CRANFIELD UNIVERSITY

DAVID J. L. THOMAS

UNDERSTANDING THE CAUSES OF TOXICITY IN TREATED LANDFILL LEACHATE THROUGH WHOLE EFFLUENT TESTING

SCHOOL OF APPLIED SCIENCES

DOCTOR OF PHILOSOPHY

SUPERVISOR: Dr. S. F. TYRREL

CRANFIELD UNIVERSITY

SCHOOL OF APPLIED SCIENCES

DOCTOR OF PHILSOPHY

ACADEMIC YEAR 2009-2010

DAVID J. L. THOMAS

AUGUST 2010

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Abstract

Landfill leachate is collected and treated before discharge to protect the environment from a potential toxic cocktail of substances. In the U.K. biological treatment is the favourite technology for rendering landfill leachate safe due its simple design, effective handling of varying chemical loads and relatively low operating costs. Biological treatment is effective at reducing the concentrations of ammoniacal-nitrogen and the biological oxygen demand (BOD) to acceptable levels for discharge. Even though the ammoniacal-nitrogen and BOD levels have been reduced there still remains a considerable quantity of refractory organic chemicals and inorganic ions. Heavy metals tend be present in very low concentrations. A view has developed that these effluents potentially pose a risk to the aquatic environment due to the presence of these compounds.

This project aims to answer a number of gaps in the scientific knowledge on the causes of residual toxicity in treated landfill leachate:

1. What levels of residual toxicity are present in effluents?
2. Are refractory organics or inorganic salts the cause of residual toxicity?
3. Are further treatment options needed to render landfill leachate safe?

These gaps are to be answered by performing whole effluent toxicity (WET) investigation to determine the levels of toxicity and identify the causes of toxicity through chemical manipulations.

A comprehensive literature review of WET and the types of bioassay used for determining the toxicity of both raw and treated landfill leachate was carried out. The review highlighted the sensitivity of each test through meta-analysis of previously published reports on the levels of toxicity. From this review a new battery of tests was proposed.

Initial experiments utilised a Toxicity Identification Evaluation (TIE) procedure in an attempt to determine whether sample manipulation could identify the causes of toxicity. On the basis

of the battery proposed in the literature review 5 species were selected for determining WET in treated leachates from three sites. The bioassays used were: *Lemna minor; Daphia magna; Thamnocephalus platyurus; Vibrio fischeri (*Microtox™); *Escherichia coli* (Toxi-ChromoPlate™). Levels of residual toxicity in treated landfill leachate were found to be low when compared to raw landfill leachate. This procedure was unsuccessful in definitively identifying the classes of compound responsible for toxicity though it did open up new avenues to explore.

A dedicated recalcitrant organic removal procedure was used to fractionate and remove specific portions of the chemical oxygen demand (COD) of treated landfill leachate. Using WET, this procedure was designed to test whether the residual COD fraction was the cause of toxicity in treated landfill leachate. In this stage only two bioassays were used: *D. magna* and *L. minor*. This procedure successfully removed >90% of the COD fraction without any significant change in toxicity.

Major ions, Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}, HCO_3^-, SO_4^{2-}, were the remaining fraction left within treated landfill leachate and were the likely cause of residual toxicity. Two methods for evaluating the role of major ions were utilised:

i. A model that can predict toxicity based on the concentration of inorganic ion was investigated. The model consistently over predicted the toxicity towards *D. magna* based on the concentrations of inorganic salts in treated landfill leachate from the sites investigated in this project.

ii. In a different approach to most WET testing it was decided to attempt to recreate toxicity by producing synthetic leachates based on the inorganic salt chemical composition of treated landfill leachate. The results from this testing demonstrated that it was possible to recreate toxicity towards *D. magna* and *L. minor* by dissolving leachate quantities of inorganic salts in a buffered water solution.

Acknowledgements

Firstly I would like to thank my family for all the love and support they have given me throughout the years. Secondly, my thanks to my wonderful wife Sadaf for keeping my hopes and dreams alive with her love and kindness. Thirdly I'd like to thank Sean Tyrrel for his time, patience, humour, keeping me focused on the work and for making this project happen. Fourthly to the amazing technicians of Building 39, including Paul Barton, for the running around, instrument training, determinations and the general keeping me stocked up with materials. And lastly the members of Building 40, who have assisted, made me welcome and helped me to enjoy the time during the PhD process.

Table of contents

Abstract II

Acknowledgements III

Table of contents 1

List of figures 7

List of tables 10

1 Introduction 14

1.1 Preface 14

1.2 Project aims and objectives 16

2 A review of bioassays for the evaluation of landfill leachate toxicity 18

2.1 Introduction 18

2.2 Basics of ecotoxicology 21

2.3 Rationale for the review 27

2.4 Bioassays using bacteria 27

2.4.1 Luminescent bacteria; Vibrio fischeri and Photobacterium phosphoreum 27

2.4.2 Activated sludge respiration inhibition test (ASRI) 31

2.5 Toxicity testing using green algal species 32

2.5.1 Pseudokirchneriella subcapitata (aka Selenastrum capricornutum) 32

2.6 Toxicity testing with invertebrates 35

2.6.1 Daphnia magna 35

2.6.2 Ceriodaphnia dubia 38

2.6.3 Brachionus calyciflorus 39
2.6.4 Artemia salina 40
2.7 Toxicity testing on fish 41
2.7.1 Toxicity testing with Lemna species 44
2.8 Genotoxicity 47
2.8.1 Ames test 48
2.8.2 umuC gene test 51
2.8.3 Toxicity testing with Escherichia coli 51
2.8.4 Bacillus subtilis rec-essay 53
2.8.5 Mutatox® 53
2.9 Discussion of review finding 54
2.9.1 Differences between raw and treated landfill leachate toxicities 54
2.9.2 Constituents of battery of tests 56
2.9.3 Sensitivity of tests 58
2.10 Gaps in knowledge 60
2.11 Conclusions 60
3 Methodology 63
3.1 Leachate sample collection 63
3.2 Physico-chemical parameter determination 63
3.2.1 Chemical oxygen demand 64
3.2.2 Biological oxygen demand 64
3.2.3 Ammoniacal nitrogen 65
3.2.4 Sulphate 66
3.2.5 Total nitrogen 66
3.2.6 Chloride 67
3.2.7 Metal analysis 68

3.2.8 Conductivity and pH 69
3.2.9 Suspended solids 69
3.3 Justification for bioassay selection 69
3.3.1 Toxicity identification evaluation procedure 70
3.3.2 Rationalisation for the selection of toxicity tests 71
3.4 Toxicity testing procedure 73
3.4.1 Daphnia magna 73
3.4.2 Thamnocephalus platyurus 74
3.4.3 Lemna minor 75
3.4.3.1 Stock culture collection 75
3.4.3.2 Stock solution preparation 76
3.4.3.3 Exposure procedure 78
3.4.3.4 Observations 78
3.4.4 Microtox 79
3.4.4.1 Procedure 79
3.4.5 Toxi-ChromoPlate™ 81
3.4.5.1 Procedure 81
3.5 Statistical treatment of toxicity testing results 83
4 Overview of selected landfill leachate chemistry and toxicity 85
4.1 Rationale for work 85
4.2 Introduction 86
4.2.1 Marston Vale leachate treatment plant 87
4.2.2 Arpley landfill leachate treatment plant 88
4.2.3 Buckden, Cambridgeshire 90
4.3 Aim and objective 91
4.4 Chemical composition of raw and treated leachate analysis 92

4.4.1 Arpley raw and treated landfill leachate 92
4.4.2 MVP raw and treated landfill leachate 100
4.4.3 Buckden raw and treated landfill leachate 108
4.5 Comparison with previously reported data 109
4.6 Conclusions 114
5 Long term toxicity responses and a toxicity identification evaluation procedure of treated landfill leachates 115
5.1 Rationale for work 115
5.2 Introduction 116
5.2.1 Toxicity identification procedure 117
5.2.2 Hypothesis 124
5.2.3 Experiment aim and objective 125
5.3 Methods and materials 126
5.3.1 Toxicity identification procedure 126
5.3.2 Bioassays for assessing toxicity 127
5.3.3 Statistics 127
5.4 Results and Discussion 127
5.4.1 Long term WET testing of MVP, Arpley and Buckden treated leachates 127
5.4.2 TIE procedure 130
5.5 Conclusions 140
6 Reduction of recalcitrant COD with a selection of XAD resins 143
6.1 Findings from previous work 143
6.2 Introduction 143
6.2.1 Background 143

6.2.2 Humic and fulvic acid description 146
6.2.3 Use of XAD to remove COD from leachate – basic approach 153
6.3 Hypothesis 156
6.4 Aims and objectives 157
6.5 Materials and methods 157
6.5.1 XAD resins 158
6.5.2 COD removal procedure 160
6.6 Results and discussion 161
6.6.1 Tests using XAD-7 164
6.6.2 Tests using XAD-7 and 4 in sequence 168
6.6.3 Tests using XAD-7, 4 and 16 in sequence 173
6.7 Limitations and implications of this approach 181
6.8 Conclusions 182
7 Major ion toxicity and synthetic leachates 183
7.1 Findings from previous work 183
7.1.1 General knowledge of major ion toxicity 185
7.1.2 Knowledge of major ion sources in landfill leachate 189
7.1.3 Mount predictor for major ion toxicity 190
7.1.4 Synthetic solutions 194
7.2 Hypothesis 195
7.3 Objective 195
7.4 Materials and methods 196
7.4.1 Mount model 196
7.4.2 Synthetic leachate 197

7.5 Results 200
7.5.1 Mount model predictions 200
7.5.1.1 MVP Mount model predictions 201
7.5.1.2 Arpley Mount model predictions 203
7.5.2 The toxicity of synthetic leachates 205
7.6 Discussion 208
7.6.1 Mount model predictions 208
7.6.2 Synthetic leachates 211
7.6.3 Discussion of the two pronged approach to understanding the role of major ions in residual leachate toxicity 218
7.6.4 Limitations of experimental approach 219
7.7 Conclusions 220
8 Overall project discussion 221
8.1 Introduction 221
8.2 General findings 221
8.2.1 Overview of selected landfill leachate chemistry and toxicity 222
8.2.2 Toxicity identification evaluation discussion and conclusions 224
8.2.3 XAD discussion and conclusions 226
8.2.4 Major ions and synthetic leachate discussion and conclusions 228
8.3 Overall achievement and contribution to science 231
8.4 Limitations of this work 233
8.5 Recommendations for further work 236
References 238
Appendix 1 253

List of figures

Figure 1-1: The additive and subtractive experimental approach to determine the causes of residual toxicity in treated landfill leachate. 17

Figure 2-1: Chemical structure of $FMNH_2$ 28

Figure 3-1: Image of the Microtox multiwell plate 80

Figure 3-2: Toxi-Chromo multiwell plate with letter and number position highlighted 81

Figure 3-3: An example of the graph formed in multiple regression analysis. Each point represents a mean of observations. The y-axis is a logarithmic scale. 84

Figure 5-1: The TIE procedure for treated landfill leachate samples. Nat=Baseline pH (Norberg-King *et al.*, 1991). 119

Figure 5-2: Phase diagram showing the speciation of Fe in water at pH values of 0-14..... 121

Figure 5-3: EC_{50} toxicity of MVP treated leachates towards *D. magna* (n=4) and *L. minor* (n=3). 95% confidence intervals shown as bars on the column..... 128

Figure 5-4: EC_{50} toxicity of MVP treated leachates towards *L. minor* (n=4) and *L. minor* (n=3). 95% confidence intervals shown as bars on the column..... 129

Figure 5-5: EC_{50} toxicity of Buckden treated leachates towards the Microtox test. No variance data was supplied by the laboratory. 130

Figure 5-6: *D. magna* average EC50 (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different. 134

Figure 5-7: *L. minor* average EC_{50} (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different..... 135

Figure 5-8: *E. coli* average EC_{50} (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different.....136
Figure 5-9: *T. platyurus* average EC_{50} (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different. 137
Figure 6-1: Idealised Buffle structure of fulvic acid (Aiken, 1985) 147
Figure 6-2: Sugar-amine condensation reaction to form n-substitued-1-amino-deoxy-2-ketose via an Amadori rearrangement (Stevenson, 1994). 148
Figure 6-3: Combination of quinones and amino acids to form humic substances (Stevenson, 1994). 149
Figure 6-4: Changes in the humic acid content of the COD in landfill leachate based on previous reports (Blakey *et al*., 1992).....150
Figure 6-5: Flow diagram of the steps for the rapid removal of humic and fulvic acids from treated landfill leachate samples. 155
Figure 6-6: XAD-4 is designed to target solvent size molecules such as toluene and isoprene (left to right). 156
Figure 6-7: XAD-16 is designed to remove antibiotic sized molecules e.g. penicillin. 156
Figure 6-8: Experimental process adopted for the reduction of the COD fraction in treated landfill leachate from MVP and Arpley.....158
Figure 6-9: Soxhlet extraction apparatus used for cleaning XAD resin. 159
Figure 6-10: The mean COD concentration (mg/L) at the start and after application of each stage of the rapid batch procedure (n=3). The percentages above each bar represent the percentage removal between the original and the application. 164

Figure 6-11: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure with samples collected from MVP (n=4). 95% confidence intervals shown as bars. 165
Figure 6-12: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure with samples collected from MVP (n=3). 95% confidence intervals shown as bars. 166
Figure 6-13: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure with samples collected from Arpley (n=4). 95% confidence intervals shown as bars. 167
Figure 6-14: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure with samples collected from Arpley (n=3). 95% confidence intervals shown as bars. 168
Figure 6-15: The mean COD concentration (mg/L) at the start and after application of each stage of the rapid batch procedure (n=3). The percentages above each bar represent the percentage difference between the original and the application. 169
Figure 6-16: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from MVP (n=4). 95% confidence intervals shown as bars. 170
Figure 6-17: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from MVP (n=3). 95% confidence intervals shown as bars. 171
Figure 6-18: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from Arpley (n=4). 95% confidence intervals shown as bars. 172

Figure 6-19: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from Arpley (n=3). 95% confidence intervals shown as bars. 173
Figure 6-20: The mean COD concentration (mg/L) at the start and after application of each stage of the rapid batch procedure (n=3). The percentages above each bar represent the COD percentage reduction between the original and the application.....174
Figure 6-21: Effect of rapid batch removal process with XAD 4, 7 and 16 on treated landfill leachate toxicity displayed towards *D. magna* (n=4). Samples collected from MVP and Arpley. Error bars represent 95% confidence intervals. 175
Figure 6-22: Effect of rapid batch removal process with XAD 4, 7 and 16 on treated landfill leachate toxicity displayed towards *L. minor* (n=3). Samples collected from MVP and Arpley. Error bars represent 95% confidence intervals. 176
Figure 6-23: Generic phthalate ester structure. 178
Figure 6-24: Left image; from left to right, Arpley treated landfill leachate, Arpley leachate following centrifugation, Arpley leachate following XAD-7 extraction. Right image; from left to right, MVP treated leachate, following centrifugation and XAD-7extraction, following XAD-16 extraction, following XAD-4 extraction. 181
Figure 7-1: Response of *D. magna* towards three repeats of the synthetic leachate based on the average Arpley treated leachate major ion concentration. Error bars represent the 95% confidence intervals. 206
Figure 7-2: Response of *D. magna* towards three repeats of the synthetic leachate based on the average MVP treated leachate major ion concentration. Error bars represent the 95% confidence intervals. 207

Figure 7-3: Response of *L. minor* to synthetic leachates based on average major ion concentrations from MVP and Arpley treated landfill leachates. Error bars represent the 95% confidence intervals. 208

Figure 7-4: The relationship between chloride concentration and toxicity by Cooman *et al* (2003). 211

Figure 7-5: Comparison of average EC_{50} toxicity for *D. magna* towards MVP synthetic and real leachates. The standard deviation is shown as a bar on the column. 212

Figure 7-6: Comparison of average EC_{50} toxicity for *D. magna* towards Arpley synthetic and real leachates. The standard deviation is shown as a bar on the column. 213

