Aerosol and Air Quality Research, 13: 1002–1008, 2013

ISSN: 1680-8584 print / 2071-1409 online
doi: 10.4209/aaqr.2012.10.0267

Toxicity Evaluation of Fly Ash by Microtox®

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ABSTRACT

Fly ash samples from a cooling tower were extracted after incinerating plastic solid waste (PSW) and organic liquid waste (OLW) by n-Hexane or dichloromethane/n-Hexane mixtures to evaluate the toxicity. The metal and PCDD/Fs were analyzed by ICP OES and HRGC/HRMS, respectively. The toxicity of the extracted fly ash was evaluated by Microtox®. The results showed the environmental risk factor (ERF) of Hg in PSW fly ash was the highest compared to other metals, by more than 60%. Additionally, the acute toxicity tests of the fly ash showed that dichloromethane/n-hexane extracts were all very toxic, except for the PSW-1 obtained through Soxhlet extraction following the column clean-up procedure. The n-Hexane extracts for OLW-1 obtained through Soxhlet extraction following the column clean-up procedure were extremely toxic. There were no significant relationships among the concentrations of the regulated heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn), PCDD/Fs concentration and the TU values in the toxicity test. Furthermore, the results of the statistical analysis showed that there were significant differences in the results of the Microtox test with regard to the solvents, solutes and various extraction methods. However, it is remains a complicated process to differentiate between the various compounds in order to produce accurate results with regard to acute toxicity in the fly ash, and thus this issue warrants further investigation.

Keywords: Microtox test; Toxicity; Extraction; Fly ash.

INTRODUCTION

Incineration is still the main treatment method for wastes in Taiwan. More than 1.2 millions of ash were produced in 2011 and nearly 60% of them will be landfilled as final treatment (EPA, Taiwan). It was unknown how many ashes are classified as hazardous wastes because of the complicated measurements. Many research efforts are focused on the investigation of the ash' physical structure (Ontiveros *et al.*, 1989; Álvarez-Ayuso *et al.*, 2008) and chemical composition (Criado *et al.*, 2004), the reuse of the fly ash (Mangialardi, 2001), or the detection of metals concentration (Karuppiah and Gupta, 1997; Prokop *et al.*, 2003; Kalderis *et al.*, 2008) or PCDD/F concentration in the fly ash (Lin *et al.*, 2011; Chin *et al.*, 2012). Chemical analysis assists in determining the anthropogenic concentration and provides estimates of their distribution (Chiu *et al.*, 2011; Huang *et al.*, 2011). However, the chemical data alone provide no direction as to the potential effects on the environment.

Toxicity bioassay has been widely applied in water (Gutiérrez *et al.*, 2002; Sarmiento *et al.*, 2011), sediment (Jacobs *et al.*, 1993; Niemirycz *et al.*, 2007) or soil (Maxam *et al.*, 2000; Loureiro *et al.*, 2005). Algae (Hörnström, 1990), daphnia (Guilhermino *et al.*, 2000) or fish (Lammer *et al.*, 2009) have been used to process many toxicity tests; however, these tests are time and cost consuming and some test organisms require further culturing. Table 1 compared the advantages of various test organisms, bioassay based on bacteria (such as Microtox®) implied a simple procedure, short testing time and high convenience. Additionally, Microtox® is distributed in many countries and is recommended by standards in America, Germany and Poland (standard methods, 1995, ISO/DIS 11348, DIN 38412) and performed as a rapid, economical monitoring tool for toxicity of environmental contaminants. It was applied in metal plating wastewater (Choi and Meier, 2001) and air

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Table 1. Comparison of testing organism in toxicity evaluation (Hsu, 2009).

testing organism	Fish, shrimp, daphnia and algae	bacteria
Testing time	long	short
Culturing technique	high	low
Testing procedure	complex	simple
cost	high	low
Testing volume	liters	milliliters
convenience	low	high

pollutant (Lin and Chao, 2002). However, limited studies were found to discuss the biotoxicity (Chakraborty and Mukherjee, 2009; Chou *et al.*, 2009) for the fly ash. In this study, Microtox® was applied to evaluate the acute toxicity of extracted fly ash and the feasibility to be a fast-prescreening tool.

