Zn, Pb, Se, and Cu. But for Cr, Fe, As, Sr, Mo, Cs, and Ba, this tendency was not so significant, implying the dissolution was one of the main factors that determine the leaching process in this study (Fig. 2).

The results of MN assay in *Vicia faba* root cells were listed in the Table 2. There were significant increases of micronuclei frequencies observed in the leachate-exposed groups compared with the negative group ($P < 0.05$), indicating that the leachates clearly had genotoxicity to the *Vicia faba* root cells. With the increase of heavy metal contents in leachates, the toxic effects on the *Vicia faba* root cells increased; for example, the MN frequency induced by the B leachate was higher than that by the A leachate. But for the C leachate, with the further increase of heavy metal amounts, the cytotoxic effects on the *Vicia faba* root cells were observed, restraining the division of the *Vicia faba* root tip cells. Therefore, the MN frequency caused by the C leachate was even lower than that by the A leachate. On the other hand, this implied that the genotoxicity of the leachates were mainly caused by the heavy metals. Since there were many kinds of contaminants in the leachates, which can induce the damage of DNA or chromosomes in the plant cells, and co-exposure may cause genotoxic effects, even if the concentration of individual contaminant was very low (Hengstler et al., 2003). It becomes very difficult to disentangle which contaminant is actually responsible for the genotoxicity of the leachates.

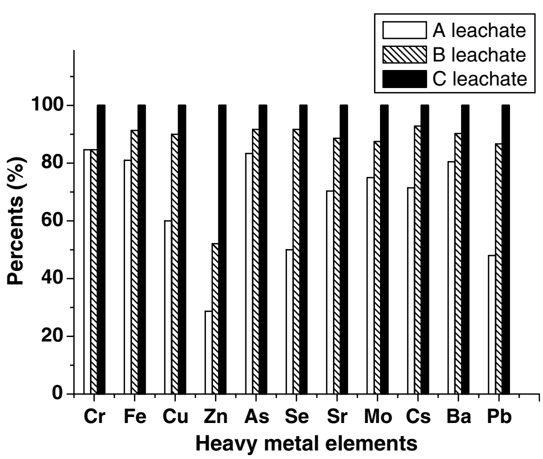

Fig. 2. The comparison of the metal elements leached by the different extraction fluids (The percent of each metal element was the ratio of its concentration in the A leachate, or it concentration in the B leachate, to its concentration in the C leachate.).

Table 2
The frequencies of micronucleus in the *Vicia faba* root tip cells

Tested materials	Numbers of cells observed	Micronucleus frequencies (‰)
A leachate	6048	6.1 ± 0.8*
B leachate	6120	7.4 ± 1.5*
C leachate	6023	4.8 ± 1.6*
Negative controls	6123	3.4 ± 0.8
Positive controls	6084	15.5 ± 2.6**

$*P < 0.05$, $**P < 0.01$, (significant differences between negative control and exposed groups by Dunnett's *t*-test).

With comparative investigation, Chang et al. (2001) pointed that the results of the TCLP test for BA can be used to characterize heavy metals mobility and simulate a field leaching scenario with which the amount of heavy metal elements leached by typical acid rain in Taiwan. The soils in Macao are the typical acidic red-soils (pH 4.5–6.5), with abundant organic materials and vigorous microbial activities. The average precipitation of a year was about 1869.4 mm in Macao in the last century (SMG, 2000), and the pH values of acid rains were among 4.0–5.0 during 1991–1997, with about a frequency of 60–80% (Li, 2003). Therefore, the extraction fluid B (pH 4.9) may better simulate the amount of heavy metal elements in the landfills leached by typical acid rain in Macao. However, the environment is very dynamic compared to a laboratory situation, and it is difficult, for example, to judge which chemical and/or biological process taking place could influence the leaching behaviors of the elements in the BA and their biological or ecological effects (Thipse and Dreizin, 2002; Pasquini, 2006). Therefore, it is worth further investigation via a field scenario.

In this study, our results clearly demonstrated that MSWIBA leachates had genotoxicity on *Vicia faba* root cells as other researches did (Radetski et al., 2004). Bekaert et al. (1999) demonstrated that the aqueous leachates from a landfill of MSWI ash had a significant genotoxicity on the amphibian erythrocytes with MN assay. However, in many countries and territories (such as USA, some OECD countries, China), BA is not included in the List of Hazardous Wastes, being dumped into landfills directly or after maturation (Gau and Jeng, 1998; Ibanez et al., 2000; Lapa et al., 2002). Therefore, we suggested that the comprehensive evaluation of the environmental impacts of BA is necessary before decisions can be made on the utilization, treatment or disposal of BA.

4. Conclusion

Different metal elements in the BA exhibited different leaching behavior. Particularly, the concentrations of Pb in the leachates were very high. The dissolution and the pH value of the extraction fluids were two of the main factors that determine the leaching process for most metal elements in the BA.

The MSWIBA leachates were found to be genotoxic with the MN assay in *Vicia faba* root tip cells.

Our results suggest that both chemical and biological approaches are necessary to evaluate the environmental impacts and risks of BA before sound decisions can be made on its utilization, treatment or disposal.

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Shaolong Feng, an associate professor in the School of Public Health, Nanhua University, China, is now involved in research on contaminants occurred in natural environment and their risks to ecosystems.