Figure 7-7: Comparison of average EC_{50} toxicity for *L. minor* towards MVP synthetic and real leachates. The standard deviation is shown as a bar on the column. 215

Figure 7-8: Comparison of average EC_{50} toxicity for *L. minor* towards Arpley synthetic and real leachates. The standard deviation is shown as a bar on the column. 216

List of tables

Table 2-1: UK LDCE requirements for the construction of landfills so to minimise leakage of leachate to the environment. 19

Table 2-2: Characteristics of leachate based on the age of the landfill (Kjeldsen *et al*., 2002) 19

Table 2-3: Toxicity tests specified by the Environment Agency and SEPA for water quality monitoring (Johnson *et al*., 2004) 26

Table 2-4: Examples of landfill leachate toxicity to luminescent bacteria 30

Table 2-5: Examples of landfill leachate toxicity to green algae species. 33

Table 2-6: Examples of landfill leachate lethal toxicity to *D. magna.* 37

Table 2-7: Examples of landfill leachate toxicity to fish species. 44

Table 2-8: Examples of landfill leachate toxicity to *L. minor*. 47

Table 2-9: Examples of landfill leachate genotoxicity towards *S. typhimurium.* 50

Table 2-10: Toxicity testing of MSW landfills around the world within the literature using different species and methods. 56

Table 2-11: Sensitivity of a number of toxicity tests based on the Sloof method. 59

Table 3-1: Dilution factor for each metal ion analysed with atomic absorption spectroscopy. 68

Table 3-2: Stock solution composition and concentrations 77

Table 3-3: Multiple regression analysis for Arpley *T. platyurus* Unfiltered pH 3 84

Table 4-1: Differences in leachate composition of young and older leachates (Kurniawan *et al*., 2006b). 86

Table 4-2: Arpley influent and effluent concentrations of COD, BOD, inorganic ions and heavy metals. Red list chemicals highlighted in red (Source: WRG chemical database). 94

Table 4-3: The List I (Red List) chemicals as identified by the Environment Agency and the 48 hr (unless stated) *Daphnia magna* EC_{50} concentration from Pesticides Database (PAN, 2010). 95

Table 4-4: The List II chemicals as identified by the Environment Agency 96

Table 4-5: Arpley "red list" organic compound average concentrations in effluent from 27.11.2006 to 29.10.2010. 'Red List' substances highlighted red in tables (Source: WRG chemical database). 97

Table 4-6: MVP influent and effluent average concentrations of COD and inorganic ions (Source: WRG chemical database). 101

Table 4-7: The average concentration of 'Red List'organic compounds in the MVP treated leachates from 27.11.2006 to 31.8.2010. 'Red List'substances highlighted red in tables (Source: WRG chemical database). 103

Table 4-8: Buckden influent and treated leachate average concentrations of COD and inorganic ions (Source: WRG chemical database). All data in (mg/L) unless stated....108

Table 4-9: Average chemical composition of both raw and biologically treated landfill leachates. All data is in mg/L. 112

Table 5-1: Mean values of chemical parameters for treated Stewartby Buckden and Arpley landfill leachates. All values in mg/L unless stated (± standard deviation).....132

Table 6-1: Average changes in COD, BOD and pH between the acetogenic phase and methanogenic phase. (Kjeldsen *et al*., 2002). 145

Table 6-2: Chemical parameter data of treated landfill leachate samples collected from MVP and Arpley (A) 163

Table 7-1: Average concentration of ions between acidogenic and methanogenic phases (Kjeldsen *et al*., 2002).....190

Table 7-2: Regression and NumCat coefficients used in the Mount model (Mount *et al*., 1997). 197

Table 7-3: Arpley synthetic leachate recipe.....199

Table 7-4: MVP synthetic leachate recipe.....200

Table 7-5: The calculated EC_{50} for MVP leachate collected on the 13.05.09.....201

Table 7-6: The calculated EC_{50} for MVP leachate collected on the 28.05.09.....202

Table 7-7: The calculated EC_{50} for MVP leachate collected on the 11.08.09.....202

Table 7-8: The calculated EC_{50} for MVP leachate collected on the 24.08.09.....202

Table 7-9: The calculated EC_{50} for MVP leachate collected on the 10.11.09.....203

Table 7-10: The calculated EC_{50} for MVP leachate collected on the 17.12.09.....203

Table 7-13: The calculated EC_{50} for Arpley leachate collected on the 7.07.09.....203

Table 7-14: The calculated EC_{50} for Arpley leachate collected on the 14.09.09.....204

Table 7-11: The calculated EC_{50} for Arpley leachate collected on the 11.08.09.....204

Table 7-12: The calculated EC_{50} for Arpley leachate collected on the 24.08.09.....204

Table 7-15: The calculated EC_{50} for Arpley leachate collected on the 28.11.09.....205

Table 7-16: Students t-test calculations to determine significance in difference between EC_{50} toxicities in MVP real (unmodified dilution series) and synthetic leachates towards *D. magna*.....213

Table 7-17: Student t-test calculations to determine significance in difference between EC_{50} toxicities in Arpley synthetic and real leachates (unmodified dilution series) towards *D. magna*.....214

Table 7-18: Students t-test calculations to determine significance in difference between EC_{50} toxicities in MVP synthetic and real leachates using the modified dilution series towards *D. magna*.....215
Table 7-19: Students t-test calculations to determine significance in difference between EC_{50} toxicities in MVP real (unmodified dilution series) and synthetic leachates towards *L. minor*.....216
Table 7-20: Students t-test calculations to determine significance in difference between EC_{50} toxicities in Arpley real (unmodified dilution series) and synthetic leachates towards *L. minor*.....217

1 Introduction

1.1 Preface

Landfill leachate is generated by the percolation of water through waste deposited within a landfill. Due to the decomposition of wastes within the landfill this water, if left uncontrolled, can transport hazardous chemicals and solids to the surrounding environment. To overcome this problem landfills are engineered with liners to stop leakage into the surrounding ground water and with sumps to collect the leachate prior to treatment before eventual return to the water environment. These precautions are necessary as landfill leachate is known as an extremely toxic cocktail.

The age and stage of decomposition of waste landfilled determines the chemical characteristics of the leachate. Stabilised landfill leachate, normally >2 years old, is the focus of this project. Stabilised leachates from different sites share a number chemical traits e.g. ammoniacal-nitrogen, biological oxygen demand (BOD) and chemical oxygen demand (COD) present in concentrations > 500 mg/L (Robinson and Barr, 1999). Inorganic cations and anions, known as major ions, in leachates tend to be present in elevated concentrations compared to natural freshwaters e.g. a range 100 - 5,000 mg/L is normal in landfill leachate (Fatta *et al.*, 1999). Heavy metal concentration in stabilised landfill leachates are generally low e.g. <1 mg/L. Evn with this low concentration there remains a concern over their presence in landfill leachate due to the potential for environment damage (Kjeldsen *et al.*, 2002).

Treatment is carried out to limit any potential damage to surrounding ecosystems. In the U.K. biologically treatment is the preferred strategy for the rendering of leachate safe. Biological treatment can effectively reduce the concentration of ammoniacal-nitrogen and BOD to levels that are considered safe for discharge. Biological treatment reduces the concentration of COD but there usually remains a considerable concentration of COD in most effluents e.g. >200

mg/L. This COD fraction is made of refractory humic and fulvic acids plus some low molecular weight hydrocarbons (Huo *et al*., 2008). There is a concern that this considerable concentration of COD could be a vehicle for toxic substances to find their way to the outside environment. This concern is due to the ability of humic and fulvic acids to transport heavy metals and harmful xenobiotic substances to the aquatic environment (Van Zomeren and Comans, 2007).

Traditional chemical analysis of this complex blend of organic and inorganic substances would be time consuming and expensive. It is unlikely that a complete separation, determination of exact concentration and resolution of state they are present would be possible. Whole effluent toxicity (WET) testing allows researchers to identify toxic risks of effluents by using a variety of species which represent the various trophic levels of ecosystems.

Residual toxicity ranges from low to moderate in treated landfill leachates whereas raw landfill leachate is normally highly toxic in even very dilute solutions. Reports exist in the literature of treated landfill leachate residual toxicity though the causes have been suggested as the dissolved organic content or inorganic ions (Okamura *et al.*, 2005; Bortolotto *et al.*, 2009). Residual toxicity is a cause for concern if these effluents are being discharged directly to the environment. Little information is available on the toxicity of U.K. treated leachates so this work aims to fill this gap with a comprehensive testing of three sites.

This project sets out to determine the levels of residual toxicity in treated landfill leachate from sites operated by the project sponsor Waste Recycling Group (WRG). WRG operates landfills with a variety of capacities and ages throughout the U.K. Due to increasing pressure from the Environment Agency over discharge consents for COD the project sponsor requires more information on the nature of treated landfill leachate toxicity and whether residual toxicity in treated landfill leachate is attributable to COD or another chemical fraction. This

work is of great benefit to overall scientific knowledge as it takes previously successfully techniques from the literature and applies them to treated landfill leachate sample WET testing.

1.2 Project aims and objectives

Project aim: Determine the causes of residual toxicity in treated landfill leachate from sites in the U.K.

Project objectives:

- Perform an extensive review of the literature to determine the differences in raw and untreated leachate toxicity. From the review a decision on the composition of a battery of bioassay species is made (Chapter 2).
- Screen treated leachate samples from a number of sites to determine toxicity levels between sites and treatment. Determine the extent, magnitude and variability in toxicity (Chapter 4)
- Resolve whether residual toxicity is attributable:
    i. To ammonia, solid particles, heavy metals and pH sensitive substances within treated landfill leachate (Chapter 5).
    ii. To organic substances that are recalcitrant to the biological treatment (Chapter 6).
    iii. To the major ions in residual toxicity. The feasibility of predicting residual leachate toxicity based on major ion concentration with modelling (Chapter 7).
- Discuss the impact effluents have on the environment and whether any need for further treatment of leachates is required (Chapter 8).

To complete these objectives a number of experimental approaches were undertaken. These experimental approaches are summarised in Figure 1-1. The diagram highlights the

subtractive methods for removing certain chemical characteristics from the leachate and then determining the toxicity of the samples. An additive approach was used later in the project to build a synthetic leachate that had toxicity similar to that of a collected treated leachate sample. Each strategies effect on the samples chemistry was determined with WET testing.

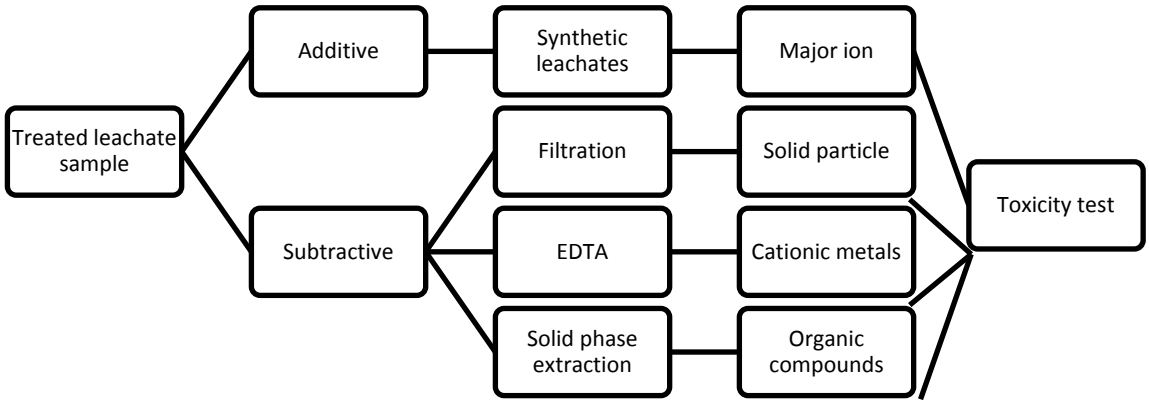

Figure 1-1: The additive and subtractive experimental approach to determine the causes of residual toxicity in treated landfill leachate.

2 A review of bioassays for the evaluation of landfill leachate toxicity

This literature review was written in November 2007 and published in the *'Journal of Toxicology and Environmental Health, Part B'* on the 1st of January 2009. The published article is presented in Appendix 1. Since publication the review has been updated to represent current developments.

2.1 Introduction

The landfilling of municipal solid waste (MSW) is the most utilized method for the disposal of waste in the UK, with 9.3M tons of UK biodegradable waste landfilled in 2007 (Defra, 2009). In 2007 the EU sent a total of 102M tons of MSW to landfill (Eurostat, 2008). Landfilling of MSW is only one of a number of technologies for the disposal of waste e.g. incineration and mechanical biological treatment.

One of the most serious impacts to the environment associated with the landfilling of MSW is the generation of leachate. Leachate is formed by water penetrating the landfill through the percolation of rainwater, the seepage of surface water and the intrusion of groundwater. As water moves through the landfill it removes both dissolved and suspended solids that might be present in the waste (Fan *et al*., 2006). Due to the degradation processes taking place within the landfill the leachate may attain a toxic nature, which if discharged to the environment might result in lethal consequences for aquatic life (Robinson *et al*., 1992).

To reduce the potential impact of landfill leachate release to the environment the EU Landfill Directive (Commission, 2000) defines engineering containment practices for landfill sites (Table 2-1). Landfills need a number of liners including clay, geosynthetic and sand to stop possible leakage of leachate to the surrounding soil.

Table 2-1: UK LDCE requirements for the construction of landfills so to minimise leakage of leachate to the environment.

LDCES component	Requirement
Basal slope:	Slope of 2% towards leachate extraction point
	300 mm thick
Drainage blanket:	Hydraulic conductivity not specified
	No fines and a carbonate content of less than 10%
Pipework:	Required and designed on a site specific basis

The biodegradation of waste leads to 3 distinct phases of degradation. These phases change over time due to the aging of the landfill (Kjeldsen *et al*., 2002). Chemical speciation of the leachate is thus dependent on the age of the landfill, temperature, and moisture levels. Some of the main features are highlighted in Table 2.2.

Table 2-2: Characteristics of leachate based on the age of the landfill (Kjeldsen *et al*., 2002)

Type of leachate	Young	Intermediate	Stabilised
Age of landfill (years)	<1	1-5	>5
pH	3.5-6.5	6.5-7.5	>7.5
BOD/COD	0.4-1.0	0.1-0.5	<0.1
COD (mg L^{-1})	15,000-60,000	3,000-15,000	<3,000
NH_3-N (mg L^{-1})	100-400	Not available	400-4,000
Heavy metals (mg L^{-1})	>2	<2	<2

Ammoniacal-nitrogen was identified as the major toxic fraction in landfill leachate (Clement and Bouvet, 1993). Ammonia is highly soluble in water and establishes equilibrium between ammonia and ammonium plus a hydroxyl ion (Horane, 1991).

$$NH_3 + H_2O \rightarrow NH_3.H_2O \rightarrow NH_4^+ + OH^-$$

The equilibrium between the ionized and unionized form of ammonia in water is controlled by both pH and temperature (Clement and Bouvet, 1993). Dissolved ammoniacal-nitrogen is toxic to many of types of fish including *Oncorhynchus mykiss* (rainbow trout), at concentrations of <0.025 mg/L (Horane, 1991). Ammoniacal-nitrogen removal from landfill leachate is generally achieved by the nitrifying bacteria though other physical processes such as ion exchange are available (Kurniawan *et al*., 2006a).

Toxicity of landfill leachate is not solely accounted for by ammoniacal-nitrogen (Clement and Merlin, 1995). The organic fraction (generally referred to as chemical oxygen demand (COD)) is an important area for consideration when assessing the toxicity of landfill leachate. Over 200 organic substances have been identified within landfill leachate (Kjeldsen *et al*., 2002). The majority of the substances within the leachate are considered to be non-toxic and those that are toxic are generally removed with existing biological and chemical treatment (Svensson *et al*., 2005). However there are a few classes of substances that are refractory to treatment processes commonly employed. The types of substances that have been identified as refractory are some of the most toxic to organisms including pesticides, phenols, halogenated hydrocarbons/aromatics, pharmaceuticals and phthalates (Slack *et al*., 2005). These types of substances are generally present at low concentrations (µg/L) thus detection may be difficult due to masking by substances present in higher concentrations (Norberg-King *et al*., 1991). In combination though there is now a view they may act in a synergistic fashion thus becoming more toxic to organisms (Baun *et al*., 2004).

