Characterization and toxicological evaluation of leachate from closed sanitary landfill

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Abstract
Landfilling is a major option in waste management hierarchy in developing nations. It generates leachate, which has the potential of polluting watercourses. This study analysed the physico-chemical components of leachate from a closed sanitary landfill in Malaysia, in relation to evaluating the toxicological impact on fish species namely Pangasius sutchi S., 1878 and Clarias batrachus L., 1758. The leachate samples were taken from Air Hitam Sanitary Landfill (AHSL) and the static method of acute toxicity testing was experimented on both fish species at different leachate concentrations. Each fish had an average of 1.3 ± 0.2 g wet weight and length of 5.0 ± 0.1 cm. Histology of the fishes was examined by analysing the gills of the response (dead) group, using the Harris haematoxylin and eosin (H&E) method. Finney's Probit method was utilized as a statistical tool to evaluate the data from the fish test. The physico-chemical analysis of the leachate recorded pH 8.2 ± 0.3, biochemical oxygen demand 3500 ± 125 mg L⁻¹, COD 10 234 ± 175 mg L⁻¹, ammonical nitrogen of 880 ± 74 mg L⁻¹, benzene 0.22 ± 0.1 mg L⁻¹ and toluene 1.2 ± 0.4 mg L⁻¹. The 50% lethality concentration (LC50) values calculated after 96 h exposure were 3.2% (v/v) and 5.9% (v/v) of raw leachate on P. sutchi and C. batrachus, respectively. The H&E staining showed denaturation of the nucleus and cytoplasm of the gills of the response groups. Leachate from the sanitary landfill was toxic to both fish species. The P. sutchi and C. batrachus may be used as indicator organisms for leachate pollution in water.

Keywords
Clarias batrachus, fish study, landfill, leachate, Pangasius sutchi, toxicity

Introduction
Environmental pressures from the generation and management of waste include emissions to air, water and soil which have potential impacts on human health and nature. Landfilling remains the dominant waste disposal method in most Asian and developing countries (Carra and Cassu, 1990). About 75% of municipal waste generated in Malaysia is landfilled and only 5% is recycled. The rest are either burned or dumped into rivers or at illegal sites and this cause significant pressure on the environment (Agamuthu et al., 2009). All landfills produce leachate by the action of ‘leaching’ when rain water percolates through the permeable waste heap. Therefore streams and other forms of water bodies can be contaminated due to the vertical and lateral migration of leachate (Jaffar et al., 2009), especially if there are no geomembrane liners.

Groundwater and other forms of water course are precious parts of the ecosystem. Most times, it is extremely difficult to assess the magnitude of risk which leachate poses to groundwater, soil and even aquatic life. Leachate composition varies based on the materials present in landfill, i.e. dissolved organic matters (alcohols, acids, aldehydes, and short chain sugars), inorganic macro-components (common cations and anions including sulphate, chloride, and ammonium), heavy metals (Pb, Ni, Cu, Hg) xenobiotic organics and polychlorinated biphenyls (Ludwig et al., 2003). It is often characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and contains other toxicants (Christensen et al., 2001). Therefore, the need for a critical study of the composition of ‘modern’ municipal waste landfill leachate had been evaluated (Murray and Beck, 1990). The study stated that leachate may contain toxic and hazardous compounds, hence there is need to properly evaluate leachate from municipal waste landfills.

The volume of leachate generated from landfills depends on the initial water content of the municipal solid waste (MSW) and the storage or disposal conditions such as temperature, humidity, and ventilation (Selic et al., 2007). Landfill leachate is also characterized by high levels of salts and NH₃-N as well as high organic loading. Higher organic loading yields greater substrate
availability for planktonic and epiphytic bacteria. This may induce inhibitory effects on sedimentary bacteria (Wendong et al., 2007). More than 200 organic compounds have been identified in municipal landfill leachate (Schwarzbauer et al., 2002), with about 35 of these compounds having the potential to cause harm to the environment and human health (Paxeus, 2000). A high level of ammonia is toxic to many living organisms in surface water. This is because it contributes to eutrophication, and dissolved oxygen depletion (Bae et al., 1997).