MATERIALS AND METHODS

Fly ash samples were taken from a laboratory waste incinerator in the southern part of Taiwan. The incinerator is equipped with the followings: first cooling tower, secondary cooling tower, bag filter and scrubber which all serve as air pollution control devices. The fly ash from the first cooling tower and secondary cooling tower were collected by two different feeding wastes: Plastic solid waste (PSW) and organic liquid waste (OLW) following Taiwan's National Institute of Environmental Analysis (NIEA) method R118.02B. The ash sample was simplified as PSW-1 to represent fly ash from the first cooling tower by feeding plastic solid waste; PSW-2 to represent fly ash from the secondary cooling tower by feeding plastic solid waste; OLW-1 to represent fly ash from the first cooling tower by feeding organic liquid waste and OLW-2 to represent fly ash from the secondary cooling tower by feeding organic liquid waste.

Two kinds of solvents: n-hexane (Hx) and dichloromethane/n-hexane (DCM-Hx) via two kinds of extraction methods: sonication and Soxhlet were chosen separately. Two grams of fly ash were extracted with 300 mL solvents. These extracted samples were divided into two parts: one was evaporated to near dryness and dissolved in dimethyl sulfoxide (DMSO). The other one was also evaporated to near dryness and then transferred to the CAPE-coupled carbon-acid silica column for cleanup. The cleanup procedure using the CAPE- coupled carbon-acid silica column was the one previously described in detail (Chen *et al.*, 2007; Lee *et al.*, 2009).

All solvents were pesticide residue grade from Merck (Darmstadt, Germany), Tedia (Fairfield, USA), or Sigma-Aldrich (St. Louis, USA). Silica gel (100–200 mesh) was obtained from Merck (Darmstadt, Germany). A rapid cleanup system by coupled acid silica column-activated carbon mini-column (CAPE-coupled carbon-acid silica column) was developed by CAPE Technologies (South Portland, USA).

Metal Analysis

Inductively coupled plasmaoptical emission spectrometry (ICP-OES, VISTA-MPX, Varian) was used to determine the presence of Ag, As, Ba, Cd, Cr, Cu, Hg, Mn, Na, Ni, Pb, Se, and Zn following U.S. EPA Method 200.7. All the dehydrated solid specimens were pretreated with microwave-assisted acid digestion, following NIEA method R317.10C (equivalent to U.S. EPA Method 3015A). The specimens were digested using a 400-W microwave MARS/MARS Xpress CEM microwave, with an 800 psi limit at 200°C for 15 minutes. Additionally, the toxicity characteristic leaching procedure (TCLP) was performed to examine the leachability of the ash samples following NIEA method R201.14C (equivalent to U.S. EPA SW846 Method 1311).

PCDD/F Analysis

Standard solutions of PCDD/Fs (1613-LCS, labeled compounds stock solution; 1613-ISS, internal standard spiking solution; 1613-CSS, cleanup standard spiking solution; 1613CVS, EPA Method 1613 calibration and verification solution) were purchased from Wellington Laboratories (Ontario, Canada). Silica gel (100-200 mesh) was obtained from Fisher (Leicestershire, England). For the HRGC/HRMS method, all samples were added with the different PCDD/F internal standards before extraction and the labeled cleanup standards for PCDD/F analysis were added before the CAPE-coupled carbon-acid silica column cleanup (Mi *et al.*, 2012). The extract was analyzed using a HRGC/HRMS (HP6890/JEOL JMS-700) equipped with a DB-5MS 60 m column. The quality of QA/QC met the criteria of Taiwanese EPA Method.

Microtox Analysis

For microtox tests, extracts were placed in 10-ml graduated concentrator tubes, and solvents was removed from the extracts in a 25°C temperature water bath by introducing a constant stream of nitrogen over the solvent surface. Once the volume was reduced to near dryness and exchanged by 1 mL DMSO (HPLC- grade). The DMSO extracts were placed in borosilicate glass vials and stored at 4°C for microtox analysis.

Microtox Model 500 analyzer (SDIx, USA) was used for analysis and all detailed procedures were followed in the Microtox Users Manual. Freeze-dried luminescent bacteria, *Vibrio fischeri* were reconstituted and exposed in duplicate to a series of four diluted DMSO extracts, osmotically adjusted to a salt content of 2% and using one saline water control. The solvent vehicle, DMSO, at volumes not exceeding 2.5% of the total assay volume did not significantly affect light emission (Kahru *et al.*, 1996). The resulting decrease in bioluminescence was measured after 5 and 15 minutes at a constant temperature of 15°C. Fifteen

minute data are reported in this study. All Microtox data were recorded and analyzed by on-line software, and results are expressed as the Effective Concentration 50% (EC_{50}) in percent of extract per 2 g ash sample.