The presence of heavy metals within landfill leachate is also of concern when assessing their toxicity. Generally heavy metals are present at low concentrations (Baun and Christensen, 2004b). The attenuation of heavy metals during the methanogenic phase of

landfilling is thought to sequester most of the soluble heavy metal species (Slack *et al*., 2005). The amount of heavy metals in an active landfill is estimated as 0.02% of the total waste whereas the concentration of almost all heavy metals in a stabilised leachate is < 1 mg/L (Kjeldsen *et al*., 2002). Attenuation is thought to proceed via precipitation and sorption. Sorption with humic and fulvic acids is considered one of the main reasons that only small quantities of heavy metals are leached from the landfill (Bozkurt *et al*., 1999). Metal-sulphide and metal-carbonate precipitates have very low solubility products and are present in landfills at concentrations of >100 mg/L (Christensen *et al*., 2000). These compounds are believed to play a major role in the limiting of heavy metals in leachate. It is estimated that up to 90% of heavy metals were attenuated through these processes and this explains why the concentration of heavy metals does not reflect the amounts of heavy metals deposited (Erses and Onay, 2003).

2.2 Basics of ecotoxicology

The 20th century saw a huge increase in the variety and quantity of chemicals produced. This rapid increase in the level of chemical technology brought untold richness to the lives of peoples throughout the world but as is usually the case there was a price to pay for the development of such chemicals. This price was the lasting damage that was done to the environment through uncontrolled release of chemicals into the natural world (Carson, 1962).

Understanding the concentration at which a chemical becomes a risk to the environment was a necessary step in understanding the effect that chemical discharge has on the environment. Ecotoxicology was defined at the Working Group on Ecotoxicology in October 1973 at Kiel, Germany as:

"Ecotoxicology is a branch of Toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human) vegetable and microbial, in an integral context"

Ecotoxicology separates itself from classical toxicology by concentrating on the macro effects of chemicals to the populations of ecosystems as opposed to toxicology's concentration on individual chemical effects on individual organisms. Through this role of identifying toxic chemicals and the concentrations at which these chemicals become toxic ecotoxicology has been able produce large databases which in turn have helped regulators limit the presence of chemicals in the environment (van Straalen, 2003).

Sources of ecosystem toxins vary widely from industrial processes, to wastewater treatment, to household waste. Each source will have its own physical and chemistry characteristics. Identification of the nature and source of a pollutant is necessary to gaining a greater understanding of the risk posed to the environment. Pollutants can enter the environment through three states: air, soil and water. For this project concentration is paid to pollutants entering surface and ground waters as these are the likely entry points for landfill leachate (Baun *et al*., 1999).

Toxicity testing using species that represent the trophic levels of an ecosystem is used to predict the effects that pollutants will have on the environment. Whole effluent toxicity testing (WET) is used to characterise and measure the aggregate impact of industrial effluents. WET does not attempt to predict the impact to an entire ecosystem but studies have shown that the results are helpful in predicting the overall impact of effluent discharge (Frithsen *et al*., 1988). This type of testing is ideal for wastewaters from a number of sources e.g. sewage treatment and paper mill effluents. WET has become a standard for describing and understanding the causes of toxicity within these types of effluents. WET measures the responses of organisms to the effluent in question and from this a toxicity can be calculated and used to to compare effluents. This tool allows for

regulators to set limits for effluent discharges and it also allows operators to understand the hazard that their effluents have on the environment.

Two types of WET are encountered regularly within the literature:

- Acute toxicity testing
- Chronic toxicity testing

Further subdivisions of these types of test are encountered e.g. whether the effluent of interest is the same throughout testing or is replaced at a certain intervals of time. For this project static acute toxicity testing was carried out due to the low cost of such tests. Acute in this context means fast acting and it is only these types of toxicants that this testing highlights. This can be considered a disadvantage of this type of testing as many chemicals are not instantly lethal and require time to bioaccumulate within an organism before becoming lethal (Isidori *et al*., 2003). The main advantages of acute toxicity testing are that the endpoint of the testing is easy to quantify i.e. is the test candidate are immobilised or die at a given concentration or not.

Within the literature there is a growing trend of more chronic toxicity testing being reported (Bloor *et al*., 2006). This type of testing aims to highlight toxicity that is slow to cause harm. Chronic flow-through toxicity testing is very expensive and requires a great deal of investment in equipment and test candidates. For example, at Buckden landfill leachate treatment plant a chronic flow-through test of treated landfill leachate was conducted with rainbow trout that cost £1,100 a month. Chronic testing was considered in this project but the cost and time implications meant that it was never possible to carry out.

A number of conditions need to be met if toxicity test results are to be considered valid. Temperature needs to be fixed and noted throughout the testing with the solutions at 20°C ± 2°C. Survival of the control candidates needs to be >90% at the end of the test. The age of the test candidates before testing needs to not exceed:

- 14 days for fish
- 24 hours for Daphnids

Determining the toxicity of an effluent using an acute test is a relatively simple procedure to carry out. The test species is selected with each species having a specific test time e.g. 15 mins for Microtox™ (ISO, 1998). A dilution series is then designed so that the effect being tested for exists between two points. In most cases the dilutions series is {OECD, 1998 #97}:

0, 6.25, 12.5, 25, 50, 100%

Toxicity is generally reported as the lethal concentration (LC) that causes 50% of the test candidates to exhibit the effect being recorded after the standard testing time e.g. 50% of the fish have died after 96 hours (OECD, 1992). From these data an LC_{50} is presented which allows comparisons between different effluents to be made. In some tests it is impossible to determine whether an organism has died e.g. *D. magna* or the investigator is determining a decrease in activity e.g. light emission from Microtox test. For these types of tests the effect is presented as an effective concentration (EC_{50}).

Toxicity of individual chemicals is the preserve of classical toxicology. Determining the concentration that a substance causes the test candidate to exhibit an effect has allowed researchers to build libraries of chemical-effect datasheets (Lloyd, 1987). Studies with mixtures of chemicals have tended to be conducted with binary mixtures (Rosal *et al*., 2010). When more than one chemical is present there is a possibility that the toxicity of one substance can modify the toxicity of the other and so affect the overall toxicity of the solution. This modification can either be an increase in toxicity (additive), toxicity of the solution remains unchanged (synergistic) or the toxicity is actually reduced (antagonistic). A simple equation can be used to determine whether a mixture of substance A and B fulfils either of these conditions:

$$xTU_A + yTU_B = 1TU_{(A+B)}$$

Where TU = Toxicity units which is defined as $(1/EC_{50})$ x 100.

These types of joint action are reliant on the values of *x* and *y* fulfilling the following conditions (Lloyd, 1987):

Values for x and y	Types of joint action
x and $y > 1.0$	Antagonistic
x and $y < 1.0$	
$x + y > 1.0$	Less than additive
$x + y = 1.0$	Additive
$x + y < 1.0$	More than additive (synergistic)

In complex mixtures like landfill leachate it is almost impossible to determine the individual interactions between each substance. To overcome such a situation a more holistic view of toxicity is required. This is done by calculating the percentage dilution where a exhibited effect on the test candidates is noted e.g. an EC_{50} of 33% requires there to be a three fold dilution of the sample.

A wide variety of toxicity tests are routinely encountered in the literature. At present the Environment Agency and the Scottish environmental protection agency recommend 7 types of bioassay for monitoring watercourses (Environment Agency 2002) (see Table 2.3). The selection of toxicity tests used attempts to cover the various trophic levels present in aquatic environments. This battery of tests could change in the near future due to an ongoing consultation on which tests remain in the guidelines and which tests should be discarded (UKEA document H1, 2007).

Table 2-3: Toxicity tests specified by the Environment Agency and SEPA for water quality monitoring (Johnson *et al*., 2004)

Test name	Type of organism	Environment type	Trophic level	Test time
Vibrio fischeri	Bacteria (Microtox)	Fresh water and marine	Primary producer	15-30 mins
Pseudokirchneriella subcapitata	Algae (Green algae)	Fresh	Primary producer	72 hours
Daphnia magna	Crustacean (Water fleas)	Fresh water	Primary consumer	24-48 hours
Tisbe battagliai	Crustacean (Copepod)	Marine	Primary consumer	48 hours
Crassostrea gigas	Crustacean (Oyster)	Marine	Primary consumer	24 hours
Oncorhynchus mykiss	Fish (Rainbow trout)	Fresh water	Secondary consumer	96 hours
Scophthalmus maximus	Fish (Flatfish, flounder)	Marine	Secondary consumer	96 hours

Toxicity assessment of landfill leachate, both treated and raw, is necessary to monitor and assess the impact that leachates exert on the environment. WET testing is generally more effective than chemical analysis as it demonstrates the complete hazard posed by a sample whereas individual chemical analysis fails to show the complete profile of chemical interactions that can take place in the complex matrix that is landfill leachate. Many different methods focus on the different trophic levels of aquatic environments. Assessment of the different trophic levels is needed as toxic substances affect the producers and consumers of aquatic environments in a variety of ways. Numerous tests are

now commercially available in easy to use pre-packaged kits. A major advantage of these commercial test kits is the reproducibility of results between labs (Johnson *et al*., 2004).

2.3 Rationale for the review

At present, there is little agreement on the types of test and the number needed to assess accurately the toxicity of landfill leachate both treated and raw. This review sets out to:

- Recommend possible improvements to the constituents of a battery of bioassays for the WET testing of landfill leachate.
- Demonstrate the difference in toxicity of raw and treated landfill leachates.
- Highlight the effects that treatments have on the chemical composition of leachates.
- Emphasise any links between toxicity and the chemistry of landfill leachates.
- Determine the relative sensitivities of each of the bioassays towards landfill leachate using Slooff's (1983) analysis.

2.4 Bioassays using bacteria

2.4.1 Luminescent bacteria; *Vibrio fischeri* and *Photobacterium phosphoreum*

The bacterium *V. fischeri* was first suggested as a suitable species for toxicity test over 20 years ago (Engebrecht *et al*., 1985). *P. phosphoreum* works in a similar manner to *V. fischeri* so is covered jointly here. Since then, *V. fischeri* has become a popular test for assessing toxicity. This has been attributed to the ability to get results quickly (5 minute test available) and the ease carrying out the test (Tonkes, 2005). The speed with which results are obtained led to a recommendation for on-site monitoring of discharges to watercourses in the UK (Johnson *et al*., 2004).

Bioluminescence within bacterial cells is controlled by the 5 gene system *lux*CDABE. The two gene system *lux*AB codes for the enzyme luciferase. Luciferase is made up of two

protein units; α and β, with the α unit being primarily responsible for the kinetics of bioluminescence (Meighen, 1993). Bacterial luciferase is the catalyst for the oxidation of the reduced flavin mononucleotide $FMNH_2$ (see Figure 2:1). In the presence of oxygen and a long chain fatty aldehyde, $FMNH_2$ is reduced and emits a blue-green light at 490 nm (Meighen and Dunlap, 1993)

Figure 2-1: Chemical structure of $FMNH_2$

The *V. fischeri* test has been standardised by ISO 11348-2 (ISO, 1998) for the assessment of water quality. Two experimental procedures exist for assessing toxicity with *V. fischeri*. Acute toxicity to the bacteria is achieved by exposure to a range of concentrations of the analyte. For confidence in the data, controls need to be conducted at the same time and also the use of triplicates is advised (Tonkes, 2005). Growth inhibition of *V. fischeri* can also be determined at the same time as acute toxicity. The test organisms are subjected to an exposure time of 7hr. After this period, growth is measured against the control group.

One limitation of *V. fischeri* was reported in the toxicity relationships of Co, Cd, Cu, and Zn in binary equitoxic mixtures (Fulladosa *et al*., 2004). The study was carried out with mathematical models and experiments. Fulladosa *et al.* (2004), were able to conclude that Cd had a lower toxicity towards bacterial cells when compared to data on mammalian cells. These results show that using *V. fischeri* by itself is not suitable to determine the

effect of effluent discharge e.g. another species needs to be included. This deficiency has been identified by many authors for the use of *V. fischeri* in toxicity screening (Isidori *et al*., 2003, Johnson *et al*., 2004) The use of different organisms with different susceptibilities has been identified as being more suitable by the EU (Umweltbundesamt, 1997).

Devare and Bahadir (1994) reported that *P. phosphoreum* showed little sensitivity to leachates collected from 2 MSW sites and a mixed Industrial-MSW site in Germany (Table 2.4). Of interest was the biologically treated leachate from the mixed Industrial-MSW site showed no toxicity towards *V. fischeri.* Similar sensitivities were reported by Isidori *et al*. (2003) and Ward *et al*. (2002) when using *V. fischeri* as a test species. All the reports were with different strength leachates and all showed low toxicities towards *V. fischeri* (Rutherford *et al*., 2000). The results of Rutherford *et al*. (2000) show standard biological treatment in many cases reduces toxicity effectively so that the risk to the environment is greatly reduced.

Fan *et al*. (2006) carried out toxicity assessment with *V. fischeri* on three treated landfill's leachate from Taiwan. One landfill (Site C) was noted to show considerable toxicity towards *V. fischeri*, with a recorded toxicity of 5-33 TU. Elemental analysis and Fourier transform infrared spectroscopy of the landfill's leachate showed the sample to have a significant aromatic characteristic. This aromatic characteristic was attributed to the presence of phenolic substances and humic substances. Phenolic substances are a constituent of many toxicants found in landfills e.g. nonylphenols. The other two landfill leachates sampled showed no toxicity towards *V. fischeri*. Fan *et al*. (2006) concluded that the landfilling of mixed wastes might help reduce leachate toxicity because the toxicity is effectively diluted by the different wastes.

Table 2-4: Examples of landfill leachate toxicity to luminescent bacteria

Location	Test species	Physicochemical parameters (mg L^{-1})				Test time (mins)	EC_{50} results (%)	Toxicity units	Reference
		COD	NH_3	pH	Alk				
1. Braunschweig (Germany)	*P. phosphoreum*	2,740	-	7.9	-	30	35	0.3	Devare and Bahadir (1994)
2. Hannover (Germany)	*P. phosphoreum*	4,200	-	7.6	-	30	18	0.6	
3. Schwicheldt (UT) (Germany)	*P. phosphoreum*	2,975	-	8.0	-	30	-	-	
4. Schwicheldt (T) (Germany)	*P. phosphoreum*	61	-	8.0	-	30	0	-	
1. Casone A (Italy)	*V. fischeri*	520	270	8.7	-	30	41.7	2.4	Isidori *et al* (2003)
2. Casone B (Italy)	*V. fischeri*	2,500	400	8.9	-	30	58.9	1.7	
2. Uttaro (Italy)	*V. fischeri*	1,100	440	8.8	-	30	14.3	7.0	
1. July, Florida A (USA)	*V. fischeri*	1,850	-	7.5	6,213	15	15*	6.6	Ward *et al*., (2002)
2. July, Florida B (USA)	*V. fischeri*	636	-	7.0	2,407	15	58*	1.7	
3. July, Florida C (USA)	*V. fischeri*	351	-	7.2	1,494	15	78*	1.3	
4. July, Florida D (USA)	*V. fischeri*	1,165	-	7.5	3,238	15	45*	2.2	
5. July, Florida E (USA)	*V. fischeri*	857		7.7	2,503	15	17*	5.9	
6. July, Florida F (USA)	*V. fischeri*	12,245		7.6	5,500	15	82*	1.2	
Raw LL; Lubna, (Poland)	*V. fischeri*	1973*	567*	-	-	15	20	4^+	Slomczynska *et al*., (2004)
Coagulation treated LL; Lubna, (Poland)	*V. fischeri*	1973*	567*	-	-	15	25	4^+	
Ozonation treated LL; Lubna, (Poland)	*V. fischeri*	1973*	567*	-	-	15	50	3^+	
Ozone/Peroxide treated LL; Lubna, (Poland)	*V. fischeri*	1973*	567*	-	-	15	50	3^+	
Treated LL (Nov1993)	*V. fischeri*	65	2.8	6.6	-	15	>100	1.	Rutherford *et al*., (2000)
Station 1 (Nov 1993)	*V. fischeri*	<40	0.4	6.3	-	15	>100	1	
Station 2 (Nov 1993)	*V. fischeri*	<40	0.8	6.6	-	15	>100	1	
Control (upriver of discharge pipe (Nov 1993)	*V. fischeri*	75	0.14	5.4	-	15	>100	1	

LL=Landfill leachate; MSW= Municipal solid waste

2.4.2 Activated sludge respiration inhibition test (ASRI)

The ASRI test is based on respiration inhibition of microbial inocula due to exposure to toxic agents. This test has a standardised methodology and interpretation of results from the OECD 209 (OECD, 1993) and ISO 15522 (ISO, 1999). Activated sludge collected from the aerobic digesters of domestic sewage treatment works is recommended in the guidelines although activated sludge from any industrial wastewater treatment works may be used in its place e.g. activated sludge from the biological treatment of landfill leachate. This test measures the inhibition of the respiration of the inoculum by O_2 uptake or CO_2 respiration. The main advantage to this test method is the generation of results within 30 min and 3 hr (Narita *et al*., 2005). This test is mainly used for monitoring influent toxicity, particularly in STW although the test may be used for any type of test solution.

