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Biotoxicity assessment on reusability of municipal solid waste incinerator (MSWI) ash

Bor-Yann Chen [a], Kae-Long Lin [b,*]

[a] *Department of Chemical and Materials Engineering, National I-Lan University, I-Lan 260, Taiwan, ROC*
[b] *Department of Environmental Engineering, National I-Lan University, I-Lan 260, Taiwan, ROC*

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Abstract

This study provides a first attempt of dose–response analysis and margin of safety using *Escherichia coli* DH5α, *Bacillus subtilis* as indicator microorganisms to put forward, in general terms and explanations, the toxicity rankings of various ashes of municipal solid waste incinerator (MSWI) for feasibility in further applications. Since the MSWI ash often contains cations of Si, Ca, Al and Fe, it is frequently considered to be recycled for construction building-materials. Growth inhibition of *E. coli* DH5α occurred at concentrations over 0.156, 0.625 and 0.0195 g/L for bottom ash (BA), cyclone ash (CA), scrubber ash (SA), respectively, suggesting the toxicity ranking of SA > BA > CA. In contrast, except for SA (ca. 0.313 g/L), almost same inhibitory levels of ashes to cell growth were also observed in *Bacillus subtilis*. Evidently, biotoxicity responses were strongly dependent upon the characteristics of indicator microorganism. Based on DH5α, the margins of safety (MOS) were thus 0.195, 1.56 and 6.25 mg/L for SA, BA and CA, respectively. Nearly identical levels of MOS were also suggested by *B. subtilis*, except for SA (3.13 mg/L). Although MSWI residual ashes qualified EPA's standard test of Toxicity Characteristic Leaching Procedure (TCLP), they might still contain other toxic residues (e.g., chloride ions and/or anions) to cause existing toxicity as indicated in this toxicity study.

Keywords: TCLP; MSWI; Margin of safety; Dose–response curve

1. Introduction

Municipal solid waste (MSW) basically composed of an organic fraction, an inorganic fraction and moisture. The organic fraction is primarily lignocellulosic material, a potential source for energy recovery. Approximately 85% of the moisture-free MSW is combustible or convertible to liquids [1]. To a highly populous and small island country (e.g., Taiwan), the worst is that the volume of MSW generated increases as a result of an increase in population and a marked rise in living standards. Thus, municipal solid waste incineration (MSWI) is usually one of most viable alternatives in place of landfill disposal, since MSWI considerably reduced the volume and weight of solid waste by 90 and 70%, respectively [2]. In addition, the waste-to-energy systems have been incorporated into MSWI management programs for energy recovery. However, the MSWI residues may still contain high-level toxic substances (e.g., heavy metals) in ashes, leading to a persistent threat to the environment. Apparently, post-treatment of MSWI residues must be carried out to ensure safety to humans. In Taiwan, landfill is still the most frequent method used to deal with solid waste up to now. However, as the persistent need to construct incinerators will significantly increase due to difficulties to obtain appropriate sites for landfill in the populous Taiwan. Thus, how to seek economically feasible ways to recycle the remaining waste is evidently the first priority subject to treatment of MSWI residues.

As MSWI residues (e.g., fly ash, scrubber ash) often contain high levels of leachable heavy metals and salts, they are usually classified as hazardous wastes worldwide. This point suggests that ashes must be appropriately treated by means of wastes intermediate treatment process (e.g., melting [3,4] and sintering [5]) prior to recycling as construction materials or safe disposal. Basically, typical MSWI residues contain chemical compositions of Si, Al, K, Ca, Fe, Mg and Na. From the perspectives of safe reuse and/or disposal, all compositions of MSWI residues must achieve the criteria of EPA's regulation to be termed as environmentally friendly and ecologically sound materials. Therefore, owing to the characteristics of MSWI residues, recycle

* Corresponding author. Tel.: +886 3 9357400x749; fax: +886 3 9364277.
E-mail address: kllin@niu.edu.tw (K.-L. Lin).

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and reuse of residues as the construction materials will be more promising to further treatment. For instance, as a result of the similar physical and chemical characteristics between the bottom ash and the nature gravel, reuse of the bottom ash from MSWI residues has become very popular [6–9]. In addition, much attention on reuse of MSWI fly ash has been paid for production of cement mortars [10], concrete mixtures [11] and fine aggregate in mortars [12].

