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Research article

An emerging pollutant contributing to the cytotoxicity of MSWI ash wastes: Strontium

Wu-Jang Huang[a,*], Hsing-Chuan Tang[a], Kae-Long Lin[b], Ming-Huei Liao[c]

[a] Department of Environmental Engineering and Science, National Ping-Tung University of Science and Technology, 912 Ping-Tung, Taiwan
[b] Department of Environmental Engineering, National I-Lan University, 260 I-Lan, Taiwan
[c] Department of Veterinary Medicine, National Ping-Tung University of Science and Technology, 912 Ping-Tung, Taiwan

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ABSTRACT

In this study, we used the multiple toxicity characteristic leaching procedure to test the long-term leaching behavior of bottom ash, scrubber residue, and baghouse ash from a municipal solid waste incinerator (MSWI). We used the short-term viability percentage of African green monkey kidney cells (Vero cells) as a bioindicator to investigate the cytotoxicity of the leachates from the MSWI ash wastes. We found that strontium was a significant contributor to the cytotoxicity of the bottom ash.

1. Introduction

In Taiwan, the degree of treatment of municipal solid waste (MSW) in incinerators is ca. 82% [1,2]. Municipal solid wastes incineration (MSWI) generates several ash wastes, including cyclone ash (CA), scrubber residue (SR), baghouse ash (BaA), and bottom ash (BoA) [3–5]. The toxicity characteristic leaching procedure (TCLP) is a widely employed method for analyzing the hazardous nature of waste materials. Previously, we reported the chemical compositions, co-leaching behavior, and cytotoxicities of pure SRs and pure BaAs from seven of Taiwan's MSWI plants [2,6,7] as well as the cytotoxicity of the flue gas obtained after heating MSWI BaA [8]. Previous studies of the *E. coil* toxicities of several of Taiwan's MSWI ash wastes [9,10] revealed that they followed the order SR > BoA > CA, although the SR samples used were mixed with BaA. In an earlier investigation, we determined the TCLP extract cytotoxicities of individual ash wastes collected from several MSWI plants [7]. The toxicity toward African green monkey kidney cells (Vero cells) followed the order BaA > SR > BoA; toward pig kidney cells (PK-15 cells; short contact time) it followed the order SR > BaA > BoA.

The genotoxicity of MSWI BoA has been studied by Feng et al. [11], Radetski et al. [12], Bekart et al. [13], and Silkow et al. [14], who found that the leachate of BoA has toxic effects on *Vicia faba* root tip cells and amphibian erythrocytes. Another two papers reported the cytotoxicity [15] and ecotoxicity [16] of healthcare incineration BoA and MSW incineration BoA, respectively.

Several researchers have investigated the mechanism of cell death caused by coal fly ash. Ali et al. [17] undertook a cytological examination of the apoptotic effect of coal fly ash leachate in fish hepatocytes. Their study of the "seven-day leachate" of coal fly ash clearly revealed an apoptosis effect toward freshwater fish (*Channa punctata Bloch*). The particle-induced cell-death by alveolar macrophages was also investigated in two other studies [18,19], which found that the toxicity increased upon decreasing the particulate size. In this present paper, the effect of particle size can be ignored because we used the extracts of MSWI ashes, all of which were filtered prior to performing the cytotoxicity tests.

Recently, when we used the multiple toxicity characteristic leaching procedure (MTCLP) on MSWI ash wastes to determine the long-term leaching cytotoxicity of MSWI ash wastes, we found that the undiluted leachates from BoA and SR had higher cytotoxicities than that from BaA (see below). Because MSWI BoA is classified as a non-hazardous material (it has been used as a recycled material in civil engineering and landfills), in this study we investigated the major contributors to the cytotoxicity of BoA.

* Corresponding author. Tel.: +886 8 7703202x7076; fax: +886 8 7740256.
E-mail address: wjhuang@mail.npust.edu.tw (W.-J. Huang).

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2. Materials and methods

2.1. Sampling of ash wastes

An MSW incineration plant located in southern Taiwan having a treating capacity of 1350 tons/day was selected as the site from which to collect combustion ash wastes. All incinerators were of the mass-burn type and the operation temperatures ranged from 950 to 1050 °C. The general industrial solid waste was mixed to within 30% into the MSW for combustion. The air pollution control device (APCD) of the incinerator was a semi-dry lime scrubbing system equipped with a fabric filter. The APCD residues, including the SR, BaA, and incinerator BoA, were sampled from the manhole separately and stored in high-density polyethylene (HDPE) bottles at temperatures below 4 °C prior to analysis. When sampled, the BoA had not been mixed with either the boiler ash or the grid ash. Seven MSWI baghouse ash samples (named P1, P2, P3, P4, P5, P6, and P7) were collected from the manholes of the baghouse at large MSWI plants.

2.2. Normal TCLP and multiple TCLP tests

The normal TCLP test, following USA EPA Method #1311, is a widely employed essential test for assessing the leaching properties of hazardous wastes. The sampled ashes were graded by passing them through a 10-mm standard sieve. Because the values of pH of the ashes were all greater than 5.0, the pH of the extractant was chosen to be 2.88. Samples were mixed with an acidic extractant in a liquid-to-solid ratio of 20:1 and agitated at a rotation rate of 30 rpm for 18 h. The MTCLP test employed the same operating conditions as those for the normal TCLP, except that the samples was extracted several times: on the first day, TCLP was performed using a fresh sample; after the first day's extraction was complete, the residual solid in the extract was filtered and used as the sample for the second day with a fresh extractant, and so on, repeated seven times (days).