In terms of solid waste management, Malaysia has many uncontrolled landfills. About 291 landfills of different sizes and ages are recognized officially while an estimated three times more is illegal dumps. With the exception of a few, most of the landfills are non-sanitary as they lack adequate leachate collection and/or treatment facilities, and infrastructure to exploit landfill gas (Fauziah and Agamuthu, 2010). Toxicological evaluations of the landfill leachate are in great demand in order to ensure safe discharge of leachate from landfills. Presently, it is gradually being incorporated into the environmental legislations in some countries (Eun-ah et al., 2009). Chemical oxidation has been developed as a method for the early-stabilization of landfills. However, the effect of by-products that are difficult to be detected by chemical analysis is better understood through toxicological evaluation. Therefore, toxicity tests have become useful tools for detecting the changes of leachate quality as a way to complement the chemical oxidation method (Eun-ah et al., 2009). Both mortality and behavioural effect of landfill leachate on the fish, *Cyprinus caprio* had been evaluated (Jaffar et al., 2009). Toxicity of municipal dump leachate was tested on zebra fish (*Brachydanio rerio*) (Sisinno et al., 2000), while different concentrations of leachate were utilized to analyse the survival ability of tilapia (*Sarotherodon mossambicus*) under leachate pollution (Wong, 1989). Another study had used juveniles and adults of Japanese Medeka (*Oryzias latipes*) to test the toxic potency of landfill leachate (Osaki et al., 2006). However, the absence of landfill toxicity data on fresh water local fishes, *Pangasius sutchi* and *Clarias batrachus* is a subject of concern since both fish species are vastly dominant and of high economic value in the tropical and some temperate countries. Therefore, this study is aimed to critically identify constituents of landfill leachate in Malaysia which might be the potential contaminants/toxicants to the aquatic environment. Additionally, it is also meant to generate landfill leachate toxic potency data for both fish species which can serve as indicator organisms for leachate pollution in Malaysian waters.

**Materials and methods**

**Site characterization**

Air Hitam Sanitary Landfill (AHSL) leachate was used in this study and it represents closed/inactive landfills. It was an active landfill from 1995–2006. AHSL is a sanitary landfill with all requirements for environmental quality assessment (Fauziah and Agamuthu, 2003).

**Leachate sampling and laboratory analysis**

For a better understanding of the toxicity study of the leachate, raw leachate sample was collected for five times (on different days) from AHSL and duly replicated to ensure coherence in analysis. The samples were collected from the pipes directly linked to the landfill cells and taken immediately to the laboratory. The leachate analyses were carried out in respect to the physical, biochemical and chemical parameters.

**Physico-chemical parameters**

Some parameters were analysed on-site or in the laboratory. Parameters like pH, dissolved oxygen and total dissolved solids were analysed using pH meter (HANNA HI 8424) probe, DO 6 dissolved oxygen palm-top meter and multipurpose Hach Sension 7, respectively. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels were obtained via the use of APHA standard methods (5-day BOD test and colorimetric method respectively) (APHA, 2008). For the purpose of analysing the chemical components (metals, volatile fatty acids, alcohols, monocyclic aromatic hydrocarbons, semivolatile organic carbon, organophosphorus pesticides, chlorinated hydrocarbons) of the raw leachate, methods [inductively-coupled plasma mass spectrometry(ICP-MS) and, gas chromatography and mass spectrometry (GC-MS)] that conform to international standards were adopted, namely from APHA (2008) and US EPA (2000).

**Fish test**

The fish species used in this study, *Pangasius sutchi* S., 1878 and *Clarias batrachus* L., 1758 were of Pangasidae and Claridae families, respectively. Both species are native to the rivers of south-east Asia and are freshwater fish although *C. batrachus* can even thrive in stagnant waters. Species used in this study were obtained from commercial aquaculture in Malaysia. Toxicity test of the raw leachate was carried out on fish species based on the following procedure.

**Acclimatization**

Before putting the fish into the aquarium, 20 g of aquarium salt was added into each of the aquarium, and aerated continuously for 1 week with an air pump of 0.04 MPa. This was to remove any trace of chlorine from the tap water. The fish was later put into the aquarium maintained at pH 7.2–8.0, dissolved oxygen concentration 7.0–8.2 mg L⁻¹, and temperature 27.3–28.2 °C. The fish were acclimatized for 7 days without further aeration to resemble the natural aquatic environment. There was much unfilled air space in the aquarium that allows for moderate natural aeration.

The test organisms were fed with commercial fish feed (mainly pellets). Feeding was stopped 48 h prior to initiating the acute toxicity testing (APHA, 2008). This was to reduce metabolic wastes from the fish during the experiment. The photoperiod was

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set at 12 h of light and 12 h of dark throughout the duration of the experiment. Proper measures were taken to ensure that less than 5% mortality was maintained 5 days prior to toxicity testing. The fish used for toxicity tests had total mean length of 5.00 ± 0.1 cm and body weight of 1.3 ± 0.2 g.

