Toxicity identification evaluation of landfill leachate taking a multispecies approach

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Abstract

In this study, the first phase of Toxicity Identification Evaluation (TIE) was used to characterise the major toxicants present in a Malaysian landfill leachate, using multispecies bioassay. Freshwater fish (*Rasbora sumatrana*), freshwater prawn (*Macrobrachium lanchesteri*) and tomato seeds (*Lycopersicon esculentum*) were chosen as test species based on local availability, as well as their broad sensitivity for environmental toxicants. Physico-chemical fractionation steps (oxidant reduction with sodium thiosulphate, EDTA chelation, pH adjustment, pH adjustment followed by filtration, aeration, extraction with solid phase C18 column (SPE), and graduated pH analyses) were carried out. The results show that the major toxicants were mostly basic in nature, precipitable under acidic conditions and contained non-polar organic compounds. The small reductions in toxicity observed when treated with sodium thiosulphate indicate the presence of oxidizers. The EDTA chelating step did not significantly reduce toxicity in the test organisms, suggesting insignificant levels of (toxic) metals. The formation of emulsion when mixed with crude oil indicates the presence of surfactant.

Keywords: toxicity identification evaluation, landfill leachate, bioassay, surfactant.

1 Introduction

Leachates are the liquid generated by the percolation of rainwater and moisture through the layers of wastes in landfills or dumping areas (Koshy [1]). Landfill leachates may contain substantial amounts of dissolved organics, xenobiotic

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organic compounds (XOCs), inorganic salts, ammonia, heavy metals and other toxicants (Christensen *et al.* [2]; Pivato and Gaspari [3]), which are potentially harmful to aquatic organisms. Runoff from heavy rainfalls, floodings, and other unlikely events can cause leachates to contaminate surface and ground water. When assimilated, some of these chemicals can bioaccumulate in aquatic organisms and be passed along the food chain (Sang *et al.* [4]), eventually reaching humans.

Successful assessment of potential impacts of landfill leachates on the ecosystem requires identification of the contaminants responsible for the toxicity observed. Identifying these contaminants, however, may be difficult because of the variety of chemicals present, the limited number of chemicals which are routinely analysed, the complexity and diversity of each leachate as well as the uncertainty of contaminants' bioavailability to the impacted organisms (Isidori *et al.* [5]). The toxicity identification evaluation (TIE) method developed by the U.S. EPA [6–8] has been found to be a useful tool for the detection and identification of the classes of chemicals present. This method is generally conducted as part of a larger program to control effluent toxicity (Novak and Holtze [9]) and has been used effectively in characterizing and identifying toxicants in samples of effluents, sediments, ambient waters, and other complex mixtures (Isidori *et al.* [5]; Kosian *et al* [10]; Ankley and Burkhard [11], Wik and Dave [12]). This method, which combines physical and chemical fractionation of the leachates with toxicity testing, can identify the main classes of toxicants present before further confirmation by instrumental analyses. The TIE method comprises three related phases. Toxicants are characterised in Phase I, identified in Phase II and their identity confirmed in Phase III.

Various species can be used for characterizing the toxicity of effluents and receiving waters (Novak and Holtze [9]). Generally a suite of organisms representing several taxa is recommended, the choice depending on the requirements of the regulatory authority and the objectives of the tests conducted (U.S. EPA [13]). Until now, Malaysia has not issued any regulations or directives for effluent toxicity testing. Furthermore, no standardised procedures for toxicity testing are in place. This paper reports the Phase 1 TIE of a sanitary landfill leachate, using a newly developed suite of toxicity testing organisms from three taxonomic levels of aquatic species: fish (representing vertebrates), prawn (representing invertebrates) and tomato seeds (representing plants).

2 Material and methods

2.1 Sampling of leachate

Raw leachate was sampled from Selangor's Jeram landfill, a municipal solid waste sanitary landfill designed to receive about 1,000 to 1,500 tonnes of solid waste per day. Opened in 2007, its operation lifespan is expected to be ten years. Leachate was collected in clean, double-stoppered polyethylene bottles and immediately transported to the laboratory on ice (4°C) to prevent chemical

degradation. In the laboratory, the leachate was stored in the dark at 4°C if analysed within one week or frozen at -80°C until needed.

Physico-chemical analysis of the leachate was performed both *in situ* and in the laboratory following the APHA method [14]. pH, DO, conductivity, and TDS were measured *in situ* (YSI 55). Alkalinity, BOD, COD, TSS and ammonical nitrogen were measured according to APHA standard methods APHA [14], and nitrate, phosphate and sulphate were determined according to the HACH Method (HACH [15]). Heavy metal contents were measured by ICP-MS (Perkin Elmer, Elan DRC 9000).

