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Toxicity identification evaluation of landfill leachate using fish, prawn and seed plant

Setia Budi [a,*], B. Asih Suliasih [b,*], Mohammad Shuhaimi Othman [b], Lee Yook Heng [b], Salmijah Surif [b]

[a] Department of Chemistry, Faculty of Mathematics and Science, Universitas Negeri Jakarta, Jl. Pemuda No 10, Jakarta 13220, Indonesia
[b] Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia

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ABSTRACT

The first phase of toxicity identification evaluation (TIE) method comprised of physic-chemical fractionation steps of pH adjustment, pH adjustment followed by filtration, aeration, extraction with solid phase C_{18} column (SPE), oxidant reduction with sodium thiosulphate and EDTA chelation was conducted to characterize the toxicants of a Malaysian landfill leachate. The battery of organisms test chosen were freshwater fish (*Rasbora sumatrana*), freshwater prawn (*Macrobrachium lanchesteri*) and tomato seed (*Lycoperson esculentum*). Toxicity reductions at each step were comparable for all test organisms. The major toxicants present in the leachate were found to be mostly basic in nature and precipitable under acidic conditions as well as containing non-polar organic compounds. A small reduction in toxicity was observed when leachate was treated with sodium thiosulphate in oxidant reduction test indicating the presence of oxidizers. The EDTA chelating step did not significantly reduce toxicity in the test organisms suggesting insignificant level of (toxic) metals.

1. Introduction

Leachate is the liquid generated by the percolation of rainwater and moisture through the layers of wastes in landfills or dumping areas (Koshy et al., 2007). This liquid may contain substantial amounts of dissolved organics, xenobiotic organic compounds (XOCs), inorganic salts, ammonia, heavy metals and other toxicants (Christensen et al., 2001; Pivato and Gaspari, 2005), which are potentially harmful to aquatic organisms. When assimilated, some of these chemicals can bioaccumulate in aquatic organisms and be passed along the food chain (Sang et al., 2006), 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 analyzed, the complexity and diversity of each leachate as well as the uncertainty of contaminants' bioavailability to the impacted organisms (Isidori et al., 2003). Moreover, chemical measurement alone is not enough to give adequate data for assessing the risks, especially pertaining to living organisms. The use of bioassay test is needed to complement the chemical data as this biological test directly shows effects on organisms or parts of the organisms.

The toxicity identification evaluation (TIE) method developed by USEPA (1991, 1993a, 1993b) has been found to be a useful tool for the detection and identification of the classes of chemicals present in effluents. This method is generally conducted as part of a larger program to control effluent toxicity (Novak and Holtze, 2005) and has been used effectively in characterizing and identifying toxicants in samples of effluents (especially for leachate sample) (Isidori et al., 2003), sediments (Kosian et al., 1998), ambient waters (Norberg-King et al., 1991), and other complex mixtures (Ankley and Burkhard, 1992; Wik and Dave, 2006). This method, which combines physical and chemical fractionation of the leachates with bioassay test, can potentially identify the main classes of toxicants present before further confirmation by instrumental analyses. The TIE method comprises three related phases; toxicants are characterized in Phase I, identified in Phase II and their identity confirmed in Phase III. With the approach of this method, the number of constituents associated with the toxicants can be reduced before analyses began and some knowledge of physical/chemical characteristics is gained. This approach simplifies the analytical problems and reduces cost.

Various species can be used for characterizing the toxicity of effluents and receiving waters (Novak and Holtze, 2005). Fish is useful as a test organism as it play a critical role in aquatic food

* Corresponding authors.
E-mail address: setiabudi@unj.ac.id (S. Budi).

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webs by top-down and bottom-up regulation of nutrient and energy flow (Lammer et al., 2009). As fish is the last chain in the aquatic food cycle, it could be the first step to show the potential risks of chemicals (Castano et al., 1996). Prawn is also a valuable test organism for toxicity testing. Its position is close to fish in food chain and is frequently used as live food for aquarium and cultivated fish (Chong and Khoo, 1988). Bioassay by using plant which is recently known as phytotoxicity test have several advantages. First, many dry plant seeds remain viable on the shelffor a long time. Second, the test is simple and does not require major equipment. Generally, a battery of organisms representing several taxa is recommended for toxicity testing. The choice depending on the requirements of the regulatory authority and the objectives of the tests conducted (USEPA, 2002). Until now Malaysia has not issued any regulations or directives for effluent toxicity testing. Furthermore, there are also no standardized procedures for toxicity tests.

This paper reports the characterization of landfill leachate in Malaysia using phase I of TIE method. This study used local species which have very high sensitivity to pollutant and widely available in the tropical countries from three taxonomic levels: fish (representing vertebrates), prawn (representing invertebrates) and seed plant (representing plants).

2. Material and methods

2.1. Sampling and physico-chemical analyses of leachate

Raw leachate was sampled from a sanitary municipal solid waste landfill located in Jeram, Selangor, Malaysia (latitude 03°11′35.0″ and longitude 101°21′57.3″). This landfill was opened in 2007 and designed to receive between 1000 and 1500 tonnes of solid waste per day with expected lifespan of ten years. The sampling of leachate was conducted once in April 2009 and the leachate was taken from the drainage pipe that flow the leachate to the collecting pond.

