OGÓLNOPOLSKA KONFERENCJA NAUKOWA

INŻYNIERIA EKOLOGICZNA

połączona z Jubileuszem 70-lecia Urodzin
oraz 48-lecia pracy naukowej
prof. dr hab. inż. Piotra Kowalika
OGÓLNOPOLSKA KONFERENCJA NAUKOWA
INŻYNIERIA EKOLOGICZNA

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pod redakcją
Hanny Obarskiej-Pempkowiak

Gdańsk, 7 września 2009
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Wydanie monografii zostało dofinansowane ze środków Wojewódzkiego Funduszu Ochrony Środowiska i Gospodarki Wodnej w Gdańsku z zadania z zakresu edukacji ekologicznej dla woj. pomorskiego

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ISBN 978-83-89293-78-7

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Regionalna Dyrekcja Lasów Państwowych w Gdańsku
SPIS TREŚCI

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Hazard assessment of untreated and biologically treated landfill leachate using toxicity tests – lessons learnt from some Swedish case studies

Sylvia WAARA¹, Mats EK², Åke FORSBERG¹, Magnus FRIDOLFSON³, Karl-Otto WAARA¹

Introduction

Many landfills in Sweden have recently been forced to shift treatment options from municipal sewage treatment to on-site treatment. Biological methods using bioreactors, bioreactors combined with gravel filters or reed beds and constructed wetlands have often been seen as preferred options in spite of the climate which should set limits on the rate of biological processes. So far, authorities have decided that the treatment methods should result in a leachate with a stated maximum level of total nitrogen, total phosphorous and BOD and/or COD, but apart from these parameters there are no other discharge limits. At the same time several studies indicate that landfill leachate may contain many xenobiotics and these might have a negative impact on the environment (Öman et al. 2000, Kjeldsen et al. 2002, Thörneby et al. 2006) alone or in combination. Synergistic effects can therefore not be ruled out. A good tool for investigating the interactive effects of identified and non-identified toxic xenobiotics in effluents is the use of toxicity tests. The results can then also be used for evaluation of the treatment technology applied and for subsequent process optimization.

Several studies indicate that untreated leachates may have high acute toxicity (Clément et al. 1997, Öman et al. 2000, Kjeldsen et al. 2002, Marttinien et al. 2002) and they might also have estrogenic and dioxin-like potency (Behnisch et al. 2001). Treatment method will generally reduce or remove the toxicity but the composition of the leachate and the treatment method will influence the extent of reduction for each specific toxicity response measured (Ek et al. 2000, Rutherford et al. 2000, Behnisch et al. 2001, Marttianien et al. 2002, Bloor & Banks 2006, Deguchi et al. 2007).

In this study the results from toxicity testing of untreated and biologically treated landfill leachate from four different sites in Sweden will be presented. The reduction of toxicity, possible influence of some confounding factors and the usefulness of the test method will be

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presented and discussed. A test battery of selected test organisms and test endpoints for use in evaluation of treatment technology in conjunction with chemical analysis of landfill leachates discharged to freshwater ecosystems is proposed based upon the experience from this work.

**Materials and methods**

**Sites studied**

A presentation of the site studied, the respective treatment methods and sampling sites is given in Table 1.

**Norsa landfill, Köping**

The Norsa landfill is located in Köping and it has been operated since 1974. Various types of waste have been deposited at the site as building and industrial waste, sludge and ash. Very little organic material has been deposited at the site because it has been combusted in an incinerator at the site. In 2000 a treatment facility consisting of a SBR, previously used for sludge treatment at the site, followed by a gravel/sand filter was taken into operation. The leachate is heated before treatment. The samples for the toxicity tests were taken the 24th of November 2000. Incoming leachate from an aerated lagoon, leachate treated in the SBR and leachate after treatment both in the SBR and the filter system were sampled.

