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# PRELIMINARY ASSESSMENT OF THE IMPACTS OF THE GREEN ISLAND LANDFILL LEACHATE ON THE RECEIVING ENVIRONMENT USING PASSIVE SAMPLERS AND TOXICITY TESTING

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# 1. INTRODUCTION

This study investigated the potential impacts from the leachate of the Green Island landfill on ground and surface waters. An effect-directed analysis was used to assess the toxicity of surface and groundwater samples collected using passive sampling devices (PSDs) deployed at a range of sites in the catchment surrounding the landfill. The PSDs used concentrate hydrophilic organic chemicals, so the resulting extracts do not contain all contaminants present in the leachate, e.g. trace metals. The toxicity of the PSD extracts was assessed using tests on an algal and bacterial test species. If toxicity is detected using this method, it triggers further investigations, which can involve the use of sophisticated chemical fractionation and analysis to identify the chemical(s) responsible for the effects.

# 2. MATERIALS AND METHODS

## 2.1. Passive sampling devices

Hydrophilic–lipophilic balance (HLB) polar organic chemical integrative sampler (POCIS) PSDs were obtained from Affinisep (Le Houlme, France). This type of PSD was selected for this application because it adsorbs and concentrates primarily polar hydrophilic organic contaminants that are mobile and transported in aquatic environments. The Affinisep POCIS provides a ring-type PSD for deployment in surface-water sites and a rectangular-shaped PSD geometry designed for deployment in groundwater wells of reduced diameter (< 100 mm). Therefore, the same HLB sorbent can be used to sample both surface-water and groundwater well sites.

The HLB adsorbent phase in the Affinisep PSD exhibits an affinity and capacity to concentrate a broad range of semi-polar to polar organic contaminants, including pesticides, phenols and industrial alkylphenols, personal care chemicals, biocides, steroid hormones and pharmaceuticals.

## 2.2. Deployment sites

Figure 1 is an aerial photo of the Green Island landfill and its surroundings. On Thursday, 28 April 2022, PSDs were deployed at four surface-water sites (GI-1, Kaikorai Stream upstream of landfill; GI-2, Abbotts Creek upstream of landfill; GI-5, downstream of the landfill; and ‘Site downstream’, on the Kaikorai Stream close to the receiving estuary, to provide an insight into stressors from other sources) and then retrieved by staff of Boffa Miskell Ltd on Friday, 20 May 2022. The deployment was for a duration of 22 days as longer periods in surface water typically result in biofouling of

the membrane, which can ultimately inhibit the uptake of contaminants (Stewart et al. 2016).

PSDs were also deployed at two groundwater well monitoring sites outside the leachate trench (Lines 2 and 4) within the Green Island landfill. A single groundwater PSD was deployed into a shallow well (C) and into a deep well (D). A duplicate set of each type of PSD (surface water and groundwater) was used as field blanks, and another single PSD of each type was used as a laboratory blank (Table 1). The groundwater PSDs were deployed on Friday, 29 April 2022 and retrieved on Friday, 20 May 2022, corresponding to a 21-day deployment period.