Acute toxicity testing

Standard recommendations (APHA, 2008; OECD, 1993; USEPA, 2000) were considered while carrying out the acute toxicity test on *P. sutchi* and *C. batrachus*. Laboratory static test was carried out to determine the median lethal concentration (LC<sub>50</sub>) of the raw leachate sample on each fish species.

A group of 10 fish (in triplicates) of similar size were selected randomly and transferred from the acclimation tank using small hand net into suitable test aquarium of 25 L capacity. This was to avoid any occurrence of mechanical injury to the test fish. The fish weight to water ratio in the aquarium was maintained at an average of 1.0 g L<sup>−1</sup>. A range finding test was carried out with widely spaced concentrations (50, 25, 12.5, 6.25, and 3.125)% v/v. Finally, 5.63, 5, 4.38, 3.75 and 3.13 (% v/v) of the leachate were chosen for the experiment on both fish species in order to have effective comparison in toxicity levels. A control group of equal number of fish was set up without addition of leachate sample. Dissolved oxygen level of not less than 5 mg L<sup>−1</sup> was observed despite the fact that no air pump was used throughout the experiment (control maintained 7.8 mg L<sup>−1</sup> without use of air pump). The water temperature was maintained at 28 ± 1 °C which is nominal for catfish in aquatic environment. All experiments were conducted on 96 h basis and no feeding was done during the experiment. The mortality and behavioural changes of both control and exposed groups were recorded daily at 12 h intervals. Zero mortality was maintained in the control group thereby allaying fear of experimental interference due to starvation. Dead fish were immediately removed from the experiment in order to prevent DO depletion (typical of static bioassay) and ensure proper preservation for tissue analysis. The 96 h LC<sub>50</sub> for both test organisms and their 95% confidence limits were generated using an EPA program, Finney’s Probit Analysis Version 1.5.

Tissue analysis (histology method)

For further examination and evaluation of leachate effect on the test organisms other than mortality rate, laboratory analysis of gills of the dead fish were conducted with histology approach. Sample abstraction and fixation were done on all the concentrations (two of each fish species per concentration) and dissected accordingly to obtain the liver and gills. Care was taken to avoid pinching or pressing hard on the gills and liver samples that were chosen for fixation. Steps observed in the preparation of these tissues for histology included; fixation in suitable solutions (formalin and bowin), dehydration and paraffin infiltration, tissue embedding and sectioning, and affixing of tissue sections onto slides. The prepared tissue slides were transferred into caplin jars for the staining process; Haematoxylin and eosin (H&E) stain method. Each slide was viewed under light microscope while alternating the magnifications to obtain clear image. An external lens, Dinoeyes (1.3 M/ Resolution 1280 × 1024, AM-423 model) was inserted into the microscope and attached to a personal computer to obtain enhanced images of the tissues stains.

Results and discussion

Results

Apparent colour of AHSL leachate was bright brown accompanied by strong ammoniacal odour (Table 1). The pH of the leachate was slightly alkaline at pH 8.2 ± 0.3 while the temperature was 29.5 ± 1 °C. Conductivity value was 20 ± 2.3 mS cm<sup>−1</sup> with corresponding DO of 5.8 ± 0.2 mg L<sup>−1</sup>. BOD and COD values were 3500 ± 125 mg L<sup>−1</sup> and 10 234 ± 175 mg L<sup>−1</sup>, respectively; hence a BOD/COD ratio of 0.34 was obtained. Total oxygen content (TOC) 110 ± 15 mg L<sup>−1</sup> was recorded, while the turbidity value of the leachate was found to be 108 ± 17 FAU.

The result showed high concentration of ammoniacal nitrogen which was 880 ± 74 mg L<sup>−1</sup> (Table 1). A total of 29.1 ± 2.5 and 2.7 ± 0.3 mg L<sup>−1</sup> of NO<sub>3</sub>-N and NO<sub>2</sub>-N, respectively, were recorded. The alkalinity content was recorded at 9000 ± 187 mg L<sup>−1</sup>. Basically heavy metals such as Pb and Cd were not detected in the landfill leachate, but 0.12 ± 0.08 mg L<sup>−1</sup> of Hg was identified (Table 1). The concentrations of other cationic components such as Cr (0.11 ± 0.03) mg L<sup>−1</sup>, Ni (0.29 ± 0.04) mg L<sup>−1</sup>, Zn (0.1 ± 0.02) mg L<sup>−1</sup>, Mn (0.12 ± 0.03) mg L<sup>−1</sup> and Fe (3.10 ± 1.1) mg L<sup>−1</sup>, among others were also obtained from the leachate analysis.

