RESEARCH ARTICLE

# Leaching toxicity characteristics of municipal solid waste incineration bottom ash

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**Abstract** The continuously increasing production of municipal solid waste incineration bottom ash (MSWIBA) has promoted its utilization as construction material and raised environmental concern. The physico-chemical properties and leaching behavior of MSWIBA were studied, and ecotoxicological testing using a luminescent bacterium bioassay was performed to assess the ecological pollution risks associated with its leached constituents. The MSWIBA was leached by two types of leachants, $H_2SO_4/HNO_3$ and HAc solution, at different liquid to solid ratios and contact times. The concentrations of heavy metals and anions in the leachates were analyzed. Multivariate statistical analyses, including principle component analysis, Pearson's correlation analysis and hierarchical cluster analysis, were used to evaluate the contributions of the constituents to the toxicity ($EC_{50}$) of the MSWIBA leachate. The statistical analyses of the ecotoxicological results showed that the Ba, Cr, Cu, Pb, $F^-$ and total organic carbon (TOC) concentrations were closely correlated with the $EC_{50}$ value, and these substances were the main contributors to the ecotoxicity of the MSWIBA leachate. In addition, the cluster of these variables indicated similar leaching behaviors. Overall, the research demonstrated that the ecotoxicological risks resulting from MSWIBA leaching could be assessed before its utilization, which provides crucial information for the adaptation of MSWIBA as alternative materials.

**Keywords** Municipal solid waste incineration, bottom ash, alternative material, leaching tests, toxicity, *Vibrio qinghaiensis* (Q67)

## 1 Introduction

Due to rapid urbanization, China faces a serious crisis in the management of municipal solid waste (MSW). Incineration is an effective and widely used solid waste treatment method that results in significant waste reduction [1,2]. The amount of incinerated MSW in China reached 27% of the collected MSW in 2012 [3], producing more than 9 million tons of MSW incineration bottom ash (MSWIBA). With the rapid increase of the MSW incineration ratio, the management of MSWIBA has become a great concern. In general, MSWIBA has a mineral composition that is similar to natural sand, and engineering characteristics that are similar to construction materials. Recently, the use of MSWIBA for civil engineering applications has been considered as an appropriate solution for reducing waste disposal and providing alternative construction materials for several applications. However, during utilization the waste products will be exposed in the environment, so the contaminants such as soluble salts, heavy metals and other trace pollutants could leach out and contaminate soil, surface water and ground water [4]. According to several field study reports, heavy metals in MSW were mostly concentrated into the bottom ash during the incineration process. For example, Zhang et al. [5] found that more than 80% of As, Cr, Cu and Ni, 74%–94% of Zn, and 46%–79% of Pb remained in the bottom ash through their two years investigation in Shanghai, China. Belevi and Langmeier [6] reported that more than 90% of Cu, Cr, Ni and more than 80% of Co were kept in the bottom ash based on a full scale incinerator investigation in Switzerland. It is also known that heavy metals in MSWIBA may have a long-term impact on the environment [7]. Therefore, it is of significance to control the release of heavy metals from MSWIBA in reuse applications.

Although MSWIBA is considered non-hazardous, it contains a relatively high concentration of heavy metals and organic compounds [7]. This is an important obstacle for recycling MSWIBA. Previous studies, which investigated the possibility of heavy metals in bottom ash being released into the environment, have provided valuable information about the leaching behavior of heavy metals [7,8]. Although the leaching concentrations of heavy metals from MSWIBA are mostly below the regulatory limits for hazardous waste, there were some cases that Pb and Cu exceeded these limits [9–11]. Furthermore, mutagenicity [7] and plant genotoxicity [12] of MSWIBA were reported. During utilization of MSWIBA products, the environmental condition may change and the release of pollutants from MSWIBA may vary greatly over the long-term, indicating a potential threat to the surrounding environment. Chemical analysis is used to quantify the pollutants and cannot account for the interaction among the pollutants in complex mixtures [13], thus providing insufficient comprehensive risk information. On the contrary, the ecotoxicity is the result of the combination of several factors, such as heavy metals, ammonia, salts and volatile fatty acids. Therefore, to reveal the feasibility of utilizing MSWIBA as construction material, the ecotoxicity risk assessment of leachates derived from MSWIBA besides their chemical composition needs to be carried out.

Toxicity measurements are often conducted to evaluate wastewater's toxicity by using microorganisms [14], invertebrate [15], small animals [16], fish [17], and plants [18]. Recently, several methods have been used to dissect the ecotoxicological effects of municipal solid waste incinerator (MSWI) ash and other solid waste [10,19–21]. Among these approaches, the *Photobacterium phosphoreum* [22,23] and *Vibrio fischeri* [24] assay have been widely accepted and used to evaluate the toxicity potential of a variety of substances [25]. This test is one of the most reliable short-term and cost-effective bacterial test systems, and is used to evaluate the acute toxicity of wastewater by measuring the reduction of light output of luminescent bacteria. However, a high concentration of sodium chloride (2%–3%) is needed to supplement to the testing solution, which may change the inherent properties of freshwater samples. *Vibrio qinghaiensis* sp. Nov. (*V. qinghaiensis*) is a kind of freshwater luminescent bacterium separated from body surfaces of *Gymnocypris przewalskii* (a fish species living in Qinghai Lake, China) [26]. Ma et al. [27] evaluated the toxicity of polluted river water samples using *V. qinghaiensis*. The results showed that *V. qinghaiensis* was effective and reliable as well as conventional, which was more suitable for freshwater bioassay due to its wide pH tolerance.

In this study, the acute toxicity potential of the leachates that were derived from MSWIBA was evaluated with a bioluminescent bacteria (*V. qinghaiensis*) bioassay. The concentrations of heavy metals and anions in the MSWIBA leachates from various extraction conditions, including extractant type, leaching time and liquid to solid ratio, were measured. The multivariate statistical analyses were adopted to evaluate the relationship between the leaching constituents and $EC_{50}$ values, and their contributions to the ecotoxicity were discussed.

## 2 Experimental sections

### 2.1 Materials

The water-quenched MSWIBA samples (MSWIBA1 and MSWIBA2) were collected from two large-scale (1000–1500 t·d⁻¹) MSW incineration plants in Shanghai that were equipped with reciprocating grate furnaces operating under mass burn processes. At these facilities, the temperatures in the furnaces are set to above 850°C to destroy dioxin. Semi-dry air pollution control system comprised of lime slurry injection, activated carbon injection and bag filter, is equipped in both plants to remove acid gas, dioxin, heavy metals, etc. The incinerators accept MSW from residential, commercial, and institutional sources, which is mainly composed of kitchen and food waste, paper, plastics, glass, textiles, metals etc. Prior to analyses, the MSWIBA samples (100–150 kg each) were air-dried, and the ferrous and non-ferrous metals were manually removed.