A comparative study between ASRI and Microtox™ with 5 common inorganic pollutants and 6 organic pollutants has been performed (Gutierrez *et al*., 2002). During the trials Gutiérrez *et al*. (2002), compared inocula collected from domestic sewage treatment and industrial water treatment. The authors concluded that Microtox™ was too sensitive and that ASRI was more suited to online STW monitoring of influents to reactors.

As a further development, carbon dioxide (CO_2) sensors were introduced for evaluating the inhibition to respiration produced by toxic agents (Chan *et al.*, 1999, Aivasidis *et al.*, 2002, Narita *et al.*, 2005). Previous studies indicated the problems in the size of O_2 meters and the need for reference cells which take up a great deal of space. CO_2 meters on the other hand may be miniaturized and offer quicker turnover of results. Narita *et al*. (2005) particularly commented on the higher sensitivity of the CO_2 compared with the O_2 meter approach.

The activated sludge test was used to compare the toxicity reduction capabilities of a number advanced oxidation processes (Cotman and Gotvajn, 2009). The authors collected leachate samples from a landfill that received waste from Europe's biggest pig tannery complex. This

sample was highly polluted with many volatile organics, ammoniacal-nitrogen >2300 mg/L and BOD > 650 mg/L. In a comparison with *D. magna, V. fischeri* and the activated sludge test was found to be the least sensitive to a particularly toxic sample. The lower sensitivity is attributed to bacteria metabolism being less affected by pollutants due to the simplier energy conversion routes {Russell, 1999 #391}.

Using the same techniques, a similar study from the same research group on the ozonation of leachates collected from a municipal landfill in Slovenia has recently been reported (Žgajnar Gotvajn *et al*., 2009). Conversely the activated sludge technique test recorded a EC_{50} of 0.1% compared to the 48 hr *D. magna* EC_{50} of 8.0%. Unfortunately the authors did not attempt to explain the differences in the test results.

Using raw landfill leachate and wastewater samples from industrial processes the authors showed that two types of microorganisms are needed for accurate assessment of wastewater toxicity i.e. heterotrophic and nitrifying bacteria (Žgajnar Gotvajn and Zagorc-Končan, 2009). Without using two species there are issues with interference with the respiration of the test bacteria and this can cause false negative results (Žgajnar Gotvajn and Zagorc-Končan, 2009).

2.5 Toxicity testing using green algal species

2.5.1 Pseudokirchneriella subcapitata (aka *Selenastrum capricornutum)*

As primary producers green algae are a key indicator to the health of aquatic environments. Effects of effluent discharges on the growth and reproduction of green algae is useful, as seen in eutrophication of watercourses due to the surplus of nutrients associated with industrial discharges. The green algae growth inhibition test has been standardised by ISO 8692 (ISO, 2004) and OECD 201 (OECD, 2006).

The test is carried out on unicellular green cultures that have reached the exponential growth stage. The minimum testing period as advised by OECD 201 is 72 hr although longer test

times may be used. Average specific growth rate and the inhibition to growth are reported as EC_{50}. Values such as no observed effect concentration ($NOEC_{acute}$) and lowest observed effect concentration ($LOEC_{acute}$) are also reported. Measurements of these responses determine the lowest concentrations which are safe for discharge. These types of measurements are vital for building a complete picture of dose response for pollutants in the environment.

Within the literature the use of *P. subcapitata* has become popular in the last 5 years. *P. subcapitata* has been packagedinto a commercial bioassay kit under the name Algaltoxkit F™. An investigation of the effects of 90 organic substances indicated that *P. subcapitata* was particularly sensitive to phenyls, aldehydes and alkenes (Tsai and Chen, 2007). High sensitivity is characteristic of chlorophyll based tests which shows the top end of organism responses to pollutants.

Table 2.5 shows selected data published by a number of authors on the toxicity of landfill leachates to different algal species. A general trend in the data is that green algae are very sensitive to the composition of raw leachate samples (Bernard *et al*., 1996). In one report ammoniacal-nitrogen does not seem to play a significant role in the toxicity of the leachates, with the highest ammoniacal-nitrogen concentration scoring the lowest toxicity (Baun *et al*., 2004).

Table 2-5: Examples of landfill leachate toxicity to green algae species.

Location	Test species	Sample type	Sample info	Physiochemical parameters (mg L^{-1})				Test time (hrs)	EC_{50} results (%)	Toxicity units	Reference
				COD	NH_3	pH	Cl				
Finland	*P. subcapitata*	Raw MSW LL	Fresh leachate	340-920	110–220	7.1–7.6	-	72	22.7	4.4	Marttinen *et al*., (2002)
France	*P. subcapitata*	Chemical industry effluent treated sludge (B_2)	1994	355	-	10.2	-	72	1.8	55.6	Lambolez *et al*., (1993)
		Fly ash from incineration of MSW (I)	1994	266	-	11.7	-	72	5.4	18.1	

Location	Test species	Sample type	Sample info	Physiochemical parameters (mg L^{-1})				Test time (hrs)	EC_{50} results (%)	Toxicity units	Reference
				COD	NH_3	pH	Cl				
		Paint waste	1994	670	-	7.6	-	72	3.1	32.3	
		Contaminated materials	1994	43	-	7.5	-	72	74.1	1.3	
Denmark	*P. subcapitata*	Raw MSW LL	Forlev	-	860	7.0	18,400	72	6.1	15.4	Baun *et al*., (2004)
			Sandholt Lyndelse	-	546	8.0	2,730	72	3.8	26.3	
			Højer	-	623	7.1	2,560	72	2.5	40.0	
			Skovsted	-	340	7.7	3,770	72	3.8	26.3	
			Logstor	-	340	7.7	4210	72	2.9	34.4	
			Esbjerg	-	546	7.0	3470	72	6.5	15.4	
			Grindsted	-	104	6.7	126	72	2.2	45.5	
			Vejen	-	154	6.7	472	72	9.4	10.6	
			Sorup	-	205	7.1	606	72	6.8	14.7	
			Arnitlund	-	110	6.9	195	72	3.3	30.3	
25 landfills, France	*S. subspicatus*	Raw MSW LL	Site L1a	-	-	-	-	120	8.3	12.0	Bernard *et al*., (1996)
			L3	-	-	-	-	120	1	100.0	
			L4	-	-	-	-	120	7.7	13.0	
			L9b	-	-	-	-	120	8.5	12.0	

LL=Landfill leachate; MSW= Municipal solid waste

Algaltoxkit F™ and a number of other toxicity tests were used to assess the toxicity of a river that receives discharges from two STW (Latif and Licek, 2004). Only the test Thamnotoxkit F™ (see Section 5.2.1) was more sensitive to this effluent. Interestingly the other three bioassays; *D. magna*, *Tetrahymena thermophila*, and *Heterocypris incongruens*, that were used in the battery of tests showed no signs of toxicity.

Other studies with *P. subcapitata* concentrated on the effects of heavy metal toxicity. Chromate was a particularly potent inhibitor to the growth rate of the cultures within the first 24 hr and this continued to 72 hr (Labra *et al*., 2007). Copper has been shown to affect the brood sizes of *D. magna* when fed with *P. subcapitata* grown in Cu solutions (De Schamphelaere *et al*., 2007). These results highlight the concerns that dietary heavy metal uptake in organisms is of particular concern to the health of watercourses.

2.6 Toxicity testing with invertebrates

2.6.1 Daphnia magna

The first published studies using *D. magna* as a test species for assessing pollution in watercourses appeared in 1937 (Ellis, 1937). *D. magna* are native to most of the world's freshwater systems and are considered as primary consumers within watercourses. Their primary food is algae. Over the past 30 years the genus *Daphnia* emerged as the most suitable invertebrate species for toxicity testing due to their sensitivity and ease of use (Selivanovskaya *et al*., 2004). The test using *D. magna* is based upon the immobilization of this species. The testing method was internationally standardised by OECD 202 (OECD, 2002) and recommended by the Environment Agency, SEPA and most other national environment authorities (Persoone, 2000).

A genetically modified luminescent *D. magna* has been developed and is marketed as IQ™ Fluotox-test. Toxic substances inhibit the reduction of a fluorometric substrate by galactosidase (Hayes *et al*., 1993). When irradiated with ultraviolet light the *D. magna* will glow. The glow is produced when an enzyme used in the metabolism of a fluorescent marked sugar is damaged. The 1 hr test time is quicker than the 24/48 hr needed for a standard *D. magna* test but the IQ™ Fluotox-test comes at a higher price. The IQ™ Fluotox-test was used in conjunction with the standard *D. magna* test (Slomczynska *et al*., 2004). The IQ™ Fluotox-test produced similar EC_{50} results as the conventional *D. magna* test when testing landfill leachate samples. Unfortunately, this system is more expensive (£87 more expensive) than the standard Daphtoxkit F marketed by SDIX, U.K.

Table 2.6 shows the results of toxicity testing of landfill leachates with *D. magna*. There is a large degree of variability between the results with the EC_{50} 1.1-58.8% with the lowest value for raw leachate and the highest for a treated sample. Jurkoniene *et al*. (2004) reported from 4 sites over three years. Over this period, there was a noticeable lowering in toxicity of the

landfill leachate samples e.g. site A reduced the EC_{50} from 23 to 65% over the course of sampling (Jurkoniene *et al*., 2004). One possible explanation for the disparity in results is seasonal variations. Winter samples may be more diluted due to rainfall than samples collected in summer months, so a winter sample may be less toxic than a summer one. Baun *et al* (1999) demonstrated that toxicity reduction of leachates was possible the further away the sample comes from the operational centre. This implies that soil is able to mitigate the toxicity of the samples through the binding capacity of humic substances.

D. magna toxicity has been shown to remain even after most COD had been removed from raw landfill leachate sample with Fenton oxidation e.g. an EC_{50} of 34% (Goi *et al*., 2010). Marttinen *et al* (2002) reported that the treatment of low strength leachates with ozonation, nanofiltration and air stripping reduced the concentration of COD by ≥60% and ammoniacal-nitrogen by ≥27%. All of these methods failed to reduce the toxicity of the leachate samples as effectively as biological treatment. Influent EC_{50} toxicity ranged from 3-29% and after treatment the maximum reduction in toxicity was ~20%. In comparison biological treatment of leachates reduces toxicity to >50%. These advanced processes should only be considered as secondary treatment options.

Isidori *et al*. (2003) (Table 2.6) reports some interesting results based on the pH of the leachate. The toxicity of each landfill leachate is significantly changed by increasing or decreasing the pH of the leachate. This was attributed to the bioavailability of certain substances at different pH values i.e. divalent cationic metals, ammoniacal-nitrogen, and apolar substances. The role of the toxicity identification evaluation (TIE) procedure in understanding the causes of toxicity in landfill leachate can be helpful to researchers. Many of the toxicants within landfill leachate are sensitive to alterations in the pH of samples. Exploiting this characteristic can help operators understand and reduce the toxicity of their effluents.

Lambolez *et al*. (1994) reported a significant difference in toxicity to *D. magna* depending on the type of waste landfilled (Table 2-6). Data suggested that the toxicity of the chemical industry effluent sludge and incineration ash might be attributed to the presence of metals and salts of metals. It was concluded that it would be "very difficult" to predict the toxicity of a leachate based on the types of waste landfilled and the chemical composition.

Table 2-6: Examples of landfill leachate lethal toxicity to *D. magna.*

Location	**Sample type**	**Sampling information**	**Physiochemical parameters (mg L^{-1})**				**Test time (hrs)**	**EC_{50} results (%)**	**Toxicity units**	**Referencne**
			COD	**NH_3**	**pH**	**Con**				
Kairiai, Lithuania	Treated MSW LL	**2001**								Jurkoniene *et al*. (2004)
		Site A	-	-	-	-	48	23	4.3	
		Site B	-	-	-	-	48	10%	10.0	
		Site C	-	-	-	-	48	25	4.0	
		Site D	-	-	-	-	48	0	0	
		2004								
		Site A	-	-	-	-	48	65	1.5	
		Site B	-	-	-	-	48	37	2.7	
		Site C	-	-	-	-	48	40	2.5	
		Site D	-	-	-	-	48	-	-	
France	Chemical industry effluent treated sludge	**1994**	355	-	10.2	-	24	2.9	34.4	Lambolez *et al*. (1994)
	Incineration ash of MSW	**1994**	266	-	11.7	-	24	16.3	6.1	
	Paint waste	**1994**	670	-	7.6	-	24	40.1	2.5	
Lubna, Poland	Treated MSW LL	**Sample 1**	1973*	567*	-	-	48	4.5	22	Slomczynska *et al*. (2004)
		Sample 2	1973*	567*	-	-	48	22.2	4.5	
		Sample 3	1973*	567*	-	-	48	3.1	32	
Vejen and Grindsted, Denmark	Raw Ground water polluted with LL	**V1**	-	-	6.3	22.1	48	30*	3.3	Baun *et al.* (1999)
		V2	-	-	6.4	21.8	48	40*	2.5	
		G1	-	-	6.5	25.8	48	60*	1.7	
Casone (C) and Uttaro (U), Italy	Raw MSW LL	**C pH 8**	2500	730	8.7	-	48	1.2	85	Isidori *et al.* (2003)
		C pH 3	2500	730	3	-	48	1.4	69	
		C pH 11	2500	730	11	-	48	0.5	18	

Location	Sample type	Sampling information	Physiochemical parameters (mg L^{-1})				Test time (hrs)	EC_{50} results (%)	Toxicity units	Referencne
			COD	NH_3	pH	Con				
		U pH 8	1150	440	8.8	-	48	1.2	83	
		U pH 3	1150	440	3	-	48	2.1	48	
		U pH 11	1150	440	11	-	48	1.7	58	
Canada	Treated MSW LL	**Treated LL (Nov1993)**	65	2.8	6.6	-	96	100	1 .	Rutherford *et al*. (2000)
		Station 1 (Nov 1993)	<40	0.4	6.3	-	96	100	1	
		Station 2	<40	0.8	6.6	-	96	100	1	
		Control (upriver of discharge pipe	75	0.14	5.4	-	96	100	1	

LL=Landfill leachate; MSW= Municipal solid waste

2.6.2 Ceriodaphnia dubia

A bioassay using the invertebrate *C. dubia* is becoming a common method for assessing adverse effects on reproduction and survival of aquatic species (Dave and Nilsson, 2005). This testing system has been standardised by the United States Environmental Protection Agency (USEPA) (USEPA, 1994). One of the main advantages of this test method is the shorter reproduction time of 3-5 days of *C. dubia* compared with 6-10 days of *D. magna* which makes it easier to culture (Tonkes, 2005). The test is commercially marketed under the name Ceriodaphtoxkit F™ by SDIX, UK. This test is routinuely used as a standard WET test in the USA though its use is less widespread in other parts of the world.