In the view of human risk and toxicity, the leaching of heavy metal and/or other toxic chemicals from residues will become a rank one problem prior to applications. In Taiwan, Toxic Characteristic Leaching Procedures (TCLP) has been adopted as a typical analytical method to inspect the concentration of leaching heavy metals. However, due to a lack of assessment guideline to guarantee the long-term safety of remaining leaching heavy metals and slats, whether MSWI residues are feasible for safe reuse is still remained uncertain. To reveal the feasibility of utilizing MSWI ash residues for reuse as construction material (e.g., brick and cement), this first-attempt biotoxicity assessment on model ashes was introduced to present a quantitative measure for the practicability to reuse in practice. Here, the model MSWI ash used was collected from the cyclone of a mass-burning incinerator located in Taipei county, northern Taiwan. The findings indicated herein that although the MSWI ashes were qualified the inspection of a standard test of TCLP for EPA regulations, significant toxicity perhaps is still lingered on the residual ashes. This study also provided a novel scheme of general guideline (e.g., dose–response analysis and margin of safety) for on-site professionals to reveal the toxicity rankings of various MSWI ashes for the feasibility.

Regarding the toxicity of inorganic and organic pollutants, Chen et al. [13] provided a first attempt from a toxicological perspective to put forward the toxicity ranking of Cd(II), Cu(II) and Zn(II) to *Thiobacillus thiooxidans* for metal bioremediation. Similar perspective [14] was adopted to reveal to explore whether there exists a noteworthy change in combined biotoxicity of phenol to *Ralstonia taiwanesis* in the presence of other carbon sources. However, whether this assessment is viable to be used in practice still remained open to be discussed. Thus, from practical perspectives this study tended to employ such aspects in pursuit of feasibility for on-site or in situ applications.

2. Materials and methods

2.1. Materials

The municipal solid waste incinerator ash used was collected from the mass-burning incinerator located in Taipei County, Taiwan. The incinerator, capable of processing approximately 1350 metric tons of local municipal solid waste per day, is equipped with air pollution control devices (APCD) consisting of a cyclone, an adsorption reactor and a fabric baghouse filter. Three residual MSWI solid ashes were obtained as follows:

1. Cyclone ash: The incinerator systems were equipped with cyclone separators that employed inertial forces to separate particles (i.e., cyclone ash) down to approximately 5 μm in size.
2. Scrubber ash: The semi-dry systems have introduced into lime slurry, activated carbon and diatomaceous earth, and removed acid gas from the gas stream. The scrubber ash was collected from the baghouse filter systems.
3. Bottom ash: The procedure was performed with water-quenched bottom ash taken from a MSWI. The ash was screened and magnetically separated to remove its coarse non-ferrous impurities and ferrous substances.

The cyclone ash, scrubber ash and bottom ash were individually dried at 105 °C for 24 h until a constant weight was reached (i.e., almost moisture-free), and then the chemical compositions were characterized.

2.2. Analytical methods

2.2.1. Chemical analysis

MSWI cyclone ash, scrubber ash and bottom ash were used for analysis by means of TCLP, and chemical composition determination as follows: chemical composition determination was conducted by inductively coupled plasma atomic emission spectroscopy (ICP- AES; Kontron, S-35). Two-gram predried samples were digested with ultra pure grade reagents through a three-step procedure to release all soluble chemicals: a concentrated hydrofluoric/nitric acid mixed at a ratio of 5 mL/5 mL was added to the sample; after evaporation, a 3 mL/9 mL mixture of concentrated nitric/hydrochloric acid was added; after another evaporation, the samples were dissolved in a 5% nitric acid solution.

Toxicity characteristic leaching procedure (TCLP) was carried out according to Taiwan EPA method NIEA R201.13 C [15]. The extraction procedure required the pre-evaluation of the pH value of the sample to estimate the appropriate amounts of extraction fluid for the experiment. Upon testing, extraction fluid (ca. pH 2.88 ± 0.05) used for the TCLP analysis was prepared by adding 5.7 mL acid to 500 mL double distilled water, and diluted to a volume of 1.0 L. A 25-g sample was prepared in a 1.0-L Erlenmeyer flask, and then well mixed with a 500-mL extraction fluid in each flask. These samples were agitated for 18 h using an electric vibrator. The slurry was filtered by 6–8 μm pore size Millipore filter paper to remove insoluble particles. The leachates were then preserved in 2% HNO_3 for further analysis.