### 2.2 Physical and chemical properties of the MSWIBA samples

The particle size distributions of the MSWIBA samples were analyzed by passing the samples through sieves with diameters of 20, 10, 5, 2 and 1 mm. The loss on ignition (LOI) of MSWIBA was determined by drying the sample at 600°C for 6 h. According to previously reported procedures [5,9,28], the elemental compositions of the MSWIBA samples were measured by X-ray fluorescence spectrometry (XRF, Bruker, SRS 3400, Germany), and inductively-coupled plasma optical emission spectrometry (ICP-OES, Agilent 720ES, USA) after performing acidic digestions with $HCl/HNO_3/HF/HClO_4$ (Sinopharm chemical reagent Co., Ltd, China). X-ray diffraction photometer (XRD, Bruker, D8 Advance, Germany) was performed to analyze the mineral compositions of the samples.

### 2.3 Leaching procedure

Acetic acid (0.1 mol·L⁻¹, pH = 2.88±0.05) and $H_2SO_4/HNO_3$ (60:40 w/w) solutions (pH = 4.20±0.05) were used in the leaching tests to simulate landfill and acid rain conditions, respectively. The MSWIBA samples were mixed with the leachants at liquid to solid (*L/S*) ratios of 1, 2, 5, 10, 20, 50 and 100 L·kg⁻¹ and shaken on a rotary shaker at 30±2 r·min⁻¹ at room temperature (i.e., 25°C) for 24 h. For the time-dependent leaching tests, the MSWIBA

samples were mixed with the leachants at an $L/S$ of 10 L·kg$^{-1}$ and shaken (30±2 r·min$^{-1}$) at room temperature for 0.1, 0.5, 1, 2, 6, 12, 24, 48 and 72 h. After extraction, the mixtures were passed through 0.45-μm glass fiber filters and divided into 4 aliquots for analysis. All the leaching tests were conducted in triplicate.

Immediately after leaching and filtration, the pH values, oxidation-reduction potentials (ORP) and electrical conductivities (EC) of the first aliquots of the leachates were determined by using pH, ORP (Inesa, PHS-2F, China) and EC (Inesa, DDS-307A, China) meters, respectively (Figs. S1–S6 in the supplementary material). The second leachate aliquots were diluted using ultrapure water. The total organic carbon (TOC) content and the anion (i.e., F$^-$, Cl$^-$, NO$_2^-$, NO$_3^-$, and SO$_4^{2-}$) concentrations in the diluted leachates were determined using a TOC analyzer (Shimadzu, TOC-Vcph, Japan) and an ion chromatography (Dionex, ICS-1000, USA) system, respectively (Tables S1–S4 in the supplementary material). The third aliquots of the leachates were acidified with HNO$_3$ to pH < 2 for storage (up to 2 weeks) at 4°C prior to analyses. The

leachate concentrations of As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, V, and Zn were analyzed using ICP-OES after HNO$_3$ digestion (5% v/v). The ecotoxicological tests were performed using the fourth aliquots of the leachates.

## 2.4   Ecotoxicological bioassay

The ecotoxicities of the leachate samples were evaluated using fresh water bioluminescent bacteria *V. qinghaiensis* sp. (Q67, Beijing Hamamatsu Photon Techniques Inc., China) as a bio-indicator. The ecotoxicological bioassays were conducted according to the ASTM D5660-96 standard [29]. Briefly, 0.05 mL of bacteria suspension and 2 mL of the leachate samples at different dilution ratios were thoroughly mixed in a test tube. The light emissions of the luminescent bacteria in the tubes at a reaction time of 15 min were measured using a luminometer analyzer (BHP9511, Beijing Hamamatsu Photon Techniques Inc., China). The luminescent inhibition ratios (*LIR*, %) of the samples were calculated using the following Eq [25]:

$$LIR/\% = \frac{\text{Relative intensity of the blank} - \text{Relative intensity of the sample}}{\text{Relative intensity of the blank}} \times 100$$

The toxicity end-point of the median effective concentration ($EC_{50}$) was determined as the test sample concentration that causes a 50% reduction in the studied biologic response (i.e., reduction in light output) [30]. The light emissions from the bioluminescent bacteria resulted from the reduction of flavin mononucleotides and the oxidation of a long chain aldehyde by the luciferase enzyme. The metabolic energy generated in this pathway was converted to chemical energy into visible light through the electron transport pathway. This pathway is intrinsically linked to cellular respiration; therefore, disruptions to the normal cellular metabolism cause decreases in light emissions [30,31].

## 2.5   Statistical analysis

Statistical analyses of the toxicity results were performed using IBM SPSS Statistics version 19 (SPSS Co. Ltd, USA), and XLSTAT software (Addinsoft Co. Ltd., USA) for Windows. The multivariate statistics were analyzed using principal component analysis (PCA), Pearson's correlation analysis (CA), and hierarchical cluster analysis (HCA), with the 32 observation sample points resulting from the leaching tests at $L/S$ ratios of 1 to 100 L·kg$^{-1}$ and leaching times 0.1 to 72 h. The interrelationships between the $EC_{50}$ values and various substances in the MSWIBA leachates were described by PCA and CA. The HCA was conducted based on the squared Euclidean distance and within-groups linkage, and the rescaled distance between two objects was considered to be minimal (i.e., a close relationship) if they belonged to the same group [32].

# 3   Results and discussion

## 3.1   Physical and chemical properties of the MSWIBA samples

The unimodal size distribution of the MSWIBA samples is shown in Fig. 1. The median grain size ($d_{50}$) values of MSWIBA1 and MSWIBA2 were 3.1 mm and 3.8 mm, respectively, and were classified as a well-graded road materials according to the European Standard EN 13285 [33]. MSWIBA1 and MSWIBA2 had LOIs of 3.31% and 1.14%, respectively, which were within the limits (< 5%) set in Chinese Standard GB 18485 [34]. The LOI of MSWIBA1 was higher than that of MSWIBA2, indicating a higher percentage of unburned carbon. The variation in the LOI values could be attributed to the different combustion temperatures and the incomplete oxidation of the combustible constituents.