The test is carried out in a similar manner to that of *D. magna*. The test candidates are exposed to at least five concentrations of the test solution, as well as controls. Analysis is made by comparison between candidates and control group reproduction or survival rates. After testing it was concluded this test was suitable 'for overall testing' although native species were felt to be more suitable for testing in New Zealand (Ruck *et al*., 2000).

Dave and Nilsson (2005) reported the use of *C. dubia* as a bioassay of landfill leachate. Studies showed that toxicity of leachate decreased significantly after treatment with activated sludge bacteria. The decrease was from four to one toxicity units after 13 days of treatment

with activated sludge. Ward *et al*. (2002) concluded that *C. dubia* was the more sensitive test for bioassays using landfill leachate samples from Florida, USA. In the report, Ward *et al*. (2002) formed a battery of tests with Microtox™, *S. capricornutum* and *C. dubia* . A EC_{50} toxicity range of 3.3-10% (estimated from graphs) was found thus showing the high sensitivity of the test species to landfill leachate.

2.6.3 Brachionus calyciflorus

Rotifers are named after the cilia that line their mouths that resemble a wheel during feeding. A commercial test kit is available under the name Rotoxkit F™ by SDIX, UK. This test kit is based on *B. calyciflorus* and is available in an acute 24 hr test kit and a 48 hr short term chronic test kit. Cysts are provided with the test kits and hatched when testing is needed. One limit of this test is the need to filter samples before testing is carried out (Rojickova-Padrtova *et al*., 1998). *B. calyciflorus* has been used in a limited number of studies for the assessment of toxicity of leachates (Bernard *et al*., 1996; Isidori *et al*., 2003).

Bernard *et al*. (1996) used *B. calyciflorus* as part of a battery of tests for assessing the toxicity of 27 landfill leachate samples from 14 sites. Two statistical tests were used for the assessment of the sensitivity of each bioassay and which combination is most suitable for a battery of tests. Analysis of each test was made using Slooff's number analysis (Slooff, 1983) method for determining sensitivities of test methods. To calculate the sensitivity of each test the arithmetic mean of all test results, is divided by $E(L)C_{50}$ of each test carried out. Bernard *et al*. (1996) concluded that *B. calyciflorus* was one of the least sensitive tests available. To select the most suitable tests to combine into a battery Bernard *et al*. (1996) used Principal Component Analysis on 18 samples. Using this method it was concluded that a bacterial assay, a protozoan test and another using a higher species were needed to form a complete test battery. This conclusion is in agreement with that of other agencies (USEPA, 1985, Johnson *et al.,* 2004).

Isidori *et al*. (2003) used *B. calyciflorusi* to determine the toxicity of three landfill leachates at sites that had been closed for different periods of time. The authors used a TIE procedure described by Norberg-King *et al.* (1991). Data indicated that *B. calyciflorus* was the second most sensitive test species to the range of pH values. This species was particularly sensitive to higher pH values i.e. pH>8.7. This indicated that it was a basic compound that was responsible for toxicity e.g. ammonia.

2.6.4 Artemia salina

Artemia salina, otherwise known as sea monkeys, has proved to be a suitable candidate for the assessment of toxicity of effluents and chemicals. This species is found within inland salt-water lakes but not the ocean. Due to adaptations to their local environment this species is essentially resilient to high saline solutions.

Svensson *et al*. (2005) collected raw landfill leachate samples from Kristianstad landfill, Sweden. The raw leachate had a low toxicity of EC_{50} of 91.2%. These can be considered as weak leachates when compared with other reported leachates, e.g., EC_{50} of 1.7% (Isidori *et al*., 2003) and EC_{50} of 14.0% (Wong, 1989). Five different treatment methods were performed on the sample: chemical oxidation, ozonation, bioreactor, peat geofilter, and peat-ash geofilter. The bioreactor reduced the EC_{50}toxicity to 100% and the peat-ash geofilter to 90.9%. The other treatment technologies had little effect on reducing the toxicity of the samples.

In a bid to better understand the types of substances causing toxicity, a number of tests were performed by Svensson *et al.* (2005). Two landfills were sampled for these tests: Kristianstad landfill and Siauliai landfill, Sweden. Two manipulations were performed to isolate the cause of toxicity. By passing a sample through an ion-exchange membrane, ammoniacal-nitrogen and heavy metals were removed from the samples. This manipulation removed all toxicity from both samples. The second manipulation carried out was passing a sample from each

landfill through activated carbon. This step allows heavy metals and ammoniacal-nitrogen through while removing the organic fraction (Svensson *et al*., 2005). This step had little effect on removing toxicity from the Siauliai sample but removed approximately 50% of toxicity from the Kristianstad samples. It was concluded that the majority of the toxicity of these samples was produced by ammoniacal-nitrogen and ammonium as the heavy metal concentration was not high enough to produce a toxic response. This conclusion on the toxicity of ammoniacal-nitrogen is in agreement with Bernard *et al*. (1996) and Wong (1989).

A comparison between coagulation/flocculation, ozonation, and membrane filtration, for their ability to reduce toxicity towards *A. salina* has been reported (Silva *et al*., 2004).From a battery of 4 tests, *A. salina* showed little sensitivity toward the raw landfill leachate sample. Treatment with the 3 methods significantly reduced the toxicity of the samples toward *A. salina*. The other species used in the report showed a lower reduction in toxicity after treatment, with only the ozonation samples becoming more toxic toward *V. fischeri*.

Another comparison of this test compared to *D. magna* has been made using leachates collected from municipal site in Brazil (Bortolotto *et al*., 2009). Raw and treated leachates were collected for this testing. *A. salina* produced a 50% lower response to the raw and treated leachates compared to *D. magna*. This lack of sensitivity was due to *A. salina* being able to tolerate high salinities which were a characteristic of these leachates. This is due to *A. salina* being a marine species tolerant of highly salinity.

2.7 Toxicity testing on fish

Fish are considered a reliable indicator of the health of watercourses. The use of *O. mykiss*, rainbow trout, for acute toxicity testing is advised by the USEPA and the UK Environment Agency (Johnson *et al*., 2004). A similar test with different species which are more representative of local environments is advised by different regulatory agencies e.g. *Oryzias*

latipes, Japan and USA (Tonkes *et al*., 2005); *Salmo gairdneri*, Canada (Atwater *et al*., 1983); *Sarotherodon mossambicus*, Hong Kong (Wong, 1989).

The test based on *O. mykiss* has a defined procedure from the OECD 203 (OECD, 1992). OECD 203 (OECD, 1992) gives instructions for the testing of 7 fish species e.g. *Cyprinus carpio*, *O. latipes* and *Pimephales promelas.* Guidelines require groups of 7 fish to be used in the testing regime. The fish are kept in tanks with a light source for 12-14 hr per day and are maintained at a defined temperature range. The fish are exposed to the test solution for a period of 96 hr. At intervals of 24, 48 72 and 96 hr mortalities (LC_{50}= lethal concentration) of the fish are measured. LC_{50} is determined due to the ease of which the operator can determine death in fish. Controls must be used in the testing regime for validity.

O. mykiss have been used to test susceptibility to endocrine disruptors, organophosphates and heavy metal pollution. A study on the effect of estrogenic substances concluded that immature male rainbow trout were more susceptible than the adults to the adverse effects of these types of substances, with marked effects on genital growth recorded (Gibson *et al*., 2005). In a toxicity screening test on the River Esk, U.K. using *O. mykiss*, Johnson *et al*. (2004) found the test fish to be resilient to the discharge from the Langholm STW. Further investigation found organophosphates (diazinon and propetamphos) as the major contributor to toxicity (Johnson *et al*., 2004).Testing has shown that *O. mykiss* were more resilient to the heavy metals Cu, Zn and Cd when compared with *Cottus bairdi* (Besser *et al*., 2007). In another report it was concluded that *O. mykiss* was sensitive to Ag due to ingestion through the gills (Webb and Wood, 1998).

A longer test for effluent toxicity was also developed using *O. mykiss*. Test duration of 28-32 days is advocated in flow through conditions within the holding tank. This type of testing was employed at Buckden leachate treatment plant on the request of the Environment Agency for reassurance on the quality of discharges to the River Ouse (Robinson and Barr, 1999). After

12 months of no toxicity response in the test candidates, the Environment Agency decided that the Microtox™ test regime might be used as a cost effective and more rapid assessment method (Robinson and Barr, 1999). The high cost of the fish and the results indicating that the new treatment works was effectively treating the leachate meant that the test no longer was best practice when an alternative was suitable.

Atwater *et al*. (1983) (Table 2.7) used *S. gairdneri* as the fish species for testing the toxicity of 7 landfill leachates in Canada. The data collected on *S. gairdneri* showed the candidates were particularly sensitive to the leachate pH. A pH change from 7.0 to 5.0 produced a 100 fold increase in the toxicity of a leachate sample to the test candidates (Atwater *et al*., 1983). Osaki *et al*. (2006) reported a treated landfill leachate sample with an EC_{50} of 100%. This toxicity was linked to the low COD concentration of 70 mg/L in the treated leachate and 7.6 mg/L in the treated leachate (Osaki *et al*., 2006). Wong (1989) reported a high toxicity of a leachate sample from March (dry season in Hong Kong). A EC_{50} toxicity of 1.4% was linked by Wong (1989) to the high ammoniacal-nitrogen concentration of 1,621 mg/L.

Table 2-7: Examples of landfill leachate toxicity to fish species.

Test species	Location	Sample type	Sample info	Physiochemical parameters (mg L^{-1})				Test time (hrs)	LC_{50} results (%)	Toxicity units	Reference
				COD	NH_3	pH	Alk				
S. gairdneri	Canada	Raw MSW LL	**Site 1**	-	-	-	-	96	24.8*	4.0	Atwater *et al*., (1983)
			Site 2	-	-	-	-	96	11.6*	8.6	
			Site 3	-	-	-	-	96	17.0*	5.9	
			Site 4	-	-	-	-	96	46.3*	2.2	
			Site 5	-	-	-	-	96	21.8*	4.6	
			Site 6	-	-	-	-	96	3.6*	27.8	
			Site 7	-	-	-	-	96	7.7*	13.0	
O. latipes	Okayam, Japan	MSW LL	**Raw**	79	-	7.7	-	72	53	1.9	Osaki *et al*., (2006)
			Treated	7.6	-	7.8	-	72	100*	1.0	
S. mossambicus	Hong Kong	Raw MSW LL	**March**	-	1053	8.0	3345	96	1.4	71.4	Wong (1989)
			July	-	256	7.8	2627	96	12.0	8.3	
O. mykiss	Canada	Treated MSW LL	**Treated LL (Nov1993)**	65	2.8	6.6	-	96	100	1.	Rutherford *et al*., (2000)
			Station 1 (Nov 1993)	<40	0.4	6.3	-	96	100	1	
			Station 2 (Nov 1993)	<40	0.8	6.6	-	96	100	1	
			Control (upriver of discharge pipe (Nov 1993)	75	0.14	5.4	-	96	100	1	

LL=Landfill leachate; MSW= Municipal solid waste Toxicity testing with plant species

2.7.1 Toxicity testing with Lemna species

Plants are a key part of many organism's diet and the impact landfill leachate has on their growth is important. *Lemna gibba* and *L. minor*, commonly known as duckweed, have been standardised for testing by OECD 221 (OECD, 2006).

The recommended test procedure is to expose different plants with known characteristics to 5 different concentrations plus controls. After a 10 day test period the $E(I)C_5$ (IC_{50} the half maximal inhibitory concentration and is a measure of the effectiveness of a compound in inhibiting biological or biochemical function), $E(I)C_{50}$, $E(I)C_{90}$, LOEC and NOEC are

calculated. The parameters that are measured include mortality, frond growth, frond mortality, chlorophyll content and frond florescence (OECD, 2006).

Toxicity of landfill leachates to *L. minor* was assessed with a total of 10 sites sampled (Table 2.8) (Clement and Bouvet, 1993). The leachate was collected at various stages of treatment with both raw and treated leachates being assessed. This was seen in frond number (ΔN), dry weight (ΔDW), discolouration of fronds and break up of colonies. At low concentrations there was a marked increase in ΔN and ΔDW. This increase was thought to be produced by an increase in nutrients available to the plants. As the concentration is increased, all test plants showed a decrease in ΔN and ΔDW leading to rise in the $E(I)C_{50}$ values. It was not possible to link these changes to the physicochemical parameters of NH_3, alkalinity and conductivity.

A further investigation used a step wise multiple regression model (see below) (Clement and Merlin, 1995). From this model it was concluded that NH_3 and alkalinity were the main causes of toxicity to plants from leachate at pH 8.

$$EC_{50}(\Delta N) = -23.1\log(ALK) - 8.9\log(NH_4^+) + 78.3$$

Clement and Merlin (1995) then tested this model with experiments. It was found that that NH_4 was non-toxic at 148 mg/L but was toxic to the plants at 372 mg/L. At pH 8, bicarbonate is the major contributor to alkalinity. It was shown that it was not until levels of 4,096 mg/L were reached that bicarbonate become toxic to the test plants. Results from other treatment works show that effluent alkalinity is not normally within the range of being toxic to *L. minor.*

Mackenzie *et al*., (2003) conducted trials using three wild specimens of *L. minor* (W1, W2, W3) and one commercial cultivated species (C1) of *L. minor*. One sample of *L. minor* collected from the wild was collected from an area close to a landfill (W3 in table 2.8). At a treated leachate concentration of 11% all samples showed a decline in growth, with a 45%

concentration of leachate producing a growth rate of zero. The sample collected from close to a landfill showed the least sensitivity to the concentration of leachate. Evidence of *L. minor's* ability to become tolerant to organic substances and heavy metals has been reported previously (Cowgill *et al*., 1991, Van Steveninck *et al*., 1992). This development of tolerance is an important consideration when assessing the impact of discharges to the environment. Overall this is positive for the health of the environment as it demonstrates that over time the negative impact of leachate discharge is lessened.

Devare and Bahadir (1993) conducted trials with *L. minor* on treated and raw landfill leachate samples collected from 5 sites. It was reported that for raw leachate samples, a 10% concentration produced a 100% inhibition on the growth rate. A treated leachate sample had only a 20% inhibition effect at 100% concentration on the growth of the test plants. These are important findings as they show that treatment can greatly reduce the toxicity of raw landfill leachate samples but that there still remains a degree of toxicity in the samples following treatment.

Table 2-8: Examples of landfill leachate toxicity to *L. minor*.

Test species	Location	Sample type	Sample info	Physiochemical parameters (mg L^{-1})				Test time (hrs)	EC_{50} results (%)	Toxicity units	Reference
				COD	NH_3	pH	Alk				
L. minor	France	MSW LL	**1***	3320	564	8.24	3620	120	5.2	19.2	Clement and Bevare (1993)
			2	349	158	7.81	585	120	29.6	3.4	
			3	467	85	8.03	2465	120	23.1	4.3	
			4	798	516	8.35	3650	120	7.4	13.5	
			5	979	587	8.55	5610	120	3.8	26.3	
			6*	4886	384	8.20	4120	120	12.7	7.9	
			7*	1432	899	8.18	8470	120	5.5	18.1	
			8*	898	1350	8.13	2350	120	26.8	3.7	
			9*	4828	34	6.56	1070	120	34.0	2.9	
			10*	1998	825	7.13	495	120	25.7	3.9	
L. minor	E. Sussex U.K.	Treated MSW LL	**W1**	690	290	7.6	-	168	13.0	7.7	Mackenzie *et al.*, (2003)
			W2	690	290	7.6	-	168	19.4	5.2	
			W3	690	290	7.6	-	168	28.6	3.5	
			C1	690	290	7.6	-	168	18.4	5.4	
L. minor	Germany	Raw MSW LL	Braunschweig (R)	2,740	-	7.9	-	30	3*	33.3*	Devare and Bahadir (1994)
			Hannover (R)	4,200	-	7.6	-	30	6*	16.7*	
			Schwicheldt (R)	2,975	-	8.0	-	30	4*	25.0*	
			Schwicheldt (T)	61	-	8.0	-	30	-	-	

LL=Landfill leachate; MSW= Municipal solid waste; T= Treated; R= Raw

2.8 Genotoxicity

Genotoxicity testing utilises the DNA repair system of bacteria to determine the toxicity of pollutants. This system is initiated when DNA is damaged. The repair system is used to produce new DNA but is prone to errors. This error prone system has been utilised into a number of tests. Over 200 different tests for the assessment of genotoxicity have been recorded (De Maagd, 2000). Only three tests are currently internationally standardised by the OECD; Ames test, UmuC assay, and chromosomal aberration.