A PerkinElmer Optima 3000XL ion-coupled plasma (ICP) detector was used to monitor the concentrations of the species in the final extracts (following US EPA Method #7420). A Fisher Scientific Accruement pH meter was used to probe the pH of the solutions.

2.3. Determination of total organic carbon in final extracts

The total organic carbon (TOC) contents of the final extracts were measured using a Shimadzu TOC-5000 instrument. Potassium hydrogenphthalate, sodium carbonate, and sodium hydrogencarbonate were used as standards; the maximum heating temperature was 680 °C.

2.4. Cytotoxicity tests

2.4.1. Principle of testing method

The cytotoxicity of each extract was measured toward African green monkey kidney cells (Vero cells), which were purchased from the Bioresource Collection and Research Center (BCRC) of Taiwan (code number: BCRC-60013). The mode of toxicity of the incineration ashes toward the digestion systems of the animal cells was tested. The measured biological endpoint was the release of lactate dehydrogenase (LDH) from cells when they died. The quantitative data were measured using a Dynex-MRX reader (Dynex Technologies, USA). Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), and trypsin versene solution (TVS) were obtained from HyClone Co. (USA). The cytotoxicity detection kit (LDH) was obtained from Roche Applied Science. The dilution of all extracted samples was performed using deionized water.

2.4.2. Cell line conditioning

Vero cells were cultured for several days in DMEM containing 1% FBS. The cultured cells were then transferred to a 96-well plate at a cell density of 10,000 cells/well and incubated continuously at 37 °C for 24 h under an atmosphere of 5% CO_2. 0.25% TVS was used as the digest solution to separate the single cell layer from the bottom of the incubation bottle to form a clear cell-suspended solution.

2.4.3. Choice of positive and negative controls

The cytotoxicity of the TCLP extracts of MSWI ash wastes and mixtures of pure semi-dry scrubber residue and baghouse ash have been tested previously toward African green monkey kidney cells (Vero), baby hamster kidney cells (BHK-21), and pig kidney cells (PK-15) [8]. Because only Vero cell exhibited high sensitivity toward the compositional changes of the TCLP extracts from MSWI ashes, they were selected for this present study. An acetic acid-based extractant was used as a negative control for the cytotoxicity test (see Section 2.4 for details). Triton X-100 was used as a positive control.

2.4.4. Assay procedure

Each cell suspension solution was diluted using an appropriate medium to adjust the cell number to 1×10^5 cells/L before being seeded into a 96-well tissue culture microplate (100 μL/well). The microplate was incubated overnight (37 °C, 5% CO_2, 90% humidity). The adherent cells were exposed to each ash extract for 2, 4, or 6 h. The microplate was then centrifuged for 5 min under a force of 250 × *g*. A portion (100 μL) of the supernatant in each well was carefully transferred into the wells of an optically clear 96-well flat-bottom microplate, and then an aliquot (100 μL) of a mixture of 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT) dye solution and Diaphorase/NAD^+ mixture (as a catalyst), in a ratio of 45:1 (v/v), was added to each well. The optically clear microplate was incubated for another 30 min at room temperature in the dark and then the absorbances of the samples were measured at 490 nm using a multi-well absorption reader. The cytotoxicity reading for each extract, based on its absorbance, was converted, using Eq. (1), into its relative efficiency potential percentage (REP%). A 2% Triton-100 solution and 100 μL of the conditioning solution (e.g., DMEM for Vero cells) were used for the high and low control experiments, respectively. The high control represented the system in which all of the cells died; in the low control, all of the cells survived. All data were triple replicates; the averaged cytotoxicities are presented herein.

$$\mathrm{REP}(\%) = 100 \times \frac{[\text{sample} - \text{low control}]}{[\text{high control} - \text{low control}]} \quad (1)$$

3. Results and discussion

The cytotoxicity is affect by multiple factors, including the TOC, pH, and contents of alkaline and heavy metals ions. We plotted regression curves for the experimental cytotoxicities and these factors. Tables 1–3 list the values of pH, TOC, and IC and the ICP data for all of the TCLP extracts from the BoA, SR, and BaA. Only the ions that were obtained in high leached concentrations (over the detection limits of ICP) are presented; other species were either not detected or had extremely low leached concentrations.

3.1. Physicochemical properties of extracts of ash wastes

For the BaA, the Pb, Zn, and Sr ions had the highest leached concentrations. The leached concentration of Pb was less than expected based on previous analysis [6] of BaA samples. The leaching of Pb returned to a higher level after 4 days. We observed similar behavior for the data for Zn and Sr, which provided leached levels of up to

Table 1
Properties of extracts of BaA.