The compositions of the MSWIBA samples are listed in Table 1. The XRF analysis showed that the MSWIBA samples contained high concentrations of Si, Ca, Fe and Al. The fractions of CaO-SiO$_2$-Al$_2$O$_3$-Fe$_2$O$_3$ contributed to more than 81% of the chemical compositions in the MSWIBA, which is similar with the common pozzolanic mineral admixtures used in cement-based materials [35]. Most of the toxic heavy metals in the bottom ashes are in the average concentration range of 0.1–5 g·kg$^{-1}$, including Cu (1.67–1.71 g·kg$^{-1}$), Cr (0.33–0.68 g·kg$^{-1}$), Mn (0.71–1.08 g·kg$^{-1}$), Ni (0.13–2.11 g·kg$^{-1}$), Pb (0.48–0.61 g·kg$^{-1}$) and Sr (0.22–0.37 g·kg$^{-1}$). Other metals, such as Cd, Co and Se were found in minor or trace amounts with
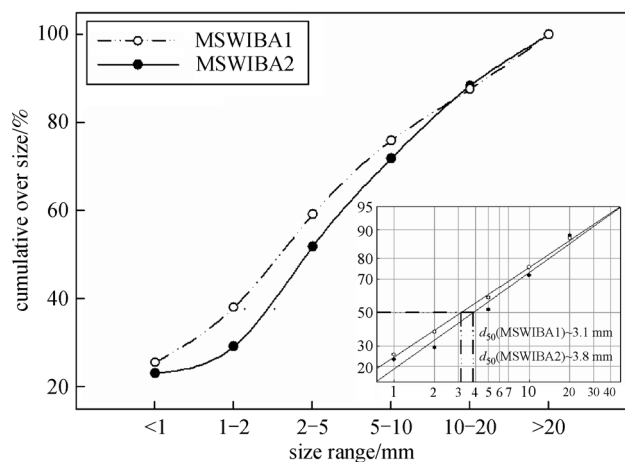
**Fig. 1**  Particle size distribution of the MSWIBA samples

concentrations $< 0.1\,g\cdot kg^{-1}$. Boron (B) and Bismuth (Bi) were below the detection limit in the analysis for both MSWIBA1 and MSWIBA2. The chemical compositions of MSWIBA depended on the waste composition, combustion temperature, furnace type, and gas velocity etc [36–38]. It can be seen from Table 1 that metal concentrations in the MSWIBA samples generated from two incinerators are quite different, probably due to the differences in incineration temperature and feeding wastes. The contents of Cd and Zn in the MSWIBA samples were two to three times lower than those in Korean and Japanese MSWIBA, and the contents of Cu and Ni were about four to five times lower than those in the MSWIBA from the two countries [9].

As shown in Fig. 2, the mineral compositions of MSWIBA1 and MSWIBA2 were similar. According to the XRD analysis, the MSWIBA samples contained a considerably high percentage of amorphous mass. As a result, a high background signal was present in the samples. The major crystalline phases were quartz ($SiO_2$) and calcite ($CaCO_3$), similar to the findings of earlier studies [4,5,38,39]. The toxicity of MSWIBA is dependent not only on the total contents of contaminants, but also on the speciation of these contaminants [4,40]. However, XRD analysis could not identify the speciation of trace heavy metals.

The physical and chemical results showed that MSWIBA can be used in civil engineering applications, such as alternative cements in concrete, aggregates in road base, structural fillers in asphalt and asphalt base products, lightweight bricks and synthetic aggregates. Nonetheless, the pollution risk needs to be assessed before the utilization.

### 3.2   Leaching behavior of the MSWIBA samples

The possible pollution risk during utilization or disposal of MSWIBA mainly results from the leaching and release of

**Table 1**  Physical and chemical characteristics of the MSWIBA samples

| properties | MSWIBA1 | MSWIBA2 |
|---|---|---|
| loss on ignition (wt.%, at 600°C) | 3.31 | 1.14 |
| density ($g\cdot cm^{-3}$) | 2.12 | 1.83 |
| chemical composition (wt.%) analyzed by XRF | | |
| SiO$_2$ | 43.10 | 40.09 |
| Al$_2$O$_3$ | 5.69 | 7.48 |
| Fe$_2$O$_3$ | 8.23 | 8.67 |
| CaO | 24.74 | 27.38 |
| MgO | 2.83 | 2.99 |
| K$_2$O | 1.61 | 1.52 |
| TiO$_2$ | 0.77 | 1.00 |
| SO$_3$ | 2.59 | 1.68 |
| Cr$_2$O$_3$ | 0.12 | 0.07 |
| Cl | 1.04 | 0.78 |
| P$_2$O$_5$ | 5.01 | 3.38 |
| Na$_2$O | 4.27 | 4.96 |
| elemental content (mg·kg$^{-1}$) analyzed by ICP-OES | | |
| As | 57.1 | 85.4 |
| B | ND | ND |
| Ba | 1240 | 2090 |
| Be | 1.78 | 2.68 |
| Bi | ND | ND |
| Cd | 4.91 | 7.52 |
| Co | ND | 12.4 |
| Cr | 330 | 676 |
| Cu | 1670 | 1710 |
| Mn | 705 | 1080 |
| Ni | 131 | 2107 |
| Pb | 482 | 609 |
| Se | 1.60 | 3.13 |
| Sr | 219 | 366 |
| V | 44.7 | 54.0 |
| Zn | 2100 | 2370 |

