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ScienceDirect
Journal of Hazardous Materials 148 (2007) 326–333
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Dose–mortality assessment upon reuse and recycling of industrial sludge

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Received 29 December 2006; received in revised form 14 February 2007; accepted 18 February 2007
Available online 23 February 2007

Abstract

This study provides a novel attempt to put forward, in general toxicological terms, quantitative ranking of toxicity of various sources of sludge for possible reusability in further applications. The high leaching concentrations of copper in printed circuit board (PCB) sludge and chromium in leather sludge apparently exceeded current Taiwan's EPA regulatory thresholds and should be classified as hazardous wastes. Dose–mortality analysis indicated that the toxicity ranking of different sources of sludge was PCB sludge > CaF_2 sludge > leather sludge. PCB sludge was also confirmed as a hazardous waste since the toxicity potency of PCB sludge was nearly identical to $CdCl_2$. However, leather sludge seemed to be much less toxic than as anticipated, perhaps due to a significant decrease of toxic species bioavailable in the aqueous phase to the reporter bacterium *Escherichia coli* DH5α. For possible reusability of sludge, maximum concentrations allowable to be considered "safe" (ca. EC_{100}/100) were 9.68, 42.1 and 176 mg L^{-1} for CaF_2 sludge, PCB sludge and leather sludge, respectively.

Keywords: Industrial sludge; Dose–mortality curve; Margin of safety; Toxicity

1. Introduction

As known, heavy-metal contamination is one of the priority problems in Taiwan due to its significant threat to human health and life. For decades, Taiwan just like other developing countries has paid her environment as a cost to promote industry, remaining a substantial number of problems (e.g., pollutions of rivers and the air, inordinate deforestation) to be resolved up to now. According to Taiwan's EPA, the annual production of hazardous heavy metal sludge has reached 31,60,000 tons in highly populous Taiwan. In particular, the annual sludge production from the processes of wastewater treatment performed by leather tanning and printed circuit board (abbreviated PCB) manufacturing industries mostly produce large amounts of sludge that contains toxic metal salt residues (e.g., copper, cadmium, lead, nickel, and zinc) are 1,86,000 tons and 36,700 tons, respectively. To remove heavy metals (denoted by *M*), chemical precipitation in alkaline solutions to form metal hydroxide sludge (e.g., $M^{2+} + 2OH^- \rightarrow M(OH_2)\downarrow$) was frequently used. However, these heavy metals in the sludge might still be released in an aqueous phase at acidic pHs (e.g., $M(OH)_2 + 2H^+ \rightarrow M_{(aq)}{}^{2+} + 2H_2O$), causing a striking impact on the environment. For example, the electroplating sludge produced in PCB milling and semiconductor fabrication contains high levels of heavy metals and apparently must be treated and/or detoxified. Moreover, the processes of wafer chemical ething commonly used hydrofluoric acid (HF) to remove layers of SiO_2, metals and polysilicon, leading to the generation of a considerable amount of wastewater primarily laden with HF and hydrofluorosilicic acid (FSA, H_2SiF_6). As a typical treatment of waste HF is transforming HF to mineral fluorite (i.e., calcium fluoride CaF_2; $K_{sp} = 3.9 \times 10^{-11}$) via calcium(II) precipitation, a noteworthy amount of CaF_2 sludge is generated. The annual sludge (dewatered) production is approximately 10,900 tons. In addition, due to low CaF_2 content (ca. 20–40%) and other impurities present in CaF_2 sludge, the reuse and recycling of the sludge for further applications might be economically unfavorable and evidently CaF_2 sludge would be an inevitable health hazard. To resolve this problem, cement solidification was commonly exploited for the further sludge treatment. Nevertheless,

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doi:10.1016/j.jhazmat.2007.02.045

appropriate landfill site is certainly needed for the disposal of generated products of cement solidification. However, due to limited landfill sites available in highly populous Taiwan, the process of cement solidification gradually becomes inappropriate. Furthermore, a remarkable rise in waste volume after cement solidification was obviously against EPA's regulation for waste minimization [1]. Plus, only minute amounts of landfill leachate might significantly pollute a reservoir of groundwater rendering it unusable as potable water. Thus, for sustainable development sludge disposal technologies (e.g., solidification and landfill) are of course not environmentally friendly or technically feasible. Therefore, immediate action through a more promising recycling and source reduction to achieve the goal of "zero-waste discharge" must be taken to reduce the production of heavy metal-laden sludge. According to the Resource Conservation and Recovery Act (RCRA), if a waste from a specific production process exhibits at least one of the four characteristics – corrosivity, reactivity, ignitability or toxicity – it is considered as a hazardous waste. Apparently, toxicity assessment upon different sources of sludge should be primarily carried out herein as all other three characteristics are not main concern.