2.2 Toxicity testing of effluent

Acute toxicity tests were carried out in static conditions using a freshwater fish (seluang; *Rasbora sumatrana*), a freshwater prawn (*Macrobrachium lanchesteri*) and seeds of tomato (*Lycopersicon esculentum*). Test species were chosen when results of preliminary experiments showed them to be more sensitive than the other species tested for each category, namely carp (*Cyprinus carpio L.*), the prawn *Macrobrachium rosenbergii* and seeds of cucumber (*Cucumis sativus*) respectively.

Adult seluang (*Rasbora sumatrana*) (~4.5 cm long and weighing ~ 0.5 g) procured from an aquarium shop in Bandar Baru Bangi, Selangor, were acclimatized to the experimental conditions in dechlorinated aerated tap water using Air Pump series RC-004 for at least 12 days before testing (OECD [16]). All aquaria were adequately aerated with air pumps and fish were fed once a day with commercial fish food (neon micro pellet®). Feeding was stopped 24 hours before initiation of the experiment, and specimens which showed active movement and no signs of injury were chosen for the test.

Leachate was diluted to 0.32, 0.42, 0.56, 0.75, 1% of the original concentrations after a preliminary range finding test. In each aquarium a total of 10 fishes were put into 10 L water containing the leachate OECD [16]. Each test was replicated twice and the toxicity testing was done for a period of 24 hours (U.S. EPA [6]). Controls without leachate addition were provided for all experimental conditions.

Dead fish were counted every 12 hours and removed immediately. Water quality parameters (pH, dissolved oxygen, temperature, and conductivity) were monitored on a daily basis throughout the experiment. The LC50 value was calculated using the EPA computer program based on Finney's Probit Analysis Method (Finney [17]).

The prawns, *Macrobrachium lanchesteri*, ~2.5 cm long and weighing ~0.1 g, procured from a similar aquarium in Bandar Baru Bangi, Selangor, were acclimatised as above, with 10 prawns in 2 L water in 5 L glass beakers (OECD [16]). The concentrations of leachates used were 0, 0.75, 1.3, 2.4, 4.2, 7.5% of original concentration, also determined by prior range finding test.

Seeds of a local variety of tomato *Lycopersicon esculentum* (Serdang 2) were obtained from the Agriculture Department of Malaysia. Only seeds with good germination potency, as determined by dormancy test, were chosen. Only the

seeds which sank in 200 ml of deionized water were then soaked in 20 ml test solution of leachate for 2 hours at 4°C to break the dormancy (Smith *et al* [19]). A total of twenty seeds were germinated on double filter paper (Whatman No.6 with diameter 12.5 cm) in a clean Petri dish, each dish containing 10 mL of the treatment solution. Concentrations of leachate used were 0 (control), 1.3, 1.8, 2.4, 3.2, 4.2, 5.6% of original concentrations, as determined by a preliminary range finding test (U.S. EPA [18]). All treatments were replicated three times. Petri dishes were then incubated for 4 days under darkness at a controlled temperature of 25 °C in a chamber box (Protech Incubator, Model Cool-200). Observations of seed germination were recorded at the end of the 4 days. Only seeds whose primary root had attained a length of 5 mm were counted as having germinated. EC_{50} calculation was done by EPA computer program based on Finney's Probit Analysis Method (Finney [17]).

2.3 TIE phase I procedures

The toxicity identification evaluation (TIE) method was carried out following the U.S. EPA guideline (U.S. EPA [6]). The following treatments were conducted: pH adjustment test, pH adjustments (at each of the three pHs tested earlier) followed by a) filtration, b) aeration and c) C_{18} solid phase extraction, ethylenediaminetetraacetic acid (EDTA) chelation test and oxidant reduction test.

In the pH adjustment tests, pHs were adjusted from the original pH of the leachate (pHi) of 8.1, to pH 3 using 5.0, 1.0, 0.1 N HCl and to pH 11 with 5.0, 1.0, 0.1 N NaOH (Merck, Darmstadt, Germany), as necessary. For the pH adjustments followed by filtration test, samples at the pHi, pH 3 and pH 11 were filtered through a 0.45 μm Whatman filter paper GF/C. The solid phase extraction (SPE) was performed using Hypersep C_{18} (octadecyl unendcapped bonded silica, 200mg/3 ml, Thermo). For the aeration tests, 290 ml of test solution was transferred to a 500-ml graduated cylinder and then moderately aerated (250 L/hr) with an air pump (Aqua Zonic Giant). For the graduated pH test, the test solution at the original pH was adjusted within a physiologically tolerable range, which was chosen to be at pH 6.5, 7.5 and 8.5.