Note: ND is for not detectable

contaminants to waters or soils. The results of the $H_2SO_4$/$HNO_3$ and HAc leaching tests are summarized in Figs. 3 and 4. Ba, Cd, Cr, Cu, Pb, and Zn were the main contributors to the leaching toxicity of the MSWIBA. The pH values of the MSWIBA samples were higher than 10.79 for all $H_2SO_4$/$HNO_3$ leaching tests, indicating that the MSWIBA samples were highly alkaline and had high acid neutralization capacities. The concentrations of Ba, Cd, Cr, Cu, Pb, and Zn were higher in the HAc leachates than in those of $H_2SO_4$/$HNO_3$. The high concentration of HAc caused the complexation of metals with Ac$^-$ as well as
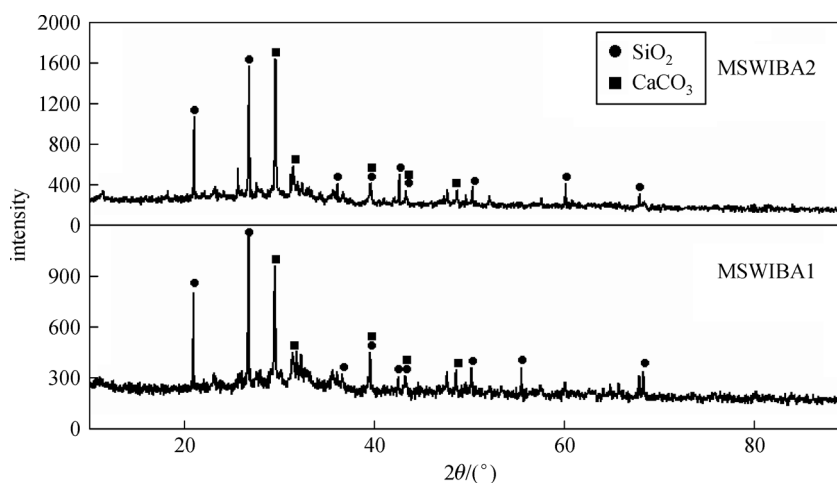
**Fig. 2**  XRD patterns of the MSWIBA samples

the lower leachate pH, thus increasing the leachability of these metals [28]. There is the possibility that the alkalinity of the MSWIBA is eventually exhausted in the environment, leading to a decrease in leachate pH, and then the leaching concentrations of the metals may increase. Acid consumption and the increase in the concentrations of some elements may also be caused by the solubility of minerals or desorption from reactive surfaces of ash matrix [8]. The high $L/S$ ratios used in the HAc leaching tests obtained leachate pH values as low as $4.07-4.38$, which could show this pattern. In addition, Cr and Pb were only detectable in the HAc leaching tests. On the other hand, these two elements could not be detected in the $H_2SO_4$/$HNO_3$ leaching tests under investigated conditions. It is known that leaching of trace elements is a very slow process and the solid and liquid phase equilibrium may not be attained even with long leaching times [41]. Furthermore, the leachability of toxic elements is often dependent on their speciation and the nature of the host phases, e.g. elements combined with the glass phases are more resistant to leachant solutions [42].

The leaching concentrations of metals show a tendency to decrease with an increasing $L/S$ ratio (Figs. 3 and 4) through solubility effect during the leaching process. When $L/S$ increased from 1 to $100 \, L \cdot kg^{-1}$, the total leached amounts of Ba, Cd, Cu, and Zn in MSWIBA samples extracted by $H_2SO_4$/$HNO_3$ solution increased by 30–41, 5–7, 23–24, and 39–45 times, and those of Ba, Cd, Cr, Cu, Pb and Zn extracted by $0.1 \, mol \cdot L^{-1}$ HAc solution increased by 37–56, 28–37, 25–38, 33–48, 5–38, and 58–62 times, respectively. The result indicated the effects of both solubility and dilution. Due to the dilution effect, the leaching concentrations of heavy metals decreased with the increase of L/S ratio. The increase of the total leaching amounts of heavy metals indicate, however, the leachability (solubility) of the heavy metals was also changed, which varied strongly with leachate pH for some metals. Overall, the heavy metal leaching concentration in this

study depended on the $L/S$ ratio and leaching solution, which is similar to the previous work [43,44]. The leaching behaviors of MSWIBA indicated that releases of the elements were controlled by solubility and strongly pH dependent. The average leaching concentration of Pb from the Korean and Japanese MSWIBA ($3 \, mg \cdot L^{-1}$) was 2–3 times higher than that in this study. On the other hand, the concentrations of Cr and Zn by $0.1 \, mol \cdot L^{-1}$ HAc leaching test in this study were higher than those in the MSWIBA from both countries [9].

Comparatively, the MSWIBA1 generally caused less environmental pollution than MSWIBA2 due to the lower concentrations of leached heavy metals. Comparatively, the metal concentrations in the leachates of MSWIBA by $0.1 \, mol \cdot L^{-1}$HAc ($L/S = 20 \, L \cdot kg^{-1}$) and $H_2SO_4$/$HNO_3$ leaching ($L/S = 10 \, L \cdot kg^{-1}$) were respectively all below the limit values for hazardous waste identified by US EPA toxicity characteristics leaching procedure (TCLP) (Ba: 100; Cd: 1; Cr: 5 and Pb: $5 \, mg \cdot L^{-1}$) [45] and China GB 5085.3 regulations (Ba: 100; Cd: 1; Cr: 15; Cu: 100; Ni: 5; Pb: 5; and Zn: $100 \, mg \cdot L^{-1}$) [46], therefore the MSWIBA samples were classified as non-hazardous material.

### 3.3  Toxicity assessment

*Vibrio qinghaiensis* Q67 can grow in fresh water with low concentrations of NaCl. It exhibits a broad pH tolerance and is suitable for detecting toxic substances in fresh water. The pH values of the leachates were adjusted to 6–9 before toxicity analyses to ensure that the measured toxicity effects were caused by the bio-available substances in the sample rather than as an artifact of pH effects [47].