Tonkes *et al*. (2005) highlighted the following reasons for the importance of assessing the genotoxicity of industry effluents:

• Genotoxicity affects fitness and reproduction of organisms.

• Higher mutation frequencies increase the instability of ecosystems.

• Genotoxic substances might be relevant to humans when contaminated surface water is used downstream for other purposes e.g. agriculture, recreation, drinking water.

2.8.1 Ames test

This test is based on the growth of revertants to quantify the induction of the bacteria's error prone DNA repair systems (Cheng Vollmer and Van Dyk, 2004). Originally the test used 4 strains of *Salmonella typhimurium* whose genes were mutated such that without an external source of histidine the cells die (McCann *et al*., 1975). Substances that induce damage to the bacteria's DNA initiate the DNA repair system. The test makes use of mutations back to the wild state where the bacteria synthesize their own histidine. Cells that do not revert back to their wild state die.

In order to facilitate a faster uptake of agonists, the cell walls of the test bacteria are genetically altered to be more absorbent (Ames *et al*., 1973). A wide range of substances have been demonstrated to act as mutagens to these types of bacteria. A toxic response is due to chemical changes in the bases that make up the bacteria DNA. Changes in the structure and pairing combinations, when transcribed during DNA replication, result in cellular malfunctioning.

Lambolez *et al*, (1994) used strains TA97a, TA98, TA100 and TA102 of *S. typhimurium* during their Ames test of 15 leachates from MSW and industrial wastes (Table 2.9). In all the types of waste from MSW and industrial sources, except the leachate from paint waste, data showed TA98 to be most sensitive strain. Lambolez *et al*. (1994) were unable to make a firm link between the chemical characteristics of each landfill leachate sample and genotoxicity

observed. Evidence indicated that heavy metal content played a role in increasing the genotoxicity observed.

Schrab *et al*. (1993) used the TA98 strain of *S. typhimurium* to test treated leachates from 4 landfills in Texas, USA. None of the landfill leachates showed any mutagenic response to *S. typhimurium.* Three of the landfill leachates did however produce acute toxicity to the bacteria cells. Further analysis of the landfill leachate using GC found the samples to contain appreciable concentrations of substances that have been reported as mutagenic in the Ames test (Schrab *et al*., 1993). Data indicated that the acute toxicity of these substances was masking a genotoxic response. In two of the samples these responses were recorded at concentrations <1%.

Rutherford *et al*. (2000) (Table 2.9) also used the Ames test to assess the toxicity of treated landfill leachate. Samples were collected before discharge and after discharge to the Sackville River, Canada. The results showed that the treated landfill leachate showed signs of genotoxicity to the test species used which were viewed as inconclusive. This suggests more study needs to be done on treated leachate to confirm any adverse responses.

Table 2-9: Examples of landfill leachate genotoxicity towards *S. typhimurium.*

Test species	Location	Sample type	Date	Physiochemical parameters (mg L^{-1})				Mutagenesis detected	Strain sensitive to landfill leachate	Reference
				COD	NH_3	pH	Alk			
S. typhimurium	France	**Chemical industry effluent treated sludge**	-	355	-	10.2	-	Yes	TA98	Lambolez *et al*., (1993)
		Fly ash from incineration of MSW	-	266	-	11.7	-	Yes	TA98	
		Paint waste	-	670	-	7.6	-	Yes	TA97	
		Contaminated materials	-	43	-	7.5	-	Yes	TA98	
S. typhimurium	Canada	**Treated LL**	(Nov 1993)	-	-	-	-	No	-	Rutherford *et al*., (2000)
		Treated LL	(Dec 1993)	-	-	-	-	Yes	TA98	
		Station 1		-	-	-	-	No	No	
		Station 2	(Nov 1993)	-	-	-	-	No	No	
		Station 2	(Nov 1993)	-	-	-	-	Yes	TA98, TA100, TA102	
		Control (upriver of discharge pipe)	(Dec 1993)	-	-	-	-	Yes	TA 97, TA98, TA100, TA102	
			(Nov 1993)							
S. typhimurium	Denmark	**Vejen**	-	-	-	6.3	22.1	No	No	Baun *et al*., (1999)
		Grindsted	-	-	-	6.5	25.8	No	No	
S. typhimurium	Denmark	**Forlev**		-	860	7.0	18,400	Yes		Baun *et al*., (2004)
		Sandholt Lyndelse		-	546	8.0	2,730	No		
		Højer		-	623	7.1	2,560	No		
		Skovsted		-	340	7.7	3,770	No		
		Logstor		-	340	7.7	4210	No		
		Esbjerg		-	546	7.0	3470	No		
		Grindsted		-	104	6.7	126	No		
		Vejen		-	154	6.7	472	No		
		Sorup		-	205	7.1	606	No		
		Arnitlund		-	110	6.9	195	No		

LL=Landfill leachate; MSW= Municipal solid waste

2.8.2 umuC gene test

The *umu*C test differs to the Ames test in the chemical pathway utilised to produce a readable response. SOS genes are present within cells in order to correct mistakes that happen during DNA replication. Under normal conditions these are negatively regulated by the LexA repressor gene but can be activated by an accumulation of single stranded DNA due to replication fault in the action of DNA polymerase (Stryer *et al*., 2002). Upon exposure to mutagens a SOS gene response is produced in the replication of bacterial DNA. This response produces an increase in the concentration of ß-galactosidase that is measured by an integrated instrument. The test leads to increasing amounts of DNA single strands and oligonucleotides: O- and N-alkylation, adducts, depurinization, depyrimidation, deamination oxidative damage of DNA and DNA dimers (Wittekindt, 2000).

Baun *et al*., (1999a, 1999b, 2000) used the *umu*C test with landfill leachate samples and reported that of the 10 sites tested, only one site's samples produced a genotoxic response in the test species which indicates these test is very insensitive. These results seem unlikely as these were landfills that were still operational.

2.8.3 Toxicity testing with Escherichia coli

The Toxi-ChromoTest™ is a commercially available test kit (EBPI Inc., Brampton, Canada) that uses mutant *E. coli* as the test organism. The mutant *E. coli* has a highly permeable cell membrane which allows for rapid testing of a variety of substances (Persoone, 2000). The test measures the effect of substances on the synthesis of β- galactosidase. The ability of the *E. coli* to recover from stress is measured by a change in colour of the colony using a colorimeter. No testing on landfill leachate has been published in the literature although these tests have been successfully used in the water industry for testing sediments (Dutka *et al*., 1995).

For a bacterial toxicity assessment of heavy metal there are two commercially available test kits; MetPAD™ and MetPLATE™. These are based on β- galactosidase synthesis within *E. coli.* Bitton *et al*. (1992) used this test to assess 9 industrial effluents and showed that when used in conjunction with Microtox™ this testing method was fast and efficient though this is the only report in the literature of this test.

Ward *et al*. (2005) used the MetPLATE™ test to assess the toxicity of 16 treated landfill leachates for toxicity of Zn, Cu and Hg. Predictions concerning the toxicity of heavy metals based upon concentration were inaccurate. This was suggested to be due to other physiochemical parameters effecting the speciation of metals e.g. alkalinity, sulphide, and organic/inorganic ligands (Ward *et al*., 2005). Site specificity was shown through heavy metal binding capacity (HMBC). This equation demonstrates the ability of MSW leachates to bind heavy metals compared to control water (deionised water plus major ions) and in doing so alter the toxicity of the leachate. The HMBC is defined as:

$$HMBC = \frac{EC_{50}\ \text{MSW landfill leachate}}{EC_{50}\ \text{Control water}}$$

Using this equation Ward *et al*. (2005) were able to identify a pattern between the highest strength leachates and the ability to reduce the bioavailability of heavy metals. An example of this is a site with a COD value of 11,339 mg/L that has a HMBC (Zn) = 93. The high HMBC value indicates that majority of Zn has been bound to the COD fraction of the landfill leachate thus detoxifying the Zn cation. This could indicate that COD can be used to bind heavy metals in effluents. Binding of metals in this manner neutralises their direct toxicity. This process is reversible with acidification so care needs to be taken during treatment to stop the release of heavy metals. An investigation into the bioavailability of other heavy metals might be performed and would be an interesting and worthwhile addition to the knowledge of landfill leachate toxicity.

2.8.4 Bacillus subtilis rec-essay

B. subtilis is a bacterium which is known to be less sensitive than other test bacterium. It is the most abundant bacterium in activated sludge used in treatment processes (Takigami *et al*., 2002). This makes it the ideal candidate for testing landfill leachate toxicity before treatment due to its high tolerance to pollutants. The bacterial resilience to chemical substances is due to its ability to form an endospore shield inside the cell walls. The test is based on the relative difference in survival of a DNA repair-recombination proficient strain and a deficient strain. *B. subtilis* test has been applied to sewage samples with great success although its application to landfill leachate has only been reported twice previously.

Schrab *et al*. (1993) used *B. subtilis* as part of a genotoxicity testing battery. Three of the four landfill raw leachate samples recorded a positive toxicity result. It was noted that two of the samples contained pollutants that were in excess of the USEPA guidelines (Schrab *et al*., 1993). These leachates had cancer risk levels of 10^{-4} which is the same as an industrial landfills leachate.

Takigami *et al*. (2002) tested the waste water effluents from a number of industries and treatment technologies. A sample collected from a landfill leachate treatment facility demonstrated a positive result towards *B. subtilis* with an S-probit value of 0.53. For comparison an effluent sample from STW yielded a response of 84% (Takigami *et al*., 2002). These results indicated that current activated sludge technologies were sufficient in treating waste water to a high enough standard for discharge.

2.8.5 Mutatox®

Mutatox® is an example of a commercially available bioassay kit. This test uses a ‘dark’ strain of *V. fischeri*. Exposure to mutagens in the environment induces the bacteria to turn on luminescence. This type of response has been dubbed ‘lights on, lights off’. This test has been used to assess the genotoxicity of landfill leachate from Kuwait (Beg and Al-Muzaini, 1998).

It was found that the species were particularly sensitive to one borehole sample from an operational part of the landfill. The non-toxic responses came from areas of the landfill that had finished accepting waste. This indicates that the decomposition of the waste is removing most of the genotoxic substances in the leachate.

2.9 Discussion of review finding

2.9.1 Differences between raw and treated landfill leachate toxicities

This review has described the most commonly encountered bioassays found in the literature, with special attention being paid to those that have been used for assessing the toxicity of landfill leachates e.g. *V. fischeri* and *D. magna*. Many other tests have been attempted at a laboratory scale but have not been standardised for assessing toxicity of treated effluents discharged to fresh watercourses.

Raw landfill leachate EC_{50} toxicity varies greatly between sites 0.01 and 100%. One reason for this variation is the types of waste that has been landfilled. COD and the linked BOD concentration (Fan *et al*., 2006), NH_3 (Clement and Merlin, 1995), heavy metals (Ward *et al*., 2005), pH (Cameron and Koch, 1980), and alkalinity (Clement and Bouvet, 1993) have been linked to toxicity in landfill leachate. These parameters can be reduced by treatment (Osaki *et al*., 2006). It is apparent that the concentrations of these parameters depends on the types of waste and will vary in each landfill site. This makes it almost impossible to predict the exact toxicity of the leachate though estimates are possible. It is advisable to perform a toxicity assessment of each landfill leachate to understand the risk it poses to the environment. In some cases the effluent can retain a toxic characteristic even following treatment (Marttinen *et al*., 2002).

The review has presented detailed information on the differences in toxicity of raw and untreated leachates from around the world. There is considerably more research carried out with raw landfill leachates which is related to their high potential to cause damage to the

environment in small amounts. There are many landfills around the world that have been built without any sort of containment is allowing raw leachate into the surrounding aquifers and rivers (Fatta *et al.*, 1999, Baun *et al.*, 2000, Cotman and Gotvajn, 2010). Raw landfill leachate toxicity is high e.g. an EC_{50} <20% is normal though this varies depending on the site and species tested. Reported EC_{50} results for the invertebrate tests were on average ~20%.The chlorophyll based tests all displayed higher responses to the raw landfill leachate samples. *L. minor* averaged an EC_{50} ~15% whereas the algae based tests had a higher average response of ~10%. Only the Microtox test demonstrates a low response to raw landfill leachate and was in general less sensitive to landfill leachate than other higher species. Isidori *et al* (2003) reported an EC_{50} for three different leachates of >15% for the Microtox test. Ward *et al* (2002) have also reported similarly low responses of Microtox towards raw landfill leachate.

Following treatment of landfill leachate there is a significant reduction in the toxicity of landfill leachate. Toxicity levels in most cases are >40%. Okamura *et al* (2005) reported that most treated leachates had EC_{50} toxicities of >50% though there were two exceptions to this with toxicities that were more similar to raw leachates i.e. <20%. Okamura *et al* (2005) determined via statistical analysis that the DOC was the cause of toxicity in the treated leachates. This lowering of toxicity is linked to the reduction of ammonia and BOD that comes with treatment. Ammonia and alkalinity have been reported as major sources of toxicity in leachates (Clement and Merlin, 1995). Reduction in the concentration of ammonia is likely to remove much of the toxic fraction from landfill leachate but there will still remain a degree of residual toxicity. To what extent this residual toxicity remains will depend on the types and age of waste landfilled. Bortolotto *et al* (2009) concluded that the causes of toxicity could be attributed to the presence of high concentrations of inorganic ions in the treated leachates.

2.9.2 Constituents of battery of tests

Many options exist for the assessment of toxicity in landfill leachates. Six main categories were identified by Kjeldsen *et al*. (2002) for landfill leachate toxicity assessment (Table 2.10). Kjeldsen *et al*. (2002) identified 19 papers on toxicity assessment between 1979 and 2001. There has been a increased interest in landfill leachate toxicity testing. A further 23 reports on landfill leachate toxicity in the past 8 years have been identified. This increase in data reported shows the variation in the levels of toxicity in global landfill leachate.

Table 2-10 shows that there has been a particular strong interest in the use of invertebrates for the assessment of landfill leachate toxicity. The majority of reports in the literature carried out assessments using invertebrates probably due to their widespread acceptance for assessing toxicity.

Table 2-10: Toxicity testing of MSW landfills around the world within the literature using different species and methods.