EDTA was added using the effluent dilutions approach. The amount of EDTA added was based on the amount of EDTA needed to chelate the Ca and Mg present in the sample. Concentrations of Ca and Mg were determined using ICP-MS. Prior to toxicity testing, each of these pHs were adjusted back to the original pH of the leachate (pH 8.1). These pH readjustments were necessary as EDTA, being acidic, can lower the pH of the sample.

In the oxidant reduction test, additions of sodium thiosulfate ($Na_2S_2O_3$) were carried out using the dilution test approach. For this test, a matrix of three leachate concentrations and three levels of thiosulfate concentrations were used. The choice of the thiosulfate concentrations to be added to the effluent was based on the thiosulfate LC_{50} value for each test species, as determined earlier. Deionised water was used to dilute samples to their appropriate concentrations. Three sets of leachate solutions (4x-LC_{50}, 2x-LC_{50}, and 1x-LC_{50}) were prepared.

For the first set, thiosulfate was added to each leachate test solution at 0.5xLC_{50} of $Na_2S_2O_3$; for the second set, it was added at 0.25xLC_{50} of $Na_2S_2O_3$; and for the third set, it was added at 0.125xLC_{50} of $Na_2S_2O_3$ (U.S. EPA [6]). Test organisms were then introduced to the leachate plus the calculated thiosulfate solution. Using this approach, the concentration of thiosulfate remains constant over each leachate dilution series. Controls comprised leachates with no added thiosulfate. The LC_{50} values were determined as described above.

For the toxicity testing, all samples were adjusted back to the original pH (pHi; pH 8.1) prior to the test except for the graduated pH and oxidant reduction tests, where the exposure concentrations were the multiples of the LC_{50}/EC_{50} value of leachate to the seluang (0.82%), prawn (1.39%) and tomato seeds (3.51%) that were obtained from the initial toxicity testing. A series of exposure concentrations were set up at 4x-LC_{50}, 2x-LC_{50}, 1x-LC_{50}, and 0.5x-LC_{50}, for each pH (pH 3, pHi and pH 11/pH 9 for SPE). Controls were made up of untreated leachate U.S. EPA [6].

The toxicity (LC50) values were subsequently converted to toxic units (TU). The TU values of treated sample (TU sample) were compared to the TU values of the untreated sample (TU control) and reported as a percentage of toxicity reduction (TR) as shown in eqn (1).

$$TR = (1- (TU\ sample/TU\ control) \times 100\% \quad (1)$$

The presence of surfactant was detected according to Cooper and Goldenberg [20] by adding 3 mL of leachate (100% concentration) to 3 ml of crude oil and vortexing at high speed for 2 min. Measurements of emulsion were made 24 hours later. The emulsion index (E24) is the height of the emulsion layer, divided by the total height, and multiplied by 100.

3 Results and discussion

3.1 Development of toxicity testing taking a multispecies approach

In toxicity testing, the use of representative organisms from different levels of the food chain more faithfully displays the range of response to toxicants than use of test organisms from one particular level Cairns [21, 22]. Furthermore, testing with several species from different taxonomic groups gives a better indication of the natural variability in the levels of the toxicants in causing an observed effect (Rand [23]). Accordingly, this study adopted a multispecies (fish, prawn and plant seed) approach, which is more indicative of the effects of particular toxicant(s) on aquatic organisms.

When tested within the toxic range of the leachates for 24 hours, the LC_{50} 24 h for the fish (*Rasbora sumatrana*), and prawn (*Macrobrachium lanchesteri*) were found to be 0.82% and 1.39%, respectively. For tomato (*Lycopersicon esculentum*) seeds, the 96 h EC_{50} of leachate was 3.51% (Table 1).

These results suggest that of the test organisms used, the fish (seluang) is most sensitive to the leachate. It is not immediately known why the fish, which is

Table 1: Estimated LC/EC_{50} of 24 h initial toxicity of leachate to fish and prawn and 96 h initial toxicity to tomato.