As industrial sludge (e.g., PCB sludge, leather tanning sludge and CaF_2 sludge) often contain high levels of leachable heavy metals and salts, they are usually classified as hazardous wastes worldwide due to toxicity. Hence, sludge must be appropriately treated by means of wastes intermediate treatment process (e.g., acid extraction–chemical cementation [2] and acid extraction–fractional precipitations [3] prior to possible recycling and reuse. For possible reuse and/or disposal, all residual compositions of sludge at least must achieve the criteria of EPA's regulation to be termed as non-hazardous materials. Thus, owing to the specific characteristics of sludge and the need of waste reduction, recycling and reuse of residues as the construction materials should be more promising. For example, much attention on reuse of industrial sludge has been paid for production of clinker [4], cement paste [5,6], lightweight aggregates [7], glazed tiles [8] and brick [9].

As sludge dumping (e.g., landfill) is banned in the international community due to new EPA regulations, this study suggested some available alternatives for waste reduction (e.g., reuse or recycling). In the view of human risk and toxicity, the leaching of heavy metal and/or other toxic chemicals from various sources of sludge will become the highest priority problem prior to further reuse and recycling (e.g., construction material(s)). In Taiwan, up to now 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 (e.g., risk and toxicity evaluation) to guarantee the long-term safety of remaining leachable heavy metals and salts, whether industrial sludge are feasible for safe reuse is still remained open to be discussed. To reveal the feasibility of utilizing industrial sludge for reuse, this first attempt in biotoxicity assessment on some model sludge was indicated to provide a quantitative measure for the practicality of reuse in practice. Obviously, without adequate toxicity figures of industrial sludge, reusability of sludge is unsafe and unreliable. Ideally, to relate toxicity to dose, one should correlate the amount of chemical (e.g., sludge here) in the probing organisms (e.g., target metabolizing cell) with the observable effect (e.g., cell mortality) [10]. Thus, this study tended to uncover the ranking of quantitative toxicity of various industrial sludge using some essential toxic chemicals as a basis for comparison and to show the feasibility for possible reuse and recycling afterwards. As a matter of fact, Chen et al. [11] and Chen and Lin [12] provided some model attempts from a toxicological perspective to put forward the toxicity ranking of metallic ions to *Pseudomonas aerugina* PU21 (Rip64) for bioremediation and toxicity series of some incineration ashes to indicator microorganisms. Similar perspective [13] was adopted to reveal whether there existed a noteworthy change in combined biotoxicity of phenol to *Ralstonia taiwanesis* in the presence of other carbon sources to stimulate bioremediation. However, whether this assessment can be applicable to be used for on-site practical cases still remained uncertain. This first-attempt study thus tended to employ such perspectives in pursuit of toxicity series of various sources of waste sludge. It also suggested what levels of toxicity attenuation (e.g., margin of safety) on sludge prior to reuse and recycling should be achieved. If the tested sludge contained toxic species, it is not bearable to viable cells for propagation on agar-plates. That is, cellular viability can be used as a direct parameter in response to the dose of "toxic" sludge or chemical. Since *Escherichia coli* was well-characterized and faster grown on agar-plates, it was feasible to be a reporter microorganism to determine percentage cell viability for toxicity evaluation. With regard to economic feasibility, our toxicity assessment is apparently more cost-effective compared to Microtox® and the rat models which are popularly used in toxicology. Here, we also intentionally selected a genetically modified *E. coli* DH5α for probing to increase the sensitivity in response to toxicity. Thus, this study could quantitatively uncover the toxicity potency of different sources of sludge in terms of dose–mortality curves and specifically suggested the present risk of tested sludge to on-site professionals.

2. Materials and methods

2.1. Sludge materials

Three sources of sludge (i.e., PCB sludge, leather sludge and CaF_2 sludge) were used for this assessment as follows:

(1) PCB sludge: the sampled sludge was obtained from a PCB manufacturing plant in Chungli Industrial Park, Northern Taiwan. Manufacturing of high-quality multilayered PCBs requires that the boards are thoroughly washed in each electro-chemical process (e.g., electroplating and etching) to prevent any cross contamination. The wash effluent contains chelating agents laden with a high level of metallic ions and special polymers in the electroplating process. The metal ions were then precipitated in alkaline solutions to form the insoluble metal hydroxide. To facilitate this precipitation, a commercial poly-electrolyte flocculent is added

to absorb/complex with the ions and form large aggregating particulates before precipitation.

(2) Leather sludge: the primary chemical sludge primarily containing chromium (III) was obtained from a physical–chemical effluent treatment plant of a garment leather tanning manufacturing plant in Chungli Industrial Park, Northern Taiwan. The sludge collected from the settling tank was dewatered through filtration and pressed to ca. 65% of total solids (TS).