Detailed chemical parameters were studied in order to fully characterize the leachate from the landfill. The chemical groups included are monocyclic aromatic hydrocarbon and semivolatile organic carbon, as shown in Table 1; while others were organophosphorus pesticides, organochlorine pesticides, volatile fatty acids, alcohols and chlorinated hydrocarbons (Table 2). The study revealed low concentrations of the aforementioned parameters. However, there were traces of benzene, toluene and ethyl benzene at 0.22 ± 0.14, 1.2 ± 0.06 and 0.86 ± 0.01 mg L<sup>−1</sup>, respectively. Although most of the parameters were less than 0.01 mg L<sup>−1</sup>, including phenol, the presence of o-cresol and p-cresol at 0.09 ± 0.02 and 0.06 ± 0.01 mg L<sup>−1</sup>, respectively, might be of significant toxicity concern to fish (Svobodova, 1993).

Based on the acute toxicity test, the result showed there was no mortality at all in the control group throughout the duration of the experiment. However, this was not the case with the exposed group. The effect of leachate concentration was demonstrated by the mortality responses. Figures 1 and 2 showed that response/mortality of *P. sutchi* and *C. batrachus* increased with increased leachate concentration. Mortality was 100% within the first 12 h of exposing *P. sutchi* to 5.63 and 5% of AHSL leachate, while at 3.13% leachate concentration, the least mortality of 16.7% was recorded (Figure 1). On *C. batrachus*, total of 50% mortality was recorded at the highest leachate concentration (5.63%). However, the peaks of mortality were at the 12th and 36th hour of exposure. The least mortality was 3.3% along the various exposure intervals (Figure 2).
Table 1. Physico-chemical parameters of the raw leachate (pronounced components).