Point	Exposure Concentration		
	Fish	Prawn	Tomato
LC/EC 1.00	0.358	0.669	0.868
LC/EC 5.00	0.456	0.828	1.307
LC/EC 10.00	0.518	0.928	1.626
LC/EC 15.00	0.566	1.002	1.884
LC/EC 50.00	**0.817**	**1.386**	**3.511**
LC/EC 85.00	1.180	1.917	6.545
LC/EC 90.00	1.288	2.070	7.584
LC/EC 95.00	1.465	2.319	9.435
LC/EC 99.00	1.866	2.871	14.208

a much bigger organism (weighing ~ 0.5 g), was more affected than the prawn, which is only a fifth of its weight. So far, there has been no report of a similar study on toxicity of leachate on these two organisms. It is, however, very likely that this difference in sensitivity is due to species differences. Unlike fish, prawns are bottom feeders, so their normal diet is also different. Different species are known to respond differently to similar toxicants, and some from the lower level taxa are seemingly less susceptible than those in the higher level taxa. Thus, it is likely the different physiological and biochemical processes within these two organisms affect the leachate toxicity.

Plant seeds are thought to absorb nutrients from the medium during germination in addition to those nutrients provided by the embryos (Cheng and Zhou [24]). The effect of toxic substance on seed germination depends on several factors, including the plant species itself and interspecific differences in seed structure, particularly the seed coat with its wide range of anatomical forms (Duffus [25]; Wierzbicka and Obidziniska [26]).

The toxicity tests for tomato was performed for only 96 hours because at that time the primary root in the control was already able to reach a length of 5 mm, the length stipulated for the seed to be counted as having germinated. While the toxicity endpoint for each of fish and prawn was mortality, calculated as LC_{50}, or the median dose that kills 50% of the population, in the case of tomato seeds, the endpoint was taken as EC_{50}, the concentration that gives 50% of the toxicants' maximal effect or where 50% of the population exhibit a response after specified exposure duration. EC_{50} is also related to IC_{50} which is a measure of 50% inhibition due to toxicants. EC_{50} is generally used for agonist/stimulator assays whereas IC_{50} is used for competition binding assays and functional antagonist assays. Amongst the test organisms, fish was found to be most sensitive, although the most insight is gained when the three species (fish, prawn and seed germination) are used together.

3.2 TIE manipulation of leachates

Table 2 shows the reduction in leachate toxicity to seluang and prawn when they were exposed for 24 h to the original leachate and to manipulated leachates at the end of Phase I testing. Germination of tomato seeds was observed after 96 h exposure to the various manipulated leachates.

Altering the pH of a toxic effluent can have a significant effect on its chemistry and toxicity. In the pH adjustment test, the effluent samples were adjusted to pH 3 and 11 and, along with the unadjusted effluent (at pH i), were processed by filtration, aeration or extracted with SPE. The processed effluent samples were then readjusted to pH i before assessment for toxicity reduction. Table 2 shows that the pH adjustment test showed higher toxicity reduction at pH 3 (range: 46.51% - 67.09%) than at pH 11 (range: -23.81% - 30.30%) for all three test organisms Higher toxicity reduction at low pH was probably due to the significantly high contents of basic substances such as ammonia, which are ionized at lower pHs. Ammonium ion (NH_4^+) is known to be much less toxic than the non-ionised ammonia (NH_3) (U.S. EPA [6]). The physico-chemical analysis of the leachate (Table 3) shows that ammonia levels in the leachate reached up to 1693 mgL^{-1}.

Reduction in or loss of toxicity may also be the result of degradation of the toxicants at these altered pH values. Inorganic and organic substances may also

Table 2: Toxicity reduction (TR) after phase I TIE manipulations (percentages are that of the control, which is the untreated leachate).

Fractionation / Manipulation	TR on *Rasbora sumatrana* (%)			TR on *Macrobrachium lanchesteri* (%)			TR on *Lycopersicon Esculentum* (%)		
	pH 3	pHi (pH 8.1)	pH 11	pH 3	pHi (pH 8.1)	pH 11	pH 3	pH i (pH 8.1)	pH 11
pH Adjustment	62.04		24.07	67.09		30.30	46.51		-23.81
pH Adjustment & Filtration	71.43	0.00	29.79	75.95	0.00	0.00	82.15	-62.44	-0.28
pH Adjustment & Aeration	57.51	-33.93	35.34	69.26	-6.36	30.92	74.79	-30.36	27.12
pH Adjustment & Solid Phase Extraction	66.03	36.77	14.89	75.10	21.37	39.71	84.65	6.32	27.44
EDTA Chelation	0.00			0.00			13.81		
Oxidant reduction Test	27.95			9.7			-38.65		
Graduated pH test	-			-			pH 6.5 = 39.41 pH 7.5 = 36.29 pH 8.5 = -6.55		

Table 3: Physico-chemical characteristics of landfill leachate.