(3) CaF_2 sludge: the sludge was obtained from a semiconductor manufacturer based in Hsinchu Science Park, Northern Taiwan. As hydrofluoric acid has been used for wafer ething and tool cleaning, the process generated a considerable quantity of waste acid solutions (e.g., hydrofluoric acid and hydrofluorosilicic acids). The HF was then converted to a significant amount of calcium fluoride (CaF_2) (i.e., a major composition of CaF_2 sludge) via calcium (II) precipitation.

The PCB sludge, leather sludge and CaF_2 sludge were individually dried at 105 °C for 24 h until a constant weight (ASTM D 2216) was reached (i.e., almost moisture-free) in order to characterize their chemical compositions.

2.2. Chemical analysis

PCB sludge, leather sludge and CaF_2 sludge were used for analysis by means of TCLP, and chemical composition determination as follows: Chemical composition determination was conducted by X-ray fluorescence (XRF) was performed with an automated RIX 2000 spectrometer. The specimens were prepared for XRF analysis by mixing 0.40 g of the sample and 4.0 g of 100 Spectroflux, at a dilution ratio of 1:10. Homogenized mixtures were placed in Pt–Au crucibles and treated for 1 h in an electrical furnace at 1000 °C. The homogeneous melted sample was recast into glass beads (ca. 2 mm thick, 32 mm in diameter). Toxicity characteristic leaching procedure (TCLP) was carried out according to Taiwan EPA method NIEA R201.13 C. The extraction procedure required the pre-evaluation of the pH value of the sample to estimate the appropriate amounts of extraction fluid for the analysis. 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 ions and anions via Metrohm 761 Compact IC. In addition, Metrosep SUPP3 column was used in IC with a flow rate of 1.0 mL min^{-1} and suppressed conductivity mode detection settings at a system pressure of 12.9 MPa. Leaching concentration was evaluated via EPA standard methods as follows: 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

The indicator microorganism *Escherichia coli* DH5α (generously provided by Professor Jo-Shu Chang, National Cheng-Kung University, Taiwan) was used for biotoxicity assessment. To achieve the optimal metabolic activity in the same growth phase for bioassay, a loopful of the indicator microbial 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. Then, 5% (v/v) cultured broth was inoculated to fresh sterile LB medium and a cell culture was harvested at approximately mid-exponential growth phase (ca. 4 h) to ensure optimal cellular viability 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.

2.4. Biotoxicity assessment

Biotoxicity assessment was specially designated through a modification of dose–mortality analysis [12,14] as follows: the sampled sludge (i.e., PCB sludge, leather sludge and CaF_2 sludge) were first sterilized via moist-heat method (121 °C at 15 psi for 20 min) to exclude the presence of unwanted microbial contaminants. As the sludge might contain mixtures of several chemicals instead of pure chemicals, the "apparent" concentration of samples defined herein was the concentration of sludge and their serial diluents well mixed with sterile saline solution (SSS; $NaCl_{(aq)}$ 10.0 g L^{-1}). Note that phosphate buffered saline (PBS) solution, which is regularly used for biological assay, was not applicable for serial dilution of sludge solutions, since metal phosphate precipitates might be formed to interfere with results. The initial concentration C_0 for toxicity tests of all sources of sludge was chosen at 20.0 g L^{-1}. 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 sludge 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 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 sludge-free control. The numbers of viable bacterium DH5α in SPCD or the control were estimated by the standard plate count method. 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. 0.10–0.20 mL) of SPCD were spread onto agar *Petri* plates. Note that all cells in SPCD would be assumed viable and culturable on LB-medium plates due to fresh preparation of cells in maximal viability for 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. The microbial population in the original RSD was calculated using the following formula (VCC: viable cell count):

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

To have quantitative toxicity for comparison, VCC_0 was chosen as the VCC at sludge-free SSS control. The ratio VCC/VCC_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 nearly equals the toxicity of SSS (i.e., "zero" toxicity). The concentration range for the ratio jumped from 1.0 to 0.0 in dose–mortality curves is defined as the "toxicity threshold" (TT) range. The TT ranges of various sources of sludge in dose–mortality curves can provide obvious figures of their toxicity ranking. For example, if the TT range for sludge A is much less than that for sludge B, sludge A is inevitable much more toxic than sludge B, indicating that much higher dilution factor must be carried out for sludge A to reach "zero" toxicity as same as control (SSS).