Leachate was collected in clean, double-stoppered polyethylene bottles for toxicity test and other physicochemical analyses beside BOD, COD and heavy metal. A few drops of concentrated sulphuric acid and nitric acid were added to the samples collected for the COD and heavy metal analysis respectively as preservative. Sample for BOD analysis were collected in dark BOD bottles. Samples were immediately transported to the laboratory on ice (4 °C) to prevent chemical degradation. In the laboratory, the leachate samples except for BOD analysis, was stored in the dark at 4 °C if analyzed within one week or frozen at −80 °C until needed.

Analysis of the leachate was performed *in situ* and in the laboratory following the APHA method (APHA, 1998). pH, dissolved oxygen (DO), conductivity, and Total Dissolved Solid (TDS) were measured *in situ* (YSI 556). Alkalinity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and ammoniacal nitrogen (NH_3-N) were measured according to the APHA standard methods (APHA, 1998). The concentrations of nitrate, phosphate and sulphate were determined according to the HACH Method (HACH, 2007). Heavy metal contents were measured by ICP-MS (Perkin Elmer, Elan DRC 9000).

2.2. Preliminary toxicity test for leachate

Acute toxicity testings were carried out in static conditions using a freshwater fish (seluang; *Rasbora sumatrana*), a freshwater prawn (*Macrobrachium lanchesteri*) and seeds of tomato (*Lycoperson esculentum*). Preliminary experiments showed them to be more sensitive and easy to acclimatize in laboratory condition than the other species tested for each category, namely carp, *Cyprinus carpio* L., prawn, *Macrobrachium rosenbergii*, and seeds of cucumber, *Cucumis sativus*.

The purpose of the test was to determine the concentrations in TIE manipulation test estimated by the 24 h/96 h LC_{50} for the test organisms. Moreover, this toxicity test was designed to provide an indication of positive or negative toxicity for TIE evaluation, thus the 24 h toxicity test is sufficient for toxicity testing using the selected organism (*R. sumatrana* and *M. lanchesteri*) (USEPA, 1991). However, toxicity test period in seed germination required up to 96 h to allow the sufficient time for seed growth to reach 5 mm (USEPA, 1996).

Adult *R. sumatrana* (~4.5 cm long and weighing ~0.5 g) procured from an aquarium shop in Kajang, Selangor, were acclimatized to the experimental conditions in 250 L tanks containing dechlorinated aerated tap water (Air Pump series RC-004) for at least 12 days before testing according to OECD guideline (OECD, 1992). All aquaria were adequately aerated with air pumps and fish were fed once a day with commercial fish food (Neon Micro Pellet®). Water was renewed every 24 h during the acclimatization period. Feeding was stopped 24 h before initiation of the experiment, and specimens which showed active movements and no sign of injury were chosen for the test.

Leachate was diluted to 0%, 0.32%, 0.42%, 0.56%, 0.75%, 1% (v/v) of the original concentrations which were determined from preliminary range finding test. Ten acclimated fish were put into the diluted 10 L leachate (OECD, 1992). Each test was replicated three times and the toxicity testing was done for a period of 24 h (USEPA, 1996). Controls without leachate were included for each experiment.

Dead fish were counted every 12 h and removed immediately. Water quality parameters (pH, dissolved oxygen, temperature, and conductivity) were monitored throughout the experiment. The LC_{50} value, the median concentration that kills 50% of the population, was calculated using the EPA computer program based on Finney's Probit Analysis Method (Finney, 1971).

M. lanchesteri (~2.5 cm long and weighing ~0.1 g) procured from a aquarium in Bandar Baru Bangi, Selangor, were also acclimatized as above. Ten prawns were put into the diluted 5 L leachate with concentration of 0%, 0.75%, 1.3%, 2.4%, 4.2%, 7.5% (v/v) of original concentration as determined by a preliminary range finding test. The acute toxicity testing was conducted as described for the fish.

Seeds of a local variety of *L. esculentum* (Serdang 2), obtained from the Agriculture Department of Malaysia were soaked in 200 ml of deionized water. Seeds used for the testing were chosen based on their ability to sink when immersed in water. Seeds were then soaked in 20 ml of the test solution of leachate which used for toxicity test, for 2 h at 4 °C to anticipate any dormant seed (Smith et al., 2002). A total of 20 seeds for experiment 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% v/v of original concentrations, as determined by a preliminary range finding test (USEPA, 1996). All treatments were replicated three times.

The petri dishes were then incubated for 4 days in the dark at 25 °C in a chamber box (Protech Incubator, Model Cool-200). Seed germination was 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. The EC_{50} value, the concentration that gives 50% of the toxicants' maximal effect or where 50% of the population exhibit a response after the specified exposure duration, was calculated by EPA computer program based on Finney's Probit Analysis Method (Finney, 1971). The design of these tests are described in Table 1.