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Evaluating the mutagenicity of leachates obtained from the bottom ash of a municipal solid waste incinerator by using a Salmonella reverse mutation assay

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HIGHLIGHTS

- The mutagenicity of leachates from the bottom ash was evaluated using an Ames assay.
- The metal contents in TCLP leachates were all below the Taiwanese regulatory limits.
- Leachate A from nonsieved and <4.75-mm-sieved bottom ash showed mutagenicity.
- Leachate A from sieved ash displayed stronger mutagenicity than that from nonsieved.
- The chemical composition and mutagenicity of leachates must be analyzed simultaneous.

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ABSTRACT

The mutagenic potential of leachates derived from the bottom ash of a municipal solid waste incinerator in Taiwan were evaluated using an Ames Salmonella mutagenicity assay with three standard tester strains, TA98, TA100, and TA1535. Three types of leachants, leachant A (pH 4.93), leachant B (pH 2.88), and leachant C (deionized water, pH 6.0), were carried out according to toxicity characteristic leaching procedure (TCLP). Moreover, two types of bottom ash, nonsieved and sieved bottom ash (particle size <4.75 mm), were analyzed with the TCLP and the Ames assay. The concentrations of five heavy metals (Cd, Cr, Cu, Pb, and Zn) in the leachates were also estimated with an ICP-OES. The results indicated that the metal concentrations in the TCLP leachates of bottom ash were all below the limits set by Taiwanese regulations. However, leachate A from nonsieved and <4.75-mm-sieved bottom ash showed mutagenicity. Moreover, leachate A from <4.75 mm-sieved bottom ash displayed stronger mutagenicity than that from nonsieved ash. The leachate A from <4.75-mm-sieved bottom ash, that were diluted by 100-fold showed no mutagenicity. In conclusion, our results suggested that the chemical composition and mutagenic potential of leachates should be monitored to evaluate the safety of bottom ash.

1. Introduction

In Taiwan, municipal solid waste is treated mainly by incineration. According to the statistics provided by the Taiwan Environmental Protection Agency (EPA), the amount of waste treated by incineration was 5.962 million metric tons (MT) in 2011. This resulted in the production of 1.237 million MT of ash, including 0.969 million MT of bottom ash and 0.268 million MT of fly ash (http://www.epa.gov.tw) Taiwan EPA, 2014. In Taiwan, the hazardous properties of solid wastes are evaluated by toxicity characteristic leaching procedure (TCLP). Incinerator fly ash and bottom ash are considered hazardous and nonhazardous, respectively. Therefore, fly ash must be solidified before it is sent to landfills. For treating the bottom ash, two approaches have been developed, including recycling and disposal in landfills. Of these, the reuse of bottom ash as materials for road construction and cement manufacturing has been widespread (Bruder-Hubscher et al., 2001; Huang et al., 2006; Lin and Lin, 2006). At present, Taiwan's incinerator bottom ash recycling rate is approximately 54%, with 46% of all bottom ash being sent to landfills.

Although incinerator bottom ash is considered nonhazardous, it contains a relatively high concentration of heavy metals and a low concentration of organic compounds (Liu et al., 2008). Previous

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studies, which have investigated the possibility of heavy metals in bottom ash being released into the environment, have provided valuable information about the leaching behavior of heavy metals (Jeong et al., 2005; Liu, 2005; Dijkstra et al., 2006; Liu et al., 2008). However, the potential environmental impact of bottom ash is not yet fully understood, irrespective of whether it is reused for construction or is sent to landfills. It is therefore important to assess the biotoxicity of incinerator bottom ash, especially the chemical composition of leachates derived from the bottom ash.

Recently, several methods have been used to dissect the ecotoxicological effects of municipal solid waste incinerator (MSWI) ash (Lapa et al., 2002; Lin and Chen, 2006; Feng et al., 2007; Huang et al., 2008; Wang et al., 2008; Chou et al., 2009; Stiernstrom et al., 2014) and other solid waste (Bekaert et al., 1999; Monarca et al., 2002; Singh et al., 2007; Lah et al., 2008; Huerta et al., 2013). Among these approaches, the Ames Salmonella mutagenicity assay has been widely accepted and used to evaluate the carcinogenic potential of a variety of substances (Bekaert et al., 1999; Monarca et al., 2002; Mouchet et al., 2006; Singh et al., 2007; Lah et al., 2008; Magdaleno et al., 2008; Chakraborty and Mukherjee, 2009; Claxton et al., 2010). This test is one of the most reliable short-term bacterial test systems, and it detects the mutagenic effects of chemicals or environmental samples by measuring the histidine-independent revertants in *Salmonella typhimurium*. More importantly, the test results are obtained rapidly and have been extensively validated (Maron and Ames, 1983; Zeiger, 1987; Shane et al., 1993; Mortelmans and Zeiger, 2000). The chemical compositions and mutagenicity of ashes from the operating municipal refuse incinerators in the United States have been evaluated (Alarie et al., 1989; Shane et al., 1990; Shane et al., 1993). However, to the best of our knowledge, the Ames assay has not been used to detect the mutagenic activity of bottom ash in Taiwan.