2.5. Dose–mortality analysis

Probit analysis [15–18] was adopted to reveal dose–mortality curves of various sources of sludge to be tested. The probit model postulates that the tolerance capacity of indicator microorganisms in response to the suspected toxic material in a given population is Log-normal distribution. Usually, the mid-point effective concentration (i.e., EC_{50}) of different material on its dose–mortality curve was selected as a comparative basis for toxicity series. This is simply because it is usually easier to interpolate the mid-point EC_{50} accurately than to make extrapolated estimates of critical 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–mortality 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:

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

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

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

where A and B denote the intercept and the Hill slope (i.e., steepness or slope factor) of dose–mortality relation, Z and Y are metal or sludge concentration (g L^{-1}) and probit unit, respectively; P is the response (%) corresponding to administered metal or sludge,erf(x) is an error function. 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 toxicity responses are determined by $P = 1 - VCC/VCC_0$, where VCC and VCC_0 denoted the viable cell count remained in the sample culture and SSS, respectively. The slope B of the dose–mortality curve also provides information which is vital in evaluating potential biotoxicity of chemicals to indicator microorganism DH5α. 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 (shallow slope), respectively. 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 remarkable rise in mortality of DH5α cells and even more severe in compete loss of cellular viability (i.e, EC_{100}).

3. Results and discussion

3.1. Characterization of sources of sludge

As indicated in Fig. 1, the fingerprint speciation of the PCB sludge identified by the XRD techniques indicated that the major components were $CuCl_2$, $CuSiO_3$ and $CaMg(SiO_3)_2$. The major components of leather sludge were $CaSO_4$, $NaAlS_2$, $CaSiO_3$, Al_2S_3 and $Cr_2(SO_4)_3$. The major species of CaF_2 sludge were $CaMg(CO_3)_2$ and CaF_2. As shown in Table 1, the plentiful compositions of PCB sludge were SiO_2 (6.02%), CaO (1.71%), Fe_2O_3 (1.97%) and Al_2O_3 (0.73%). Table 2 also revealed that the major fingerprint ion species of the PCB sludge, identified by IC techniques, were NH_4^+, Ca^{2+} and Mg^{2+} comprised 3652.2 mg L^{-1}, 1286.8 mg L^{-1} and 421.6 mg L^{-1}, respectively. The major anions were nitrite, nitrate and chloride comprised 162.2 mg L^{-1}, 16574.5 mg L^{-1} and 39.9 mg L^{-1}, respectively.

For leather sludge, the most abundant components contained SiO_2, Al_2O_3, Fe_2O_3 and CaO were 25.93%, 22.08%, 16.92%

Table 1
Chemical composition and total heavy metal of PCB sludge, leather sludge and CaF_2 sludge

Composition	PCB sludge	Leather sludge	CaF_2 sludge
SiO_2 (%)	6.02	25.93	1.21
Al_2O_3 (%)	0.73	22.08	1.13
Fe_2O_3 (%)	1.97	16.92	0.05
CaO (%)	1.71	4.45	55.52
MgO (%)	N.D.	1.33	0.72
SO_3 (%)	0.01	0.78	0.02
K_2O (%)	0.01	1.19	0.78
Cu (mg kg^{-1})	292000	800	N.D.
Zn (mg kg^{-1})	5800	120	0.35
Pb (mg kg^{-1})	1540	N.D.	N.D.
Cr (mg kg^{-1})	N.D.	25000	N.D.
Others (%)	59.62	24.74	40.57

N.D.: not detected (i.e., below the detection limit).

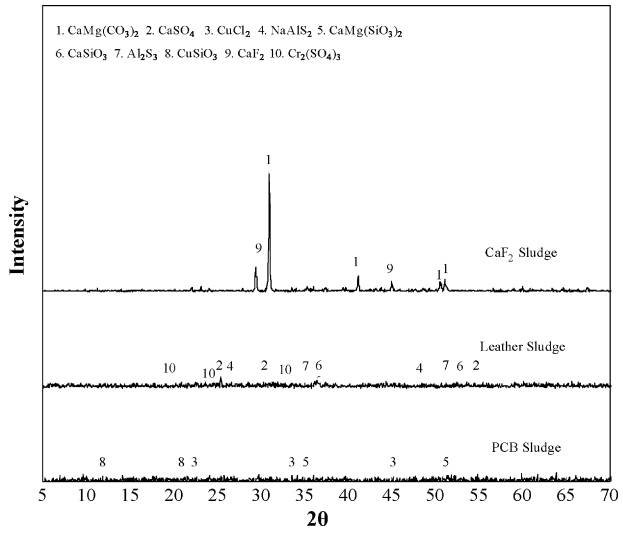

Fig. 1. XRD patterns of PCB sludge, leather sludge and CaF_2 sludge.

and 4.45%, respectively. The second richest components are MgO, K_2O and SO_3 contributing about 1.33%, 1.19% and 0.78% (w/w), respectively. The main ions were Ca^{2+}, NH_4^+ and Na^+ comprised 38402 mg L^{-1}, 12314 mg L^{-1} and 5176 mg L^{-1}, respectively. Moreover, the main anions were nitrate, sulfate and chloride comprised 107074 mg L^{-1}, 14114 mg L^{-1} and 48 mg L^{-1}, respectively.