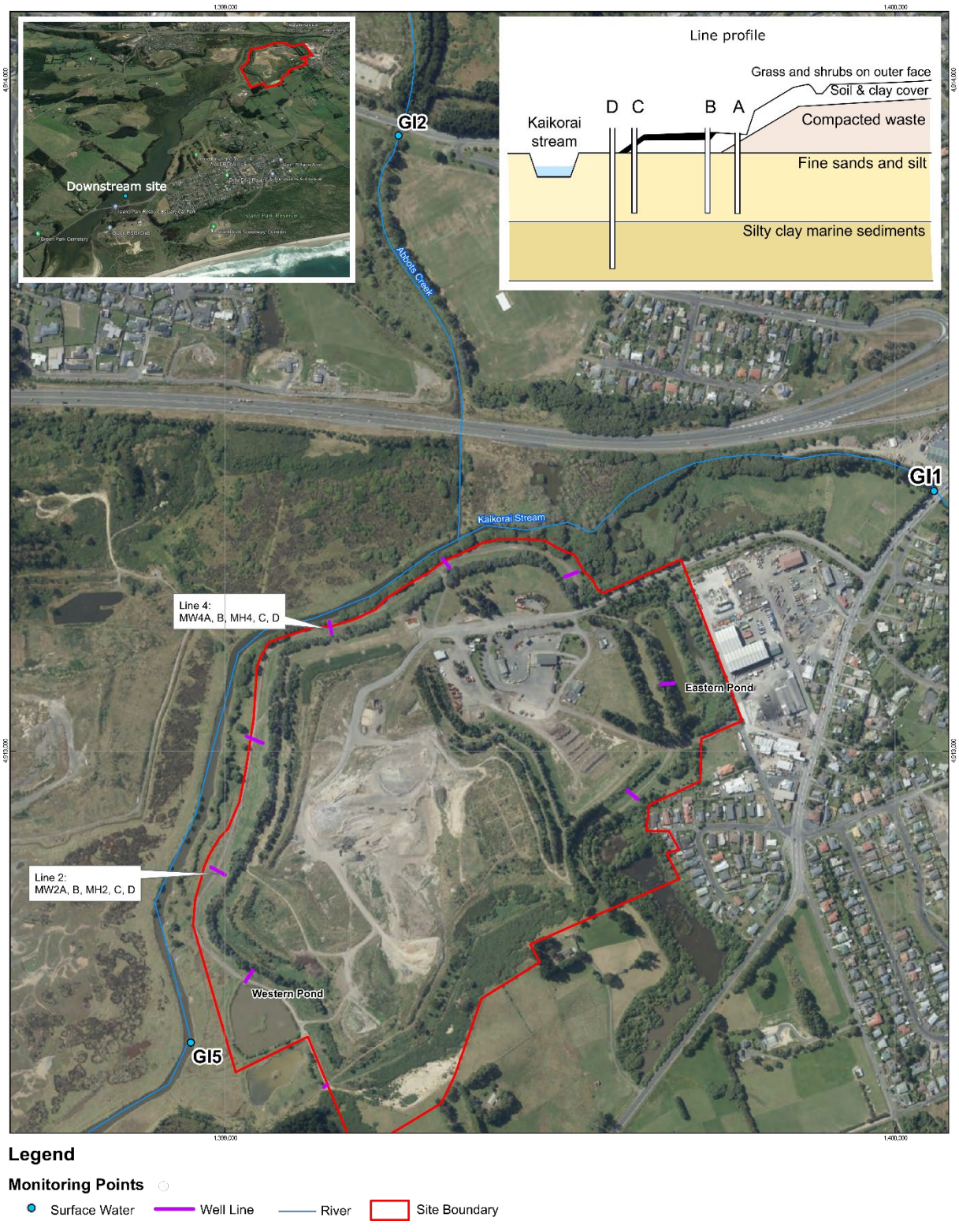


Figure 1. Map of Green Island landfill and surroundings, including the locations where passive sampling devices were deployed and groundwater-line well bore profiles near Kaikorai Stream. Source: GHD and Dunedin City Council.

Table 1. Details of sampling sites and where the PSDs were deployed, along with deployment durations.

| Sampling site | Deployment period (days) |
|---|---|
| Green Island groundwater field blank | Not applicable |
| Green Island groundwater, Line 2C, shallow well | 21 |
| Green Island groundwater, Line 2D, deep well | 21 |
| Green Island groundwater, Line 4C, shallow well | 21 |
| Green Island groundwater, Line 4D, deep well | 21 |
| Green Island surface-water field blank | Not applicable |
| Green Island surface water, Site GI-1 | 22 |
| Green Island surface water, Site GI-2 | 22 |
| Green Island surface water, Site GI-5 | 22 |
| Green Island surface water, Site downstream | 22 |

The surface water PSDs were mounted within plastic burley cages to protect them from debris in the stream water (Figure 2). The burley cages were attached to a deployment rope with plastic cable ties, and a 0.5 kg lead diving weight was attached to the end of the rope approximately 1 m from the burley cage.

At the GI-2 and GI-5 sites and Site downstream near the estuary, the PSD units and weights were placed in water at a depth > 1 m, with the lead weight placed downstream so that the burley cage was positioned parallel to the flow of water. At the downstream estuary site, where the burley cage would not be exposed to potentially high flow rates, the unit was placed perpendicular to the edge of the estuary bank. A steel waratah (1.6 m long) was driven firmly into the side of the bank and the rope end of the deployment unit was securely tied off to it.

Special care was taken to ensure the deployment system was not in plain sight unless stumbled upon. Sampling site GI-1 on the Kaikorai Stream is located within the suburb of Green Island where the Brighton Road bridge crosses the stream. The stream banks at this site were too steep to secure a waratah and were easily seen from the adjacent footpath. At this site, the deployment rope was secured to utility piping fixtures within the concrete-box bridge culvert passing under the roadway. The burley cage containing the PSDs was submerged in water within the culvert and was visible only if a person physically walked down the stream and halfway into the culvert.

Prior to deployment of the groundwater PSDs, a lead weight was attached to fishing line and run to the base of the well, and the depth from the top of the well casing to the well base was recorded. The height of the water column in the well was determined by deploying a moisture probe attached to a tape measure and lowered into the well.

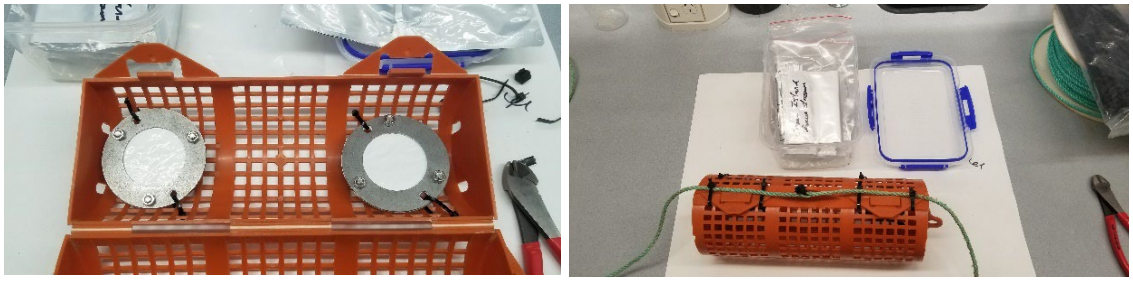

Figure 2. Preparation of surface water passive sampler devices.