Ion chromatograph (IC) was employed to analyze chloride anions via Metrohm 761 Compact IC. In addition, Metrosep SUPP3 column was used in IC with a flow rate of 1.0 mL/min and suppressed conductivity mode detection settings at a system pressure of 12.9 MPa. Leaching concentration was evaluated via EPA standard methods as the following: Cd (NIEA R302.20T), Pb (NIEA R306.20T), Zn (NIEA R307.20T), Cu (NIEA R305.20T) and Cr (NIEA R303.20T). Mineralogy was determined by XRD analysis: the XRD analyses were carried out by a Siemens D-5000X-ray diffractometer with Cu Kα radiation and 2Θ scanning, ranging between 5° and 70°. The XRD scans were run at 0.05° steps, with a 1 s counting time.

2.3. Microorganisms and culture conditions

Escherichia coli DH5α and *Bacillus subtilis* 16048 (generously provided by Professor Jo-Shu Chang, NCKU, Taiwan) were used as indicator strains for biotoxicity assessment. A loopful of the indicator strain seed taken from an isolated colony in LB-streak plate was precultured in 50 mL Luria-Bertani medium (LB broth, Miller, Difco) for 12 h at appropriate temperature (30 °C for *B. subtilis*, 37 °C for *E. coli* DH5α), pH 7.0, 200 rpm. To ensure the synchronous growth activity and maximum metabolic functioning in the same growth phase for bioassay, 5% (v/v) cultured broth was then inoculated to fresh sterile LB medium and a cell culture was harvested at approximately mid-exponential growth phase (ca. 4 h) for further toxicity assessment. The 1.0 mL cell culture was then serially diluted with 9.0 mL sterile saline solution (SSS; NaCl 10.0 g/L) and only the diluent with appropriate cell concentrations (ca. 1500–15,000 cells/mL) was chosen as the test seed (TS) for later uses.

2.4. Biotoxicity assessment

Biotoxicity assessment was specially designated through a modification of dose–response analysis [13,14] as follows: the sampled ashes (i.e., cyclone ash, scrubber ash and bottom ash) were first sterilized via moist-heat method (121 °C at 15 psi for 20 min) to exclude the presence of unwanted bacterial contaminants. As ashes were mixtures instead of pure chemicals, the "apparent" concentration of samples defined here was the concentration of ashes and their serial diluents well mixed with sterile saline solution (SSS; $NaCl_{(aq)}$ 10.0 g/L). Note that phosphate buffered saline (PBS) solution, which is regularly used for biological assay, was not used here for ashes, since phosphate precipitates might be formed in serial dilution due to metals suspected in high concentrations. The initial concentration C_0 for toxicity tests of all ashes was chosen at 20.0 g/L. Serial-half dilution of initial concentration C_0 (i.e., $1/2C_0$, $1/4C_0$, $1/8C_0$, $1/16C_0$, $1/32C_0$, ..., $1/2^nC_0$) was carried out by using 50 mL ash solution or its derived diluents mixed with 50 mL SSS. The 9.0 mL resulted serial diluents (RSD) were all placed in sterile test tubes for use in quantification of viable cells afterwards. The 1.0 mL freshly harvested TS was then well shaked with RSD ca. 20 times through a 35-cm arc elbow motion to form serial plate count diluents (SPCD). Meanwhile, 1.0 mL fresh TS mixed with 9.0 mL pure SSS was chosen as the ash-free control. The numbers of survival bacterium (i.e., *E. coli* DH5α or *B. subtilis*) in SPCD or the control were estimated by the standard plate count method [16]. Standard plate count in LB medium was carried out as follows: SPCD were serially diluted with SSS immediately after sampling, and then appropriate volumes (e.g., 0.20 mL) of SPCD were spread onto agar *Petri* plates. Note that all cells in SPCD would be assumed metabolically viable and culturable on LB-medium plates [17] due to fresh preparation of fast-growing cells in all steps. The LB-medium plates were then incubated at 30 °C (*B. subtilis*) or 37 °C (*E. coli* DH5α) for ca. 16–24 h to form observable colonies for enumeration. Plates with between 30 and 300 colonies are statistically appropriate for counting. Serial dilution-agar plating procedures were carried out in duplicate for quality assurance and control (QA/QC). The microbial population in the original RSD can then be calculated using the following formula (CC: cell count):

$$\text{cells per liter of broth (CC)} = \frac{\text{number of colonies}}{\text{amount plated} \times \text{dilution factor}}$$