In addition, the major component of the CaF_2 sludge was CaO (55.52%). Next abundant components were Fe_2O_3, K_2O, MgO, SiO_2 and Al_2O_3 at about 0.05%, 0.78%, 0.7%, 1.2% and 1.13%, respectively. The major cations were Ca^{2+} and Mg^{2+} comprised 1306.6 mg L^{-1} and 126.2 mg L^{-1}, respectively. The major anions were nitrite, nitrate and F^- comprised 23.4 mg L^{-1}, 138.2 mg L^{-1} and 15210 mg L^{-1}, respectively.

Major metal species in PCB sludge was copper at concentration 292,000 mg/kg (ca. 0.292 g/g ~ 29.2% (w/w)), very likely causing a striking toxicity to be classified as a hazardous sludge (mentioned later). The reasons to cause such a high level of copper are straightforward. The electroless copper plating process usually included several stages for use in plating through-holes in PC substrates and depositing copper on the surfaces. In addition, an electroless copper line sequence might include many electroless depositing baths (ca. 20–25 tanks in serial operations) and a metal depositing apparatus for the electroplating process. Note that over 95% of metal ions are subsequently removed by metal hydroxide precipitation in an effluent treatment plant employing a sequential process of flocculation, pH adjustment and alkaline precipitation. To facilitate the precipitation, a commercial polyelectrolyte flocculent was added to absorb/complex with the ions and form large aggregated particulates prior to precipitation.

Table 2
Ion and anion of PCB sludge, leather sludge and CaF_2 sludge

Composition	PCB sludge	Leather sludge	CaF_2 sludge
Ion (mg L^{-1})			
Na^+	N.D.	5176	N.D.
NH_4^+	3653	12314	N.D.
K^+	N.D.	N.D.	N.D.
Ca^{2+}	1287	38402	1307
Mg^{2+}	422	N.D.	126
Anion (mg L^{-1})			
Cl^-	40	48	N.D.
NO_2^-	162	N.D.	23
NO_3^-	16575	107074	138
SO_4^{2-}	N.D.	14114	N.D.
F^-	N.D.	N.D.	15210

N.D.: not detected (below the detection limit).

Table 3
Leaching concentration of various sources of sludge

Sample	Cu (mg L^{-1})	Zn	Pb	Cr	Cd
PCB sludge	713	7	0.45	N.D.	N.D.
Leather sludge	N.D.	N.D.	N.D.	575	N.D.
CaF_2 sludge	N.D.	N.D.	N.D.	N.D.	N.D.
Regulatory limit	15	–	5	5	1

N.D.: not detected (below the detection limit).

3.2. Total heavy metal and leaching concentration

To reveal sludge reusability, the heavy metal concentrations in the TCLP leachates were examined (Table 3). Compared to PCB sludge and CaF_2 sludge, the leather sludge still contained noticeably higher level of chromium (25,000 mg kg^{-1}; Table 1). The tanning agents (e.g., trivalent chromium) were used in leather tanning processes to react with collagen fibers in the hides. As the wastewater from a tannery contained +3 chromium, the dissolved oxygen in wastewater effluents might oxidize Cr^{3+} to dichromate ion, $Cr_2O_7^{2-}$ (i.e., $4Cr^{3+} + 3O_2 + 8H_2O \leftrightarrow 2Cr_2O_7^{2-} + 16H^+$ in acidic pHs). In particular, leaching concentrations of copper in PCB sludge and chromium in leather sludge were 713 mg L^{-1} and 575 mg L^{-1}, respectively, exceeding current Taiwan's EPA regulatory thresholds (Table 3). Thus, they should be classified as hazardous wastes.

3.3. Toxicity assessment of various sources of sludge

To provide detailed toxicity figures of various sources of sludge, a comparison of dose–mortality curves of different sources of sludge with typical fingerprints of ionic compounds [12] was made (Figs. 2 and 3). The reasons why cellular mortality could be directly used as an indicator for toxicity evaluation are straightforward. First, cellular mortality simply suggested the overall metabolic outcome of tested population in response to the toxicant-laden hostile environment. In addition, there might still exist some toxicants that were difficult to be identified during sludge formation. Apparently, selection of reporter bioassay to reveal the feasibility for reuse and recycling of industrial sludge is required. We intentionally used prokaryotic *E. coli* as a reporter microorganism, since prokaryotes are genetically simpler than eukaryotes and mRNA is formed via transcription without further modification (e.g., cap and tail, RNA splicing). Thus, any genetic mutations in bacterial cells