Extractant	Element	Taiwan EPA	Days						
			1	2	3	4	5	6	7
AcOH	Ca	–	4220	2460	1840	1650	860	185	78
	Si	–	N.D.	N.D.	32.3	35.9	97.6	90.1	53
	Al	–	N.D.	N.D.	N.D.	N.D.	42.5	62.4	42.1
	Na	–	1060	92.9	15.1	10.7	22.9	26.5	13.2
	K	–	1380	113	12.1	5.14	7.95	10.8	6.31
	Mg	–	N.D.	N.D.	119	68.3	53.1	20.2	8.88
	Pb	5.0	6.91	1.64	N.D.	0.51	11.8	8.2	4.64
	Zn	–	1.79	2.86	1.45	75.4	50.4	15.9	5.13
	Cr	2.5	0.175	0.148	0.243	N.D.	0.116	0.113	0.063
	Cd	1.0	N.D.	N.D.	N.D.	3.04	1.38	0.142	N.D.
	Sr	–	5.16	1.15	2.41	2.01	1.19	0.549	N.D.
	pH	12.5	12.0	11.95	7.95	7.03	4.52	3.79	3.48
	TOC	–	2.60	N.D.	4.20	N.D.	N.D.	N.D.	N.D.
	IC	–	59.4	21.0	77.8	23.0	N.D.	N.D.	25.0
	Cl^-	–	7910	1260	350	140	70	70	70
DI water	Ca	–	2800	1080	795	829	680	627	424
	Si	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.024
	Al	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Na	–	1800	178	15	1.17	1.22	2.39	0.193
	K	–	1520	167	14.9	2.35	1.05	1.01	0.416
	Mg	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Pb	5.0	3.75	3.29	2.88	2.14	1.42	1.59	0.415
	Zn	–	0.516	0.843	0.294	1.36	1.11	0.506	0.493
	Cr	2.5	0.205	0.108	0.04	0.015	0.02	0.024	0.042
	Cd	1.0	N.D.	0.001	N.D.	0.001	N.D.	N.D.	N.D.
	Sr	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	pH	12.5	12.09	12.35	12.35	12.38	12.36	12.34	7.72
	TOC	–	17.42	16.55	12.36	10.37	7.75	6.82	28.77
	IC	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Cl^-	–	7840	1680	980	1120	210	470	280

Unit: mg/L.

Table 2
Properties of extracts of BoA.

Extractant	Element	Taiwan EPA	Days						
			1	2	3	4	5	6	7
AcOH	Ca	–	1580	1520	1240	503	232	161	125
	Si	–	0.884	46.5	64.7	95.1	72.2	53	38.2
	Al	–	N.D.	N.D.	0.291	16.1	20.3	16.4	13.3
	Na	–	462.5	79.5	35.9	37	25.3	16.4	10.1
	K	–	200.8	44	19.7	14.9	8.66	4.9	2.83
	Mg	–	77.5	26.6	27.3	47.6	43.1	15.2	9.12
	Pb	5.0	N.D.	N.D.	0.071	0.204	0.133	0.089	0.057
	Zn	–	N.D.	N.D.	33.7	27.6	15.1	7.69	4.28
	Cr	2.5	N.D.	N.D.	N.D.	0.038	0.046	0.012	N.D.
	Cd	1.0	N.D.	N.D.	107	26.9	6.96	3.12	1.83
	Sr	–	2.45	3.18	3.16	1.85	0.941	0.565	0.351
	pH	12.5	7.04	7.40	5.11	4.22	3.84	3.63	3.53
	TOC	–	9.40	6.40	1.60	N.D.	N.D.	N.D.	N.D.
	IC	–	329.6	151.6	18.4	N.D.	N.D.	N.D.	N.D.
	Cl^-	–	7000	490	180	70	140	70	70
DI water	Ca	–	148	34.8	29.4	29.4	28.8	27.4	29.9
	Si	–	0.518	0.139	N.D.	N.D.	1.97	1.15	2.83
	Al	–	0.178	0.048	2.63	1.73	5.86	1.88	3.27
	Na	–	384	56.1	588	337	230	180	145
	K	–	182	34.9	32.8	21.9	17.4	14.6	13.1
	Mg	–	5.21	1.25	0.771	0.806	0.9	0.793	0.669
	Pb	5.0	N.D.	N.D.	0.02	N.D.	0.034	0.055	N.D.
	Zn	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Cr	2.5	0.105	0.044	0.049	N.D.	0.002	N.D.	0.016
	Cd	1.0	N.D.	N.D.	0.018	0.008	0.008	0.004	0.013
	Sr	–	0.455	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	pH	12.5	7.49	7.78	7.73	7.82	7.93	7.92	7.8
	TOC	–	61.72	29.50	30.16	20.11	23.41	15.94	19.28
	IC	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Cl^-	–	1050	230	120	120	230	230	230

Unit: mg/L.

Table 3
Properties of extracts of SR.