<table>
<thead>
<tr>
<th>Basic components</th>
<th>Unit</th>
<th>Anionic components</th>
<th>Unit (mg L(^{-1}))</th>
<th>Metal components</th>
<th>Unit (mg L(^{-1}))</th>
<th>Monocyclic aromatic hydrocarbons</th>
<th>Unit (µg L(^{-1}))</th>
<th>Semivolatile organic carbon</th>
<th>Unit (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent colour</td>
<td>Bright brown</td>
<td>Chloride</td>
<td>4150 ± 118</td>
<td>Mercury</td>
<td>0.12 ± 0.08</td>
<td>Benzene</td>
<td>0.22 ± 0.14</td>
<td>1,8-cineol</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Odour</td>
<td>Stench ammoniac</td>
<td>Sulfate</td>
<td>37.1 ± 6.3</td>
<td>Cadmium</td>
<td>&lt; 0.001</td>
<td>Toluene</td>
<td>1.2 ± 0.06</td>
<td>Alpha thujone</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>pH</td>
<td>8.2 ± 0.3</td>
<td>Phosphate</td>
<td>70.2 ± 14.8</td>
<td>Chromium</td>
<td>0.11 ± 0.03</td>
<td>Ethyl benzene</td>
<td>0.86 ± 0.01</td>
<td>Camphor</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>29.5 ± 1</td>
<td>Nitrate nitrogen</td>
<td>29.1 ± 2.5</td>
<td>Copper</td>
<td>&lt; 0.001</td>
<td>m + p-xylene</td>
<td>&lt; 0.01</td>
<td>n-butyl-benzenesulfamide</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Salinity</td>
<td>8.3 ± 2.6%</td>
<td>Nitrite nitrogen</td>
<td>2.7 ± 0.3</td>
<td>Nickel</td>
<td>0.29 ± 0.04</td>
<td>o-xylene</td>
<td>&lt; 0.01</td>
<td>a-cresol</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Conductivity</td>
<td>20 ± 2.3 mS cm(^{-1})</td>
<td>Alkalinity</td>
<td>9000 ± 187</td>
<td>Zinc</td>
<td>0.1 ± 0.02</td>
<td>p-cresol</td>
<td>0.06 ± 0.01</td>
<td></td>
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</tr>
<tr>
<td>Turbidity</td>
<td>108 ± 17 FAU</td>
<td>Ammonium nitrogen</td>
<td>880 ± 74</td>
<td>Lead</td>
<td>&lt; 0.001</td>
<td>Phenol</td>
<td>&lt; 0.01</td>
<td></td>
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<tr>
<td>Dissolved oxygen</td>
<td>5.8 ± 0.2 (mg L(^{-1}))</td>
<td></td>
<td></td>
<td>Manganese</td>
<td>0.12 ± 0.03</td>
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<tr>
<td>BOD</td>
<td>3,500 ± 125 (mg L(^{-1}))</td>
<td></td>
<td></td>
<td>Iron</td>
<td>3.1 ± 1.1</td>
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<tr>
<td>COD</td>
<td>10,234 ± 175 (mg L(^{-1}))</td>
<td></td>
<td></td>
<td>Calcium</td>
<td>25.6 ± 7.2</td>
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<tr>
<td>BOD/COD</td>
<td>0.34</td>
<td></td>
<td></td>
<td>Potassium</td>
<td>440 ± 57</td>
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<tr>
<td>Total dissolved solid</td>
<td>830 ± 104 (mg L(^{-1}))</td>
<td></td>
<td></td>
<td>Magnesium</td>
<td>20.3 ± 4.4</td>
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<tr>
<td>Suspended solid</td>
<td>97 ± 19 (mg L(^{-1}))</td>
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<td></td>
<td>Sodium</td>
<td>48.6 ± 9.3</td>
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<tr>
<td>Total organic carbon</td>
<td>110 ± 15 (mg L(^{-1}))</td>
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<tr>
<td>Cation exchange capacity</td>
<td>10.3 ± 1.7 (mg L(^{-1}))</td>
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<tr>
<td>Oil and grease</td>
<td>7 ± 2 (mg L(^{-1}))</td>
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<tr>
<td>Organophosphorus pesticides</td>
<td>Unit [µg L⁻¹]</td>
<td>Organochlorine pesticides</td>
<td>Unit [µg L⁻¹]</td>
<td>Volatile fatty acids</td>
<td>Unit [µg L⁻¹]</td>
<td>Alcoholic components</td>
<td>Units [µg L⁻¹]</td>
<td>Chlorinated hydrocarbons</td>
<td>Unit [µg L⁻¹]</td>
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<tr>
<td>Chlorpyrifos</td>
<td>&lt; 1</td>
<td>Aldrin</td>
<td>&lt; 0.01</td>
<td>Ethanoic</td>
<td>&lt; 5</td>
<td>Methanol</td>
<td>&lt; 5</td>
<td>1,1-dichloromethane</td>
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<td>Diazinon</td>
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<td>α-BHC</td>
<td>&lt; 0.01</td>
<td>Propanoic</td>
<td>2100 ± 350</td>
<td>Ethanol</td>
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<td>Iso-butyric</td>
<td>&lt; 5</td>
<td>2-Butanol</td>
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<td>Malathion</td>
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<td>χ-BHC</td>
<td>&lt; 0.01</td>
<td>Butanoic</td>
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<td>Propanol</td>
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<td>&lt; 1</td>
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<td>1,2-dichlorobenzene</td>
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<td>Endrin aldehyde</td>
<td>&lt; 0.1</td>
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<td>Hepachlor epoxide</td>
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<td>Heptachlor</td>
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The LC50 values were 3.2% v/v for \textit{P. sutchi} and 5.9% v/v for \textit{C. batrachus} after 96 h of exposure to leachate. The corresponding lower and upper 95% confidence limits of this leachate on \textit{P. sutchi} were 2.9 and 3.4% v/v, respectively. For \textit{C. batrachus}, the lower and upper 95% confidence limits of AHSL leachate were 5.3 and 7.2% v/v, respectively.

Figure 3 showed the stained gills from the control (non-exposed) group of \textit{P. sutchi}. The result reflects a proper detection of nucleus and cytoplasm. This was characterized by the deep blue and pink coloration which represents the nucleus and cytoplasm, respectively. The gills of extreme-situation exposed fish showed significant difference when compared with the stained gills of the control group. Figure 4 represents the stained gills of exposed \textit{P. sutchi}. The result was characterized with poor coloration or sparse colour distribution. Also, the gill of non-exposed \textit{C. batrachus} stained under H&E is shown in Figure 5. The coloration of the lamella with deep blue and pink is typical of nucleus and cytoplasm of any tissue identified to be in a normal condition. Similarly, Figure 6 shows the stained gills of exposed \textit{C. batrachus}. It was observed under microscopic view that the lamella was much lifted. The sparse coloration showed much denaturation of the nucleus and cytoplasm. This showed severe effect of exposure when compared with the gills from the control group.