To have quantitative toxicity for comparison, CC_0 was chosen as the CC at ash-free control. The ratio CC/CC_0 of 0 and 1 directly indicated complete inhibition and no inhibitory toxicity to bacterial cell, respectively. The unity of this ratio simply suggests that the present toxicity of this diluent at this concentration is nearly equal to the toxicity of SSS (i.e., "zero" toxicity). The concentration range for the ratio jumped from 1.0 to 0.0 in dose–response curves (e.g., Figs. 4 and 5) is defined here as the "toxicity threshold" (TT) range. The comparison on TT range can provide an obvious diagram of toxicity ranking for various ashes. For example, if the TT range for ash A is much less than that for ash B, ash A is inevitable much more toxic than ash B, indicating that much higher dilution factor must be carried out for ash A in order to have "zero" toxicity as same as control (SSS).

3. Results and discussion

3.1. Characterization of MSWI cyclone, scrubber and bottom ash

The chemical composition of the MSWI cyclone ash is shown in Table 1. SiO_2, CaO and Al_2O_3 comprised 28.3, 22.8 and 12.6%, respectively. The next most abundant components were K_2O and Fe_2O_3, contributing ca. 6.1 and 3.1%, respectively. As indicated in Fig. 1, the fingerprint speciation of the cyclone ash, identified by XRD techniques, revealing that the major components were SiO_2, $CaSO_4$, $KAlSi_3O_8$, $CaCl_2$, KCl and NaCl.

Moreover, the most abundant component in the MSWI scrubber ash (Table 1) was CaO in 42.3%. The next plentiful components are SO_3, Na_2O, K_2O and Cl^-, contributing about 7.3, 3.0, 3.5 and 2.1% (w/w), respectively. XRD analysis (Fig. 2) showed that the major components were CaClOH, $Ca(OH)_2$, $ZnSO_4$, C and PbO.

Table 1
Chemical composition of MSWI cyclone ash, scrubber ash and bottom ash

Chemical composition (%)	Cyclone ash	Scrubber ash	Bottom ash
SiO_2[a]	29.12	0.74	25.79
Al_2O_3[a]	12.76	<0.01	6.33
Fe_2O_3[a]	3.43	0.8	10.33
CaO[a]	22.77	42.5	26.14
MgO[a]	2.51	0.35	0.82
SO_3[a]	3.12	7.25	0.47
K_2O[a]	6.05	3.02	1.13
Na_2O[a]	<0.01	3.25	3.92
Cl^-[b]	0.20	2.12	3.20

[a] Analyzed by ICP-AES after $HF/HClO_4/HNO_3$ digestion.
[b] Analyzed by IC.

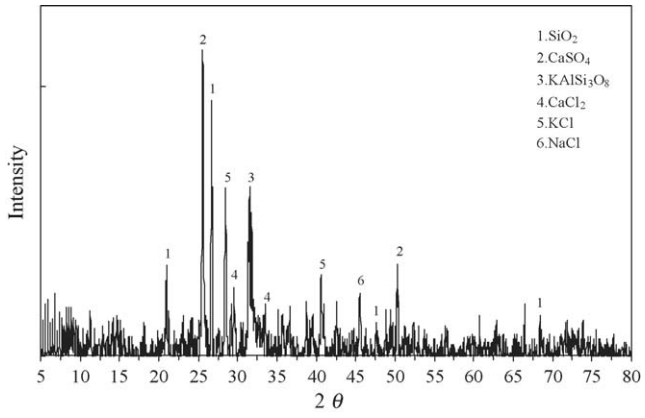

Fig. 1. XRD pattern of cyclone ash.

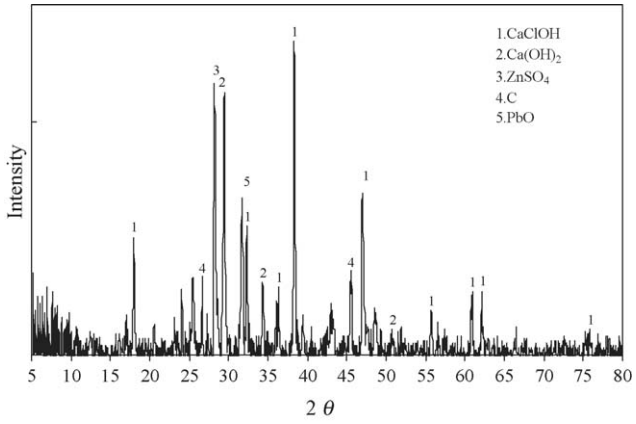

Fig. 2. XRD pattern of scrubber ash.