Extractant	Element	Taiwan EPA	Days						
			1	2	3	4	5	6	7
AcOH	Ca	–	1610	1160	855	608	242	148	109
	Si	–	60.5	136	130	117	68.3	50.4	42.1
	Al	–	N.D.	0.3	4.78	17.5	10.1	10.2	13.3
	Na	–	823	94.4	49.7	38.9	20.4	12.6	9.32
	K	–	91.2	79.9	26.5	17.5	8.25	5.07	N.D.
	Mg	–	135	150	61.1	35.2	17.8	10.6	7.63
	Pb	5.0	N.D.	0.104	0.075	0.078	N.D.	0.008	0.012
	Zn	–	N.D.	48.1	40.6	15.5	6.26	3.94	2.88
	Cr	2.5	1.93	0.083	N.D.	N.D.	N.D.	N.D.	N.D.
	Cd	1.0	N.D.	0.525	0.186	N.D.	N.D.	N.D.	N.D.
	Sr	–	3.09	2.05	1.91	1.6	0.758	0.603	0.447
	pH	12.5	7.37	5.37	4.37	3.88	3.64	3.48	3.39
	TOC	–	1.40	1.01	N.D.	N.D.	N.D.	N.D.	N.D.
	IC	–	74.6	46.0	20.0	N.D.	2.0	40.1	N.D.
	Cl^-	–	2450	420	210	140	70	70	70
DI water	Ca	–	562	411	243	125	63.1	46.3	44.1
	Si	–	2.03	1.44	N.D.	N.D.	N.D.	N.D.	N.D.
	Al	–	N.D.	0.178	0.264	2.84	9.37	1.24	10.5
	Na	–	1030	148	19.5	4.44	2.51	1.93	1.53
	K	–	1230	172	N.D.	N.D.	N.D.	N.D.	N.D.
	Mg	–	0.462	0.5	0.277	0.174	0.097	0.059	0.111
	Pb	5.0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Zn	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Cr	2.5	1.4	0.42	0.136	0.035	0.014	0.008	N.D.
	Cd	1.0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Sr	–	2.56	1.29	0.593	0.241	0.078	0.04	0.015
	pH	12.5	10.48	10.35	8.50	10.07	10.43	10.26	10.39
	TOC	–	9.93	6.35	20.01	7.82	6.66	34.81	5.62
	IC	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Cl^-	–	292	700	230	120	230	120	120

Unit: mg/L.

5.16 mg/L. For BoA, the Cd and Sr ions had higher leached concentrations from BaA, with the former reaching up to 107 mg/L after 3 days. The leached level of Sr was also high, reaching 2.5 mg/L. For the SR, the Sr ions had a higher leached concentration; it decreased from 2.5 to 0.01 mg/L upon increasing the number of days of extraction.

According to the first-day extraction data (so-called "one-day" TCLP) in Tables 1–3, the three tested ash wastes from the MSWI plant could be classified as non-hazardous materials because they all passed the regulatory levels of the Taiwan EPA. Although Sr ions leached from the ash wastes under both acidic and neutral aqueous conditions, Sr is not a regulated species in the TCLP test of the Taiwanese EPA.

The BaA extracts had very high values of pH and relatively higher concentrations of Ca, Pb, and Zn than did the SR and BoA. The detected TOC values of the three extracts ranged from 2300 to 2600 mg/g; the background TOC value of the acetic acid-based extractant was ca. 2185 mg/L. The TOC values listed in these Tables are the net TOC values contributed from the ashes. The TOC of the BoA extract was higher than those of the BaA and SR extracts. Brocca et al. [4] reported a similar observation. The detected [Cl^-] ranged from 70 to 7900 mg/L; it was more predominant in the BoA and BaA. Increasing the leaching time decreased the amount of leached Cl^-; this behavior also occurred for alkali ions.

3.2. Cytotoxicity order of MSIW ashes

In a previous study [7], we observed that, from 2 to 24 h, only BaA exhibited a significant toxic effect. We have also examined the contributing factors determining the cytotoxicity of ten-fold-diluted acetic acid extracts of BaA, SR, and BoA [7,8]. The short-term (2 h) cytotoxicity presumably arose from the contribution of the TOC of the acetic acid extractant. Because the TOC caused by the acetic acid extractant was 2185 mg/L in the extract and the total content of heavy metals was less than 100 mg/L in the extract, from the viewpoint of changing the pH of the incubation media dramatically we suggest that the major contributor to the short-term cytotoxicity was the TOC. The effect of the TOC caused by the acetic acid extracts on long-term cytotoxicity (24 h) can, however, be ignored by considering the results of the control experiment in Table 4, which reveals that the acetic acid extracts provided higher ERP% values than did the DI water extracts. The Cl, Na, Cr, Sr, and Ca contents displayed near-linear relationships with the cytotoxicities of the water extracts. We suspect that the presence of Cl, Na, Ca, Sr, and Cr was the cause of the cytotoxicity of the water extract from the SR. We note that the Sr ion content contributed significantly to the cytotoxicity of the undiluted water extract of the RP. No such correlations existed for the water extracts of the BaA and BoA.