After establishing the height of the water column above the base of the well, the PSD unit was attached to stainless-steel wire with an aluminium crimp (Figure 3A and B) and a length of wire measured off so that the PSD unit could be sited at the midpoint of the water column. The top end of the measured length of stainless-steel wire was fixed to the top well with another aluminium crimp (Figure 3C). The PSD was then deployed into the body of the well by hand.

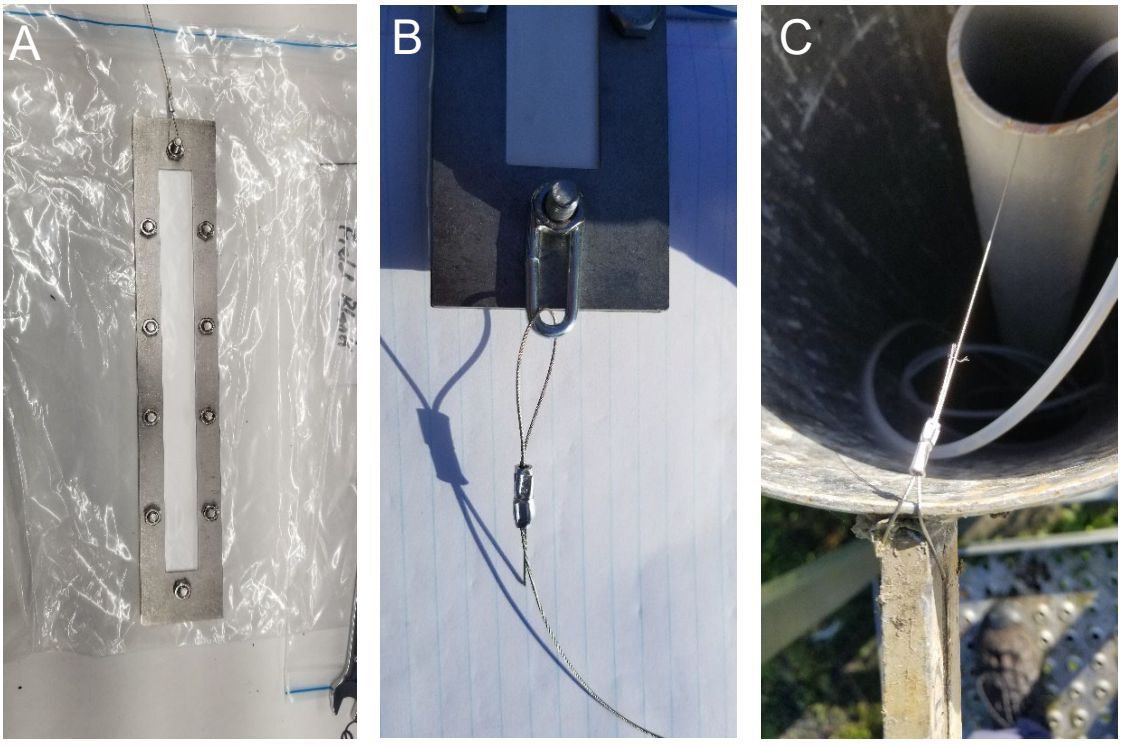


Figure 3. Securing ground water passive sampling devices with attachment to the frame (A and B), and to the body of the well (C).

The duplicate PSDs used as field blanks were removed from their packaging and exposed to the air for the duration of time that the sample PSDs were being prepared and deployed. In this way the field blank PSDs were exposed to the same sources of

any potential background environmental contaminants as the deployed PSDs would have been during their preparation and deployment at a sampling site.

## 2.3. Extraction of passive samplers and preparation for biological testing

The retrieved and quality assurance field and laboratory blank PSD samples were disassembled at the Plant and Food Research Ruakura laboratories in Hamilton. The outer membranes were washed with purified water to remove adhering particulate material before the PSDs were opened. The adsorbent media were then transferred into empty 6 mL solid-phase extraction (SPE) cartridges. A porous polypropylene frit was inserted into the barrel of the SPE cartridge and pushed down to compress the adsorbent media. The organic contaminants accumulated by the adsorbent media were eluted with acetone, methanol and ethylacetate, which was collected in a glass flask.

The raw sample extracts were concentrated by evaporation under a stream of nitrogen gas and the concentrated solutions were filtered through a small bed of Hyperflo-Supercell celite filtration aid into tapered glass gas chromatography (GC) vials. The extracts were evaporated to dryness under a stream of nitrogen gas and reconstituted in dimethyl sulfoxide (DMSO) for bioassay testing.

The prepared extracts were shipped to the Cawthron Institute Ecotoxicology Laboratories for toxicity assessment.