In addition, the major components in the MSWI bottom ash (Table 1) are SiO_2, CaO, Fe_2O_3 and Al_2O_3 comprising 25.8, 26.1, 10.3 and 6.3%, respectively. The next most abundant components are Na_2O, K_2O, MgO and Cl^- at about 3.9, 1.1, 0.8 and 3.2%, respectively. The X-ray diffraction patterns of the MSWI bottom ash (Fig. 3) revealed that the major components were SiO_2, $CaCO_3$, Ca_2SiO_3Cl and $Al_6Ca_2O_{13}{\cdot}H_2O$.

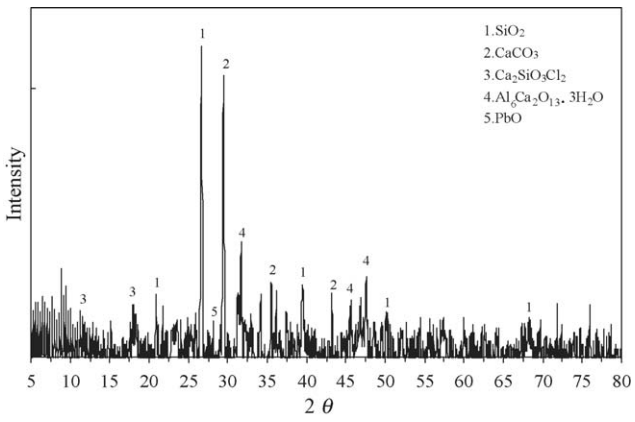

Fig. 3. XRD pattern of bottom ash.

3.2. Total metal and leaching concentrations of cyclone ash, scrubber ash and bottom ash

As summarized in Table 2, the most abundant metals in various ashes were Pb and Zn. As presented in Table 2 for the leaching concentrations of ashes obtained by TCLP, in particular, the leaching concentration of Cd in scrubber ash reached 1.04 mg/L was slightly higher than the Taiwan EPA's current regulatory thresholds (e.g., 1.00 mg/L) to be classified as hazardous. The TCLP leaching concentrations of cyclone ash and bottom ash for the target metals were all under the EPA's current regulatory thresholds (Table 2).

3.3. Biotoxicity assessment

3.3.1. E. coli DH5α

1. Bottom ash: As indicated in Fig. 4(1), cell viability was completely inhibition at the concentration greater than ca.

Table 2
Total metal and leaching concentrations of cyclone ash, scrubber ash and bottom ash

Heavy metal	Cyclone ash	Scrubber ash	Bottom ash	Taiwan regulatory limits
Total metal (mg/kg)				
Pb	650 ± 14.8	1229 ± 84.1	1230 ± 33.2	–
Cd	53 ± 1.7	130 ± 5.9	93 ± 0.6	–
Cr	274 ± 24.6	26 ± 1.7	248 ± 12.4	–
Cu	850 ± 89.1	740 ± 13.8	1130 ± 47.9	–
Zn	5540 ± 163.0	7780 ± 256.0	8210 ± 184.0	–
Leaching concentration (mg/L)				
Pb	0.03 ± 0.01	0.06 ± 0.02	ND[a]	5.00
Cd	ND[b]	1.04 ± 0.02	ND	1.00
Cr	0.07 ± 0.03	ND[c]	0.02 ± 0.01	5.00
Cu	ND[d]	0.08 ± 0.01	0.53 ± 0.03	15.0
Zn	0.89 ± 0.30	4.08 ± 0.10	0.18 ± 0.08	–

Mean ± standard deviation ($n = 5$).
[a] Pb: detection limits < 0.016 mg/L.
[b] Cd: detection limits < 0.014 mg/L.
[c] Cr: detection limits < 0.014 mg/L.
[d] Cu: detection limits < 0.013 mg/L.