The results of the ecotoxicity tests for MSWIBA1 and MSWIBA2 are shown in Fig. 5. The trends were similar to those of the chemical compositions. The $EC_{50}$ values decreased as the $L/S$ ratios decreased and were less affected by contact times. As shown in Figs. 5 (a–b), both the MSWIBA1 and MSWIBA2 showed acute toxicity. The
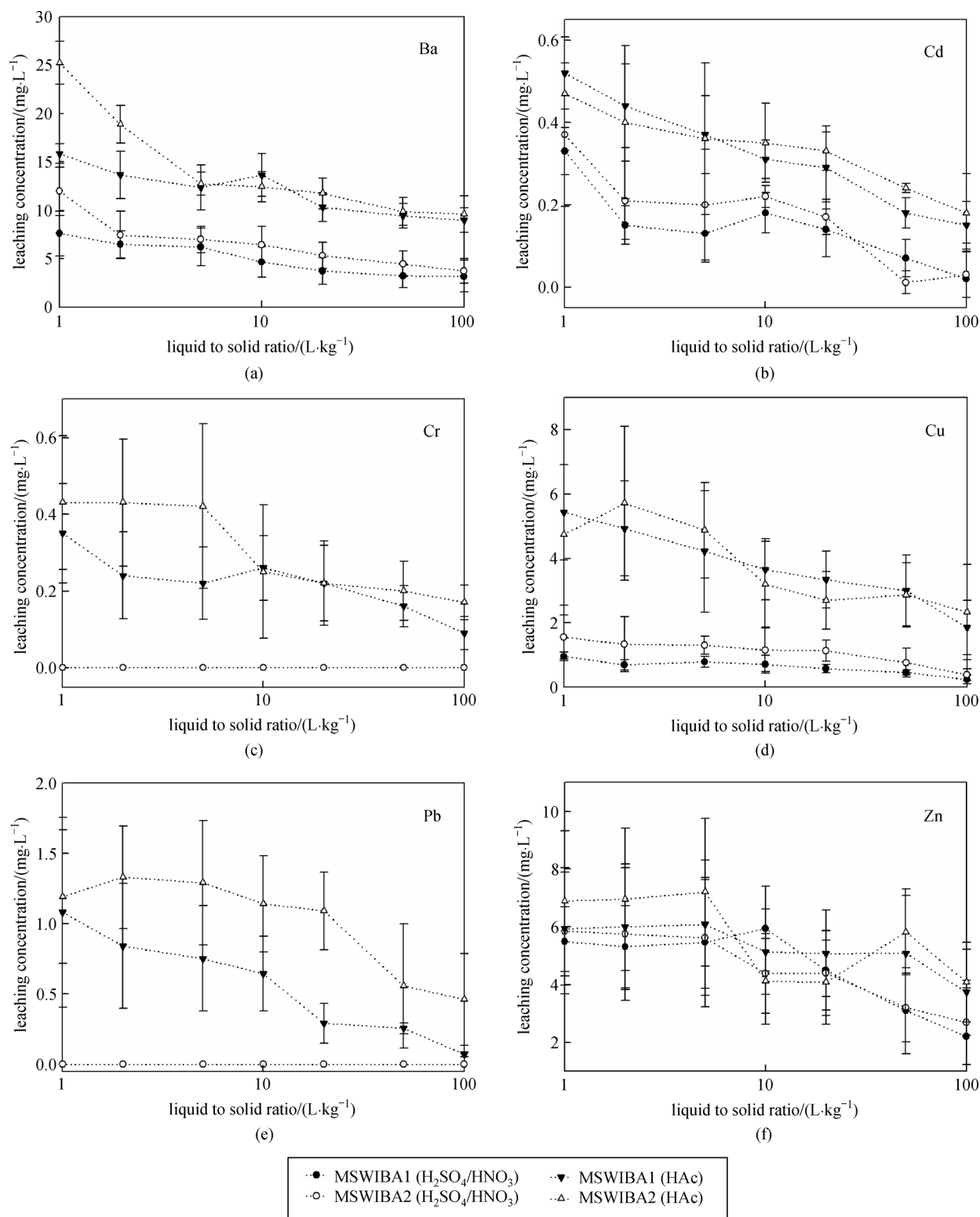
**Fig. 3** Leaching concentrations of heavy metals as a function of $L/S$ ratios. (a) Ba, (b) Cd, (c) Cr, (d) Cu, (e) Pb and (f) Zn

data indicated that leachate by $H_2SO_4/HNO_3$ tests showed no toxicity activity as $EC_{50} > 10\%$ [13]. The leachates from HAc extraction were more toxic ($EC_{50} < 10\%$) with $L/S$ 1 L·kg$^{-1}$ for MSWIBA1 and MSWIBA2 ($EC_{50}$ were

9.4% and 7.6%, respectively), as well as with leaching times of 24 h and 72 h for MSWIBA2 ($EC_{50}$ were 9.3% and 8.6%, respectively).

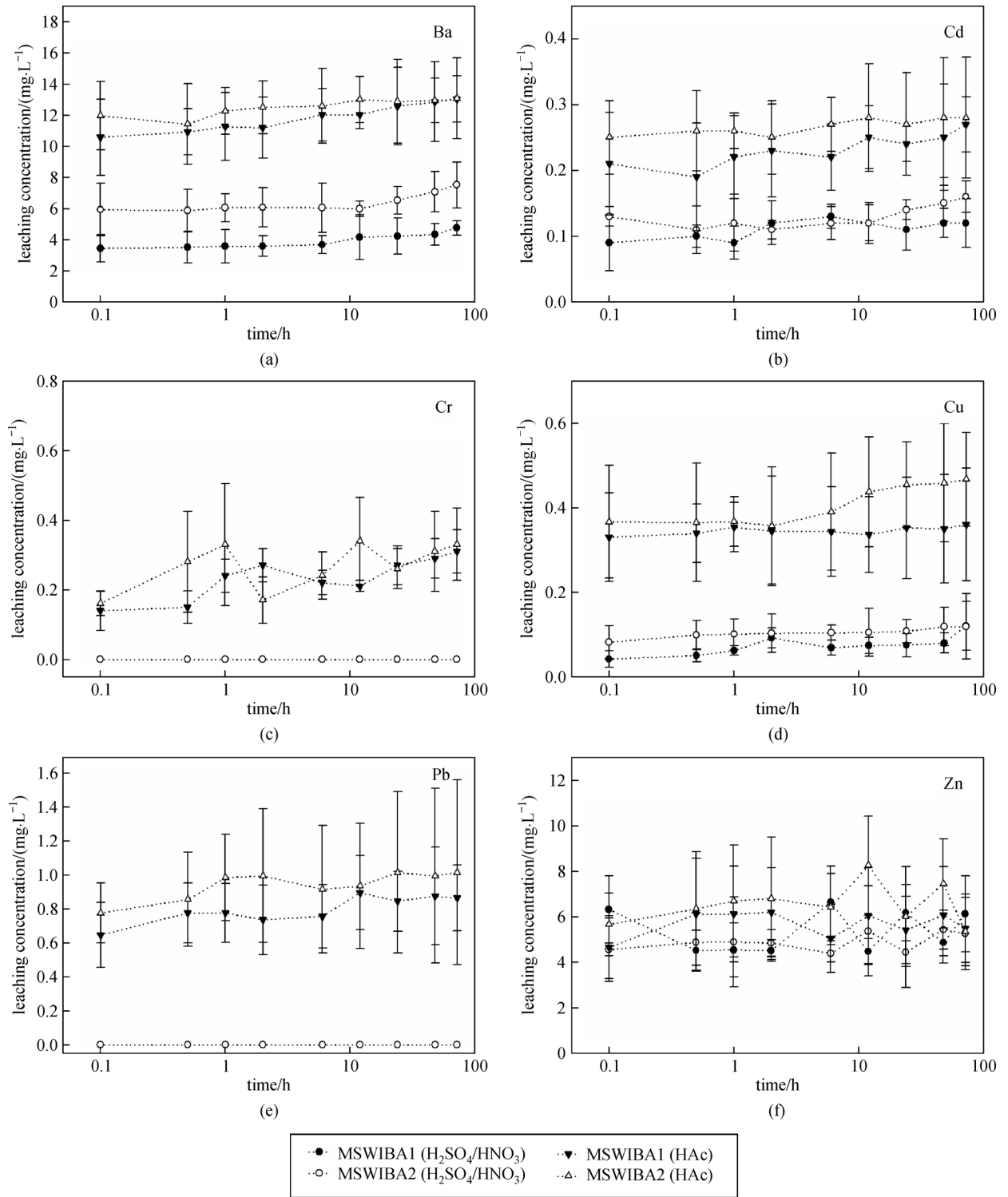As can be seen in Fig. 5, The $EC_{50}$ and associated