In this present study, we also re-investigated the effect of dilution on the cytotoxicity of the acetic acid and water extracts of MSWI ash wastes. Fig. 1 compares the cytotoxicity data of the acetic acid extracts of the BaA, SR, and BoA. In Fig. 1(A), the contact time between the extracts and the Vero cells was controlled at 2 h; the order of ash cytotoxicities was BaA > BoA > SR under undiluted conditions, but it changed to SR > BoA > BaA under ten-fold-diluted conditions; it changed again when the dilution ratio was 20. In Fig. 1(B), the contact time between the extracts and the Vero cells was controlled at 6 h; the order of ash cytotoxicities was SR > BaA > BoA under undiluted conditions, but it changed to BaA > SR > BoA under the ten-fold-diluted conditions; when the dilution ratio was 20 or more, the cytotoxicities of the three ash waste samples were almost identical. In Fig. 1(C), the contact time between the extracts and the Vero cells was controlled at 24 h; the order of ash cytotoxicities was BoA > SR > BaA under undiluted conditions, but it changed to BaA > SR > BoA under the ten-fold-diluted conditions; when the dilution ratio was 20 or more, the cytotoxici-

Table 4
Cytotoxicity data for MSWI ash wastes.

Condition		Days						
Ash	Extract (contact time)	1	2	3	4	5	6	7
BoA	Water (2 h)	7.35 ± 0.26	6.87 ± 0.52	9.25 ± 0.56	4.83 ± 0.69	5.09 ± 0.30	4.53 ± 0.07	5.20 ± 0.46
	Water (6 h)	6.55 ± 0.05	7.25 ± 0.86	10.37 ± 2.08	7.11 ± 0.44	5.85 ± 0.3	8.09 ± 1.86	6.44 ± 0.19
	Water (24 h)	6.89 ± 0.45	2.67 ± 0.44	2.03 ± 0.39	1.69 ± 0.51	1.58 ± 0.26	2.18 ± 0.35	3.73 ± 0.33
	AcOH (2 h)	26.96 ± 1.14	1.82 ± 0.19	1.11 ± 0.09	19.12 ± 0.98	1.34 ± 0.63	0.59 ± 2.53	−2.19 ± 0.28
	AcOH (6 h)	24.1 ± 3.77	84.94 ± 1.65	18.77 ± 0.19	−1.33 ± 1.05	−1.58 ± 1.12	−3.61 ± 0.18	−3.82 ± 0.16
	AcOH (24 h)	18.45 ± 1.92	61.32 ± 1.46	5.61 ± 0.86	−4.12 ± 0.03	−4.09 ± 0.06	−4.19 ± 0.02	−4.32 ± 0.04
BaA	Water (2 h)	62.05 ± 0.17	53.84 ± 1.43	14.18 ± 2.71	2.34 ± 0.48	2.93 ± 0.71	49.87 ± 3.32	6.57 ± 1.47
	Water (6 h)	34.82 ± 4.45	20.35 ± 0.96	6.34 ± 1.34	−1.33 ± 0.30	2.0 ± 1.37	26.97 ± 0.54	6.13 ± 0.46
	Water (24 h)	−3.65 ± 0.58	−2.67 ± 2.02	−3.39 ± 1.5	−5.05 ± 0.93	−3.8 ± 1.08	19.99 ± 1.92	1.96 ± 1.83
	AcOH (2 h)	62.53 ± 0.95	61.53 ± 2.73	6.13 ± 0.06	1.78 ± 0.19	24.95 ± 0.89	0.59 ± 1.80	−1.97 ± 0.43
	AcOH (6 h)	46.2 ± 0.46	42.14 ± 1.33	7.25 ± 0.14	11.21 ± 0.72	22.73 ± 1.12	−1.19 ± 1.58	−3.71 ± 0.21
	AcOH (24 h)	−1.76 ± 0.33	5.68 ± 2.41	3.82 ± 1.13	30.68 ± 2.21	0.41 ± 1.72	−4.26 ± 0.04	−4.02 ± 0.31
SR	Water (2 h)	14.47 ± 3.53	14.37 ± 3.12	5.53 ± 0.15	5.35 ± 1.06	4.68 ± 1.05	5.16 ± 0.82	5.72 ± 0.93
	Water (6 h)	8.37 ± 0.84	11.17 ± 3.45	8.44 ± 0.75	6.65 ± 0.82	6.62 ± 0.30	5.88 ± 0.02	8.83 ± 2.17
	Water (24 h)	13.37 ± 2.39	5.27 ± 1.48	4.37 ± 1.37	2.79 ± 1.58	4.03 ± 0.28	2.6 ± 0.48	3.16 ± 0.68
	AcOH (2 h)	5.38 ± 0.43	2.53 ± 0.17	36.7 ± 1.76	20.09 ± 1.24	−1.52 ± 0.56	−2.53 ± 0.22	−0.82 ± 0.17
	AcOH (6 h)	6.80 ± 0.58	7.25 ± 0.40	64.59 ± 2.45	6.41 ± 1.47	−3.68 ± 0.14	−4.03 ± 0.05	−3.33 ± 0.05
	AcOH (24 h)	13.65 ± 1.85	−0.30 ± 0.23	61.21 ± 2.76	−4.29 ± 0.04	−4.43 ± 0.07	−4.46 ± 0.02	−4.43 ± 0
Control	Water				−23.65 ± 3.76			
	AcOH				−15.81 ± 2.60			

Units: %.

ties of the three ash wastes were almost identical. We conclude that the order of the cytotoxicities of the ash wastes is strongly affected by the contact time and the dilution ratio.