Toxicity Unit (TU) was recommended and identified as follows:

$$TU = (1/EC_{50}) \times 100\% \quad (1)$$

TU is unitless. The high TU value indicates high toxicity. However, TU is relative toxicity classified into four categories as Table 2.

To ensure reliability of the Microtox method and reagents, a toxicity test using an aqueous solution of phenol (100 mg/L) was done to confirm each day prior to beginning sample tests. This check was then compared with the Microtox quality assurance product data accompanying the reagent. Procedural blanks were also prepared from cleaned filters and cartridges extracted as described above and were tested to determine if any toxicity was being contributed by the residual extracts and glassware. In this study no toxicity was detected in these blanks.

Statistical Analysis

Analysis of variance conducting by SPSS 11.0 was applied in this study to investigate the effect of experimental parameters on the analysis results. The test was performed to compare the variation of TU values in various solvent extracts, extraction methods and clean-up over four various ash samples.

RESULTS AND DISCUSSION

Environmental Risk Factor

To evaluate the environmental risk factor (ERF) for the regulated metals in the ash, ERF (Sarmiento *et al.*, 2011) was defined as:

$$ERF = (C_n - C_{SQV})/C_{SQV} \quad (2)$$

Table 2. Category of TU (Kahru *et al.*, 2000).

TU	Toxicity
< 1	Non toxic
1–10	Toxic
10–100	Very Toxic
> 100	Extremely Toxic

C_n is the toxic element concentration; C_{SQV} is the highest concentration of the studied element non-associated with biological effects defined by Sarmiento *et al.* (2011). It's hard to define the highest concentration of the studied element not associated with biological effects in ash samples, so the regulated concentration in soil was adopted in this study to represent C_{SQV}. Fig. 1 showed the environmental risk factors for the ash. Incinerating PSW led to high Hg ERF warning about a potential adverse biological effect associated with Hg and warrants further investigation in the future.

Solvent Blank

To understand the effects of various solvents on TU, a solvent blank was tested. Hx, DCM-Hx and DMSO were tested following the procedure of extracted samples to compare the TU by individual solvent. Table 3 showed no toxic for DMSO, toxic for n-Hexane (Hx) and very toxic for dichloromethane/n-Hexane (DCM-Hx). Accordingly, all extracts were transferred into DMSO to avoid the interference of extracting the solvent's toxicity. Furthermore, the water extracted samples were also tested to simulate the rainfall condition, however, no any toxic result was found.

Toxicity Test

Tables 4 and 5 summarizes the acute toxicity of the ash samples based on Table 1. To understand the effects of various solvent extracts on TU, n-Hexane and dichloromethane/n-Hexane solvents were chosen to extract ash samples. These two kinds of solvents were always used to study the organic compounds in samples. Table 4 showed that the toxicity of

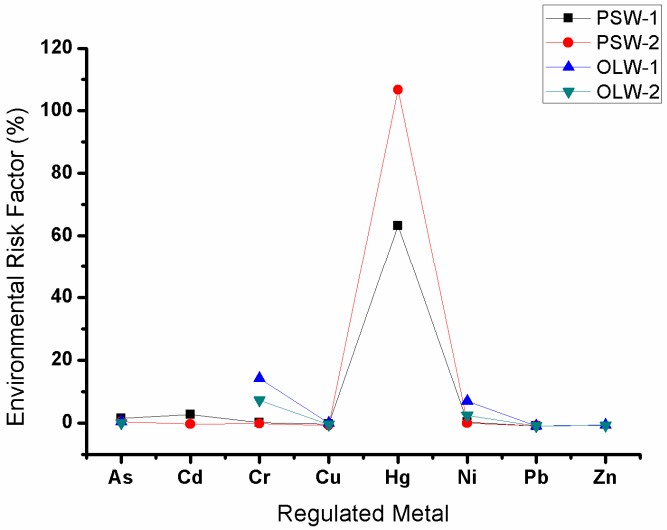

Fig. 1. Environmental risk factors for the ashes.