**Fig. 4**  Leaching concentrations of heavy metals as a function of contact times. (a) Ba, (b) Cd, (c) Cr, (d) Cu, (e) Pb and (f) Zn

constituent concentrations for the $H_2SO_4/HNO_3$ and HAc leaching tests were found to change with the $L/S$ ratio and were less affected by contact time. When the $L/S$ ratio increased from 1 to 100 L·kg$^{-1}$, the $EC_{50}$ increased by 60%–76% for $H_2SO_4/HNO_3$ leaching and 179%–201% for

HAC leaching, respectively. While the contact time changed from 0.1 h to 72 h, the $EC_{50}$ decreased by 37%–45% for $H_2SO_4/HNO_3$ leaching tests and 37%–42% for HAc leaching tests, respectively. The reported ecotoxicity results ($EC_{50}$) of MSWIBA using marine and fresh water
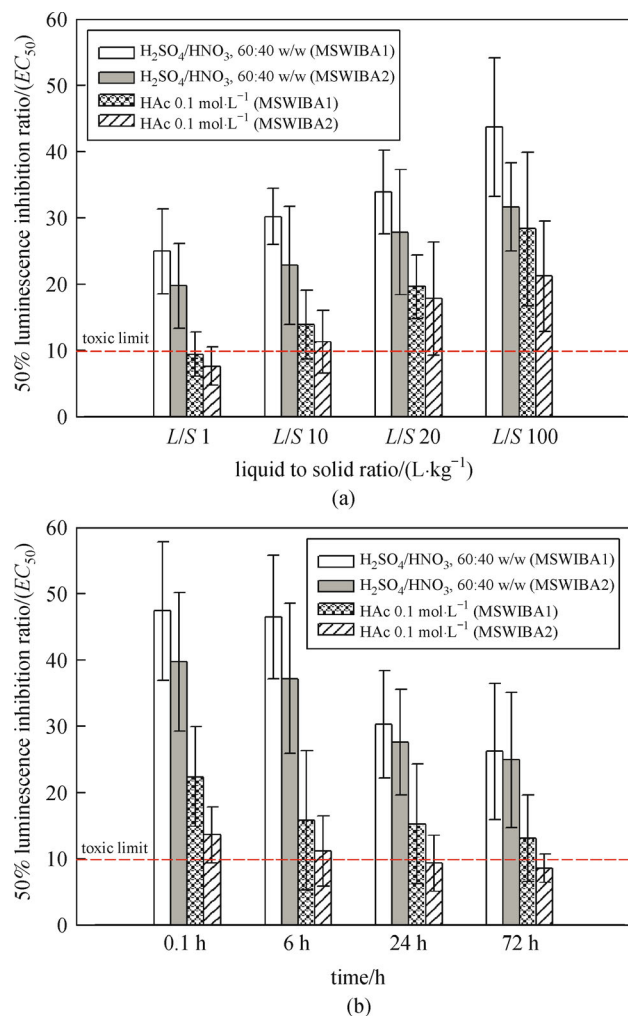
**Fig. 5**  $EC_{50}$ of the MSWIBA leachates as a function of (a) $L/S$ ratio and (b) contact time

organisms *V. fischeri*, *A. franciscana*, *P. tricornutum*, *S. capricornutum* and *D. magna* are similar [48]. The results indicate that the greater toxic effects of the MSWIBA2 leachates on the Q67 cells than those of the MSWIBA1 leachates may be attributed to their higher heavy metal concentrations. Toxic pollutants in leachate can destroy its metabolic process and inhibit the luminescence. The inhibition extent is correlated with the total amount of the toxic pollutants, which can be used to evaluate the leachate's toxicity [25]. The results indicated that although the use of MSWIBA for engineering applications is feasible based on its physiochemical properties, the potential toxicity of the MSWIBA needs to be considered.

### 3.4   Statistical analysis results

#### 3.4.1   PCA analysis

The sources of toxicity ($EC_{50}$) were identified using PCA.

The Varimax rotation technique was used because an orthogonal rotation minimizes the number of variables with a high load on each component and facilitates the interpretation of results. Three principal components (PCs) were extracted, which explained 82.3% of the total variance. A PCA bi-plot was used to represent the scores of the sample observations and vectors were used to represent the coefficients of the variables (constituents) on the PCs, as shown in Fig. 6 (a). The sample numbers 1 to 4 were $H_2SO_4/HNO_3$ leaching tests with $L/S$ ratios of 1, 10, 20 and 100 L·kg$^{-1}$ at contact time of 24 h for MSWIBA1, and the sample numbers 17 to 20 for MSWIBA2, respectively. The sample numbers 5 to 8 were $H_2SO_4/HNO_3$ leaching tests with $L/S$ ratio 10 L·kg$^{-1}$ at contact times of 0.1, 6, 24 and 72 h for MSWIBA1, and the sample number 21 to 24 for MSWIBA2, respectively. The sample numbers 9 to 16 and 25 to 32 were the same as above, but for HAc leaching tests.