**Isättra landfill, Sala**

The Isättra landfill is located in Sala and it has been operated since 1973. It has received various types of waste such as municipal waste, industrial waste, sludge and demolition waste and the waste is deposited in cells. In 1999 a SBR was taken into operation and it can treat 85 m³ leachate/day and a full treatment cycle takes 12 h. The samples for the toxicity tests were taken on the 5th of April 2001. The samples taken were incoming water to the SBR from an aerated lagoon, after nitrification, after denitrification and after settling (outgoing water). An evaluation of the treatment efficiency of the plant has previously been presented by Johansson Westholm (2003).

**Gryta landfill, Västerås**

The untreated leachate was either a landfill leachate from the older parts of the landfill, here named percolate, or a mixed landfill leachate from an equilibration pond, here named leachate.

The percolate was derived from a small part of the deposit that received different types of waste such as industrial waste, sludge and household waste during 1982–1991. The mixed landfill leachate was derived from an aerated equilibration pond that receives leachate from several parts of the landfill including the percolate, leachate from an ash deposit used since 1981, leachate from biocells constructed in 1994 containing household waste and organic waste and contaminated ground water from a shaft well (VAFAB 2000).
The treatment of the two landfill leachates was conducted in a pilot scale plant owned and maintained by PEAB at the landfill during August to December 2001. The plant has been described in detail elsewhere (Larsson et al. 2002). The percolate was pumped from a leachate well and the pond water was pumped directly from the pond. The plant consisted of several parts that could be connected or disconnected. It contained; a) an aggregate where the water could be heated to 20°C, b) a mixing tank (2 m³) where the leachate could be aerated and additions of NaHCO₃ to increase the alkalinity was possible, c) 2 bioreactors (each 2 m³) for nitrification, filled to 40% with carrier material. In these reactors the water could be aerated or oxygenized for oxidation of organic material and for nitrification, d) a bioreactor (1.1 m³) for denitrification and to this reactor acetic acid or Na-acetate was added as a carbon source, e) 2 drum filters for retention of particulates, f) a drum filter, a reservoir, an ozone/UV-unit and charcoal filter in an uninterrupted sequence (ozone/CF), g) several other reservoirs. For biological treatment of percolate and leachate the following sequence was used: Heating – Mixing tank – Nitrification in Bioreactor 1 – Nitrification in Bioreactor 2 – Reservoir– Denitrification – Drum filter. For biological treatment with ozone and charcoal filter treatment of the percolate the ozone/CF unit was connected after the 2 nitrification bioreactors. For biological treatment and ozone/CF of the leachate the ozone/CF unit was introduced in between the two bioreactors for nitrification. Further details of this study can be found in Ek & Waara (2002) and Waara et al. (2003).

**Tab. 1. Sites and the treatment methods used in the study**

<table>
<thead>
<tr>
<th>Site</th>
<th>Treatment method</th>
<th>Sampling points</th>
<th>Reference (year of study)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After SBR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After SBR+filter</td>
<td></td>
</tr>
<tr>
<td>Isätra landfill, Sala</td>
<td>SBR</td>
<td>Before SBR</td>
<td>Unpublished (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After SBR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After denitrification</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After settling</td>
<td></td>
</tr>
<tr>
<td>Gryta landfill, Västerås</td>
<td>Pilot plant Biological treatment with or without ozonation and activated charcoal</td>
<td>In</td>
<td>Ek &amp; Waara 2002</td>
</tr>
<tr>
<td></td>
<td>2 types of landfill leachates tested</td>
<td>After</td>
<td>Waara et al. 2003</td>
</tr>
<tr>
<td>Atleverket landfill, Örebro</td>
<td>Free surface flow wetland</td>
<td>Into the wetland</td>
<td>Waara et al. 2008</td>
</tr>
</tbody>
</table>

