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Dose–mortality assessment on municipal solid waste incinerator (MSWI) ash

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Abstract

This study provides a novel attempt to put forward, in general toxicological terms, quantitative series of toxicity of various ashes of municipal solid waste incinerator (MSWI) for reusability in various applications. Previous study disclosed that growth inhibition of *Escherichia coli* DH5α occurred at concentrations above 0.156, 0.625 and 0.0195 g L^{-1} for bottom ash (BA), cyclone ash (CA), scrubber ash (SA), respectively, suggesting the toxicity series of SA > BA > CA. However, the severity of such a toxicity series was not clearly revealed, thus whether ashes were still feasible for reuse in further applications was still remained uncertain. Compared to $NaNO_3$, $CrCl_2$ and $CdCl_2$, the existing toxicities of ashes were apparently significant even these ashes were all satisfied by the TCLP guidelines for EPA regulations. Dose–response analysis based upon loss of cell viability (e.g., EC_{50}) stated a toxicity series of SA > $CrCl_2$ > BA > $CdCl_2$ > CA > $NaNO_3$. The ranking of Hill slope *B* in BA > SA > CA > $NaNO_3$ > $CrCl_2$ > $CdCl_2$ clearly suggested the smallest tolerance (e.g., ranges from EC_{20} to EC_{50}) for ashes very likely due to synergistic toxicity of multiple species present in ashes. The findings showed that toxicity attenuation of ashes should be the first-ranking task prior to practical reuse and recycle in applications.

Keywords: MSWI ash; Toxicity; Dose–mortality curve; Margin of safety

1. Introduction

In Taiwan, as there was a marked rise in population for decades due to rapid economic development, it was getting difficult to find sites for landfill to treat significantly increased municipal solid waste (MSW). Thus, incineration certainly becomes a major refuse disposal method in particular on this populated island. In MSW, 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]. Thus, 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]. Additional benefit is that the waste-to-energy systems have been incorporated into MSWI management programs for energy recovery. However, the MSWI residues in reuse may still contain high-level toxic substances (e.g., heavy metals and organic chemicals like dioxins), causing a persistent threat to all species in ecology. Critics upon the industrial approach for reusability of MSWI residues have frequently pointed out that problems in evaluating toxicity and depleting risk for sustainable management needed to be clearly disclosed. Apparently, post-treatment of MSWI residues must be carried out to ensure safe reuse and recycling of residues accepted to the community. In the populous Taiwan the persistent need to construct incinerators will significantly increase due to difficulties to obtain appropriate sites for landfill. Thus, this dose–mortality assessment using a well-characterized *Escherichia coli* as our reporter microorganism tended to present the feasibility for reuse of ashes in further applications. Moreover, we intentionally selected the genetically defective *E. coli* DH5α as an environmentally susceptible bacterium to increase the sensitivity of toxicity indications.

In Taiwan, toxic characteristic leaching procedures (TCLP) has been adopted as a typical analytical method to inspect the concentration of leaching heavy metals. However, a lack of assessment guideline (e.g., risk and toxicity evaluation) to guarantee the long-term safety of remaining residues (e.g.,

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leaching heavy metals and slats) makes MSWI residues for safe reuse and recycling unpredictable and unreliable. To reveal the viability of utilizing MSWI ash residues for the practicability in reuse (e.g., construction material brick and cement), previous attempt in biotoxicity assessment [3] was introduced to present a quantitative measure on the model MSWI ashes collected from the cyclone of a mass-burning incinerator located in Taipei County, northern Taiwan. This follow-up study tended to quantitatively uncover the toxicity potency of ashes in terms of dose–mortality curves and to specifically suggest the present risk of ashes to on-site professionals. Dose–mortality curve refers to the ability of the tested chemical species to turn out systemic damages to reporter cells as a result of an exposure of a designated period of time. Thus, this is termed herein "chronic toxicity". In addition, experiments using a well-characterized bacterium (*E. coli*) to determine biotoxicity were relatively easy to conduct (e.g., traditional plate count method) and were very economically-feasible (e.g., much cheaper than Microtox® and rat model). As a matter of fact, Chen [4] provided a model attempt from a toxicological perspective to put forward the toxicity ranking of aromatic amines to *Pseudomonas luteola* for cell growth and biodecolorization. Similar perspective [5] was also adopted to explore whether there exists a noteworthy change in combined biotoxicity of phenol to *Ralstonia taiwanesis* in the presence of other nutrient sources. Thus, from practicality perspectives this study tended to extend such aspects to uncover suspected toxic sources present in ashes for further treatments.