There are two mechanisms for cell death: necrosis, where cell lines interact with toxicants over long contact times (several hours to days), and apoptosis, where cell lines die within very short contact times (<10 h). Measurement of apoptosis can be helpful in evaluating the overall impact of stress caused by environmental chemicals in solid wastes. The technique that we used in this study involved combining a cytotoxicity kit with an ELISA reader; this metrology suggested that the cells died through a necrosis pathway and that environmental stress (e.g., surface damage or peroxidation by oxidative species; imbalanced osmosis pressure in the cells caused by water-soluble ions) was the reason for the cell death. We also detected oxidative species, such as Cl_2, ClO_2, and SO_2, but only in the acetic acid extracts of the ash wastes. The detected total oxidant concentration ranged from 0.05 to 1.7 mg/L (data not shown); the detection limit was ca. 0.03 mg/L. Our results suggest that the formation of these oxidative species required acidic conditions; the contribution of these oxidative species can, therefore, be ignored in this study. Thus, we screened out all but one of the analyzed elemental ions; the remaining factor was the contribution of Sr.

When studying toxicants, the results from dilution tests are often used to obtain LC_{50} values. Unfortunately, the curves in Fig. 1(A)–(C) are not suitable for calculating accurate LC_{50} values for the extracts of MSWI ash wastes, due to the fact that they were not normal exponential functions. This behavior is consistent with the studies of Lin et al. and Chen et al. [9,10], who could determine only the LC_0 and LC_{100} values of such MSWI ash particles.

3.3. Strontium as the emerging pollutant in MSWI BoA

Table 5 presents the data statistical analysis by SPSS for the Sr concentration and cytotoxicity in BoA; only the acetic acid extracts provided enough Sr data for computation. The Pearson correlation factor was in the range from 0.3 to 0.7; the significance ranged from 0.5 to 0.05. Table 6 lists the analytical results for the Sr concentrations and cytotoxicities in all of the ash wastes subjected to a contact time of 24 h; it reveals that the SR had a highly correlation factor for its water extract, but the change of REP% was very limited (<15%).

In Fig. 2, we use a log–log scale plot to present the relationship between the content of Sr in the extracts and the cytotoxicity. In Fig. 2(A), a linear relationship exists between the content of Sr in the extracts and the cytotoxicity of the BoA at various contact times. In Fig. 2(B) and (C), we observe fewer correlations between the contents of Sr in the extracts and the cytotoxicities of the SR and BaA, respectively. In the linear region of these three plots, the maximum cytotoxicity effect occurred at a Sr concentration of 2.0 mg/L for the SR; for the BaA, it ranged from 1.2 to 5.16 mg/L, although the major contributor to the high toxicity of Sr at 5.16 mg/L would most likely be Pb at 6.9 mg/L (Table 1). Comparing the correlation coefficients after fitting first-order equations, the plot for the BoA had the highest value of R^2. Fig. 3 reveals the cytotoxicity of a Sr

Table 5
Data statistical results of Sr concentration and cytotoxicity in BoA.

Condition		Sr	
Ash	Extractant (contact time)	Pearson correlation factor	Significance
BoA	Water (2 h)	–	–
	Water (6 h)	–	–
	Water (24 h)	–	–
	AcOH (2 h)	0.303	0.508
	AcOH (6 h)	0.745	0.055
	AcOH (24 h)	0.691	0.085

(–) Insufficient data for analysis.

Table 6
Data statistical results of Sr concentration and cytotoxicity in three ash wastes.

Condition		Sr	
Ash	Extractant (contact time)	Pearson correlation factor	Significance
BoA	Water (24 h)	–	–
	AcOH (24 h)	0.691	0.085
BaA	Water (24 h)	–	–
	AcOH (24 h)	–0.460	0.931
SR	Water (24 h)	0.951	0.001
	AcOH (24 h)	0.420	0.348

(–) Insufficient data for analysis.