In this study, sample numbers 10–16 and 26–32 were close to each other and corresponded to observations that had similar component scores (displayed in the right part of the plot). The left plot contains sample numbers 1–8 and 18–24. To the extent that these components fit the data well, the points corresponded to observations that had similar values for the component scores. Both the direction and length of the vectors can be interpreted. The group of the Ba, TOC, Pb, Cu, Cr and F$^-$ vectors points in the same direction and corresponds to variables that have similar response profiles, indicating similar leaching behavior. Thus, these profiles can be interpreted as having similar properties. The constituents for Cd, Cl$^-$, Fe, Se, Ni, As, $SO_4^{2-}$ and $NO_3^-$ were very similar for each group. For example, PC1 was dominated by Ba, TOC, Pb, Cu, Cr and F$^-$, which accounted for 41.2% of the total variance. In this case, the Cr, Ba TOC and Cu loadings (0.945, 0.919, 0.917 and 0.913, respectively) were as high as the loadings of the other elements of the group. PC2 was dominated by Fe, Se Cd, Ni, $SO_4^{2-}$, As, $NO_3^-$ and Cl$^-$ and accounted for 32.4% of the total variance. In addition, PC3 was dominated by Zn and $NO_2^-$, accounting for 8.7% of the total variance. From Fig. 6 (a), Zn and $NO_2^-$ were separated by a large distance in the PCA loading plot, which suggested that the two elements were poorly correlated and had different sources of $EC_{50}$. Scores of Zn and $NO_2^-$ in the groups PC1 and PC2 were low and did not significantly differ between the two groups. So these components did not present an environmental risk when considering the reuse of this material for construction [49].

Moreover, the $EC_{50}$ was located in quadrant three of the bi-plot. Thus, Ba, TOC, Pb, Cu, Cr and F$^-$ (on PC1) significantly contributed to the $EC_{50}$ [7,49]. The $EC_{50}$ (lower $EC_{50}$ indicates higher toxicity) was negatively correlated with the concentrations of Ba, TOC, Pb, Cu, Cr and F$^-$ in the leachates, indicating that these constituents clearly had toxicity to the Q67.
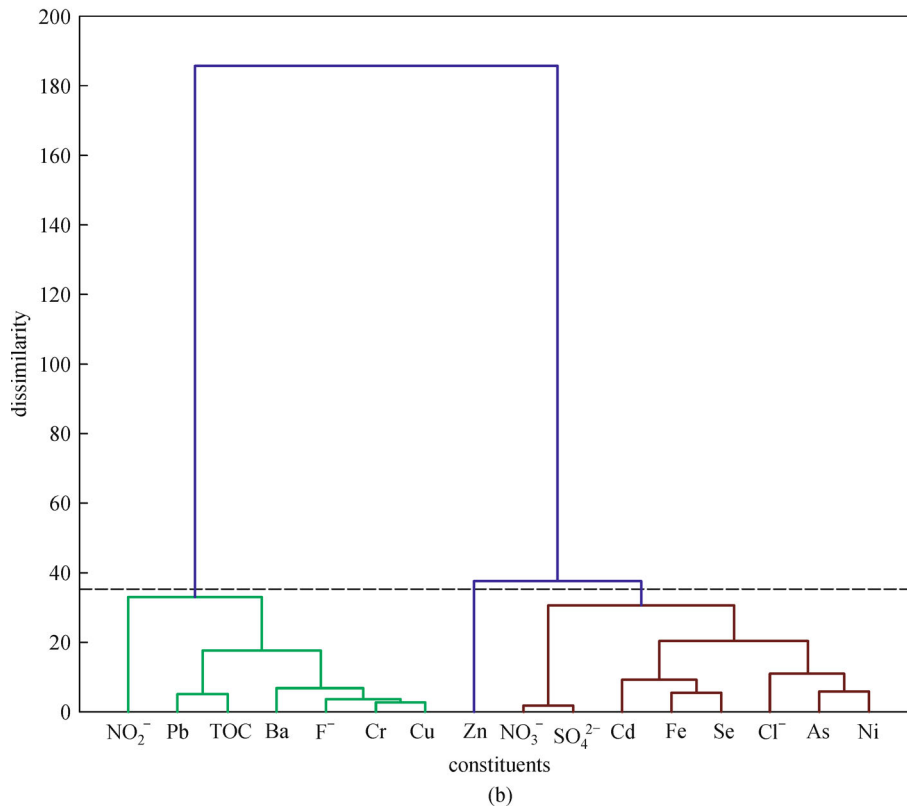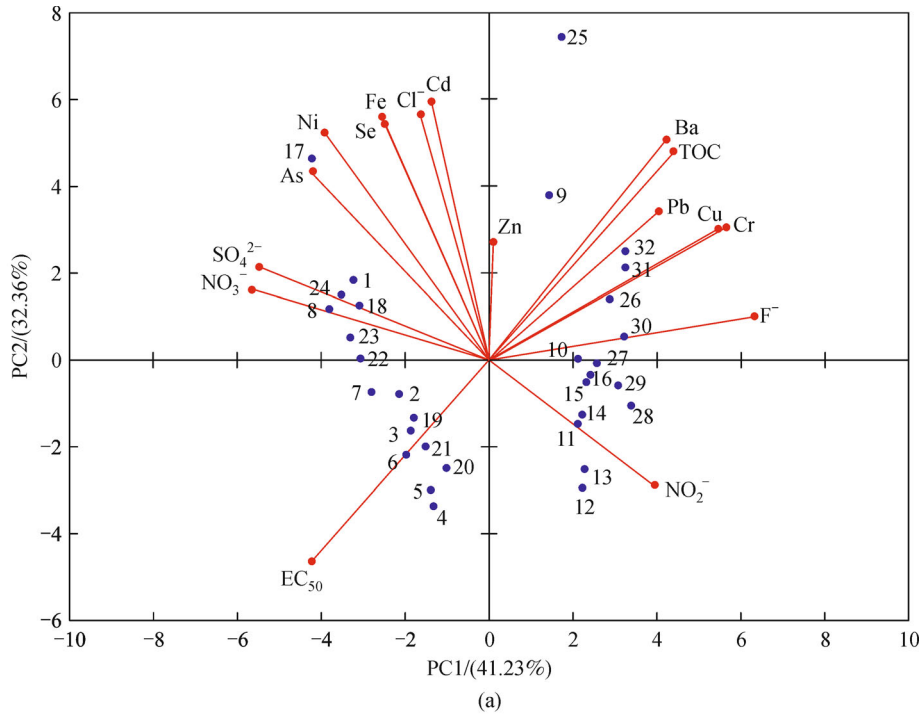
**Fig. 6** PCA and HCA results. (a) PCA score scatter plot (bi-plot) with inter-relationships between the MSWIBA samples and the constituents. (b) HCA analysis represented by dendrograms of the constituents in the MSWIBA leachate samples.

