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SCIENCE DIRECT®

Environment International 31 (2005) 269–273

ENVIRONMENT INTERNATIONAL

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Acute and sublethal toxicity tests to monitor the impact of leachate on an aquatic environment

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Available online 19 December 2004

Abstract

In this study, a specific landfill leachate (1200 mgl^{-1} COD and 600 mgl^{-1} BOD_5) was used to develop a standardised short-term acute and longer-term sublethal ex-situ toxicity testing programme, in order to determine the potential ecological implications of leaching contaminants reaching the water table. Bioassays were undertaken with juvenile *Gammarus pulex* and *Asellus aquaticus* macro-invertebrates. Preliminary acute test variables included static and static renewed flow rates for 96-h, starved and fed specimens, and aerobic and oxygen depleting conditions. However, regardless of any test variable, the lethal concentration (LC_{50}) for *A. aquaticus* remained at 12.3% v/v leachate in deionised water, whilst that for *G. pulex* was only 1%. Sublethal toxicity was judged on the basis of frequency of births and the growth rate of newly born individuals. Tests showed that even a dilution as high as 1:66- would influence the fecundity of a *Gammarus* population, whilst a dilution of 1:20 would affect the size of an *Asellus* breeding colony.

Keywords: Landfill leachate; Toxicity testing; Acute toxicity; Sublethal toxicity; *A. aquaticus*; *G. pulex*

1. Introduction

EU legislation (80/86/EEC) highlighted key contaminants (e.g. ammoniacal-nitrogen, various toxic compounds and heavy metals) in terms of their impact on ground and surface water. In the UK, there are a substantial number of unlined landfills situated on major aquifers and the potential environmental impact of such contaminants on the water table needs to be considered.

Toxicology has become a fundamental tool for monitoring the affect of pollutants on the ecology of aquatic environments. By undertaking toxicity tests, it is possible to determine what concentrations of a particular substance have a toxic or sublethal effect on a range of organisms so that standards for the protection of the aquatic ecosystem can be developed. The amphipod and isopod crustaceans *Gammarus pulex* and *Asellus aquaticus* are frequently used as comparison specimens for mixed species toxicity tests as both macro-invertebrates have differing responses to several classes of pollutants, which enable pollution boundaries to be established (Pascoe and Edwards, 1989). *G. pulex*, for example, is sensitive to toxicants, such as, ammonia that is less toxic to *A. aquaticus* (Maltby, 1995).

It has previously been suggested (Buikema et al., 1982), however, that the animals response to toxicants is dependent upon whether the testing regime is flow-through (used to test episodic pollution), static or static renewed and if the animals are fed or supplied with oxygen during the tests. The aim of the current research, therefore, was to develop a standardised short-term acute and longer-term sublethal ex-situ toxicity testing programme, in order to determine the potential ecological implications of leaching contaminants reaching the water table. A specific leachate (1200 mgl^{-1} COD and 600 mgl^{-1} BOD_5) was used for the study.

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doi:10.1016/j.envint.2004.10.010

2. Materials and methods

2.1. Leachate

The leachate (full composition unknown) was collected as a one-off, bulk sample from a covered drain within an unlined, disused (industrial waste) UK landfill site. The sample was siphoned into a 500-l header tank using a petrol driven pump and mixed thoroughly (to eliminate sample variability) before dispensing into 25-l lidded containers. On return to the laboratory, each container underwent COD and BOD_5 analysis (Standard Methods for the Examination of Water and Wastewater, 1998). All samples were then frozen until needed. Individual containers were defrosted when required, and COD and BOD_5 analyses were carried out. Freezing kept the samples COD and BOD_5 constant. Therefore, the leachate used in this study had an invariable 1200 mgl^{-1} COD and 600 mgl^{-1} BOD_5. Unused, defrosted leachate was immediately discarded.

2.2. Test species

Pollution and disease free test animals (*G. pulex* and *A. aquaticus*) were obtained from a laboratory based breeding programme. Offspring were separated from the adult population at birth, to prevent cannibalism. Both adults and juveniles were fed on conditioned alder leaves (soaked in river water and detritus for 10-days) and supplied with aerated, charcoal filtered water by a flow-through system, operated at a 4-h change over rate.

2.3. Acute toxicity tests

Ten juvenile (2-week-old) *G. pulex* were added to each test chamber (100-ml sterile plastic pots with a screw lid), which contained a series of 100-ml acute test media concentrations that ranged from 100% to 5% v/v leachate in deionised water and deionised water controls, maintained at a constant temperature of 15 °C. The same procedure was also performed with *A. aquaticus*.

Acute toxicities (LC_{50}) were assessed using static and static renewed tests with a 96-h duration. At 24-h intervals, the static renewed test solutions were replaced with dilutions of a single leachate sample, which were made up at the start of the experiment and frozen in 500-ml lidded plastic bottles. Other test variables included a combination of starved and fed specimens under both aerobic and oxygen depleted conditions. Observations were made at 24-h intervals, and immobilised specimens were removed and placed in deionised water for 24-h to monitor recovery. All experiments were replicated five times with both test species to obtain mean values and calculate the standard deviation.

2.3.1. Application of nutrition and aeration

A 5-cm^3 disk of conditioned alder leaf (soaked in river water and detritus for 10-days) was added to each test pot. The disk was replaced at 24-h intervals during static renewal experiments, whilst remaining unchanged for static tests.

2.3.2. Application of aeration

A flexible silicone tube (1-mm diameter) attached to a regulated air supply (operated at a flow rate of 12 bubbles of air per minute—to minimise VOC removal and animal stress) was inserted through the test chambers lid and remained in place throughout the test period.

2.4. Sublethal toxicity tests

A series of six 10-l tanks was established for *A. aquaticus*. All contained specific leachate dilutions, which did not affect mortality during acute bioassays (Table 1). The same procedure was also performed with *G. pulex*. Thirty juveniles (2-week-old) of unknown sex were added to each tank. They were fed on conditioned alder leaves and subjected to 5-l partial 'water' changes on a weekly basis. All dilution media was made up from a single leachate sample at the start of the experiment and frozen (until required) in 5-l lidded plastic bottles. For a 4-month period, daily observations were made on the animals' ability to reproduce.

Offspring were retrieved from the tanks and placed in 350-ml sterile plastic pots (with a screw lid), containing the same leachate dilution from which they were extracted. On a weekly basis, the animals' body length was measured (excluding antennae and other appendages). Specimens were individually extracted with approximately 20-ml of liquid media from their holding container and placed in a sterile 100-ml transparent plastic pot. A millimetre ruler was positioned underneath the container, the animals' length was measured, and then, they were transferred back to the holding container. After 1-month, the juveniles were relocated into their original 10-l sublethal tank.

Table 1
Sublethal leachate concentrations for *A. aquaticus* and *G. pulex*

Tank number	Leachate concentration (% v/v with deionised water)	
	A. aquaticus	*G. pulex*
1	40	2.50
2	30	2.25
3	20	2.00
4	10	1.75
5	5	1.50
6	Deionised water (control)	Deionised water (control)

3. Results and discussion

3.1. Acute toxicity tests

It has previously been suggested that static renewed systems ensure that the toxicants concentration remains at a constant level, whilst static tests lose their potency over the