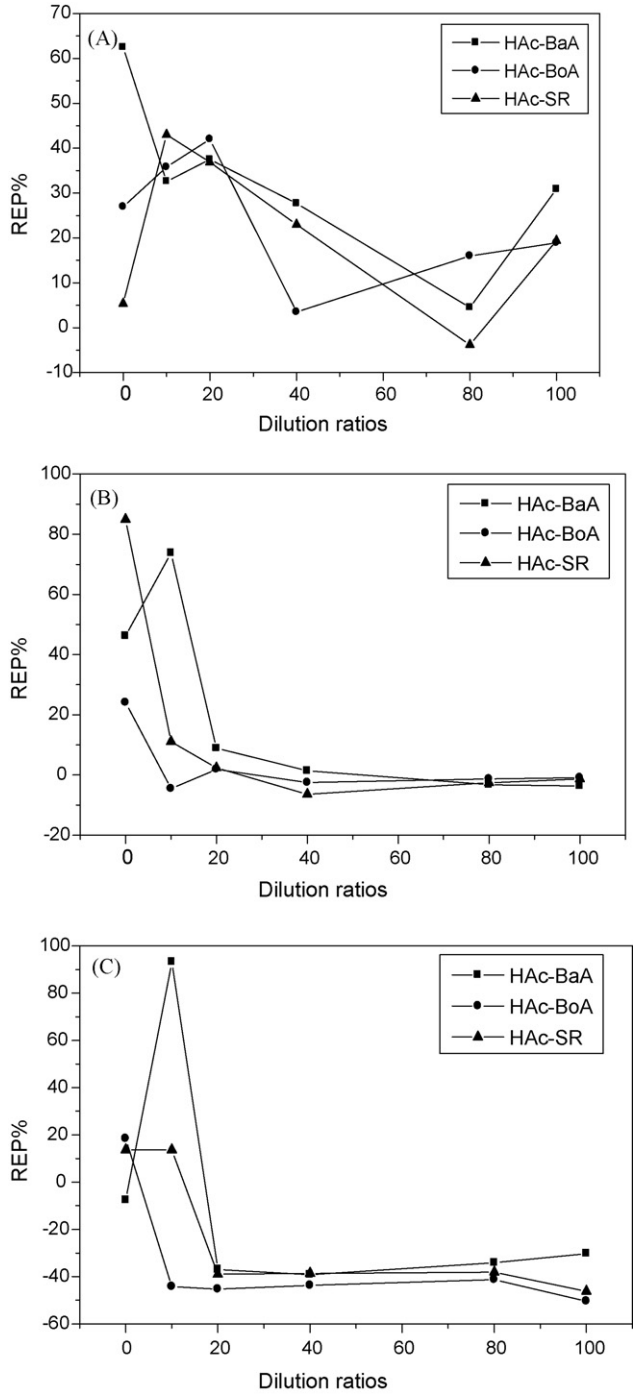

Fig. 1. Relationships between the cytotoxicities (toward Vero cell lines) of 1-day leachates and the dilution ratios for contact times of (a) 2 h, (b) 6 h, and (c) 24 h.

standard solution toward Vero cells. The cytotoxicity reached as high to 70% when the concentration of Sr was 5 mg/L; the trend in the value of REP% was to decay when the Sr concentration was greater than 10 mg/L, consistent with our observations in Fig. 2.

3.4. Literature review of the behavior ecotoxicity of Sr in solid wastes

This paper is not the only one to report that MSWI ash wastes have high leaching levels of Sr. Using a six-step sequential extraction procedure, Huang et al. [1] reported recently that Pb, Cd, Cu, and Sr have remarkable mobility in MSWI fly ash. Sivula et al. found that Sr can be observed in the landfill leachate of BoA [20]. From elemental speciation analyses, Huang et al. [5] reported that ca. 28% of the Sr dissolved into pure water and 14% into $CaCl_2$ solution; these values are almost comparable with those for Pb (61 and 18%, respectively). Their data are not consistent with our present results, but they are consistent with those reported by Feng et al. [11]. In Table 1, we indicate that Sr could be extracted only when using acetic acid as the extractant—it could not be extracted using pure water; in contrast, Pb could be extracted using either pure water or acetic acid. In an earlier paper, we studied [21] the speciation of BaA using the same six-step sequential extraction method as that used by Huang et al. [5]; we found that the leached percentage of Pb from the BaA into pure water was ca. 60–70% of that obtained when acetic acid was used as the extractant of the TCLP, due to Pb being an amphoteric ion in such a highly alkaline ash waste [21].