No. of landfills sampled	F	I	P	A	B	GM	Other	Reference
1	1							(McBride *et al*., 1979)
7	2	1						(Cameron and Koch, 1980)
								(Atwater *et al*., 1983)
1	1	1		1	1			(Plotkin and Ram, 1984)
1	1							(Wong, 1989)
8						1		(Omura *et al*., 1992)
2		1			2			(Gotvajn *et al*., 2009)
19	1	1			1			(Kross and Cherryholmes, 1993)
4					1	3		(Schrab *et al*., 1993)
2				4				(Cheung *et al*., 1993)
1		1						(Goi *et al*., 2010)
2			1		1		2	(Devare and Bahadir, 1994)
1	2	1						(Ernst *et al*., 1994)
1						1		(Bortolotto *et al*., 2009)
1							1	(Amahdar *et al*., 2009)
3	1							(Alkassasbeh *et al*., 2009)
1		1			2			(Cho *et al*., 2009)
1					1			(Nohava *et al*., 1995)
9			1					(Clement and Bouvet, 1993)
35		1						(Assmuth and Penttila, 1995))
2						4		(Helma *et al*., 1996)
8		3	1	1	1		2	(Clement and Merlin, 1995)

No. of landfills sampled	F	I	P	A	B	GM	Other	Reference
1						1		(Beg and Al-Muzaini, 1998)
1						3		(Cabrera and Rodriguez, 1999)
10			1	1	1			(Ledin *et al*., 2005)
2		2			1		1	(Isidori *et al*., 2003)
6		1		1	1			(Ward *et al*., 2002)
7		1			1			(Wolska *et al*., 2006)
1	1	2			1			(Silva *et al*., 2004)
3					1			(Fan *et al*., 2006)
1		1		1				(Marttinen *et al*., 2002)
1		2		1	1			(Slomczynska *et al*., 2004)
1	2							(Osaki *et al*., 2006)
1		1						(Dave and Nilsson, 2005)
1						1		(Sang and Li, 2004)
1						1		(Sang *et al*., 2006)
3			3					(Feretti *et al*., 2009)
1						1		(Takigami *et al*., 2002)
2						4	2	(Talorete *et al*., 2008)
1						3		(Cabrera and Rodriguez, 1999)
1		1			1	1		(Baun *et al*., 2000)
1	1	2			1	1		(Rutherford *et al*., 2000)
1		1			1		1	(Kuczynska *et al*., 2006)
10				1	1			(Baun *et al*., 2004)
2		1		1		1		(Baun *et al*., 1999)
7		2			1			(Wolska *et al*., 2006)
1							1	(Mackenzie *et al*., 2003)

Note. F, fish; I, invertebrates; P, plant; A, algae; B, bacteria; GM, genotoxicity and mutogenocity; Other, rotifers, activated sludge, etc.

Bacteria are another prevalent test due to ease of use and reproducibility. Bacteria testing are also considered to be a cost effective and rapid technique of testing (Fan *et al*., 2006). There has been an increase in genotoxicity testing reports within the literature, with many different tests reported. Issues over the lack of international standards available for all the different tests reported.

Selection of the components to form a battery of tests for use in assessing toxicity needs to incorporate a variety of test species and time spans. From this review it has become apparent that *D. magna* is the most popular test species for short term testing due to its sensitivity towards a variety of leachate strengths. One drawback to *D. magna* is that the shortest test is 24 hr. *V. fischeri* is useful for a quick determination of toxicity even though problems exist with its low sensitivity towards landfill leachate (Marttinen *et al*., 2002).

A view is present in a number of studies for the inclusion *S. ambiguum*, *B. calyciflorus* and *C. dubia* instead of *D.magna* (Johnson *et al*., 2004; Bernard *et al*., 1996; Isidori *et al*., 2003). This view is due to these species of invertebrates being more sensitive to landfill leachate toxicity and thus providing a better test. Certainly any battery needs the inclusion of one type of invertebrate along with a bacteria test and higher species.

In any battery of bioassays there needs to be a higher organism. At present the UK Environment Agency suggests the use of the fish (*O. mykiss*) and algae (*P. subcapita*). Both of these tests have international standard procedures for testing and have been reported as acceptable for the testing landfill leachate toxicity. But there is no provision in the UK Environment Agency guidelines for the use of a plant species such as *L. minor*. This plant has had fewer reports of bioassays of landfill leachates than the other two higher organisms. *L. minor* shows potential to be included in a future battery due to being part of the diet of many organisms.

Genotoxicity is not included in the current Environment Agency guidelines. With changes in waste streams coming to landfills e.g. hazardous waste landfills and incinerator ash deposits high in heavy metals, a more thorough understanding of the effects on cellular damage is needed. Commercial bioassays are available and easily incorporated into a battery of bioassays.

2.9.3 Sensitivity of tests

When selecting which tests should form the battery, the sensitivity of the test must be understood. To calculate the Slooff (1983) sensitivity of each test the arithmetic mean of all test results, is divided by E(L)C_{50} of each test carried out (Bernard *et al*., 1996). Of the papers reviewed, 8 were selected for the high standard of data presentation. Table 2.11 shows the results of the Slooff's analysis from the 8 reports. *V. fischeri* was seen to be the least sensitive test available for which there is much evidence in the literature already. This is important as

V. fischeri is used as a standard test for the assessment of toxicity. This lack of sensitivity could mean that discharges which were considered safe may actually be toxic to aquatic life.

The results show a close grouping of results for *D. magna* 48 hr tests, with only Isidori *et al*. (2003) showing deviation from this trend. The *D. magna* 21 day test shows a great deal of variability but the low Slooff numbers of Wolska *et al*. (2006) show the test to be particularly sensitive. Due to the lack of reported toxicity data in the studies it is hard to compare the sensitivities of the other types of tests. Isidori *et al*. (2003) noted in a comparison that *T. platyurus* was the most sensitive of the 5 tests used in their battery. This test is an obvious area for future research as to whether it really is more sensitive to landfill leachate than *D. magna*.

The results for the chlorophyll based species show their sensitivity towards landfill leachate. This is in agreement with Bernard and Clement (1996). These results further reinforce the argument for inclusion of chlorophyll-based species into toxicity testing battery.

Table 2-11: Sensitivity of a number of toxicity tests based on the Sloof method.

V. f	D.m 24h	D. m 48h	D. m 21d	O. m	S.f	O.n	S. s	L.m	S.a	B.c	T.p	C.d	S.c	Reference
1.38		1.41	0.16											**Wolska *et al*., (2005)**
		1.16	1.06			0.77								**Atwater *et al*., (1983)**
1.77		1.16	0.06											**Kuczynska *et al*., (2006)**
		1.21		0.84	1.21									**Ernst *et al*., (1994)**
0.8		1.1					1.56	0.27	0.12	1.27	0.27	0.33		**Bernard *et al*., (1996)**
4.37	0.31	0.17								0.1	0.04			**Isidori *et al*., (2003)**
1.76												0.97	0.27	**Ward *et***

	V. f	D.m 24h	D. m 48h	D. m 21d	O. m	S.f	O.n	S. s	L.m	S.a	B.c	T.p	C.d	S.c	Reference
															***al*., (2005)**
Average	2.016	0.31	1.035	0.43	0.84	1.21	0.77	1.56	0.27	0.12	0.685	0.155	0.65	0.27	

V.f= V. fischeri; D.m= D.magna; O.m= O. mykiss; S.f = Salvelinus fontinalis; O.n= Oncorhynchus nerka; S.s= S. subspicatus; L.m= L. minor; S.a= S. Ambiguum; B.c= B. calyciflorus; T.p= T. platyurus; C.d= C. Dubia; S.c= S. capricornutum

It is therefore suggested that further use of Slooff numbers in assessing the sensitivity of the bioassays is needed. This requires authors to fully report their results in all details, either in graphs or tables.

2.10 Gaps in knowledge

- Is there a place for *L. minor* in a battery of tests for determining landfill leachate toxicity?
- Treated leachate displays a variety of toxicities, are any of the effluents from landfills in the U.K. toxic and if so how toxic.
- Assuming any residual toxicity does exist within treated landfill leachate, what are the causes of this toxicity? Is it the organic fraction (Okamura *et al*., 2005) or is it the inorganic ion fraction (Bortolotto *et al*., 2009)
- Is there a way to recreate the levels of toxicity being recorded in landfill leachate in a controlled environment e.g. by determining the chemical composition of a sample and making a solution with those constituents?

2.11 Conclusions

The review has highlighted the general findings on landfill leachate:

- Toxicity of landfill leachate has been well researched, in particular the hazard posed by raw landfill leachate to the environment. Treated landfill leachate has received less attention with this due to its benign nature.

- Raw landfill leachate is normally very toxic to most organisms i.e. EC_{50} = <5% . This toxicity is attributed to the high concentration of ammoniacal-nitrogen and organic pollutants within the leachate. Treatment of the landfill leachate is able to effectively reduce the toxicity e.g.≥EC_{50} = 50%. Even after treatment there remains a degree of residual toxicity within treated landfill leachate that needs investigation.
- An inconsistent picture is evident from the literature about levels of toxicity within landfill leachate. For one leachate there is no toxicity to some species but then another test this species is displaying the highest sensitivity. This phenomenon is due to the complex physico-chemical nature of landfills and the leachate is individual to each landfill. This makes it impossible to make predictions on toxicity without conducting assessment with a number of species.
- A number of causes of toxicity have been identified by authors. These are: ammoniacal-nitrogen, heavy metals, organic substances and major ions. The concentration of each of these pollutants is dependent on the types of waste that have been landfilled. Ammoniacal-nitrogen, BOD and alkalinity concentrations are reduced by biological treatment. Heavy metals and recalcitrant organic substances can be difficult to eliminate with biological treatment and might require a more advanced chemical or physical treatment to remove the potential hazard.

The selection of a battery of bioassays from the evidence gathered in this review needs to include the following:

- A number of tests are needed to accurately assess the hazard landfill leachate can pose to the environment. *V. fischeri* is a popular choice in the literature due to its speed of results and ease of use. A number of authors have highlighted a lack of sensitivity of this test as has been shown in the Slooff (1983) number analysis in (Table 2-11).

- An invertebrate test is recommended by many authors (Johnson *et al*., 2004; Isidori *et al*., 2003; Clement and Bouvet, 1993). The choice of invertebrates is between three species: *T. platyurus, S. ambiguum,* and *D. magna*.
- One chlorophyll based species is a minimum. Green algae are well documented for use as bioassays. *L. minor* is less documented and offers a more complete understanding of the effects of landfill leachate toxicity towards aquatic environments and soils. Ideally a battery of tests would include both.
- Genotoxicity is becoming an important area for the understanding of landfill leachate toxicity. Most genotoxicity was carried out by Baun *et al*., (1999a; 2000; 2004) and this further investigations are needed so a greater knowledge can be gained on any possible hazard posed by landfill leachate.
- Statistical analysis of results should be used more in reports. Particularly sensitivity analysis which is rapid and offers a great insight into which tests are suitable for which type of waste.
- Statistical analysis to determine significant difference between results is needed and Isidori *et al* (2003) demonstrated the value of such analysis in a TIE procedure. For understanding chemical causes principal component analysis is excellent of separating multiple chemical effects e.g. (Bernard *et al*., 1996).

3 Methodology

This chapter outlines the methods and practices that were adopted in collecting, storing and analysing samples. The chemical and toxicity characteristics of the samples are explained in detail along with the statistical analysis of the results.

3.1 Leachate sample collection

Leachate samples were collected in flexible 1 L plastic bottles from the effluent discharge tank or tap at each landfill leachate treatment plant. To minimise the head space in the bottles the bottles were squeezed to overflowing and the cap tightly screwed on. Samples were stored at 4°C and allowed to warm to room temperature before experiments.

3.2 Physico-chemical parameter determination

In order to understand the physico-chemical nature of the leachates a number of parameters for analysis were selected:

- Chemical oxygen demand was assessed in order to determine the concentration of organic compounds remaining after treatment.
- Biological oxygen demand is a key determinant for assessing the amount of organic matter readily biodegradable by aerobic processes in leachate and is a measure of the suitability of treated leachate for discharge to receiving waters.
- Ammoniacal nitrogen was assessed as it is considered a highly hazardous pollutant that is the focus of biological treatment of landfill leachate.
- Chloride concentration was determined as it was identified early on as being present in very high concentrations and a potential cause of toxicity.
- Suspended solids and total nitrogen were assessed to give a complete a picture as possible of the makeup of the landfill leachate.

- Group 1 and 2 metal ions were identified later in the project as being a possible source of toxicity in landfill leachate and for this reason assessment was needed.
- Bicarbonate concentration was assessed for the same reason as metal ions.

3.2.1 Chemical oxygen demand

The COD of a sample is an indirect expression of the concentration of organic compounds that are readily oxidised by a known amount of potassium dichromate. The test relies on the assumption that most organic compounds can be oxidised to CO_2 and H_2O under acidic conditions with a strong oxidising agent. The oxidation is performed at 150°C with a silver catalyst. The overall equation is:

$$C_nH_aO_bN_c + (n + \frac{a}{4} - \frac{b}{2} - \frac{3}{4}c)O_2 \rightarrow nCO_2 + (\frac{a}{2} - \frac{3}{2}c)H_2O + cNH_3$$

Chloride can interfere in the performance of this test due to chloride being oxidised to chlorine. Due to the high concentration of chloride in the treated landfill leachate samples a 2-fold dilution was performed. To determine the COD concentration in treated landfill leachate a 5 ml sample was dispensed to a Merck cell test with a concentration range of 0-1,500 mg/L (VWR International Poole, UK). The cell was shaken and heated at 150°C for 2 hours. After 2 hours the cell was removed from the heating block and allowed to cool. After 10 minutes the cell was swirled to homogenise the liquid. The COD concentration was determined (mg/L) photometrically (Spectroquant Nova 400, Merck, Germany). In this work, the COD concentration is a mean from three replicates using new cells each time (APHA, 2006).

3.2.2 Biological oxygen demand

The metabolic activities of aerobic bacteria are responsible for the decomposition of organic compounds in waterways. Aerobic bacteria require oxygen to process these compounds. These metabolic processes deplete the dissolved oxygen that is present in the water. Higher concentrations of organic compounds encourage a growth in bacterial communities. Increases

in the sizes of these communities deplete the levels of dissolved oxygen in the water that can cause oxygen starvation in water that can lead to the inhabitants leaving an area or dying (Hassell *et al*., 2006).

The BOD measures this effect as a depletion of dissolved oxygen (DO) in water sample. The test empirically measures the quantity of DO that is utilised by bacteria in a 5-day period. A nitrification inhibitor is added to the solution so that the nitrification is not included the measurement. The decomposition of nitrogenous compounds was inhibited by the addition of 2-chloro-6-(trichloro methyl) pyridine (Fisher Scientific Chemicals, U.K.).

The procedure adopted in this project comes from 'Standard methods for the examination of and wastewater 21st Edition' (APHA, 2006). No dilution was needed for this testing as in a range finding test it was found that the BOD_5 was within the range of the oxygen meter i.e. 0-10 mg/L. A 250 ml glass bottle was filled to overflowing with a landfill leachate sample in a temperature controlled room (set to 20°C). At the start of the test the DO was measured and after 5 days the final DO was measured. Using the equation the BOD_5 was calculated:

$$BOD_5 \text{ (mg/L)} = \frac{D_1 - D_2}{P}$$

Where:

- D_1 = DO of the sample immediately after preparation for testing (mg/L)
- D_2 = DO of the sample after 5 day incubation at 20°C (mg/L)
- P = decimal volumetric fraction of sample used

3.2.3 Ammoniacal nitrogen

Ammoniacal nitrogen (NH_4-N) in water exists as ammonium (NH_4^+) and ammonia (NH_3) (Metcalf and Eddy, 2002). The specific ratio of each species is dependent on pH. At acidic pH values, NH_4^+ is the dominant species and conversely at alkaline pH values ammonia is the dominant species (Horane, 1991).

Ammonium reacts with hypochlorite to form monochloramine (see below) which when mixed with substituted phenol forms an indophenol derivative which is a deep blue colour (APHA, 2006). The colour forms a gradient dependent on concentration that can be measured photometrically.

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O$$

Ammonium cell tests with a concentration range of 0.20-8.00 mg/L were used in this project (VWR International Poole, UK). To the reaction cell containing sodium hypochlorite, 1.0 ml of treated landfill leachate was pipetted. The reaction cell was closed, shaken and allowed to settle. One dose of the substituted phenol was added to the reaction cell and shaken for a second time. After 15 minutes the concentration of ammonium (mg/L) was determined photometrically (Spectroquant Nova 400, Merck, Germany).