Parameter	Value	industrial effluents Std B
DO sat (%)	0.7	
DO (mg/L)	0.05	
pH	8.67	5.5 - 9
Temp	32.86	40
BOD (mg/L)	834.27	50
COD (mg O2/L)	3583.33	100
Nitrate (mg/L)	53.75	
Phosphate (mg/L)	62.33	
Sulphate (mg/L)	112.5	
Ammonia (mg/L)	1693.33	20
Chloride (mg/L)	3199.01	
Alkalinity (mg/L)	13066.67	
TSS (mg/L)	1391.11	100
TDS (mg/L)	14680	
conductivity (μs/cm)	25982	

be precipitated out if the pH is changed, although the precipitated chemical may or may not be the toxicant (U.S. EPA [6]).

The filtration test following pH adjustment investigates whether the solubility of the toxicant is pH dependent. As Table 2 shows, filtration tests showed slightly higher toxicity reductions at pH 3 (71.43% - 82.15%) than with pH adjustment alone, suggesting the presence of some toxic chemicals which are not soluble and are precipitated out at acidic pHs. Carboxylic acid for example donates a proton to water, forming carboxylate ions. When the pH is lowered i.e. when protons are added, the carboxylate ion become protonated, becoming less soluble and therefore can be removed by filtration (Timberlake [27] and Borowiec [28]). Solubilities of heavy metals are also affected by pH. Toxic metal cations such as Cd^{2+} and Pb^{2+} may be converted to insoluble hydroxides at high pHs. However, as Table 4 shows the metals are present at low concentrations, and that may partly explain why there is less reduction of toxicity at pH 11.

Table 2 shows higher toxicity reduction (ranging from 57.51% to 74.79%) in the aeration test at pH 3 for all test species. Aeration may alter toxicity by any of three mechanisms: removal of toxicant by volatilisation, toxicant removal by sublation or chemical alteration of toxicant by oxidation (Reimer Analytical and Associates [29]). Hydrogen sulphide can be removed by volatilisation. Compounds such as surfactants, which tend to concentrate at the air/water interface, may be physically removed within the microdroplets that are formed as the air bubbles leave the water surface (Ankley and Burkhard [11]).

pH adjustment followed by SPE with the C_{18} column caused toxicity reduction to as much as 66.03% - 84.65% at low pH (Table 2). The C_{18} column

Table 4: Heavy metal contents in the landfill leachate.

Parameter	Value (ppm)	industrial effluents Std B (ppm)
Cr	0.092	0.05
Mn	0.026	1.0
Fe	1.199	5.0
Co	0.014	1.0
Ni	0.072	1.0
Cu	0.006	
Zn	0.105	2.0
As	0.160	0.1
Ag	0.003	1.0
Cd	0.001	0.02
Sn	0.006	
Hg	0.001	0.05
Pb	0.007	0.5

packing presents a hydrophobic surface to the effluent. As the toxicant passes through the column, any relatively polar toxicant will remain in the aqueous phase, whereas a relatively nonpolar toxicant may be retained on the column (Norberg-King *et al* [30]). Results suggest the very strong likelihood of the presence of nonpolar organic compounds, as shown by the significant loss of toxicity when manipulated in this way.

EDTA chelation did not reduce toxicity in fish and prawn and only caused small toxicity reduction in tomato (13.81%), suggesting that metals were present in small concentrations and were not a major toxicant. This confirms the finding of low concentrations of all toxic metals in the leachate (Table 4).

The addition of sodium thiosulphate, $Na_2S_2O_3$, a reducing agent, to the leachate for 24 hours caused small reductions in toxicity, 27.95% in seluang and 9.7% in prawn. This reduced toxicity suggests the presence of toxic oxidizers such as chlorine, which takes away electrons from sodium thiosulphate to form the less toxic sodium chloride and sodium tetrathionate. It is not known why addition of this reducing agent increased toxicity in the tomato seed germination test by as much as 38.65% (Table 1).

From the observations thus far, it can be concluded that that the major toxicants are basic, unstable and thus filterable under acidic conditions, and they also contain significant amounts of non-polar organic compounds, very likely surfactant(s). Another test was conducted to confirm the presence of surfactant in which leachate (100% concentration) was mixed with crude oil (1:1; v/v). Surfactant was identified in terms of emulsion index (E_{24}), which was found to be 59.03%. Work is in progress to further identify all the contaminants.

4 Conclusion

Taking a multispecies approach in this Phase I Toxicity Identification Evaluation study of a sanitary landfill leachate in Malaysia, it was found that the leachate contained toxicants which are basic in nature, unstable under acidic pHs, possibly organic acids, some oxidants as well as nonpolar organic compounds, which includes surfactant.

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