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ACUTE AND SUB-LETHAL TOXICITY OF LANDFILL LEACHATE TOWARDS TWO MACRO-INVERTEBRATES
Assessing the Remediation Potential of Constructed Wetlands

M. C. BLOOR* and C. J. BANKS

School of Civil Engineering and the Environment, University of Southampton, Southampton, UK

A specific leachate that contained 1.036 $mgl^{-1}$of 2-chlorobiphenyl was used in the study (255 mgl^{-1} COD and 133 mgl^{-1} BOD_5). When operated on a 10 day hydraulic retention time (RT), reed beds planted with *Juncus effusus* removed 60% of the leachates COD, compared to 25% in unplanted beds. The constructed wetlands proved effective at reducing the level of acute toxicity to *A. aquaticus* and *G. pulex*. In untreated leachate, the LC_{50} for *A. aquaticus* was 57% v/v leachate in deionized water and 5% for *G. pulex*. When reed beds were operated on a 1–10 day RT, the LC_{50} for *Asellus* increased from 69% to below the LC_{50} threshold. The *Gammarus* LC_{50} also rose from 10% to 50%. The maximum toxicity reduction achieved by unplanted beds was 10% towards *A. aquaticus* and 5% for *G. pulex*, when operated on a 10 day RT. However, in sub-lethal concentrations of reed bed effluent (100%, 80% and 60% dilutions, obtained from planted beds on a 10 day RT), the final length of *Asellus* was significantly reduced, in comparison to a deionized water control. It is also speculated that chronic leachate stress may have affected the fecundity of *Gammarus*, however, insufficient data was collected to statistically validate this hypothesis.

Keywords: Asellus aquaticus*; 2-chlorobiphenyl; constructed wetlands;* Gammarus pulex*; landfill leachate; toxicity tests.*

INTRODUCTION

Leachate generated from landfilling can contain materials that pose a threat to the surroundings and in the worst cases, may cause ground and surface water contamination (Ground Water Directive 80/86/EEC). Leachate stress has previously been shown to modify the population dynamics of both *A. aquaticus* (pollution tolerant) and *G. pulex* (pollution sensitive) macro-invertebrates (Bloor *et al.*, 2004a). Both riverine species are commonly found together throughout the British Isles, Europe and North America (Maltby, 1995). A specific leachate was used in the study (255 mgl^{-1} COD and 133 mgl^{-1} BOD_5), which was obtained from an undisclosed UK landfill site. After short term exposure to the leachate, juvenile (two-week old), laboratory bred *A. aquaticus* had an LC_{50} value of 57% v/v leachate in deionized water and 5% for *G. pulex*. It was also found that prolonged exposure to a 1 : 20 sub-lethal dilution affected the breeding colony size of *A. aquaticus* and a 1 : 66 dilution appeared to influence the fecundity of a *G. pulex* population but insufficient data was obtained to corroborate this supposition (Bloor *et al.*, 2004a).

The leachates toxic component was identified as 1.036 mgl^{-1} of 2-chlorobiphenyl (Bloor, 2004). The LC_{50} value of 2-chlorobiphenyl towards freshwater species (including fish) ranges between 0.01–25 mgl^{-1} (Hayes, 1987). In the aforementioned study, *G. pulex* (2-chlorobiphenyl LC_{50} of 0.0518 mgl^{-1}) showed 11.4 times more sensitivity to 2-chlorobiphenyl, after short term exposure, than *A. aquaticus* (2-chlorobiphenyl LC_{50} of 0.59 mgl^{-1}), (Bloor, 2004). The documented toxicological symptoms of 2-chlorobiphenyl exposure (on a sub-lethal level) also concur with the studies findings. These signs manifest themselves as infertility, reduced population growth and diminished growth rates (Mayer *et al.*, 1977; Bridgham, 1988).

Current landfill engineering practice and regulatory control have gone a long way in protecting the environment through reducing pollution loads, by the use of leachate treatment systems and by controlling leachate migration.

**Correspondence to*: Dr M. C. Bloor, Department of Civil and Environmental Engineering, University of Southampton, Highfield, Southampton, SO17 1BJ, UK.
E-mail: m.c.bloor@soton.ac.uk

There are, however, a considerable number of closed landfill sites that were constructed and operated without engineering containment, and from which leachate is likely to arise over the next several decades (Rowe, 1998). At these sites, consideration needs to be given to methods for reducing the environmental impacts. In such cases, the use of passive treatment systems has some advantages and has raised interest in the potential of constructed wetlands for the diminution of pollutant loads, including toxic components, such as 2-chlorobiphenyl (Crowley and Borneman, 2004).