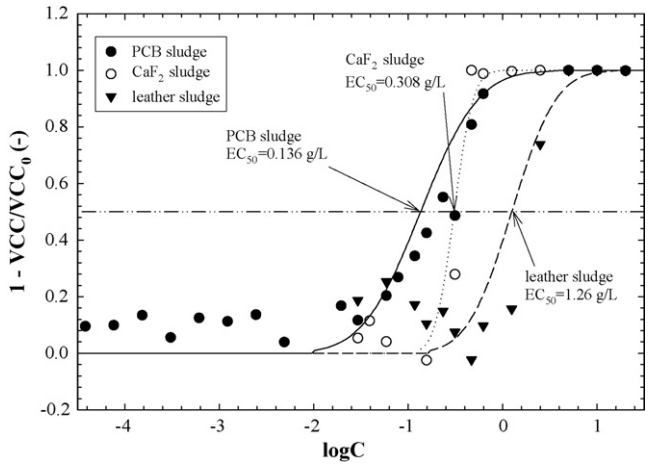

Fig. 2. Dose–mortality curves of toxicity of various sources of sludge predicted from the probit model (lines) (unit of C: g L^{-1}).

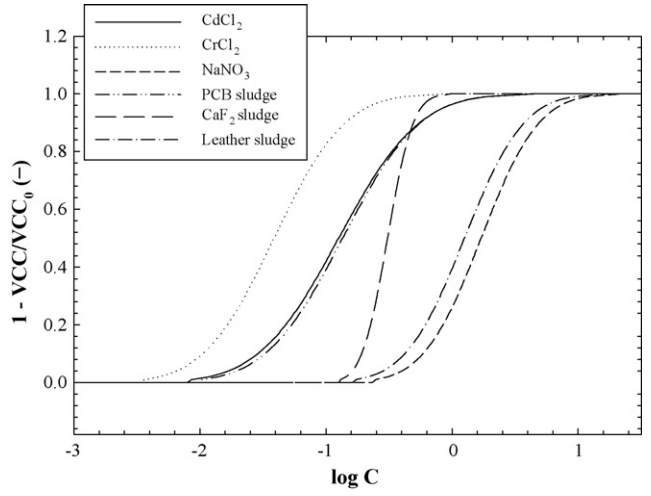

Fig. 3. Dose–mortality curves of various sources of sludge in comparison of some model compounds (unit of C: g L^{-1}).

for long-term culture might be directly due to the presence of toxicants in the culture. Moreover, this study is a first attempt to present a quantitative assessment for toxicity ranking of various sources of sludge. The toxicity of a tested material can be expressed by the effective concentration (EC_x) administered to the population to provoke x% response (e.g., $x = 50$ for EC_{50}). The EC_0 and EC_{100} can be termed as the maximum concentration to have a detectable response (i.e., 0^+%) and minimum concentration to have 100% response (i.e., complete inhibition), respectively. EC_x value is useful for comparative analysis of various "toxic" responses; the smaller the EC_x, the more toxic the tested material [12]. As lower EC_x clearly implied higher toxic characteristics of the tested material, the toxicity ranking, in increasing order, based on EC_0, EC_{20}, EC_{50} and EC_{100} (unit: g L^{-1}) can be listed as follows (Figs. 2 and 3, and Table 4):

EC_0: $CrCl_2$ $(3.38 \times 10^{-3}) > CdCl_2$ $(7.94 \times 10^{-3}) >$ PCB sludge $(9.92 \times 10^{-3}) > CaF_2$ sludge $(0.127) >$ leather sludge $(0.163) > NaNO_3$ (0.233),
EC_{20}: $CrCl_2$ $(0.0165) > CdCl_2$ $(0.0479) >$ PCB sludge $(0.0536) > CaF_2$ sludge $(0.226) >$ leather sludge $(0.615) > NaNO_3$ (0.850),
EC_{50}: $CrCl_2$ $(0.0388) > CdCl_2$ $(0.126) >$ PCB sludge $(0.136) > CaF_2$ sludge $(0.308) >$ leather sludge $(1.26) > NaNO_3$ (1.70),
EC_{100}: $CrCl_2$ $(0.908) > CaF_2$ sludge $(0.968) >$ PCB sludge $(4.21) > CdCl_2$ $(4.49) >$ leather sludge $(17.6) > NaNO_3$ (22.2).