Dose–response relations (e.g., dose–mortality curves herein) in toxicology [6] uncover the quantitative criteria of maximum treatment concentration (denoted as EC_{100}), 50% effective concentration EC_{50} (or median effective dose) and threshold concentration (i.e., maximal "no-response" concentration or EC_0) allowable for reuse of ashes. Note that EC_x indicates the effective concentration supplemented to DH5α cells to provoke x% response ($\forall x \in [0, 100]$). According to Masters [7], the fundamental goal of a dose–response assessment is to obtain a mathematical relationship between the concentration of a toxicant to which bacterial cells are exposed and the risk that there will be a mortality response to that concentration. It was postulated that in this study dose–response curves still follow a standard sigmoidal shape which can be described by a log-probit model [8].

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 tonnes 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. The tested materials (e.g., three residual MSWI solid ashes and chemicals) 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.
(4) Chemicals $CdCl_2$ (99+%, A.C.S. reagent), $CrCl_2$ (anhydrous powder, 99.99%), $NaNO_3$ (>99.0%, A.C.S. reagent) and $NaNO_2$ (97+%, A.C.S. reagent) were purchased from Aldrich.

2.2. Microorganisms and culture conditions

E. coli DH5α (generously provided by Professor Jo-Shu Chang, National Cheng-Kung University, Taiwan) was used as a probing microorganism 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 37 °C, pH 7.0, 200 rpm. To ensure the synchronous growth activity and maximum metabolic function 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^{-1}) and only the diluent with appropriate cell concentrations (ca. 1500–15,000 cells/mL) was chosen as the test seed (TS) for later uses.

2.3. Biotoxicity assessment

Biotoxicity assessment was specially designated through a modification of dose–response analysis [4,5] (in dose–mortality curves) as follows: the tested chemicals (e.g., ashes, $NaNO_3$, $NaNO_3$, $CrCl_2$, $CdCl_2$) were first sterilized via moist-heat method (121 °C at 15 psi for 20 min) to exclude the presence of unwanted bacterial contaminants. The concentration of samples defined herein was the concentration of tested chemicals and their serial diluents well mixed with sterile saline solution (SSS; $NaCl_{(aq)}$ 10.0 g L^{-1}). It was postulated that the sodium chloride solution in SSS used for serial dilutions of various anions and cations was a toxicity-free control. It was also postulated that no marked combined toxic interactions between sodium chloride and tested chemicals. For example, the toxic effects of nitrate and cadmium ion were studied in the presence of sodium ion (e.g., $NaNO_3$) and chloride ion (e.g., $CdCl_2$), respectively. Note that

to exclude confounding interferences phosphate buffered saline (PBS) solution, which is regularly used for biological assay, was not used herein, since metallic phosphate precipitates might be formed in serial dilution. The initial concentration C_0 for toxicity tests of all chemicals was chosen at ca. 20.0 g L^{-1}. Serial-half dilution of initial concentration C_0 (i.e., (1/2) C_0, (1/4) C_0, (1/8) C_0, (1/16) C_0, (1/32) C_0, . . . ,(1/2^n) C_0) was carried out by using 50 mL ash solution or its derived diluents mixed with 50 mL SSS. The 9.0 mL serial diluents (RSD) resulted 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 used as the toxicity-free control. The numbers of survival bacterium (i.e., *E. coli* DH5α) in SPCD or the control were estimated by the standard plate count method [9]. Standard plate count in LB medium was carried out as follows: SPCD were serially diluted with SSS immediately after sampling, and then appropriate volumes (ca. 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 [10] due to fresh preparation of fast-growing *E. coli* cells in all steps. The LB-medium plates were then incubated at 37 °C 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 could then be calculated using the following formula (CC, cell count):

$$\text{Cells per liter of broth (CC)} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution factor}}.$$