In Taiwan, the resource recovery program of municipal solid waste has been promoted since 1998. The resource (including natural resources, kitchen waste, and bulk waste) recovery rate reached 65.41% in 2012 (http://www.epa.gov.tw) Taiwan EPA, 2014. After the recovery, the surplus of municipal solid waste is treated mainly by incineration. As a result, the chemical properties of MSWI bottom ash in Taiwan are different from those of the ashes processed in other countries. In this study, the mutagenic potential of the leachates that were derived from MSWI bottom ash was evaluated with an Ames assay with three standard *Salmonella typhimurium* tester strains, TA98, TA100, and TA1535. It is well known that samples composed of fine particles usually contain higher levels of heavy metals and organic compounds than do the samples comprising coarse particles (Chang et al., 2000; Liu et al., 2008; Shen et al., 2013). Therefore, nonsieved and sieved bottom ash (particle size less than 4.75 mm) were examined using the TCLP and Ames analysis. We selected particles of sizes less than 4.75 mm because they constitute the bottom ash that is used in road construction and cement manufacturing in Taiwan (http://depweb.ksepb.gov.tw/waste/d.htm) Environmental Protection Bureau of Kaohsiung City Government, 2014. Moreover, leachant A (pH 4.93), leachant B (pH 2.88), and leachant C (deionized water, pH 6.0) were carried out with the TCLP test that has been recommended by the Taiwan EPA. Leachant C was examined as a template of acid rain because the pH of acid rain in Taiwan ranged between 5.0 and 6.0 in 2012 (http://acidrain.epa.gov.tw/now/04.htm) Acid Rain in Taiwan, 2014. It is also known that heavy metals in bottom ash may have a long-term impact on the environment. Therefore, the concentrations of five heavy metals (Cd, Cr, Cu, Pb, and Zn) in the leachates were measured using inductively coupled plasma-optical emission spectrometry. The possible relationship between the concentrations of heavy metals and the mutagenic effects are also discussed in this study.

2. Experimental

2.1. Sampling of bottom ash

The MSWI bottom ash used in this study was collected directly from an incinerator located in Taiwan, which has been operational since 1995. The incinerator is a mass-burning type, and its treatment capacity is 1350 tons d^{-1}. The combustion temperature is 850–1050 °C, and the air-pollution control device is a dry scrubber that is integrated with a baghouse. The fly ash is solidified and is then sent to landfills. The bottom ash is recycled for road construction and cement manufacturing. For this study, the bottom ash was dried in an oven at 105 °C, crushed, screened, and sieved. The sizes of particles of the bottom ash used in the TCLP procedure were categorized as nonsieved and sieved (particles <4.75 mm).

2.2. Leaching procedure

The leaching procedure, which followed the TCLP test (NIEA R201.14C) as recommended by the Taiwan EPA, was specifically modified to include the use of deionized water as a leaching agent instead of acetic acid (CH_3COOH). Briefly, three replicate samples were leached by three different leachants, including two acidic leachants (A: pH 4.93 and B: pH 2.88; all preparing by using acetic acid) and deionized water (C: pH 6.0). Leachant C was used as a stand-in for the acid rain in Taiwan. The solid/liquid ratio was 1:20 following the leaching procedure with a speed of 30 rpm for 18 h. The leachates were then filtered through a 0.45-μm filter. Each leachate was immediately analyzed for metal content and was evaluated for mutagenicity with the Ames test, as described below. The concentrations of five heavy metals (Cd, Cr, Cu, Pb, and Zn) in the leachates were determined with an inductively coupled plasma-optical emission spectrometer (Optima 2100DV, PerkinElmer Inc., Waltham, MA, USA). The r^2 values of the calibration curves of Cd, Cr, Cu, Pb, and Zn were greater than 0.995. The analysis of each leachate was conducted three times, and the average concentrations of the heavy metals were obtained.

2.3. Ames test/Salmonella mutagenicity test

The Ames/*Salmonella* mutagenicity test was performed with a standard plate incorporation assay with the *Salmonella typhimurium* strains TA98, TA100, and TA1535 with and without metabolic activation (Mortelmans and Zeiger, 2000). Briefly, a preliminary toxic dose range experiment was conducted first to select an appropriate dose range for the mutagenicity test. TA98, TA100 or TA1535 were growth in nutrient broth (NB) at 37 °C for 18 h, and then being diluted for 10^6 and 10^7 folds by PBS. Harvested leachates of 5, 50, 100, 200, 250 or 500 μL were mixed with diluted bacteria (100 μL) and soft agar (2 mL). Then, mixed solutions were spread on to NA plates and incubated for 24 h. Cytotoxicity effects of leachates were determined by comparing the bacterial counts between leachates and blank control (deionized water) groups. The results have shown that 500 μL/plate of different leachates can be used for Ames assay. Thus, five volume levels (100, 200, 300, 400, and 500 μL/plate) of leachates were selected according to a previous report and being subjected to mutagenicity test (Radetski et al., 2004).

3. Results and discussion

3.1. Metal concentrations in the leachates

Incinerator bottom ash contains a low concentration of organic compounds and a relatively high concentration of heavy metals. It