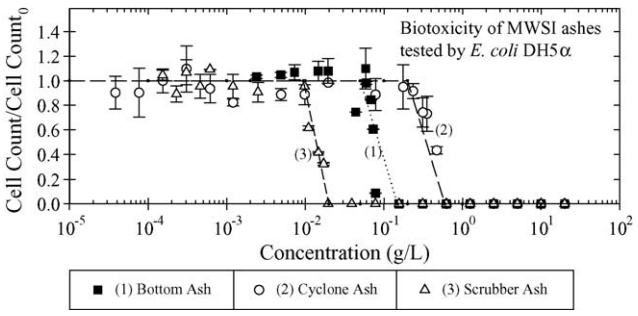

Fig. 4. Dose–response curve of bottom ash, cyclone ash and scrubber ash using *Escherichia coli* DH5α as the indicator microorganism.

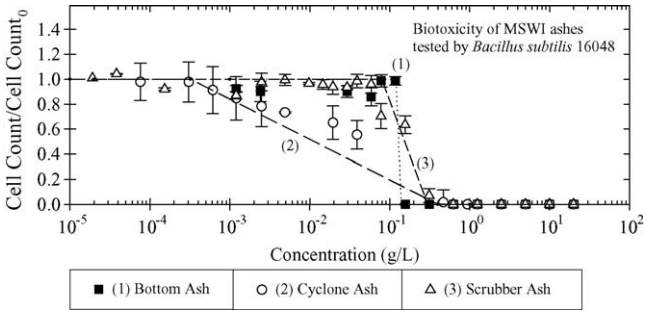

Fig. 5. Dose–response curve of bottom ash, cyclone ash and scrubber ash using *Bacillus subtilis* as the indicator microorganism.

0.156 g/L. This concentration may be termed EC_{100} (i.e., the effective concentration at which there is 100% mortality (i.e., 0% cell viability) of the indicator microorganism (i.e., DH5α; [18]). Approximately between 0.156 and 0.051 g/L, cells gradually redeemed from their significant viability loss for survival. This range (i.e., TT) is approximately the range of increasing mortality with increasing concentration of dose–response curve [18]. At the concentration below 0.0439 g/L (i.e., EC_0), cells were grown normally as the same as the control, suggesting cell growth in a nearly toxicant-free environment. This concentration may be called the "threshold" [18]. Below this threshold no detectable cell mortality was produced; in contrast, above the threshold, loss of cellular viability started to appear. Once the concentration exceeded EC_{100}, cells would no longer tolerate and cell mortality supervenes.

2. Cyclone ash: As shown in Fig. 4(2), complete inhibition to cellular growth was observed at the concentration over 0.625 g/L (i.e., EC_{100}). At the concentration less than 0.625 g/L, cell growth on plates gradually started to appear. The range of TT was approximately at the concentration ca. 0.625–0.210 g/L. At the concentration below 0.210 g/L (EC_0), complete cell growth phenomena nearly the same as control were occurred, suggesting that zero-toxicity characteristics was obtained.
3. Scrubber ash: As shown in Fig. 4(3), complete inhibition of cellular growth was observed at concentration over 0.0195 g/L (i.e., EC_{100}); a marked increase in inhibitory phenomena were gradually taking place with concentrations increased from 9.8×10^{-3} to 0.0195 g/L. The "zero-toxicity" condition was obtained at concentration less than 9.8×10^{-3} g/L (i.e., EC_0).

3.3.2. *Bacillus subtilis*

1. Bottom ash: As indicated in Fig. 5(1), cell viability was completely inhibited at the concentration greater than ca. 0.156 g/L (i.e., EC_{100}). At the concentration below 0.117 g/L (EC_0), bacterial cell growth remained nearly the same as the control. Above the EC_0, loss of cellular viability gradually increased. Once the concentration exceeded EC_{100} (0.156 g/L), bacterial cells could no longer survive.
2. Cyclone ash: As shown in Fig. 5(2), complete inhibition to cellular growth was observed at the concentration over 0.625 g/L (i.e., EC_{100}). At the concentration less than EC_{100}, cell growth on plates gradually started to appear. The range of TT is approximately at the concentration ca. 0.625–2.98×10^{-4} g/L. At the concentration below 2.98×10^{-4} g/L, complete cell growth phenomena nearly the same as control were shown, suggesting that zero-toxicity characteristics was almost obtained.
3. Scrubber ash: As shown in Fig. 5(3), complete inhibition of cellular growth was observed at concentration over 0.313 g/L (EC_{100}); a marked increase in inhibitory phenomena was taking place with concentration decreased from 0.313 to 0.085 g/L. The "zero-toxicity" condition was obtained at concentration less than 0.085 g/L (EC_0).