Note that all of the experiments were carried out in triplicate for data reproducibility of statistical significance. To have quantitative toxicity for comparison, CC_0 was chosen as the CC at zero toxicity control (i.e., SSS). The mortality response in the ratio $(1 - CC/CC_0)$ of 1 and 0 directly indicated complete repression and no inhibitory toxicity to cellular growth, respectively. The zero mortality response simply suggested that the present toxicity of this diluent at this concentration is nearly equal to the toxicity of SSS (i.e., "approached zero toxicity"). The concentration range for the ratio jumped from 0.0 to 1.0 in dose–mortality curves [3] is defined here as the "threshold toxicity" (TT) range. The comparison on TT range could provide an obvious diagram of toxicity ranking for various chemicals. For example, if the TT range for chemical A is much less than that for chemical B, chemical A is inevitable much more toxic than chemical B, indicating that much higher dilution factor must be carried out for chemical A in order to have "zero" toxicity as same as control (SSS).

2.4. Dose–mortality analysis

Probit analysis [7] is adopted to reveal dose–response curves of various toxic materials (e.g., chemicals and ashes to be tested). Probit model postulates that the tolerance capacity of individuals in response to toxic material in a given population is log-normal distribution. It is assumed that the response is strongly proportional to the concentration of the chemical bound to the cellular site of the defense action (e.g., resistance). Here, we selected the mid-point effective concentration (i.e., EC_{50}) on the dose–mortality curve for indication of toxicity series of various materials to be inspected. This is simply because it is usually easier to interpolate the mid-point EC_{50} accurately than to make extrapolated estimates of some other EC_x (e.g., EC_0, EC_{100}). Semilogarithmic plot of toxic-species concentration versus the provoked response is assumed to reveal a linear relation. Probit model converts sigmoid-shaped dose–response curve into a linear normal equivalent deviation (NED) scale. For example, the 50% and 84.1% response correspond to the NED scale in 0 and 1, respectively. In addition, probit unit in the model equals NED scale plus 5. The conversion formulae are shown as follows:

$$Y = A + B \log Z, \tag{1}$$

$$P = \frac{1}{2}\left\{1 + \text{erf}\left(\frac{Y-5}{\sqrt{2}}\right)\right\}, \tag{2}$$

$$\text{erf}(x) \equiv \frac{2}{\sqrt{\pi}}\int_0^x e^{-\xi^2}\,d\xi, \tag{3}$$

where A and B denote the intercept and the Hill slope (i.e., steepness or slope factor) of dose–response relation, Z and Y are metal concentration (mg L^{-1}) and probit unit, respectively, P the response (%) corresponding to administered metal, and erf(x) is an error function. The procedures of transform and inverse transform between the probit unit and the response were described elsewhere [4,5]. Note that the response variable is normalized to be located between 0 and 1. The conversion relation between the probit unit and provoked response is listed in Table 1. For example, 55% and 85% of toxic response correspond to probit unit of 5.13 and 6.04, respectively. The dose should be defined in a continuous positive real domain (i.e., $C \in R^+$). The toxicity responses are determined via $P = 1 - (VCC/VCC_0)$, where VCC and VCC_0 denoted the viable cell count survived in the tested culture and SSS, respectively. The response is defined to be any real number between 0 and 1. The slope B of the dose–mortality curve also provides information which is vital in evaluating potential biotoxicity of chemicals for humans. The Hill slope B may be steep (e.g., $B > 1$) or shallow (e.g., $B < 1$), indicating that the effective concentration range (i.e., TT or the range from EC_0 to EC_{100}) is narrow (i.e., steep slope) or wide

Table 1
Critical effective concentrations of toxicity predicted from the probit model

	EC_0	EC_{20}	EC_{50}	$Y = A + B \log C$
Bottom ash	0.0602	0.0781	0.0898	$Y = 19.5 + 13.85 \log C$
Cyclone ash	0.191	0.287	0.358	$Y = 8.935 + 8.817 \log C$
Scrubber ash	0.00831	0.0111	0.0130	$Y = 28.27 + 12.34 \log C$
$NaNO_3$	0.233	0.849	1.703	$Y = 4.357 + 2.781 \log C$
$CdCl_2$	0.00794	0.0479	0.126	$Y = 6.796 + 1.998 \log C$
$CrCl_2$	0.00338	0.0165	0.0388	$Y = 8.195 + 2.264 \log C$

The response variable P is defined as $1 - (VCC/VCC_0)$ and C denotes the concentration of tested material (g L^{-1}). The Y value corresponds to its response variable P via probit transformation.