3.4.2 Pearson correlations analysis

The Pearson correlation coefficients of the constituents are shown in Table 2. It was found that the $EC_{50}$ was positively correlated only with As, $NO_3^-$ and $SO_4^{2-}$, but the correlation was not significant. The remaining constituents,

**Table 2** Pearson correlation coefficients between the constituents and $EC_{50}$ ($n = 32$)

| | As | Ba | Cd | Cr | Cu | Fe | Ni | Pb | Se | Zn | $F^-$ | $Cl^-$ | $NO_2^-$ | $NO_3^-$ | $SO_4^{2-}$ | TOC | $EC_{50}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| As | | 0.046 | 0.515** | −0.240 | −0.270 | 0.625** | 0.809** | −0.303 | 0.662** | 0.286 | −0.538** | 0.636** | −0.681** | 0.625** | 0.631** | −0.111 | 0.023 |
| Ba | | | 0.372* | 0.887** | 0.805** | 0.278 | 0.088 | 0.614** | 0.218 | 0.283 | 0.741** | 0.444* | 0.139 | −0.417* | −0.345 | 0.821** | **−0.847**** |
| Cd | | | | 0.105 | 0.093 | 0.741** | 0.634** | 0.418* | 0.712** | 0.104 | −0.076 | 0.598** | −0.299 | 0.412* | 0.432* | 0.374* | −0.334 |
| Cr | | | | | 0.906** | −0.063 | −0.220 | 0.612** | −0.015 | 0.209 | 0.899** | 0.120 | 0.288 | −0.678** | −0.645** | 0.795** | **−0.811**** |
| Cu | | | | | | −0.062 | −0.175 | 0.573** | −0.010 | 0.207 | 0.868** | 0.113 | 0.352* | −0.584** | −0.534** | 0.779** | **−0.833**** |
| Fe | | | | | | | 0.726** | 0.207 | 0.818** | 0.031 | −0.299 | 0.547** | −0.334 | 0.493** | 0.570** | 0.243 | −0.204 |
| Ni | | | | | | | | −0.078 | 0.709** | 0.212 | −0.465** | 0.737** | −0.644** | 0.646** | 0.708** | 0.040 | −0.109 |
| Pb | | | | | | | | | 0.156 | 0.107 | 0.607** | 0.049 | 0.241 | −0.402* | −0.360* | 0.836** | **−0.601**** |
| Se | | | | | | | | | | 0.064 | −0.286 | 0.452** | −0.426* | 0.477** | 0.498** | 0.211 | −0.214 |
| Zn | | | | | | | | | | | 0.038 | 0.365* | −0.477** | 0.071 | 0.025 | 0.315 | −0.128 |
| $F^-$ | | | | | | | | | | | | −0.133 | 0.583** | −0.727** | −0.692** | 0.687** | **−0.750**** |
| $Cl^-$ | | | | | | | | | | | | | −0.559** | 0.294 | 0.379* | 0.292 | −0.284 |
| $NO_2^-$ | | | | | | | | | | | | | | −0.454** | −0.426** | 0.071 | −0.229 |
| $NO_3^-$ | | | | | | | | | | | | | | | 0.940** | −0.434** | 0.345 |
| $SO_4^{2-}$ | | | | | | | | | | | | | | | | −0.375* | 0.253 |
| TOC | | | | | | | | | | | | | | | | | **−0.823**** |
| $EC_{50}$ | | | | | | | | | | | | | | | | | |

Notes: The bold values represent statistically significant correlation with $EC_{50}$.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Ba, Cr, Cu, Pb, F⁻ and TOC, were significantly and negatively correlated with the $EC_{50}$ ($P < 0.05$). These results and the PCA results indicated that these constituents in the MSWIBA primarily and significantly influenced the toxicity of Q67 relative to the other constituents.

### 3.4.3  Hierarchical cluster analysis

An HCA was performed to further classify and group the constituents of different sources of toxins ($EC_{50}$) based on the similarities of their chemical properties. This method describes the similarity of these points by using a dendrogram, as shown in Fig. 6 (b), to reveal any clustering behaviors of the constituents in the samples. All the samples were separated into three clusters, As-Ni-Cl⁻-Cd-Se-Fe-NO₃⁻ -SO₄²⁻ (shown in red), Ba-Cr-Cu-F⁻-Pb-TOC-NO₂⁻ (shown in green), and the remaining constituent (shown in blue). The distance cluster represents the degree of association between constituents. The lower value on the distance cluster, the more significant is the association.

The second cluster was at a relatively higher level and had very strong mutual correlations to form primary cluster pairs. In the effluent samples, Ba-Cr-Cu-F⁻-Pb-TOC primary clusters were formed. Three clusters were observed for the constituents in the effluent samples that had significant linkage distances. For Zn and NO₂⁻ which did not belong to any of the clusters, these constituents were separated from the other constituents by even longer distances. Figure 6 (b) shows that HCA can be used in preliminary toxicity screening to the constituent groups in the MSWIBA leachate samples. These results corresponded with the results of the PCA. Additionally, both the PCA and the HCA identified the sources of toxic ($EC_{50}$) and were able to categorize the constituents of the leachate samples [49].

## 4   Conclusions

The leaching tests and Q67 bioassay were conducted to evaluate the ecotoxicity of leachates from the MSWIBA. The results showed that the heavy metal leaching concentrations in the $H_2SO_4/HNO_3$ and HAc leachates of MSWIBA were all below the regulatory limits for hazardous waste, however, the leachates showed acute toxicity by $EC_{50}$ at 9.4% and 7.6% for 0.1 mol·L⁻¹ HAc extraction ($L/S$ = 1 L·kg⁻¹ and 24 h) of MSWIBA1 and MSWIBA2, respectively, as well as 9.3% and 8.6% for 0.1 mol·L⁻¹ HAc extraction ($L/S$ = 10 L·kg⁻¹) of MSWIBA2 at 24 h and 72 h, respectively. Moreover, the toxicity ($EC_{50}$) was primarily influenced by Ba, Cr, Cu, Pb, F⁻ and TOC in the leachates from MSWIBA. The statistical analyses showed similar leaching behaviors of the grouping of the variables Ba, TOC, Pb, Cu, Cr and F⁻. The

results also suggest that the Q67 (*V. qinghaiensis*) bioassay could be used as an efficient ecotoxicity test for MSWIBA leachate and as a monitor of environmental contamination. It is necessary to evaluate the environmental impacts and risks of MSWIBA before their utilization. Effective environmental monitoring and protection must be carried out to ensure that MSWIBA utilization and disposal does not become an environmental hazard.

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