**Atleverket landfill, Örebro**

Atleverket landfill and wetland is located 7 km south of Örebro, Sweden. The landfill was started in 1978 and has received different types of wastes. In the area today there is a facility for extracting gas, a composting plant, an area for recycling and an aerated lagoon followed by a wetland system. Around the landfill there are ditches where landfill leachate is collected and pumped into the lagoon. From the lagoon, pre-treated leachate is loaded partly to the
municipal sewage treatment plant and partly to the wetland. The wetland is intermittently loaded generally when the N-NH4+ concentration is below 100 mg/l. The wetland consists of a series of 10 ponds covering an area of 8 ha. The depth of the ponds is generally between 0.4–0.6 m with the exception for the 1st pond which has a depth of 1 m. The samples were taken at the inlet of the wetland after the water was treated in an aerated lagoon (F2) and at the outlet of the wetland (k1). The samples were taken on the 19th of April 2005 and on the 9th of August 2006. Further details of this study can be found in Waara et al. (2008).

**Sampling and sample storage**

Sampling and transport of water for toxicity tests was been conducted according to SS-EN-ISO 5667-3:2004 Water quality – sampling – Guidance on the preservation and handling of water samples. The samples were stored frozen at -20°C and at the day of testing they were thawed at 20°C.

**Toxicity tests & data treatment**

Rotifer toxicity testing with *Brachionus calyciflorus*

Tests with the freshwater rotifer *Brachionus calyciflorus* were conducted using the Rotoxkit™ following the ASTM Standard guide E14490-1. Immobilization was analyzed after 24 h of incubation. Effect values were calculated with the trimmed Spearman–Karber method using ToxCalc. 5.0 (Tidepool Scientific Software, U.S.A.). The highest tested sample concentration was 100 vol-%.

Crustacean toxicity test with *Daphnia magna* and *Thamnocephalus platyurus*

Tests were performed with the Daphtoxkit™magna or Thamnotoxkit™ from MicroBio Test Inc., Denize, Belgium according to the manufacturer’s manual. The Daphtoxkit™magna test was conducted essentially according to SS-EN ISO 6341 Water quality – Determination of the inhibition of mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test. Toxicity was measured as inhibition of mobility after 48 h for *Daphnia magna* and 24 h for *Thamnocephalus platyurus*. 24h-EC50 and 48-h EC50 were calculated with the trimmed Spearman–Karber method using ToxCalc. 5.0 (Tidepool Scientific Software, U.S.A.). The highest tested sample concentration was 100 vol-%.

Algal toxicity test with the green algae *Pseudokirchneriella subcapitata*

The water was filter sterilized through a 0.45 μm filter prior to use. The tests were performed with *Pseudokirchneriella subcapitata* (previously named Selenastrum capricornutum or Raphidocelis subcapitata) using the Algaltoxkit™ from MicroBio Test Inc., Denize, Belgium according to the manufacturer’s manual or, as in the Norsa study, using algal flask culture according to ISO standard 8692:1989 Water quality – Fresh water algal growth inhibition
test with Scenedesmus subspicatus and Selenastrum capricornutum. Toxicity was measured as percentage inhibition of growth rate (μ) compared to a control calculating μ with linear regression using an Excel spread sheath in the Gryta and Norsa study or, as in the Atleverket study, by using the area under the curve method. Both methods are described in the ISO standard 8692:1989. 72h- IC50 was calculated with ICp using ToxCalc. 5.0 (Tidepool Scientific Software, U.S.A.). The highest tested sample concentration was 90 vol % (flask tests) or 99 vol-% (toxkits).

Macrophyte toxicity test with the angiosperm Lemna minor

The test was performed according to SS 0283 13 Water quality – determination of growth inhibition (7-d) Lemna minor, duckweed. pH of the samples were adjusted to 6.5. The percentage inhibition of growth compared to the control was calculated based upon dry weight measurements. 7d- IC50 was calculated with ICp using ToxCalc. 5.0 (Tidepool Scientific Software, U.S.A.). The highest tested sample concentration was 97 vol-%.