(shallow slope). The steep slope means that, within the bacteria, there is only a small difference between the concentration that is lethal for the most susceptible cells and the concentration that is lethal for the most resistant cells. If the concentration of toxic materials exceeded EC_0 (i.e., threshold), only a slightly increase in the concentration might result in a significant rise in mortality to DH5α cells and even more severe in compete loss of cellular viability (i.e., EC_{100}).

3. Results and discussion

3.1. Toxicity assessment

As lower effective concentration (EC_x) could clearly reveal higher toxic characteristics of tested material to the indicator *E. coli* DH5α, the toxicity ranking, in increasing order, based upon loss of cell viability (e.g., EC_0, EC_{20} and EC_{50}) are listed as follows (Table 1 and Figs. 1–3 [11]):

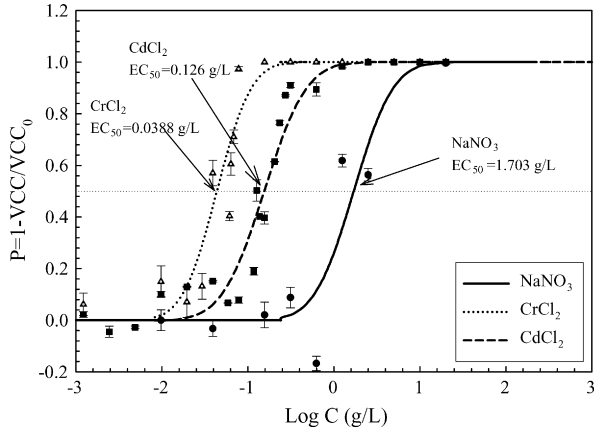

Fig. 1. Dose–mortality curves of typical chemical species using *E. coli* DH5α as the indicator microorganism. Error bars indicated the data variations of experiments in triplicate.

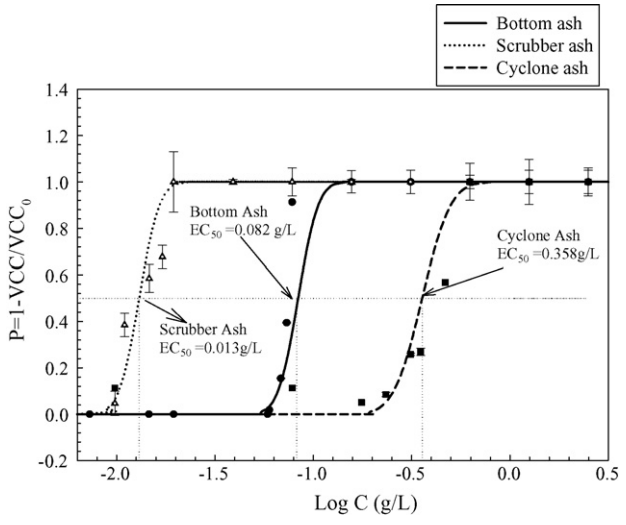

Fig. 2. Dose–mortality curves of ashes using *E. coli* DH5α as the indicator microorganism. Error bars indicated the data variations of experiments in triplicate.

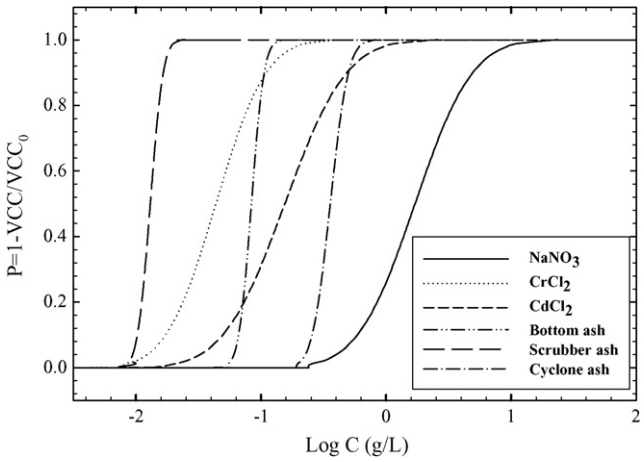

Fig. 3. Comparison of toxicity potency for all tested materials (i.e., typical chemicals and ashes). Steep-shapes of dose–mortality curves for ashes clearly indicated the synergistic interactions of multiple toxic species present in ashes.