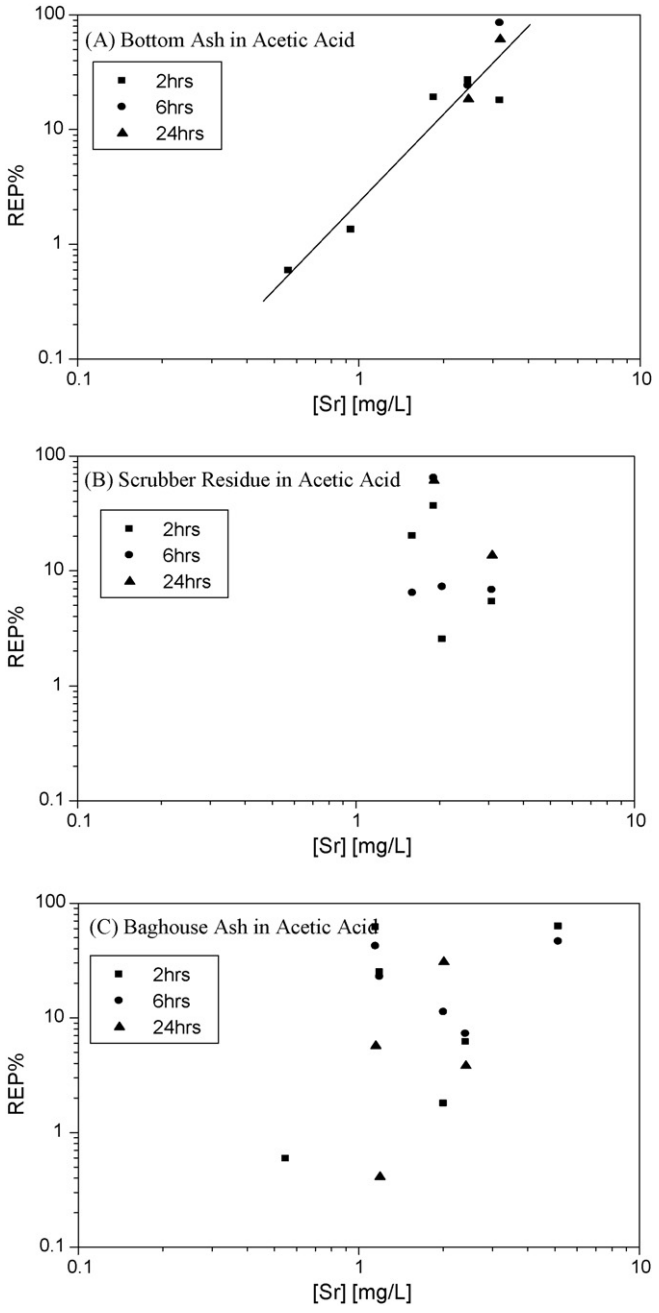

Fig. 2. Relationships between the concentrations of Sr ions and the Vero cytotoxicities of the (a) BoA, (b) SR, and (c) BaA extracts.

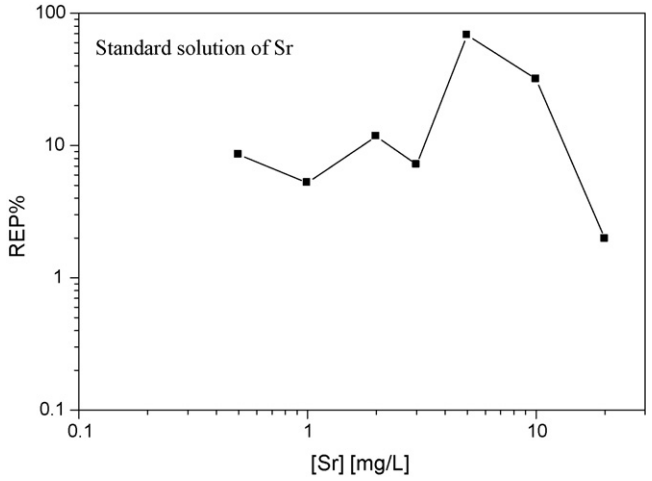

Fig. 3. Relationship between the concentration of a standard solution of Sr and the Vero cytotoxicity.

Because the Sr(II) ion has a fully filled electron configuration, much like Kr, it does not dissolve in basic solutions. It is notable that the final pH of the water extracts of the BaA was greater than 12.5.

From their normal TCLP tests, Feng et al. [11] reported that the leached concentrations of their tested BoA into pure water and acetic acid extractants were 1.54 and 2.19 mg/kg, respectively; these values are consistent with our data (Table 2). We detected levels of 0.455 and 2.45 mg/L for Sr released into the pure water and acetic acid extractants, respectively, from our BoA sample. Notably, the final pH from the BoA after 1-day TCLP was almost neutral. Table 3 reveals that Sr was extractable from the SR using both acetic acid and water if the final pH of the water extracts was less than 10.5. Although these results suggest that the Sr leaching behavior was related to the pH, we are unable to provide a plot of the solubility of strontium hydroxide with respect to the pH.

Many literature reports have described the cytotoxicity of Sr and its compounds [22–25]. Keller reported that ^{89}Sr could suppress the spontaneous cytotoxicity in mouse tissues [22]. In recent years, Sr-incorporated hydroxyapatite cement [23] and calcium phosphate cement [24] have been developed as new biomedical materials, which exhibit good compatibility and low cytotoxicity, for use in human bones. An in vitro test revealed that strontium ranelate is non-toxic toward human periodontal ligament fibroblasts [25]. Several papers describe the genotoxicity [11,14], cytotoxicity [7,15], and ecotoxicity [16] of MSWI BoA; most have concluded that the MSWI BoA possesses relatively low toxicity.

Most of the existing data suggest that Sr is safe for human contact. Sharma et al. [26] predicted, however, that among the analyzed elemental components in the fine particles from an incineration energy plant and urban air, only Mn and Sr might influence DNA damage statistically significantly.

Our present study suggests that if the cytotoxicity of BoA is high relative to those of BaA and SR, it arises mainly from the presence of Sr ions. The contributions of Sr toward the cytotoxicities of BaA and SR were not particularly high because of the high alkalinities of these two ashes. Huang et al. [5] reported that the Taiwanese EPA's official regulated level (1.0 mg/kg) for Sr, measured using TCLP, is inaccurate. At present, Sr is not regulated by TCLP regulations in Taiwan; therefore, we recommend that the regulated level for Sr should be less than 5.0 mg/L.

4. Conclusion

The cytotoxicities of undiluted MTCLP acetic acid extracts of BaA, SR, and BoA do not correlate well with their elemental concentrations. In contrast, the cytotoxicity of the water extract of SR is caused by the presence of Cl, Na, Ca, Sr, and Cr. This paper is, therefore, the first to report that Sr is a significant contributor to the cytotoxicity of the water extract of MSWI BoA. A ten-fold dilution ratio is optimal when performing cytotoxicity analyses of the MSWI BaA extracts; the BoA and SR extracts do not need dilution. The main finding from this present study is that Sr ions are a hitherto unknown and potentially dominating species affecting the cytotoxicity of MAWI BoA.

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