## 2.4. Bioassays

Two bioassays were used in standardised test species. A bacteria-based bioluminescence test was used to detect general toxicity in the PSD extract samples. This test also provides insights into the presence of contaminants with antimicrobial activities. The algal-based assay was used to indicate the presence of herbicidal toxicity in the PSD extracts.

Both tests were carried out with a reference toxicant[1] to ensure consistency of the response observed. Summary of the test's conditions are reported in Table 2.

### *2.4.1. Luminescence inhibition assay*

Microtox™ (ISO 11348-3) is an *in vitro* testing system that uses a strain of naturally occurring luminescent marine bacteria (*Aliivibrio fischeri*, syn. *Vibrio fischeri*) sensitive to a range of toxicants to determine the acute toxicity in an aqueous suspension

[1] Chemical used to assess the constancy of response of a given species of test organisms to that chemical. It is assumed that any change in sensitivity to the reference substance will indicate the existence of some similar change in degree of sensitivity to other chemicals / effluents whose toxicity is to be determined.

(Environment Canada 1992; ISO 2007). Briefly defined, the test measures light changes produced naturally by the luminescent bacteria when they are exposed to the samples under standard conditions over 15 minutes. Zinc as $Zn^{2+}$ was used as the reference toxicant.

The testing of the PSD extracts at 1:100 (maximum concentration of carrier solvent) resulted in a total extinction of the bacterial luminescence, likely due to the colour of the extracts (Figure 4). Extracts from the groundwater Line 2D and Line 4C had dark brown colour. Extracts from surface-water site GI-1 had a slightly lighter brown colour, while groundwater Lines 2C and 4D, surface-water sites GI-2 and GI-5, and Site downstream extracts had the lightest brown colour. Groundwater field blank and surface-water field blank extracts were transparent. The Line 2D sample extract was used to determine the dilution required to achieve a suitable colouring of the solution for the test. A further dilution of the extracts 1:32 in DMSO was carried out to reduce colour-quenching impact during measurement.

For the assay, a final concentration of DMSO in the test tube was 1:100, with the final extract dilution being 1:3,200 (0.0313%).

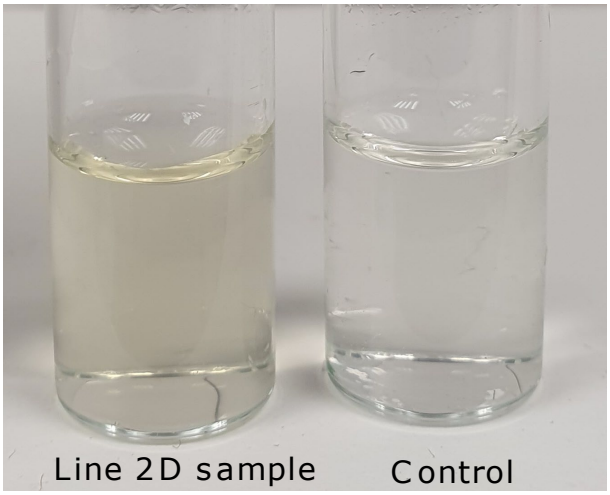


Figure 4. Example of a sample of passive sampling device extract at 1% in test tubes before dilution.

### *2.4.2. Algal growth inhibition assay*

The 96-hour chronic toxicity test uses the green microalga *Dunaliella tertiolecta* according to the American Society for Testing and Materials standard (ASTM 2012) and is recommended by the Ministry for the Environment as a method for direct toxicity assessment (DTA) testing (Hall & Golding 1998). During their exponential growth phase, the algae are exposed to dilutions of a test solution under static conditions (i.e. no renewal of the test solutions) and at constant temperature and salinity over a period

of 96 hours. Over that period, the algae can produce several generations and their growth while exposed to the test solution is compared to the growth of the control. A test solution is considered toxic when statistically significant dose-dependent inhibition of algal growth occurs. Potential effects from the solvent DMSO were assessed by testing solutions with and without the carrier solvent. Copper was used as the reference toxicant.

## 2.5. Statistical analysis

Comparison in toxicity between PSD sample extracts (level of statistical significance of $P < 0.05$) and calculation of the $EC_{50}$ (the concentration that produces an effect on 50% of the test organisms) with associated 95% confidence intervals (CI) followed Hall and Golding (1998) using CETIS™ (Tidepool Scientific, LLC, USA) and Statistica™ (TIBCO software, California, USA).

Table 2. Summary of the test conditions.