EC_0: $CrCl_2 > CdCl_2 >$ scrubber ash > bottom ash > cyclone ash > $NaNO_3$, (4)

EC_{20}: scrubber ash > $CrCl_2 > CdCl_2 >$ bottom ash > cyclone ash > $NaNO_3$, (5)

EC_{50}: scrubber ash > $CrCl_2 >$ bottom ash > $CdCl_2 >$ cyclone ash > $NaNO_3$. (6)

The maximum concentration at which "no-effects" are observed is called the threshold concentration and no-observed effect level (i.e., EC_0 [12]). A threshold may also be considered as the minimum concentration that does produce an effect (e.g., a decrease in cellular viability). That is, the threshold is the turning point between no-effect and effect levels of exposure. Moreover, the slope factor B of probit model for dose–response curves (see Eq. (2)) is also a significant indicator to the toxicity potency of a material to be tested. In Eqs. (5) and (6), the lowest EC_{20} and EC_{50} for scrubber ash (SA) indicated that SA was rank-1 toxic ash of all. It might reveal that in incinerators significant amounts of toxic metals (originally vaporized at 750–1000 °C) were condensed to ca. 450 °C and concentrated onto the surface of SA in the flue gas cooling system. The highest metal concentration was observed in the bag filter very likely due to collected ash particles sized <100 μm provided a relatively larger specific surface area for metal attachment [13]. The toxicity ranking, in increasing order, based upon Hill slope B is listed as follows (Table 1 and Figs. 1–3):

Slope B: bottom ash (13.85) > scrubber ash (12.34) > cyclone ash (8.817) > $NaNO_3$ (2.781) > $CrCl_2$ (2.264) > $CdCl_2$ (1.998). (7)

Due to the steep-shape (i.e., higher slope B value) response curves for ashes, scrubber ash and bottom ash tended to be

more toxic than tested metals (e.g., $CrCl_2$ and $CdCl_2$; toxicity series (4)–(7), Figs. 1 and 2). For pure chemicals, there is a particular receptor site or intracellular binding site for each specific toxicant. In contrast, as ashes might contain multiple toxic species, toxic responses of ashes would be more sensitive than responses of pure chemicals. This is very likely due to abundant energy requirements and enzymatic activities to trigger several receptors and active sites for resistance concurrently. Mineralogical studies [14] have also shown that bottom ash in MSWI is primarily composed of fine ash material (ca. size <20 mm) and melted components, including half in crystallized form and only minute quantities of metallic components, ceramics and stones. Mineral species (e.g., calcite, ettringite, hematite, quartz, gypsum and silicate) have also been identified [14]. However, the bottom ash still contains potentially toxic elements (e.g., metal chlorides) which could be leached into soils and sediments to threaten the environment. For example, laboratory and field studies have indicated that these leachates contained high concentrations of soluble salts (e.g., IA, IIA element-bearing) [15,16] and the fine-particle fraction (ca. <20 mm) often contained heavy metals (e.g., Cu and Zn) [17]. It is noted that in comparison a standard dose–response curve has a Hill slope B of unity. Since all slope factors B of the curves were greater than unity, these steeper curves thus emphasize the toxic characteristics for all tested cases. Apparently, significantly higher values of Hill slope B (ca. 9–14 Table 1) for ashes simply implied that DH5α cannot provoke effective cellular defense mechanisms to resist the toxicity of these toxic species once the concentration of ashes exceeded their threshold levels (EC_0). For instance, previous study [3] indicated that significant amounts of Cd and Pb were found in the scrubber ash. This point might suggest that the air pollution control devices removed a significant portion of the metals (e.g., volatile metals Cd and Pb in incineration) and organic chlorides (e.g., dioxins) via deposition into solid particles (SA), leading to noteworthy toxicity in SA. This is very likely due to complicated compositions present in ashes to augment synergistic interactions of multiple species to DH5α. Evidently, the largest value (13.85) of slope B for bottom ash (Table 1) suggests the smallest tolerance range from EC_0 to EC_{100} to the indicator cells DH5α (Fig. 3). In contrast, the smallest value (1.998) of slope B for $CdCl_2$ directly implies the widest range of tolerance to phenol toxicity from the threshold dose (EC_0) to a maximum effect dose (EC_{100}). Owing to the smallest tolerance ranges of toxicity for ashes, toxicity attenuation prior to reuse and recycling of ashes is critically important for further applications. Therefore, the safety criteria as a guideline for feasibility of ash reuse were provided afterwards.