Bacterial toxicity tests with the marine bioluminescent bacteria Vibrio fischeri

The tests have been conducted with three different methods using equipment, bacteria and utensils from Microtox®. The highest tested sample concentration was 80 vol-%.

In the Norsa study samples were centrifuged for 5 min at 4000 rpm to remove particulate material. The supernatant was recovered and used for testing according to the comparison test MicrotoxOmni™ Software (Azur Environmental, U.S.A.).

In the Gryta study the samples were centrifuged at 5000 rpm for 30 min. The supernatant was used for toxicity tests. To the samples, which had a salinity of 2–4 ppt, NaCl was added to a final concentration of 20 ppt (excluding the samples salinity). The samples were adjusted to pH 7.5 by adding 50 μl of 0.12 M MOPS buffer to 10 ml of salinity adjusted sample. Untreated and treated percolate were tested with the basal test with 2 replicates and the highest test concentration was 80 vol-% and the data were colour corrected. Untreated and treated leachates were tested with the comparison test. The tests were performed according to the MicrotoxOmni™ Software (Azur Environmental, U.S.A.).

In the Atleverket study the samples were centrifuged at 3000 rpm for 15 min. The tests were conducted according to ISO 11348-3 Water quality – Determination of the inhibitory effect of water samples on light emission of Vibrio fischeri (luminescent bacteria) Part 3: method using freeze dried bacteria The tests were performed using the MicrotoxOmni™ Software (Azur Environmental, U.S.A.).

Toxicity classification

A sample has been classified as being toxic if one or more tested concentrations of the sample show an inhibition that is higher than 20 % compared to the control. For the plant bioassays a sample has been classified as stimulating growth if growth in one or more tested concentration is 20 % higher or more than in the control.
Results and discussion

Toxicity reduction in the treatment plants

The results of the toxicity tests are presented in Table 2 (Norsa landfill), Table 3 (Isätra landfill), Table 4–5 (Gryta landfill) and Table 6 (Atleverket landfill).

In the Norsa study 4 different toxicity tests were conducted (Table 2). The landfill leachate was toxic to three of the test organisms but not toxic to V. fischeri. The most sensitive organism was T. platyurus. Toxicity was reduced but not completely removed after the different treatment steps. This can be partly due to that the SBR was not in operation during 3 days before the test due to pump failure but also due to the high sensitivity of T. platyurus to unionized ammonia and chloride (see confounding factors below) but the present of toxic persistent compounds cannot be ruled out.

Tab. 2. Acute toxicity to selected test organisms before and after treatment of leachate from Norsa landfill. Effect values are expressed in vol-%

<table>
<thead>
<tr>
<th></th>
<th>Before SBR (after aerated lagoon)</th>
<th>After SBR</th>
<th>After SBR + filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>168</td>
<td>9.1</td>
<td>0.7</td>
</tr>
<tr>
<td>chloride</td>
<td>3400</td>
<td>3460</td>
<td>3520</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test organism/endpoint</th>
<th>Before SBR (after aerated lagoon)</th>
<th>After SBR</th>
<th>After SBR + filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daphnia magna 48h-EC₅₀</td>
<td>30 (23–38)¹</td>
<td>87 (68–111)</td>
<td>88 (71–110)</td>
</tr>
<tr>
<td>Pseudokirchneriella subcapitata 72 h-EC₅₀</td>
<td>20 (14–27)</td>
<td>&gt; 90</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Thanatephorus platyurus 24 h-EC₅₀</td>
<td>17 (16–18)</td>
<td>23 (20–26)</td>
<td>54 (47–63)</td>
</tr>
<tr>
<td>Vibrio fischeri comparison test 15 min response</td>
<td>&gt; 80</td>
<td>&gt; 80</td>
<td>&gt;80</td>
</tr>
</tbody>
</table>