The fish behaviour in the control experiment was normal, including the swimming pattern. However, the fish exposed to leachate showed some behavioural variations which are typical of neurotoxin toxicity. These included the tendency of the fish to gather at the surface and lose equilibrium.
Characterized leachate

Leachate quality is often influenced by biochemical changes, physiochemical processes, including dissolution, precipitation, adsorption, dilution and volatilization. Therefore characterization of leachate is complicated based on the fact that its composition may vary as a function of landfill age (Dorota and Ewa, 2008). It has been demonstrated that large variations in leachate quality exist for different landfills and even at different locations within the same landfill (Tatsi and Zouboulis, 2002).

The value of pH is an important parameter in wastewater quality and effluent discharge. It is often used to represent the aggressiveness of leachate and biochemical conditions in solid waste (Jaffar et al., 2009). The pH of the landfill leachate was within the neutrality range (8.2 ± 0.3) and permissible limits. Mature landfills show pH values greater than 7 whereas more neutral pH values are expected in the leachates that have already undergone some stabilization (Chian and Dewelle, 1976; Jaffar et al., 2009). The 29.5 ± 1 °C temperature value of the landfill leachate depicts resemblance to normal lithospheric temperature. Excluding the fact that seasonal variation in temperature severely affects the nitrification of leachate treatment (Dong-Jim et al., 2006), it is also important in the metabolic activities of microbes that enhance degradation in landfills. Conductivity value obtained in this study is typical of values obtained in Malaysian sanitary landfills (North Jinjang and Kelana Jaya) (Agamuthu, 2001), and this depicts the degree of dissolved inorganic components or total concentration of cations and anions present in a substance.

Sanitary landfills leachate in Malaysia are characterized with very high BOD and COD but AHSL leachate showed 3500 ± 125 mg L⁻¹ BOD and 10 234 ± 175 mg L⁻¹ COD due to its non-operational status. The alkalinity which was 9000 mg L⁻¹ might be as a result of long time degradation of the organic components of the waste into organic acids like humic acids. The organic acids may not have formed salts which increases alkalinity as the pH increases. The calcium content might have led to increase in alkaline level as well. Such are common with landfill leachates in the early phases of waste stabilization (Kjeldsen et al., 2002).

Both ammonia and alkalinity are known to be potential toxicants in landfill leachates.

Despite the absence of most heavy metals in the leachate sample, Hg value was above Malaysia discharge standard (EQA, 1974, 2007). This may be as a result of deposition of some waste substances that have mercuric compound in their formulation such as cosmetics containers, battery components, gasoline, paints, rubber and plastics that were discarded to the waste stream. Also waste type deposited may have led to the detection of semivolatile organic carbon compounds especially o-cresol and p-cresol at 0.09 ± 0.02 and 0.06 ± 0.01 mg L⁻¹, respectively. O-cresols are used as solvents, disinfectants and chemical intermediate, while p-cresol is utilized in the formulation of antioxidants, fragrance and dye industries. Improper waste disposal option might have led to the presence of such compounds in the MSW stream. The leachate characterization gives an idea of the possible toxicant associated with the leachate from landfill.

Toxicity test and tissue analysis

Generally, it was observed that increase in the leachate concentration, led to significant mortality of the test organisms. However, increase in time did not give the same trend, as increase in concentration. The lethality time increased with decrease in concentration. The leachate exhibited the highest degree of toxicity with 100% absolute mortality on P. sutchi and 13.3% mortality on C. batrachus within 12 h of exposure. This implied that the leachate is toxic to both species and maybe attributed to the concentration of dissolved organic matters such as BOD and COD. Pangasius sutchi being a stricter freshwater fish than C. batrachus might have experienced difficulty in taking up oxygen due to the presence of organic components of the leachate.

Based on the calculated LC₅₀ values, the leachate is more toxic on P. sutchi. Although the overall mean LC₅₀ values recorded in this study for P. sutchi and C. batrachus differed from those reported by other researchers, yet it showed that leachate from Malaysian landfills are toxic. Study had evaluated the acute toxicity of C. carpio known as common carp (weighed 0.92 ± 0.24 g with length of 3.83 ± 0.19 cm) with mean LC₅₀ values of 1.13, 2.0 and 3.82% for leachate from AHSL, Ampar Tenang landfill (ATL) and Sungai Sedu landfill (SSL), respectively (Jaffar et al., 2009). The difference between the LC₅₀ values of AHSL leachate in this study and that from previous study (Jaffar et al., 2009) may be due to higher tolerance ability of the test species used in this study as compared to that of C. carpio. Furthermore, ATL and SSL are non-sanitary landfills unlike AHSL; hence it can influence the disparity in degree of toxicity. Another study on Sarotherodon mossambicus showed LC₅₀ values of 1.4 and 12% v/v at two different sampling months (Sisinno et al., 2000). Furthermore, considering fish size, the acute toxicity of ammonia on larvae and fingerlings of Nile tilapia (weight of 0.056 ±0.008 g and 10.114 ± 0.045 g, respectively) showed LC₅₀ values of 1.007–1.01 mg L⁻¹ and 7.39–7.41 mg L⁻¹ in two different replicates, respectively (Benli and Koksal, 2005).