3.4. *Margin of safety*

3.4.1. *DH5α*

As shown in Fig. 4, cellular growth started to appear at ca. $EC_{100} = 0.0195$, 0.156 and 0.625 g/L for scrubber, bottom and cyclone ash, respectively. Interpolation also revealed that EC_{50} values for scrubber, bottom and cyclone ash were 0.0136, 0.0958 and 0.352 g/L, respectively. In addition, EC_0 values for scrubber, bottom and cyclone ash were 9.8×10^{-3}, 0.0439 and 0.210 g/L, respectively. Thus, toxicity ranking to DH5α is scrubber ash > bottom ash > cyclone ash. If the so-called "100-fold margin of safety" was adopted as a standard for an acceptable quantity for reuse of the MSWI residues [18], the maximum concentrations allowable to be considered "safe" were 0.195, 1.56 and 6.25 mg/L for scrubber, bottom and cyclone ash, respectively. The postulates behind this 100-fold margin are that (1) humans are 10 times more susceptible to the adverse effects of ashes than the indicator microorganism, (2) the weak in human population (e.g., young, old, etc.) are 10 times more sensitive than healthy adult humans and (3) multiplication law is applicable to have 100-fold margin (i.e., 10×10).

3.4.2. *Bacillus subtilis*

As shown in Fig. 5, cellular growth started to appear at ca. $EC_{100} = 0.313$, 0.156 and 0.625 g/L for scrubber, bottom and cyclone ash, respectively. Interpolation also revealed that EC_{50} values for scrubber, bottom and cyclone ash were 0.155, 0.132 and 0.0107 g/L, respectively. Thus, toxicity ranking to *B. subtilis* based upon EC_{50} is cyclone ash > bottom ash > scrubber

ash. Thus, the margin of safety (i.e., the maximum concentrations allowable to be considered "safe") for reuse of the MSWI residues were 3.13, 1.56 and 6.25 mg/L for scrubber, bottom and cyclone ash, respectively. It is noted that the discrepancy of toxicity ranking (e.g., EC_{100} and EC_{50}) to *B. subtilis* is due to differences in "defense" response range increased from EC_0 to EC_{100}. In particular, a small TT range for bottom ash (Fig. 5(1)) simply indicates that *B. subtilis* cannot tolerate to such a hostile source ash. Compared with *B. subtilis*, DH5α is more sensitive to respond the present toxicity (i.e., less level of margin of safety), since all the EC_0, EC_{50} and EC_{100} in DH5α showed in tandem to reveal parallel toxicity rankings of ashes. In summary, DH5α is thus more likely to be a best indicator microorganism for toxicity assessment to MSWI ashes. This first-attempt study apparently provided a feasible strategy to quantitatively evaluate the present risk of ashes for further applications. Follow-up study will be focused on the toxic species (e.g., cations and anions) present in ashes from various MSWI sources and may suggest cost-effective means to reduce toxicity for reuse of all MSWI residues. In addition, it was suspected that the presence of dioxins in the fly ash might have an adverse effect, thus further follow-up study will be inevitable for conclusive discussion.

4. Conclusion

This study clearly suggested that using TCLP as a standard leaching test may be feasible to other metal-bearing pollutants as criteria for safety regulation. However, low values of margin of safety (ca. 0.20–0.63 mg/L) stated herein for all ashes simply implied that ashes might still contain other toxic residues and evidently TCLP cannot guarantee the feasibility of reusability of ashes. It is suspected that high levels in anions are very likely to cause the residual toxicity, in particular chloride ions. The toxicity ranking to *B. subtilis* was different from that to DH5α, revealing that some toxic compositions in ash samples might repress the growth of specific microorganisms. DH5α was the most appropriate to be an indicator microorganism for toxicity evaluation of ashes, since the rankings of effective concentrations were all shown in tandem. Biotoxicity responses were strongly dependent upon the characteristics of indicator microorganism. Based on DH5α, the margins of safety (MOS) were thus 0.195, 1.56 and 6.25 mg/L for SA, BA and CA, respectively. Nearly identical levels of MOS were also suggested by *B. subtilis*, except for SA (3.13 mg/L).

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