¹ confidence limits in brackets

In the Isätra study only 2 different toxicity tests were performed (Table 3). The incoming water before the SBR was toxic to D. magna but this toxicity was removed already after the nitrification step. None of the samples were toxic to L. minor but growth stimulation was observed in many of the samples except the incoming and the outgoing water. Morphological investigation of the plants showed distorted root growth and accumulation of sludge on the roots in many of the treatment steps. These changes were not seen in the control and in the outgoing water.
Tab. 3. Acute toxicity to selected test organisms before and after treatment of leachate from Isätra landfill. Effect values are expressed in vol-%

<table>
<thead>
<tr>
<th></th>
<th>Before SBR (After aerated lagoon)</th>
<th>After Nitrification</th>
<th>After nitrification + denitrification</th>
<th>After nitrification + denitrification + settling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical and chemical characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>6.9</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td>NH₄-N (mg/l)</td>
<td>82¹</td>
<td>3.5</td>
<td>3</td>
<td>&lt;2</td>
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<tr>
<td>Chloride (mg/l)</td>
<td>212</td>
<td>331</td>
<td>325</td>
<td>347</td>
</tr>
<tr>
<td><strong>Test organism/endpoint</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48h-ECₑ₂₀</td>
<td>67</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>48h-ECₛ₀₂₀</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Lemna minor</em></td>
<td></td>
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<tr>
<td>7d-ICₑ₂₀</td>
<td>&gt;97</td>
<td>&gt;97</td>
<td>&gt;97</td>
<td>&gt;97</td>
</tr>
<tr>
<td>7 d-ICₛ₀₀</td>
<td>&gt;97</td>
<td>&gt;97</td>
<td>&gt;97</td>
<td>&gt;97</td>
</tr>
</tbody>
</table>

¹ mean value from October to December 2001 (Johansson Westholm 2003),
² stimulation of growth observed in several concentrations and samples.

Tab. 4. Acute toxicity to selected test organisms before and after treatment of the percolate from Gryta landfill. Effect values are expressed in vol-%

<table>
<thead>
<tr>
<th></th>
<th>Biological treatment</th>
<th>Only nitrification and Ozone/charcoal filter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In</td>
<td>Out</td>
</tr>
<tr>
<td><strong>Physical and chemical characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>9.0</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>530</td>
<td>0.4</td>
</tr>
<tr>
<td>chloride</td>
<td>850</td>
<td>700</td>
</tr>
<tr>
<td><strong>Test organism/endpoint</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48h-ECₑ₂₀</td>
<td>n.a.¹</td>
<td>&gt;100</td>
</tr>
<tr>
<td>48h-ECₛ₀₂₀</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Vibrio fischeri</em></td>
<td>60 (55–65)²</td>
<td>&gt;100</td>
</tr>
<tr>
<td>15min-ECₛ₀₀</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ not analysed because a previous study has shown that this leachate is highly toxic
² confidence limits in brackets

In the Gryta study two different types of leachates were tested, one percolate from the old part (Table 5) and one leachate from the aerated lagoon (Table 6). Due to the incomplete treatment of the percolate in the pilot plant only a few toxicity tests were performed with this sample. Furthermore, a previous study of this leachate (Waara unpublished) has shown it to
be highly toxic partly because high concentrations of ammonia. After treatment the percolate was not found to be toxic. The leachate showed a higher treatability in the pilot plant and the toxicity was removed after treatment independent of treatment method used (Table 6).

**Tab. 5.** Acute toxicity to selected test organisms before and after treatment of the leachate from Gryta landfill. Effect values are expressed in vol-%

<table>
<thead>
<tr>
<th></th>
<th>Biological treatment</th>
<th>Biological treatment and Ozone/charcoal filter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In</td>
<td>Out</td>
</tr>
<tr>
<td>Physical and chemical characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>9.1</td>
</tr>
<tr>
<td>NH₃-N (mg/l)</td>
<td>230</td>
<td>0.27</td>
</tr>
<tr>
<td>Chloride (mg/l)</td>
<td>990</td>
<td>990</td>
</tr>
<tr>
<td>Test organism/endpoint</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio fischeri</td>
<td>31 (25–38)²</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Daphnia magna 48h-EC₅₀</td>
<td>25 (21–29)²</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pseudokirchneriella subcapitata 72h-EC₅₀</td>
<td>23 (22–24)²</td>
<td>Int.²</td>
</tr>
</tbody>
</table>