3.2. Criteria for ash reuse and recycling

As known, TCLP is the most common leaching test generally accepted by US EPA for toxicity evaluation under Resource Conservation and Recovery Act (RCRA) of 1976. Characteristic hazardous or toxic wastes are determined by evaluating their ignitability, corrosivity, reactivity and toxicity. Apparently, compared to other factors, the residual toxicity of MSWI ashes is the sole requirement to satisfy for the feasibility of ash reuse and recycling. Leachability reflects the rate and potential at which a constituent is released from the material and enters into the environment via the leachate. Thus, our findings herein were compared with TCLP regulations to reveal the conclusive safety criteria. According to TCLP parameters (refer to Table 5-5 in [18]), the regulatory levels (RL) of cadmium and chromium are 1.0 and 5.0 mg L^{-1}, respectively. If the threshold levels (EC_0; Table 1) of tested species in dose–mortality curves (Figs. 1 and 2) were selected as regulatory criteria for toxicity, the estimates should be converted into the correlation relationships with RL. For example, the ratios of EC_0 divided by the RL (i.e., EC_0/RL) for cadmium and chromium were approximately 7.94 and 0.676, respectively. Therefore, as indicated in Table 1 safety criteria of ashes to be reused could be set at convention of a 10-fold margin of safety. The purpose of this 10-fold margin of safety (instead of ca. 7.94-fold) is to allow an extra deviation for safety concern to humans. This 10-fold margin was considered appropriate, as similar setting of margins of safety were also popularly used in toxicology [6]. Of course, the hypothesis that doses are equivalent if the dose per unit of body weight in the tested bacterium (i.e., DH5α) and humans is the same is not valid. However, according to Ottoboni [6], the assumptions behind this 10-fold margin are that: (1) humans are 10 times more sensitive to the adverse effects of toxic chemicals than tested organisms (e.g., *E. coli* DH5α), and (2) the 10-fold margin of safety is a postulated separation between the highest level of a chemical that produces no adverse effect (e.g., EC_0) in the probing organism and the level of exposure estimated to be safety for humans. Thus, regulatory levels of bottom ash, cyclone ash and scrubber ash were ca. 6.02, 19.1 and 0.831 mg L^{-1}, respectively. Obviously, the toxicity of scrubber ash is the first rank to be considerably reduced in comparison with to all ashes. In contrast, cyclone ash should be the most appropriate for further reuse purposes.

4. Conclusions

In summary, compared to prior study [3] toxicity rankings of ashes and tested chemicals (e.g., EC_{50}: scrubber ash > $CrCl_2$ > bottom ash > $CdCl_2$ > cyclone ash > $NaNO_3$ and EC_0: $CrCl_2$ > $CdCl_2$ > scrubber ash > bottom ash > cyclone ash > $NaNO_3$) strongly suggested that synergistic interactions of multiple toxic species in ashes due to steep nature of response curves for ashes. This analysis also suggested that any means (e.g., acid wash) to remove toxic species for toxicity attenuation should be requisite prior to reuse and recycling of MSWI ashes. This study is a first screening criterion upon tested materials for feasibility and further evaluation upon human receptor for risk assessment should be carried out afterwards. Follow-up study will be focused on how to significantly reduce residual toxic species present in MSWI ashes by means of toxicity attenuation strategy for safe reuse of MSWI residues. In addition, more typical toxic metals of major interest to EPA (e.g., Ni, Al) will be selected for toxicity evaluation to construct a data bank of finger-print profiles as a basis of comparison on toxicity figures of various ashes and unknown materials. In addition, according to risk assessment the criteria proposed herein also provide a

guideline of the performance index to be achieved for toxicity attenuation.

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