The toxicity rankings indicated that the toxic characteristics of PCB sludge is almost identical with that of $CdCl_2$, suggesting a role of PCB sludge as a hazardous waste [19]. Apparently, PCB could not be used for reuse and recycling unless further toxicity attenuation was carried out. It is suspected that PCB sludge might contain significant amount of precipitates of unidentified toxicants. Serial dilution of PCB sludge still released trace amounts of soluble toxic species due to solubility products, leading to background levels of toxicity (ca. 0.1–0.2; Fig. 2). It also suggested that for possible reuse and recycling of PCB sludge, removal of such toxic precipitates would be inevitable. However, dose–mortality curves (Fig. 3) and the toxicity series seemed to suggest the less toxic characteristics of leather sludge. As the *E. coli* DH5α cells were grown in cultures of nearly neutral pH for toxicity analysis, trivalent chromium might be precipitated in solid phase ($K_{sp}(Cr(OH)_3) = 1.6 \times 10^{-30}$) and apparently was not bioavailable to indicator cells. In contrast, leaching chromium of leather sludge was in a high level (Table 3), since acidic pHs in TCLP could convert nearly all metal ions in soluble form. In addition, +6 chromium ion was possibly converted to Cr^{3+} in higher pHs. This was very likely why leather sludge could not express its actual toxicity in dose–mortality curves. If the concept of "100-fold margin of safety" was adopted as a standard for an acceptable quantity for reuse of different sources of sludge

Table 4
Critical effective concentrations and related parameters of toxicity predicted from the probit model (unit of EC_x: g L^{-1})

	EC_0	EC_{20}	EC_{50}	EC_{100}	$Y = A + B\log C$
PCB sludge	9.52×10^{-3}	0.0536	0.136	4.21	$Y = 6.802 + 2.079\log C$
Leather sludge	0.163	0.615	1.26	17.6	$Y = 4.730 + 2.705\log C$
CaF_2 sludge	0.127	0.226	0.308	0.968	$Y = 8.188 + 6.242\log C$
$CdCl_2$	7.94×10^{-3}	0.0479	0.126	4.49	$Y = 6.796 + 1.998\log C$
$CrCl_2$	3.38×10^{-3}	0.0165	0.0388	0.908	$Y = 8.195 + 2.264\log C$
$NaNO_3$	0.233	0.850	1.70	22.2	$Y = 4.357 + 2.781\log C$

[15,20], the maximum concentrations allowable to be considered "safe" (ca. $EC_{100}/100$) were 9.68, 42.1 and 176 mg L^{-1} for CaF_2 sludge, PCB sludge and leather sludge, respectively. Note that the postulates behind this 100-fold margin are that (1) humans are 10 times more susceptible to the adverse effects of sources of sludge than the indicator bacterium DH5α, (2) the weak in human population (e.g., young, old, etc.) are 10 times more sensitive than healthy adult humans and (3) multiplication rule due to independence of events (i.e., $P(A \cap B) = P(A) \times P(B)$) is applicable to have this 100-fold margin (i.e., 10×10).

Moreover, the slope factor B of the probit model for dose–mortality curves (Eqs. (1) and (2)) is also significant to indicate tolerance to the tested material. As shown in Table 4, the toxicity ranking, in increasing order, based on the slope B is shown as follows: CaF_2 sludge (6.24) > $NaNO_3$ (2.78) > leather sludge (2.71) > $CrCl_2$ (2.26) > PCB sludge (2.08) > $CdCl_2$ (2.00).

As all of the slope factors B of the curves were greater a slope factor of B of unity (i.e., a standard slope to classify toxic or non-toxic characteristics), the behaviors of steeper curves clearly revealed the toxicity characteristics for all cases. The largest value (6.24) of the slope B for CaF_2 sludge suggested the smallest tolerance range from EC_0 to EC_{100} for dose–mortality relationship. In contrast, the smallest value (2.00) of slope B for $CdCl_2$ simply implied the widest range of tolerance for toxicity of $CdCl_2$ from the threshold dose EC_0 to a maximum effect dose (EC_{100}).

4. Conclusions

According to dose–mortality assessment upon some mode3l sources of industrial sludge, the conclusive remarks can be drawn as follows:

1. As the high leaching concentrations of copper in PCB sludge and chromium in leather sludge apparently exceeded current Taiwan's EPA regulatory thresholds, these sludges should be classified as hazardous wastes.
2. Dose–mortality analysis indicated that the toxicity ranking of different sources of sludge was PCB sludge > CaF_2 sludge > leather sludge.
3. The toxicity evaluation suggested that the toxic characteristics of PCB sludge are almost identical with that of $CdCl_2$, confirming a role of PCB sludge as a hazardous waste. Leaching chromium of leather sludge was in a high level, since acidic pHs in TCLP might convert nearly all metal ions in soluble form. Moreover, +6 chromium ion was possibly converted to Cr^{3+} in higher pHs.
4. Leather sludge seemed to be much less toxic than as anticipated, perhaps due to a marked decrease of toxic species bioavailable in the aqueous phase to the indicator bacterium *E. coli* DH5α.
5. For possible reusability of sludge, maximum concentrations allowable to be considered "safe" (ca. $EC_{100}/100$) were 9.68, 42.1 and 176 mg L^{-1} for CaF_2 sludge, PCB sludge and leather sludge, respectively.
6. To consider possible sludge reusability, sludge must be treated by means of wastes intermediate treatment processes (e.g., acid extraction-chemical cementation and acid extraction-fractional precipitations) prior to possible recycling and reuse.