¹ toxicity assessed with the comparison test
² confidence limits in brackets
³ interference due to excessive growth of bacteria

**Tab. 6.** Acute toxicity to selected test organisms before and after treatment of the leachate in a constructed wetland from the Atleverket landfill. Effect values are expressed in vol-%

<table>
<thead>
<tr>
<th>Test organism</th>
<th>F2 (into the wetland)</th>
<th>k1 (out of the wetland)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2005-04-19</td>
<td>2 2006-08-09</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
<td>8.0</td>
</tr>
<tr>
<td>NH₃-N (mg/l)</td>
<td>200</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Chloride (mg/l)</td>
<td>630</td>
<td>638</td>
</tr>
<tr>
<td>Physical and chemical characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachyonus calyciflorus 24-h EC₅₀</td>
<td>33</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Daphnia magna 48-h EC₅₀</td>
<td>27</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Lemna minor 7d-EC₅₀</td>
<td>65</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pseudokirchneriella subcapitata 72h-EC₅₀</td>
<td>18</td>
<td>Toxic¹</td>
</tr>
<tr>
<td>Vibrio fischeri 30 min-IC₅₀</td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td>30 min-IC₉₀</td>
<td>21</td>
<td>&gt;80</td>
</tr>
</tbody>
</table>

¹ Inhibition above 20 % was only observed in the highest tested concentration. It inhibited growth by 34 %.
² Sample is toxic at all tested concentrations but no dose response was observed. Undiluted sample inhibited growth by 64 %.
In the Atleverket study the sample entering the wetland taken during 2005 was toxic to all test organisms, while the sample taken during 2006 was only toxic to algae. The samples taken at the outlet were not toxic, except for the sample taken during 2005, which was toxic to the algae.

In general the studies revealed that the biological treatment was a highly effective technique for removing toxicity in landfill leachate but in some cases toxicity was observed even though the treated leachates complied with issued discharge limits. This shows that the toxicity tests are a valuable tool, preferentially in combination with extensive chemical analysis, in order to properly evaluate and optimize treatment technology and to protect the recipient aquatic ecosystem from toxic discharges.

Validity of tests

In the majority of the cases, the presented test results have fulfilled the validity criteria stipulated in respective standard. However, sometimes in the plant bioassays it was difficult to maintain pH within the desired values. For tests with L. minor the pH of the samples should be adjusted to 6.5±1.0 but even though the growth media was buffered with MOPS it was not possible to maintain the pH below 7.5 in many landfill leachate samples during the 7 day test because a continuous upward drift of pH was observed. This is thought to be related to the high alkalinity of many landfill leachates (Clement et al. 1997, Öman et al. 2000). For the algal tests with P. subcapitata it was sometimes difficult to maintain the pH within the stipulated value of less than 1.5 units of variation in the control. It is possible that this problem can be avoided if the vessels are shaken during incubation and/or if the vessels have a larger head space promoting oxygen exchange because this phenomenon is generally not observed in the flask test. In one test with the leachate from the Gryta landfill extensive bacterial growth was observed during incubation even though the sample was filter sterilized before used (Table 6). This phenomenon was observed in three independent tests of the sample.

In some of the plant bioassays a complicated pattern of stimulation/toxicity was observed and no clear concentration-response curve could be obtained. Growth stimulation often occurred at low concentration and growth inhibition at high concentration of the sample. There is today a great concern for the presence of endocrine disruptors in ecosystems and they may cause stimulation of biological responses by mimicking signal substances however; in the plant bioassays, we interpret the growth stimulation as an effect of the higher nutrient levels in the sample water compared to the controls.