Wetland plants have the ability to grow in environments where their root system (rhizophere) is submerged. The plants transport air through specialized tissues to their root and leakage of air from the roots creates aerobic conditions within the water-saturated soil, which would otherwise be mainly anaerobic. The soil itself provides a habitat for a wide range of macro-organisms, including bacteria, yeasts and fungi, which under natural conditions are responsible for the decay of organic matter, releasing nutrients back into the soil (Price and Probert, 1997).

In a wetland system developed for wastewater remediation, these same organisms are responsible for the breakdown of organics within the waste. The soil rhizophere hosts both aerobic and anaerobic organisms, thus, providing many different reaction mechanisms that can promote a very high rate of degradation. This feature is fundamental to the degradation of anthropogenic substances in the reed bed (Cobban *et al.*, 1997). In addition, the reed bed may act as a 'sink' for non-degradable recalcitrant organics and for chemical species, such as, heavy metals. This phenomenon is a result of a number of factors: adsorption reactions on microbial films and charged mineral particles; adsorption into the rhizome and other vascular structures of the reeds themselves; and precipitation reactions resulting from changes in localized redox potential (Tanner, 1996).

The rate and level of remediation achieved by constructed wetlands is influenced by several factors, such as, the type of media used (e.g., sand, gravel, clay or soil), (Gray and Biddlestone, 1995; Cobban *et al.*, 1997; Price and Probert, 1997) the choice of plants (e.g., monoculture or mixed beds), (Tanner, 1996; Coleman *et al.*, 2000) the type of bed (e.g., horizontal, sub-surface flow or downflow), (Bubba *et al.*, 2003; Garcia *et al.*, 2003). The aim of this study, therefore, is to identify if constructed wetlands could remediate the leachate and minimize the toxicological effects of 2-chlorobiphenyl on *A. aquaticus* and *G. pulex*, which may inhabit the receiving waters.

METHODS AND MATERIALS

Leachate

The leachate used in this study was also used in Bloor *et al.* (2004a, b). The leachate was collected as a one-off, bulk sample from a covered drain within an undisclosed landfill site, which is located in the UK. The sample was siphoned into a 500 litre header tank using a petrol driven pump and mixed thoroughly (to eliminate sample variability) before dispensing into 25 litre 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 255 mgl^{-1} COD and 133 mgl^{-1} BOD_5. Unused, defrosted leachate was immediately discarded.

Reed Bed

The basic reed bed reactor was a 400 × 300 mm cylinder closed at the bottom and drained by means of a tap (downflow system). This was filled with 10 mm pea gravel, giving an effective pore volume of 10 litres. Nine beds were planted with *Juncus effusus*, whilst the tenth was not planted and acted as a control. The author chose to use *Juncus effusus* for the study, as it grew naturally at the undisclosed landfill site, whilst being submerged in the leachate and has previously been used within leachate treatment systems (Tanner, 1996). The beds were operated as individual wetlands and followed the same protocol. All 10 beds were simultaneously batch fed with the same leachate dilutions (100%–10% v/v leachate in deionized water) and operated on a 1–10 day hydraulic retention time (RT). All experiments were replicated five times (with all 10 beds) and effluent from the beds underwent COD analysis (Standard Methods for the Examination of Water and Wastewater, 1998).

Acute Toxicity Tests

The acute toxicity testing methodology used in Bloor *et al.* (2004a and b) was utilized. Pollution and disease free test animals (*A. aquaticus* and *G. pulex*) were obtained from the laboratory based breeding programme. Ten juvenile (two-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%–5% v/v reed bed effluent in deionized water (from beds operated on a 1–10 day RT) and deionized water controls, maintained at a constant temperature of 15°C. All dilution media was made up from effluent samples, which were collected from the beds at the start of the experiment. 96 hour, static LC_{50} tests were undertaken in oxygen depleted conditions without nutritional supplement. The same procedure was also performed with *A. aquaticus.* Observations were made at 24 hour intervals and immobilized specimens were removed, and placed in deionized water for 24 hours to monitor recovery. All experiments were replicated five times with both test species. Bloor (2004) showed that both test species could survive under these conditions for several weeks, in a deionized water test media.

Sub-lethal Toxicity Tests

The sub-lethal toxicity testing methodology employed in Bloor *et al.* (2004a and b) was used. A series of six, ten litre tanks were established for *A. aquaticus* and *G. pulex.* All contained specific effluent dilutions, which did not affect mortality during the acute bioassays. 100%, 80%, 60%, 40% and 20% v/v reed bed effluent in deionized water