Table 3. The toxicity of solvent blank.

Solvent \ Toxicity	EC_{50} (%) 15 min	TU (%) 15 min
DMSO	NA	NA
n-Hexane	26.5	3.77
Dichloromethane/n-Hexane	8.98	11.1

NA: not available

concentrated samples (without cleanup) was evidently higher than that of the cleanup samples by sonication extraction based on n-Hexane. However, the result for soxhlet extraction is different. The toxicity of cleanup samples was evidently higher than that of concentrated samples by the soxhlet extraction based on n-Hexane solvent.

The result of Table 5 showed the toxicity for all samples were very toxic, except PSW-1 for cleanup samples by soxhlet extraction based on dichloromethane/n-Hexane solvent. The toxicity of these four dichloromethane/n-Hexane extracted samples showed no obvious difference. To further investigate the results, the regulated metal concentration and PCDD/Fs was compared with the results of toxicity.

Fig. 2 showed the acute toxicity mean value (TU%) for the concentrated samples and the corresponding PCDD/Fs concentration. Only n-Hexane sonicated extraction sample revealed to have a similar trend with PCDD/Fs concentration.

Fig. 3 showed the acute toxicity mean value (TU%) for the clean-up samples and the corresponding PCDD/Fs concentration. No similar trend was found in Fig. 3. Nor in the metal concentration and acute toxicity, where no obvious correlation was found.

The analysis of variance (ANOVA) was applied to interpret the effect of the solvent, extraction method and cleanup process on acute toxicity. The result was reported in Table 6. The p-values for the three factors: solvent, solute and extraction were all smaller than 0.01. This implies that the different levels of the three factors affected the observed amount of acute toxicity. The mean effect of clean-up on the observed amount of acute toxicity is not statistically different (p-value > 0.3) whether the sample is clean-up or not. It is also seen from the table that, except (1) cleanup *extraction and (2) solute *extraction, almost all two-factor

Table 4. Acute toxicity of ash sample extracted by n-Hexane.

Toxicity/TU	n-Hexane	PSW-1	PSW-2	OLW-1	OLW-2
Sonication extraction	Concentrated	very toxic 35.7	very toxic 39.5	very toxic 36.5	very toxic 95.9
	Clean-up	toxic 5.47	toxic 5.85	toxic 6.75	toxic 4.96
Soxhlet extraction	Concentrated	toxic 9.55	very toxic 19.2	toxic 2.64	very toxic 63.8
	Clean-up	very toxic 99.0	very toxic 66.4	extremely toxic 103	very toxic 74.1

Table 5. Acute toxicity of ash sample extracted by dichloromethane/n-Hexane.

	Dichloromethane/ n-Hexane	PSW-1	PSW-2	OLW-1	OLW-2
Sonication extraction	Concentrated	very toxic 24.2	very toxic 33.3	very toxic 48.7	very toxic 24.8
	Clean-up	very toxic 32.3	very toxic 39.0	very toxic 28.6	very toxic 41.0
Soxhlet extraction	Concentrated	very toxic 16.3	very toxic 97.0	very toxic 39.3	very toxic 27.8
	Clean-up	toxic 7.20	very toxic 10.3	very toxic 12.6	very toxic 27.4

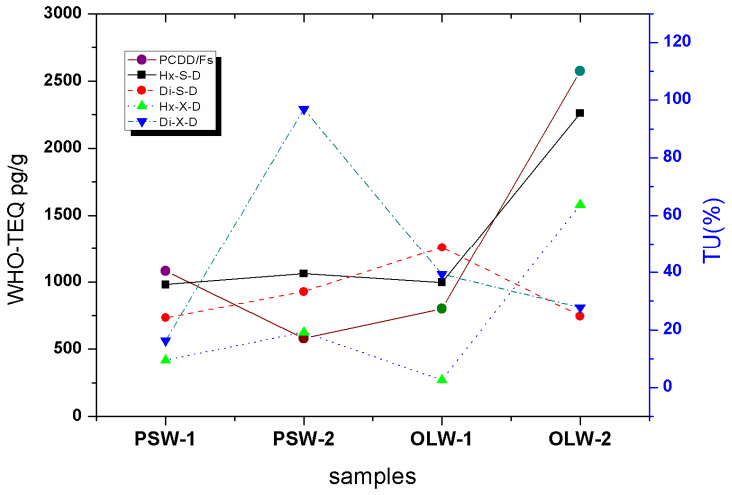

Fig. 2. Acute toxicity mean value (TU%) for concentrated samples and corresponding PCDD/Fs concentration (Hx-S-D: n-Hexane solvent-sonication-concentrated; Di-S-D: Dichloromethane/n-Hexane solvent-sonication-concentrated; Hx-X-D: n-Hexane solvent-soxhlet-concentrated; Di-S-D: Dichloromethane/n-Hexane solvent-soxhlet-concentrated).