Figure 6. Stained gills of the exposed group of C. batrachus. A microscopic view after Harris haematoxylin and eosin staining of the gills of C. batrachus that was exposed to raw leachate.

Discussion

Characterized leachate

Activity and response of fish to leachate was examined and presented in Table 3. Both ammonia and alkalinity are known to be potential toxicants in landfill leachates.

Despite the absence of most heavy metals in the leachate sample, Hg value was above Malaysia discharge standard (EQA, 1974, 2007). This may be as a result of deposition of some waste substances that have mercuric compound in their formulation such as cosmetics containers, battery components, gasoline, paints, rubber and plastics that were discarded to the waste stream. Also waste type deposited may have led to the detection of semivolatile organic carbon compounds especially o-cresol and p-cresol at 0.09 ± 0.02 and 0.06 ± 0.01 mg L⁻¹, respectively. O-cresols are used as solvents, disinfectants and chemical intermediate, while p-cresol is utilized in the formulation of antioxidants, fragrance and dye industries. Improper waste disposal option might have led to the presence of such compounds in the MSW stream. The leachate characterization gives an idea of the possible toxicant associated with the leachate from landfill.

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The mortality might be as a result of the ammonia content and degree of alkalinity in the leachate. Despite being less turbid, lower TDS and SS, yet it is possible that the oxygen uptake by the fishes was affected by the presence of components like benzene, toluene and ethylbenzene which are potential toxicants to fish. Furthermore, the concentration of o-cresol, p-cresols and propanoic acid might have contributed significantly. Due to the variations in the leachate toxic components among different landfills, it is difficult to make a direct comparison with the available results. Also the methods adopted for calculating the LC50 vary and fish size with species used are often different (Jaffar et al., 2009). In view of these, there are no similar studies on toxicity of landfill leachate on P. sutchi and C. batrachus.

The stained gills of the control group showed deep blue and pink coloration of the lamella which reflects a normal tissue condition for nucleus and cytoplasm, respectively, which will allow proper metabolic activities. Since the gills were in contact with only normal water in the aquaria, the stain showed absence of cellular impairment. However, this was the reverse in case of the exposed group as it was characterized of poor or sparse colour distribution. This may be due to the penetration of the components of the leachate into the internal system of the fish. It might have occurred consequent to the fact that fish utilizes the gills for breathing; hence it becomes a target site for any contaminant. Therefore, it is possible that the toxic components like dissolved ions, propanoic acid and other compounds had initiated complex reactions on the cells of the gills. This then caused lysis of the cell due to attack on the nucleus and cytoplasm. Therefore, it depicts the internal toxicity potential of the leachate and might indicate some degree of bioaccumulation.

Limited study has been carried out on tissue analysis of fish as it relates landfill leachate involvement. However, gills of Atlantic Salom exposed to Skerries Brook water contaminated with leachate from nearby landfill, exhibited histopathological changes. The study revealed a lamella lifting which was a lesion not found in the control group (Mathieu et al., 2007). The fish gill is a morphologic and physiologic complex organ involved in respiration, acid-base regulation, osmoregulation and excretion (Evans et al., 2005). Its complexity and constant contact with the external environment makes the gill the first target of waterborne pollutants. This was typical of the fish gills exposed in this study. Both species demonstrated lifting of the lamella (Figures 3 and 5) when compared with the control group. This may be due to much accumulation of the toxic constituents in the leachate onto the gills as a result of its level of contact with the contaminated water in the aquaria.