| | **Microtox™** | **Green microalgae** |
|---|---|---|
| Test start to end dates | 11/01/2023 | 19/01/2023 to 23/01/2023 |
| Standard | ISO 11348-3 (2007) | ASTM E1218-04 (2012) |
| Test species | *Aliivibrio fischeri* | *Dunaliella tertiolecta* |
| Source | BioLight Aqua-Science (Lot 10641121) | Laboratory culture (CS3/7) |
| Density, number per test container | n/a | $10.6 \pm 3 \times 10^3$p/mL |
| Type of test container | 5 mL glass tube | 96-well plate round bottom |
| Exposure time (h) | 0.25 | 96 |
| Concentrations (%) | 0, 0.03% | 0, 0.03% |
| Replicates | 2 | 10 for controls, 5 for treatments |
| Light | n/a | Continuous 200 µmol/$m^2$/sec |
| Temperature (°C) | 15 | $18.3 \pm 1.4$ |
| Dissolved oxygen (at the beginning of the test) (mg/L) | n/m | n/m |
| pH | n/m | 7.8 |
| Dilution water | Brackish water | Artificial seawater |
| Aeration | None | None |
| Salinity (at the beginning of the test, PSU) | 20 | 26 |
| Endpoint | Luminescence | Growth inhibition |
| Sensitivity ($EC_{50}$ with 95% CI) | 1.43 (1.14–1.79) mg $Zn^{2+}$/L | 0.141 (0.132–0.150) mg $Cu^{2+}$/L |
| Control quality for sensitivity (mean±2 standard deviation) | 1.33 (1.06–1.6) mg $Zn^{2+}$/L ($n$ = 2) | 0.128 (0.041–0.215) mg $Cu^{2+}$/L ($n$ = 47) |
| Test acceptability (in controls) | Yes | CV < 20%, 16-fold increase |
| Note | – | Age of culture: 6 days |

Abbreviations: n/m = not measured, CI = confidence interval, CV = coefficient of variation, h = hour.

# 3. RESULTS

## 3.1. Luminescence inhibition assay

The results of the luminescence inhibition test with the bacterium *Aliivibrio fischeri* are presented in Figure 5. The results are relative to the control (DMSO and diluent water). The groundwater and surface-water field blanks were not statistically different from each other. The groundwater sample extracts from Line 4C and Lines 2C and 2D were significantly different from their field blank ($P < 0.05$). The surface-water sample extracts from site GI-5 and Site downstream are significantly different from the field blank ($P < 0.05$).

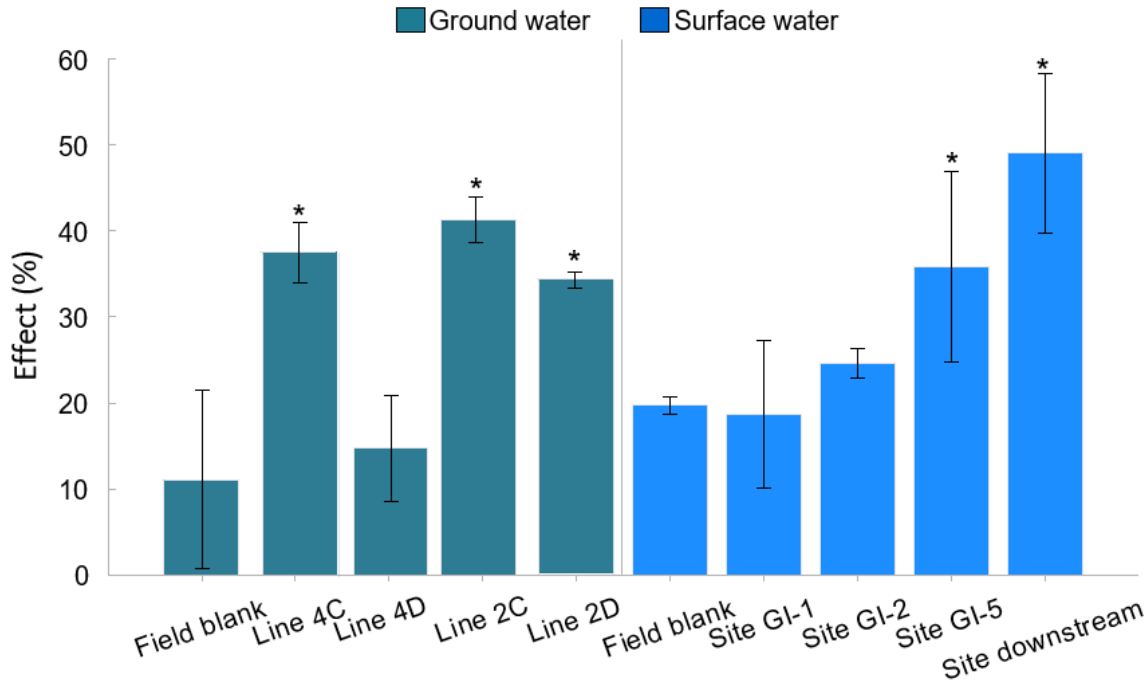


Figure 5. Acute toxicity effect (reduction of bacterial luminescence expressed as %) after a 15-minute exposure to passive sampling device extracts (0.0313%). Asterisks (*) indicate a significant difference ($P < 0.05$) from the related field blank.

## 3.2. Algal growth inhibition assay

No statistical difference was found between the field blanks and DMSO control (Table 3), indicating no effect of the solvent used with the PSD extracts. The results of the algal growth inhibition test with the marine green microalgae are presented in Figure 6. Field blanks (groundwater and surface water) were not significantly different from the DMSO control. A significant difference from the related field blank was found only for groundwater samples from Line 2C, Line 4C and Line 4D ($P < 0.05$). The sample from Line 4C was also significantly different from the DMSO control ($P < 0.05$). No statistical difference was observed between surface-water samples and their respective DMSO control or field blank.