Confounding factors

It is well known that the presence of ammonium ions (i.e. unionized ammonia) and salts (such as chloride) can cause toxicity in landfill leachates (Clement & Merlin 1995, Clement et al. 1997, Öman et al. 2000). Therefore, where E(1)C50 concentrations of the sample could be
determined calculation of concentrations of unionized ammonia and chloride at E(I)C50 was made in order to compare those values to E(I)C50 values of the substances using databases or data generated from own experiments (Waara et al. 2005). The toxicity of the majority of the untreated samples (i.e. often pretreated in aerated lagoons) unionized ammonia alone or in combination with salts could explain most of the toxicity observed. For the treated samples some of the toxicity observed can also be explained by the presence of unionized ammonia and salts. This is the case for the Norsa sample and the test organism T. platyurus which has been shown to be very sensitive to both unionized ammonia and chloride (Persone oral com.). In the Atleverket study P. subcapitata was the only one of the test organisms used with known sensitivity to the salt concentrations in all the samples (Waara et al. 2005) and therefore the presence of salt(s) might explain the toxic response of P. subcapitata. It should however be noted that, this does not suggest that persistent substance(s) alone or in combination are not present at toxic concentrations but confounding factors might complicate the interpretation and use of the data for hazard assessment and process optimization.

Selection of test battery & further studies

The test methods used in this study were initially selected based upon several criteria (see Ek and Waara 2002) such as:

1. level of standardization and use,
2. ease of use in initial screenings i.e. short test duration and cost of labor,
3. sensitivity of organisms to pollutants,
4. ecological relevance of test organisms (the final freshwater recipient of the treated waters is lake Mälaren, the 4th biggest lake in Sweden),
5. availability of effect values for reference substances and knowledge about confounding factors.

After studying recommendations for selection of a suitable test battery from other sources (e.g. Keddy et al. 1995, Johnson 2000); using and evaluating the different test methods; and by participation in different European intercalibration studies we conclude that the test organisms and test endpoints used in the Atleverket study are highly recommended for use in both hazard assessment and process optimization of landfill leachate because they represent species in different functional groups and they have high relevance for Swedish freshwater ecosystem. As we have experienced some problems with the use of the spectrophotometric assay of algal growth (as in the algal toxkit) with waste water with strong color and bacterial growth the use of flask tests is encourage following the new standardized procedure issued 2005 (EN ISO 8692:2005). The acute toxicity tests with Brachionus calyciflorus could also easily be exchange for or supplemented with the new chronic test with the same test species following the procedures described in ISO/CD 20666 Water quality – determination of the chronic toxicity to Brachionus calyciflorus in 48 h. Furthermore, it could be advantageous to include additional algal species for example some with higher tolerance to salinity in order to more clearly separate the toxic effects of salts from the toxic effects of persistent pollutants.
The proposed test battery should also be complemented with other test system for enabling the detection of the presence of carcinogens, mutagens and compounds disturbing reproduction. In addition during the treatment of landfill leachate in for example constructed wetlands and SBRs also sediment and sludge is produced but the toxicity of these treatment by products are not yet known. To fill this gap presently a study on the toxicity of the sediment in different parts of the wetland at Atleverket is being conducted using a test battery of different organisms and endpoints (Waara et al. 2009).

Acknowledgements

The authors are grateful to Christina Ingwall Johansson and Ann-Sofie Magnusson for carrying out some of the toxicity tests and for keeping order in the laboratory. The discussion and assistance by Per-Erik Persson and Anna Thuresson VAFAB MiljöAB, Västerås in the Gryta, Isättra and Norsa study is greatly acknowledge. We also thank Dr. Tommy Odelstöm, Mälardalen University for valuable discussions and on freshwater ecosystems. Many students, national and international, have through the years contributed with data to this project through their bachelor and master theses and for this we are truly grateful.

References