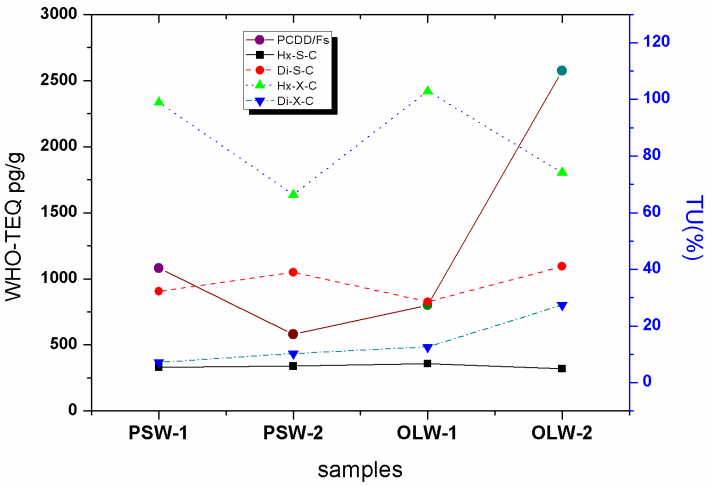

Fig. 3. Acute toxicity mean value (TU%) for clean-up samples and corresponding PCDD/Fs concentration.

Table 6. The result of ANOVA.

sources of variation	sum of square	df degree of freedom	Mean square	F	significance
Cleanup or not	220	1	220	1.09	0.301
Solvent	26540	2	13270	65.3	0.000
Solute	2679	3	893	4.39	0.006
Extraction	1588	1	1588	7.81	0.006
Cleanup or not * solvent	7991	2	3995	19.7	0.000
Cleanup or not * solute	6256	3	2085	10.3	0.000
Cleanup or not * extraction	473	1	473	2.33	0.131
solvent * solute	10031	6	1671	8.22	0.000
solvent * extraction	9579	2	4789	23.6	0.000
solute * extraction	1063	3	354	1.74	0.164
Cleanup or not * solvent * solute	9082	6	1513	7.45	0.000
Cleanup or not*solvent*extraction	43263	2	21631	106	0.000
Cleanup or not *solute*extraction	3824	3	1274	6.27	0.001
solvent * solute * extraction	5321	6	886	4.36	0.001
Cleanup or not * solvent * solute * extraction	5533	6	922	4.54	0.000
error	17282	85	203		
Sum	280876	133			

a. $R^2 = 0.938$ (adjusted $R^2 = 0.904$).

interactions have an effect on the acute toxicity. Finally, all three-factor interactions and the four-factor interactions are also valid from the p-values reported in the table. It would be warranted as a future research topic to understand why interactions affect the acute toxicity.

ACKNOWLEDGEMENTS

The study was supported by the National Science Council, Taiwan, under grant NSC 100-2221-E-033 -009 -MY2.

CONCLUSIONS

1. The toxicity test only provided reference data for acute toxicity, not for qualification and quantification.
2. The analysis indicated solvents, solutes and extraction methods showed the statistically significant on acute toxicity.
3. For sonication samples, TU was very toxic for concentrated samples; however, TU was very toxic for samples with the clean-up process based on dichloromethane and n-Hexane mixtures. An acute toxicity test for the n-Hexane extracted sample showed toxic.
4. For soxhlet samples, TU was very toxic for concentrated samples with dichloromethane and n-Hexane mixtures. Only PSW-2 and OLW-2 showed to be very toxic. Clean-up samples by dichloromethane and n-Hexane mixture showed very toxic except PSW-1; samples by n-Hexane showed toxic except OLW1.

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Received for review, October 5, 2012
Accepted, January 7, 2013