Table 3. Average algal density (×1,000/mL) in DMSO and artificial seawater (ASW).

| Controls | *n* | Average | Standard deviation | Coefficient of variation |
|---|---|---|---|---|
| DMSO control | 15 | 250.1 | 16 | 6% |
| ASW (control) | 15 | 257.6 | 14 | 5% |

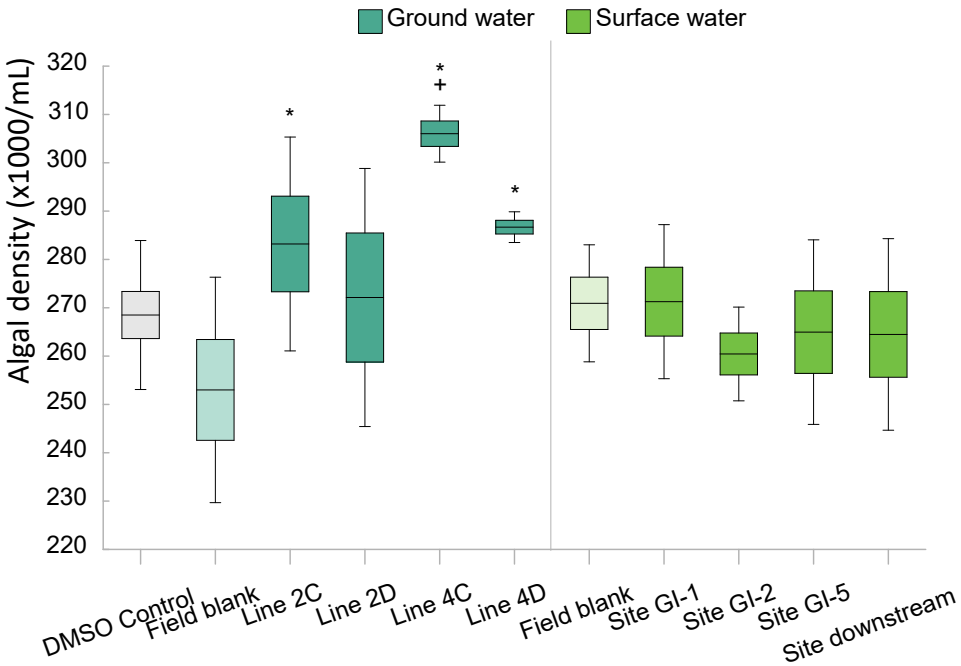


Figure 6. Box plots showing effects of passive sampling device extracts (0.0313%) on algal growth density after a 96-hour exposure. Asterisks (*) indicate a significant difference from the related field blank and the plus sign (+) a significant difference from the control ($P < 0.05$).

# 4. DISCUSSION

Environmental samples are often complex mixtures containing a range of molecules. The PSDs used in this study absorb organic compounds present in the water column, so the extracts tested did not contain metals or highly hydrophobic molecules, which would partition mainly in suspended solids and sediment. The PSD extracts from Lines 2C and 4C had some impact on the populations of the two test model organisms used.

The Microtox™ results show a pattern of increasing toxicity from the surface sample sites upstream of the landfill (GI-1, GI-2) to the GI-5 site and Site downstream. The higher inhibition at Site downstream compared to GI-5 suggests that there are sources other than the landfill contributing to the toxicity. The bacterial luminescence results of the groundwater samples indicate a potential pattern of transport and distribution of the leachate from the landfill site. The sample from Line 4C, closer to the top of the landfill, had higher toxicity than the Line 4D sample, suggesting that the leachate is present in the fine sand and silt portion at this location but has not yet migrated into the deeper silty clay marine sediment portion. The toxicity results from the Lines 2C and 2D samples, further downstream from the site, indicate that the leachate plume has now migrated across both these environmental compartments.

The bacterial luminescence data from the surface-water samples show toxicity at the sites downstream of the landfill, suggesting that toxic leachate is seeping out. The extract from Site downstream was more toxic than that at GI-5. Although the difference is not significant, this suggests that some of the toxicity is from sources other than the landfill.

Some of the groundwater extracts tested showed a stimulation of algal growth instead of the expected inhibition. This indicates that some organic compounds present in the extract samples can be used by the algae as a source of nutrient and can stimulate their growth (Yu et al. 2015). This suggests that it is unlikely that the extracts contain herbicidal activity.

The interpretation of the results of this study warrants some caution as they use data from a one-off sampling event. The results suggest that there is leachate seeping out from the landfill, but this conclusion is based solely on PSDs that concentrated hydrophilic organic chemicals. The toxicity at the site is likely to be higher when accounting for other contaminants such as trace metals, which were not captured by the type of PSD used. It would be advisable to gain a better understanding of the dynamics and composition of the leachate from the landfill to assess any long-term impacts. It would also be advisable to adopt an approach for capturing both the spatial and temporal variations of landfill leachate (Butt et al. 2008) to account for factors such as seasonality and the increase in the occurrence of extreme rain events.