Implications of the physico-chemical parameters on toxicity of the leachate

It can be inferred that the studied leachate exceeded the standard limits of 50, 100 and 150 mg L\(^{-1}\) in BOD, COD and ammoniacal-nitrogen (NH\(_3\)-N) (mainly in AHSL) respectively, as prescribed by both Malaysian environmental and health authorities (EQA, 1974, 2007). Ammonia and alkalinity are strong indicators of toxicity in aquatic environment. The high concentration of NH\(_3\)-N in AHSL contributed significantly to its degree of acute toxicity on both fish species. Of the toxic pollutants that are present in landfill leachate, NH\(_3\)-N, resulting from the decomposition process of organic nitrogen, has been identified not only as a major long-term pollutant (Kurniawan et al., 2006a), but also as the primary cause of acute toxicity (Kurniawan, 2009). Since NH\(_3\)-N is stable under anaerobic conditions, it typically accumulates in the leachate. Any untreated NH\(_3\)-N above 100 mg L\(^{-1}\) is highly toxic to aquatic organisms, as confirmed by toxicity tests using zebrafish (Kurniawan et al., 2006b).

Despite the inability of ammonia ion (NH\(_3^+\)) to penetrate cell wall of organisms, yet it can be potentially toxic to fish because at molecular form (NH\(_3\)), it can readily permeate into the tissue especially when concentration gradient exists (Svobodova et al., 1993). Water-tissue surface is characterized of acid-base balance; hence any form of alteration will allow the attraction of more molecular ammonia to the side with lower pH (Svobodova et al., 1993). This mechanism is typical of the migration of molecular ammonia from water via epithelium of the gills to the blood en route to the tissues. Nervous symptoms are prevalent in cases of ammonia toxicity of fish because ammonia is toxic to fish brain (Alabaster and Lloyd, 1980).

Nitrite may not really be the cause of mortality of the test organisms. Nitrite toxicity to fish can be affected by certain water quality characteristics (Lewis and Morris, 1986). Consequent upon the chloride content of the dilution water, their study showed that 96 h LC50 for rainbow trout ranged from 0.24 to 12.2 mg L\(^{-1}\) (the chloride content in the study was 0.35 to 40.9 mg L\(^{-1}\)). The impact of chloride on nitrite toxicity was very significant (Svobodva et al., 1993). Therefore estimation of safe nitrite concentration for particular locations requires the measurement of chloride to nitrite ratio. For rainbow trout and fish of low economic importance, the chloride to nitrite ratio of 17 and 8, respectively, is recommended which is expressed as mg L\(^{-1}\) Cl\(^{-}\) : mg L\(^{-1}\) N-NO\(_2^-\) (Svobodova et al., 1993). However, the Cl : N-NO\(_2^-\) in this study is approximately 1500 and as such may not have contributed significantly to the acute toxicity of the used leachate on the fishes.

Toxicity of leachate from various landfills in the world in countries such as Sweden, Lithuania and Brazil has been attributed to ammonia (Pivato and Gaspari, 2005; Silav et al., 2004; Svensson et al., 2005), yet the presence of phenolic compounds which is a semivolatile organic carbon in leachate sample cannot be ignored. Phenols can occur as either monobasic (creosol, naphthol, xylanol, etc.) or polybasic (pyrocatechol, hydroquinone, pyrogallol etc.). This study revealed the presence of o-cresol and p-cresol at 0.09 and 0.06 mg L\(^{-1}\), respectively in AHSL leachate. The maximum concentrations admissible for fish culture are 0.001, 0.003, 0.004 and 0.001 mg L\(^{-1}\) of chlorophenol, cresol, resorcinol and hydroquinone, respectively (Svobodva et al., 1993). Although there is no study concerning o-cresol and p-cresol effect on fish, but animal studies have reported effects to
the liver, kidney and central nervous system (CNS) from acute inhalation of mixed cresols (ATSDR, 1990). The CNS of fish can be affected by the presence of phenol as it is an unacceptable taint to water and fish. Basically fish demonstrate signs of phenolic intoxication by leaping out of water, loss of balance and muscular spasms, increased activity and irritability (Svobodova et al., 1993). This has been reflected in this study with regards to the fish behaviour.

Conclusion

This study concludes that AHSL leachate contained toxic components, most significantly ammonia, dissolved organic matters, some semivolatile organic carbon compounds and monocyclic aromatic hydrocarbons. The leachate was very toxic to both species of fish with more severity on P. sutchi. Thus P. sutchi and C. batrachus may possibly be used as indicators of leachate contamination in freshwater. Ammonia-nitrogen present in the leachate is considered the principal cause of the fish mortality in this study. However, benzene, toluene and ethyl benzene must have contributed to the leachate toxicity. There is need to review the current waste disposal practices with view to minimize leachate generation in order to allay the fear of potential groundwater and surface water contamination, while enhancing sustainability in the use of natural resources.

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