Acknowledgements

Partial financial supports (NSC 95-2221-E-197-005, 94-2214-E-197-003, and NSC 94-2622-E-197-004-CC3, 92-2211-E-197-003) from National Science Council, Taiwan, ROC for this first-attempt study are gratefully acknowledged. The authors also sincerely appreciate Tseng I-Hsiang, Hsu Hung-I, Cheng Ching Jung and Huang Chin-Chung (Department of Environmental Engineering) and Chia-Yi Yen (Department of Chemical & Materials Engineering) for their experimental data analysis.

References

[1] H.M. Freeman, J. Lounsbury, Waste minimization as a waste management strategy in the United States, in: H. Freeman (Ed.), Hazardous Waste Minimization, McGraw-Hill, Inc, New York, 1989, pp. 3–14 (Chapter 1).
[2] E.A. Alvarez, M.C. Mochon, J.C. Sanchez, R.M. Ternero, Heavy metal extractable in sludge from wastewater treatment planes, Chemosphere 47 (2002) 765–775.
[3] C. Naoum, D. Fatta, K.J. Haralambous, L. Maria, Removal of heavy metals from sewage sludge by acid treatment, J. Environ. Sci. Health A36 (5) (2001) 873–881.
[4] K.L. Lin, C.Y. Lin, Hydration characteristics of waste sludge ash utilized as raw cement material, Cem. Concr. Res. 35 (2005) 1999–2007.
[5] W.T. Kuo, K.L. Lin, W.C. Chang, H.L. Luo, Effects of nano-materials on properties of waterworks sludge ash cement paste, J. Ind. Eng. Chem. 12 (5) (2006) 702–709.
[6] H.L. Luo, D.F. Lin, Study of the surface color of sewage sludge mortar at high temperature, Construct. Build. Mater. 21 (1) (2006) 90–97.
[7] D.F. Lin, H.L. Luo, Y.N. Sheen, Glazed tiles manufactured from incinerated sewage sludge ash and clay, J. Air Waste Manage. Assoc. 55 (2005) 163–172.
[8] K.L. Lin, K.Y. Chiang, D.F. Lin, Effect of heating temperature on the sintering characteristics of sewage sludge ash, J. Hazard. Mater. B128 (2006) 175–181.
[9] J. L. Chen, A study on resource recovery of heavy metal sludge as brick material, National Taipei University of Technology, Department of Materials & Mineral Resources Engineering, Master thesis, 2004.
[10] S.W. Richard, Biological markers of chemical exposure, dose and burden, in: L.J. Cralley, L.V. Cralley, J.S. Bus (Eds.), Patty's Industrial Hygiene and Toxicology, vol. 3, third ed., John Wiley & Sons Inc., New York, 1995, pp. 79–158 (Chapter 3, Part B).
[11] B.Y. Chen, C.H. Wu, J.S. Chang, An assessment of the toxicity of metals to *Pseudomonas aeruginosa* PU21 (Rip64), Bioresource Technol. 97 (15) (2006) 1880–1886.
[12] B.Y. Chen, K.L. Lin, Dose–mortality assessment on municipal solid waste incinerator (MSWI) ash, J. Hazard. Mater. A139 (2007) 19–24.
[13] B.Y. Chen, J.S. Chang, Phenol degradation and toxicity assessment upon biostimulation to an indigenous rhizobium *Ralstonia taiwanensis*, Biotechnol. Prog. 21 (2005) 1085–1092.
[14] C. Ewards, Environmental monitoring of bacteria. Methods in biotechnology Some Problems Posed by Natural Environments for Monitoring Microorganisms, vol. 12, Human Press, Totowa, NJ, USA, 1999, p. 1 (Chapter 1).
[15] M.A. Ottoboni, Chronic toxicity, in: The Dose Makes the Poison, second ed., ITP Company, New York, 1997, pp. 79–90 (Chapter 8).

[16] J.V. Rodricks, Dose and response, in: Calculated Risks, Cambridge University Press, 1992, pp. 158–179 (Chapter 9).
[17] D.J. Finney, Probit Analysis, Cambridge University Press, London, 1971.
[18] D. Connell, P. Lam, B. Richardson, R. Wu, Dose and concentration response relationships, in: Introduction to Ecotoxicology, Blackwell Science Ltd., Cambridge, 1999 (Chapter 6).
[19] S.M. Testa, Laboratory considerations, in: The Reuse and Recycling of Contaminated Soil, Lewis Publ., CRC Press LLC, Boca Raton, 1997, pp. 81–101 (Chapter 5).
[20] B.Y. Chen, K.L. Lin, Biotoxicity assessment on reusability of municipal solid waste incinerator (MSWI) ash, J. Hazard. Mater. B139 (2006) 741–746.