# 5. RECOMMENDATIONS

- Establishing the chemical characterisation of the leachate would be valuable to identify the more toxic components and inform whether remedial actions are required to reduce the risk to exposed biota.
- Ongoing monitoring of the biota in the aquatic environment would provide valuable information on the ecological impacts of the leachate.
- Establishing the complementary characterisation of other sources of stressors in the catchment would assist in more effective management and protection of this area.

# 6. REFERENCES

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# Appendix 1. Complete datasets for both bioassays

## A1.1 Microtox™

Table A1.1. Luminescence, gamma values and effect (as % of control) per replicate after a 15-minute exposure to the PSD extracts.

| Extract samples | Luminescence (relative unit) | | Gamma | Effect (%) |
|---|---|---|---|---|
| | t0 | t15min. | | |
| Control diluent | 87 | 71 | – | – |
| Control diluent | 91 | 90 | – | – |
| Control DMSO 1 | 90 | 88 | – | – |
| Control DMSO 1 | 103 | 101 | – | – |
| Control DMSO 2 | 95 | 81 | – | – |
| Control DMSO 2 | 101 | 93 | – | – |
| Groundwater field blank | 76 | 61 | 0.395 | 3.80 |
| Groundwater field blank | 94 | 64 | 0.226 | 18.40 |
| Groundwater Line 2C, shallow well | 87 | 44 | 0.650 | 39.39 |
| Groundwater Line 2C, shallow well | 95 | 45 | 0.761 | 43.23 |
| Groundwater, Line 2D, deep well | 92 | 51 | 0.505 | 33.56 |
| Groundwater, Line 2D, deep well | 94 | 51 | 0.539 | 34.97 |
| Groundwater, Line 4C, shallow well | 83 | 45 | 0.538 | 35.02 |
| Groundwater, Line 4C, shallow well | 98 | 49 | 0.669 | 40.07 |
| Groundwater, Line 4D, deep well | 79 | 59 | 0.118 | 10.49 |
| Groundwater, Line 4D, deep well | 89 | 60 | 0.238 | 19.20 |
| Surface water field blank | 87 | 68 | 0.257 | 20.44 |
| Surface water, Site GI-1 | 93 | 74 | 0.235 | 19.01 |
| Surface water, Site GI-1 | 78 | 67 | 0.144 | 12.57 |
| Surface water, Site GI-1 | 96 | 71 | 0.328 | 24.72 |
| Surface water, Site GI-2 | 85 | 64 | 0.305 | 23.36 |
| Surface water, Site GI-2 | 92 | 67 | 0.349 | 25.87 |
| Surface water, Site GI-5 | 82 | 58 | 0.389 | 28.01 |
| Surface water, Site GI-5 | 94 | 52 | 0.776 | 43.69 |
| Surface water, Site downstream | 92 | 52 | 0.738 | 42.47 |
| Surface water, Site downstream | 94 | 41 | 1.252 | 55.60 |

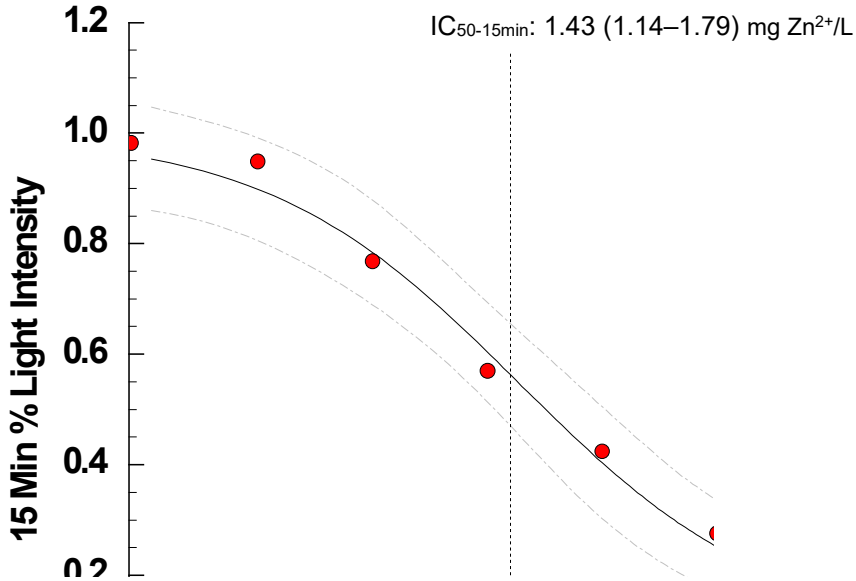


Figure A1.1. Average (red dots) light intensity after a 15-minute exposure, with 95% confidence interval (dashed line) for the dose response to the reference toxicant (zinc as $Zn^{2+}$).

## A1.2 Algal growth

Table A1.2. Algal density (×1,000/mL) per replicate after a 96-hour exposure to PSD extracts. Abbreviations: nm = not measured.

| Control | Groundwater | | | | | Surface water | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DMSO | Field blank | Line 2C | Line 2D | Line 4C | Line 4D | Field blank | GI-1 | GI-2 | GI-5 | Down-stream |
| 247.2 | 240.7 | 265.9 | 290.7 | 301.3 | 289.3 | 267.7 | 263.1 | 252.1 | 243.2 | 230.4 |
| 245.0 | 284.6 | 284.6 | 298.7 | 304.3 | 290.9 | 260.0 | 288.2 | 267.7 | 295.0 | 269.7 |
| 261.2 | 266.6 | 265.4 | 243.7 | 300.2 | 284.6 | 291.6 | 288.1 | 273.8 | 259.0 | 277.4 |
| 265.6 | 248.8 | 280.4 | 255.4 | 310.7 | 284.1 | 265.7 | 263.5 | 254.1 | 259.1 | 265.8 |
| 266.3 | 224.3 | 319.7 | n/m | 313.6 | 284.5 | 269.6 | 253.4 | 254.5 | 268.5 | 279.1 |
| 287.5 | | | | | | | | | | |
| 273.9 | | | | | | | | | | |
| 281.6 | | | | | | | | | | |
| 265.9 | | | | | | | | | | |
| 290.8 | | | | | | | | | | |

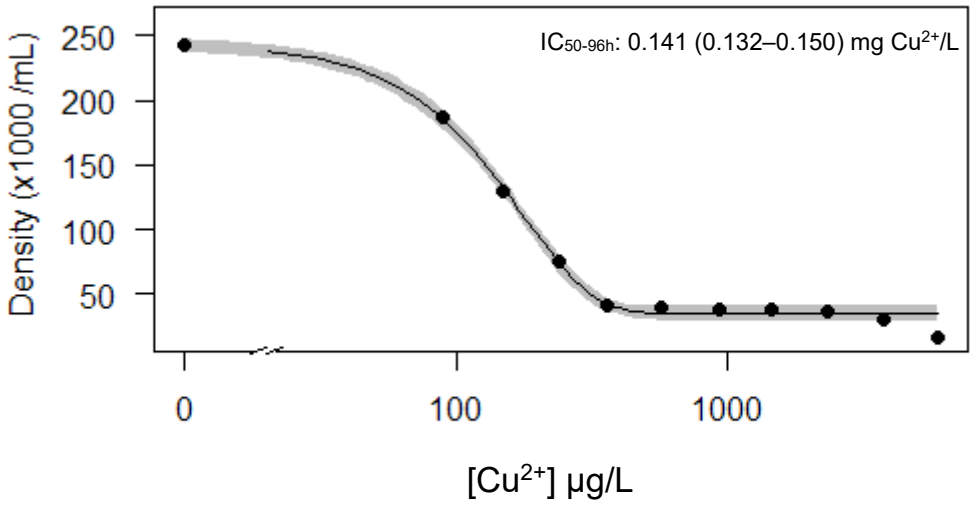


Figure A1.2. Average (black dots) algal density with 95% confidence interval (greyed surface) for the dose response to the reference toxicant (copper).

# Appendix 2. Acronyms and definitions

| Acronym | Definition |
|---|---|
| ASTM | American Society for Testing and Materials |
| ASW | Artificial seawater |
| CETIS™ | Comprehensive Environmental Toxicology Information System™ |
| CI | Confidence interval |
| $Cu^{2+}$ | Ion of copper with a double positive charge |
| CV | Coefficient of variation |
| DMSO | Dimethyl sulfoxide |
| DO | Dissolved oxygen |
| DTA | Direct toxicity assessment |
| $EC_{x-t}$ | **Effective concentration** is the generic term for a concentration of a substance or material that is estimated to cause some defined effect on a proportion (x%) of the test organisms after a defined period of exposure (t). This kind of end point allows the classification and the comparison of the toxic potency or intensity of different chemicals. More terms can be derived to describe specific effects (e.g. lethality, inhibition):<br>• **$LC_{x-t}$ (lethal concentration)** is the concentration of a substance or material that is estimated to be lethal to a proportion (x%) of the test organisms after a defined period of exposure (t). This is an acute toxicity indicator.<br>• **$IC_{x-t}$ (inhibitory concentration)** is the concentration of a substance or material that is estimated to have an inhibitory effect (e.g. algal growth) on a proportion (x%) of the test organisms after a defined period of exposure (t). This is a chronic toxicity indicator. |
| GC | Gas chromatography |
| LOEC | **Lowest observed effect concentration** is the lowest concentration of a test substance or material that is observed to have a statistically significant adverse effect on the test organisms after a defined time of exposure and under the test conditions, relative to the control. |
| NOEC | **No observed effect concentration** is the highest concentration of a test substance or material that is observed not to have a statistically significant adverse effect on the test organisms after a defined time of exposure and under the test conditions, relative to the control. |
| PFOA | Perfluorooctanoic acid |
| PFOS | Perfluorooctanesulfonic acid |
| POCIS | Polar organic chemical integrative sampler |
| PSD | Passive sampling device |
| PSU | Practical salinity unit. A unit based on the properties of seawater conductivity to measure salinity. It is equivalent to parts per thousand (‰), or to g/kg. |
| SD | Standard deviation |
| SPE | Solid-phase extraction |
| $Zn^{2+}$ | Ion of zinc with a double positive charge |