3.2.4 Sulphate

Sulphate ions react in the presence of barium ions to form the sparingly soluble solid barium sulphate under acidic conditions (see below). The quantity of solid can be inferred from a change in turbidity and thus the concentration of sulphate can be calculated.

$$SO^{2-}_{4(aq)} + Ba^{2+}_{(aq)} \rightarrow BaSO_{4(s)}$$

A 2-fold dilution was needed to bring the sample sulphate concentration into the range of the cell tests. Sulphate concentration in this project was determined using Merck cell tests with a concentration range 2-250 mg/L (VWR International Poole, UK). To the reaction cell 5.0 ml of treated landfill leachate was pipetted and the reaction cell shaken. One dose of barium chloride was added and the reaction cell shaken vigorously until all barium chloride was dissolved. After exactly 2 min the sulphate concentration (mg/L) was determined using a spectrophotometer (Spectroquant Nova 400, Merck, Germany) (APHA, 2006).

3.2.5 Total nitrogen

Both inorganic and organic nitrogen can be converted to nitrate by addition of an oxidising agent and heating at 120°C. In an acidified solution (both sulphuric and phosphoric) and the presence of 2,6-di-methylphenol, nitrate is converted to 4-nitro-2,6-dimethylphenol which can be determined photometrically (APHA, 2006).

The total nitrogen content of treated landfill leachate samples in this project were determined with Merck total nitrogen cell tests with a concentration range of 10-150 mg/L. The process for determining total nitrogen is a 2 stage operation:

I. 1.0 ml of sample and 9.0 ml of distilled water are mixed in an empty reaction cell. To this mixture, 6 drops of sulphuric/phosphoric acid solution and 1 dose of the $HgSO_4$ were added. The reaction cell was heated at 120°C for 1 hour. After heating the cell was removed and left to cool with regular swirling of the reaction cell.

II. A 1.0 ml aliquot of the reaction mixture is pipetted into the reactor cell and 1.0 ml of 2,6-di-methylphenol added. After 10 mins cooling the reaction cell the total nitrogen was determined photometrically (DR 5000 UV-Vis Spectrophotometer, Hach, U.S.A.).

3.2.6 Chloride

Liberation of thiocyanate from mercuric thiocyanate is achieved by the formation of mercuric chloride. Thiocyanate can be precipitated in the presence of ferric iron as iron thiocyanate. Iron thiocyanate is bright red that has a colour concentration proportional to the concentration of the chloride.

Due to the high concentration of chloride, a 200-fold dilution was carried out. In this project Hach chloride cell tests with a concentration range of 0.1-25.0 mg/L were used. A cell was filled with 25.0 ml of sample to which 2.0 ml of mercuric thiocyanate was pipetted. The reaction cell was swirled and left to settle. To the reaction cell 1.0 ml of ferric ion solution was added and the top added before shaking. After 5 mins the concentration of chloride was

determined photometrically (DR 5000 UV-Vis Spectrophotometer, Hach, Germany) (APHA, 2006).

3.2.7 Metal analysis

Atomic absorption spectroscopy is able to determine the concentration of metal ions by atomising the metal ions with a flame. The instrument uses a flame to promote electrons into empty orbitals of a metal atom. Each element has a 'fingerprint' orbital band gap that takes a known amount of energy to promote its electrons (Atkins and De Paula, 2006). As the electrons lose this energy, they release the energy absorbed in the form of light. Each element releases energy at a fixed wavelength, so identification of individual elements is possible. Knowledge of the amount of energy being produced by the flame and measuring the amount of energy coming to the instrument's detector it is possible from the Beer-Lambert law to determine the concentration of a element.

Calcium, potassium, sodium and magnesium were determined with atomic absorption spectroscopy (AAnalyst 800, Perkin Elmer, U.K.). Due to detector limits each metal to be analysed needed an individual dilution (Table 3-1). Dilution was performed with 0.2% HNO_3 (Fischer Chemicals, U.K.) (APHA, 2006).

Table 3-1: Dilution factor for each metal ion analysed with atomic absorption spectroscopy.

Metal ion	Dilution factor
Ca	100
Mg	300
K	1,200
Na	100

For the 300 and 1,200 dilutions the process was carried out in 2 stages so that accurate measurements could be made. Lanthanum (III) chloride (Fisher Scientific Chemicals, U.K.)

added to achieve a 0.1% concentration in all analysis. Lanthanum (III) chloride is added to increase the sensitivity of the instrument to rare earth metals (Cantle, 1982). Between each measurement a blank of 0.2% HNO_3 is used to clean the instrument.

3.2.8 Conductivity and pH

Conductivity and pH were determined with a pH and conductivity meter (Jenway 3450, Dunmow, UK).

3.2.9 Suspended solids

The procedure followed is outlined in detail in Standard Methods for the examination of and wastewater 21st Edition (APHA, 2006). Three 20 ml aliquots of ultra pure deionised water were used to wash 0.45 µm glass fibre filter papers (Munktell Filter AB, Sweden). The filter papers were dried for 24 hours at 105°C overnight. The dried filter papers were then stored in a silica desiccator

Before filtration, the weight of the dried filter paper was recorded. The sample was shaken vigorously before being filtered through the dried filter paper under vacuum. The vacuum was used to dry the filter papers before being weighed. Using the following equation total suspended solids (mg/L) were determined:

$$\text{Total suspended solids (mg/L)} = \frac{(A-B)X100}{samplevolume(ml)}$$

Where:

- A = weight of filter paper + dried residue (mg)
- B = weight of filter paper at start (mg)

3.3 Justification for bioassay selection

The range of bioassays used throughout this project evolved as new insights and understandings of sensitivities and usefulness became apparent. This section explains the changes made to the battery and the reasons for the changes.

3.3.1 Toxicity identification evaluation procedure

The literature review recommended a minimum number of test species that should form a battery of tests for assessing the toxicity of landfill leachate. This recommendation was based on the theory that individual species display a specific range of sensitivities towards the components of landfill leachate. The review recommended a battery formed from one species from each of the following kingdom: bacteria, invertebrate, plant, and a fish. Genotoxicity testing was identified as an active area of research for the assessment of landfill leachate toxicity and could be a beneficial addition to a battery of tests. Due to the need for an animal testing license the fish test could not be included at any stage during this project.

The initial screening exercise of this project was based on the Toxicity Identification Evaluation procedure (Chapter 4) (Norberg-King *et al*., 1991). A battery of bioassays was selected to include previously mentioned species.

The species used in this test battery were:

Vibrio fischeri: The Microtox® system utilises the reduction of bioluminescence when the toxicant interferes with the metabolism of the bacteria.

Daphnia magna: are invertebrates and considered as primary consumers within watercourses (Bernard *et al*., 1996).

Thamnocephalus platyurus: are invertebrates and considered as analogous with *D. magna* in the role they play in the aquatic environment.

Lemna minor: are an aquatic plants and considered to be primary producers in freshwater aquatic environments (Bernard *et al*., 1996).

Escherichia coli: Genotoxicity testing was carried on mutant *E. coli* using the Toxi-ChromoPlate™ system (EBPI, Ontario, Canada).

The Microtox system is commonly used in toxicity testing (Toussaint *et al*., 1995). Test time is very fast, ~15-30 mins, and the software presents the results in an easy to understand

format. This system was included even though there is a growing concern of the range of sensitivities of this system.

D. magna and *T. platyurus* were selected as the invertebrate candidates. *D. magna* is commonly found within the literature. *T. platyurus* is a similar a similar test to the *D. magna* test but has a shorter test time which makes it an attractive alternative. One report in the literature has suggested the *T. platyurus* is more sensitive to landfill leachate than *D. magna* (Isidori *et al*., 2003). This increased sensitivity could be beneficial for identifying pollutants in landfill leachate.

As mentioned previously genotoxicity is a growing area of interest in the assessment of toxicity in landfill leachate (see Literature review). The Toxi-ChromoPlate™ system colorimetrically assesses the damage toxins cause to the repair function of pre-stressed *E. coli*. This system has the advantage that it comes in a pre-packaged kit and a relatively short test time of 2 hours.

L. minor is a key constituent of many organism's diet and is a higher species. Previous reports on toxicity assessment of landfill leachate had shown *L. minor* to have a high sensitivity. Higher sensitivity is a desirable feature in a toxicity testing battery as it shows the maximum potential for hazard.

3.3.2 Rationalisation for the selection of toxicity tests

The results of the TIE procedure (Chapter 5) led to a re-evaluation of the composition of the battery of tests. It was clear that some tests provided little additional information and some were not suitable or gave inconclusive results. There was a cost implication of running such a large battery of tests which the project sponsor was not keen to meet.

The TIE procedure demonstrated there was little difference in the sensitivity of *D. magna* and *T. platyurus* with treated landfill leachate. The initial hypothesis that *T. platyurus* would demonstrate a greater sensitivity towards landfill leachate was unsupported. A lack of

sensitivity was in contradiction to the findings of Isidori *et al*. (2003) though that report had used raw leachates which gave very high responses. Due to the small difference in sensitivity, a decision was made that both tests were not needed in any further testing. For these reasons it was decided to retain *D. magna* in the testing regime and remove *T. platyurus.*

L. minor displayed the highest sensitivity to the treated landfill leachate samples. The response in some cases was four times higher than the other tests used. Due to *L. minor* being a key food for many animals further testing to understand the reasons and the possible effects on the population of *L. minor* exposed to effluents from treatment of landfill leachate was needed.

In the context of this testing the Microtox™ test produced no response to the treated landfill leachate samples. The literature review suggested this test did not show a high sensitivity towards treated landfill leachates. A decision to keep this test in the initial stages of the XAD work (Chapter 6) to confirm the lack of response towards treated landfill leachate was required. During the course of XAD testing the Microtox™ test was removed as the lack of response was repeated in further samples and the high cost of the test made its continued inclusion unsupportable.

The genotoxicity test produced inconsistent results that did not correlate with the responses of the other tests. This lack of correlation could be an interesting side investigation but did not fit within of this project's frame of reference. For these reasons this test was removed from the battery for the following phases of work.

The composition of bioassays for this phase of testing:

- *L. minor*
- *D. magna*
- Microtox™ (discontinued after XAD-7hp testing)

In the major ion and synthetic leachate work (Chapters 7) the bioassay choice remained the same as they showed consistent results throughout. *D. magna* is also the test used by Mount *et al*. (1997) in their work on major ion toxicity (Chapter X) which will allow comparisons between this key source and the present work easier.

3.4 Toxicity testing procedure

3.4.1 Daphnia magna

The test was supplied as Daphtoxkit F™ Magna (SDIX, Segensworth East, UK). The procedure used in this testing followed OECD 211 (OECD, 1998). This is a pre-packaged kit with all the required test candidates, chemicals and plates needed to carry out the tests.

A standard freshwater with the following composition was made in 2 L of autoclaved deionised water:

- 294 mg /L Ca $C1_2$.2H,O
- 123 mg/L $MgSO_4.7H_2O$
- 65 mg/L $NaHCO_3$
- 6 mg/L KCl

Aeration of the solution was carried out by an aquarium airpump for >15mins.

D. magna are supplied as a vial of dormant ephippia in a storage medium (exact composition unknown) with enough specimens in each vial for one complete test with 4 replicates. Hatching of the ephippia commenced 72 hours before testing was to begin. The contents of each vial were emptied into a microsieve and washed with tap water to remove all traces of the storage medium. Fifty millilitres of standard freshwater was transferred to a petri dish and the ephippia placed in the petri dish. The ephippia were incubated at 22°C and 6,500 Lux for 72 hours. After 72 hours, the hatchlings were pre-fed with a suspension of algae *Spirulina* microalgae to ensure they were not energy starved during testing.

A dilution series was used to allow calculation of an EC_{50}. Dilutions were made using Standard Freshwater. The test candidates were transferred to 1 well of a multiwell plate filled with Standard Freshwater in order to rinse the neonates before testing. The four other wells of the multiwell plate were filled with suitable dilutions. The neonates were transferred from the washing well to the test wells with 6 neonates per testing well. The dilution series, unless stated in the work, was 100, 50, 25, 12.5, and 6.25%. The multiwell plate was incubated in the dark at 20°C. At 24 and 48 hours immobilised were recorded. A neonate was considered immobilised if after gentle agitation of the liquid it did not move for 15secs. A control was used that was made up with standard freshwater. Four replicates were carried out for each dilution.

3.4.2 Thamnocephalus platyurus

The test species come pre-packaged as Thamnotoxkit F™ (SDIX, Segensworth East, UK). Testing followed the procedure outlined in (Persoone, 1999). This is a prepackaged kit with all the required test candidates, chemicals and plates needed to carry out the tests.

One litre of standard freshwater with the same chemical composition as the *D. magna* test used in this testing. Aeration of the solution was carried out by an aquarium air pump for >15mins.

T. platyurus are supplied as a vial of cysts in a storage medium (exact composition unknown) with enough specimens in each vial for one complete test with 3 replicates. Hatching of the cysts commenced 24 hours before testing was to begin. Twenty millilitres of diluted Standard Freshwater was made up by adding 17.5 ml of deionised water to 2.5 ml of freshwater. The 1 ml of diluted freshwater was added to the vial and shaken for 20 mins. The vial was shaken and then emptied into a hatching dish containing 10ml of diluted freshwater. The cysts were incubated at 22°C and 6,500 Lux for 22 hours.

A dilution series was used to allow calculation of an EC_{50}. Dilutions were made using Standard Freshwater. The dilution series, unless stated in the work, was 100,50,25,12.5, and 6.25%. Controls were run with every test in order to validate results throughout.

For this test approximately 50 larvae were transferred to a rinsing well. Three wells of the multiwell plate were filled with suitable dilutions. The larva were transferred from the rinsing well to the test wells with 10 larvae per testing well. The multiwell plate was incubated in the dark at 25°C. At 24 hours observations on death recorded. A larva was considered dead if no movement was noted after 10 seconds of observation. A control was used that was made up with standard freshwater. Three replicates were carried out for each dilution.

3.4.3 Lemna minor

3.4.3.1 Stock culture collection

The procedure adopted in this testing follows the guidelines of OECD 221 (OECD, 2002). Cultures were grown from specimens collected from a Bedford garden pond. All equipment used with this biotest was first sterilised in an autoclave (Priorclave P5, U.K.).

It was necessary to disinfect the specimens before culturing. Sterilisation was performed by first washing in Standard Freshwater (Table 3-2), followed by a 10 second soak in (90%) ethanol (Fisher chemicals, Loughborough, U.K.), followed by another washing with Standard Freshwater, followed by a 30 second soak in 20% hypochlorite solution (Unilever, Leatherhead, U.K.) and finally a last washing with Standard Freshwater.

One bacterial infection of the stock culture occurred in October 2009. This required the entire laboratory and incubator to be sterilised and fresh cultures collected and disinfected. The new stock cultures required 8 weeks to adapt before further testing could be carried out. Another infection in the stock happened in February 2010 which limited supplies of test candidates. This limited the amount of tests that could be carried out with synthetic solutions.

3.4.3.2 Stock solution preparation

The growth media used in this procedure is a modified Swedish standard (SIS) Lemna growth medium. Stock solutions were made by dissolving the following in 1L (Table 3-2). Solutions I-V were sterilised by autoclaving and solutions VI-VII were sterilised using membrane filter papers with 0.45 µm pore size (Whatman, U.K.). MOPS buffer is an optional addition for work with *L. minor* and it was decided that it was a more suitable buffer than a phosphate buffer and hence was included in this work.

Table 3-2: Stock solution composition and concentrations

Stock solution No.	Substance	Concentration in stock solution (g/L)	Concentration in the prepared medium (mg/L)
I	$NaNO_3$	8.5	85
	KH_2PO_4	1.34	13.4
II	$MgSO_4.7H_2O$	15	75
III	$CaCl_2.2H_2O$	7.2	36
IV	Na_2CO_3	4.00	20
V	H_3BO_4	1.0	1.0
	$MnCl_2.4H_2O$	0.20	0.20
	$Na_2MoO_4.2H_2O$	0.010	0.010
	$ZnSO_4.7H_2O$	0.050	0.050
	$CuSO_4.5H_2O$	0.0050	0.0050
	$Co(NO_3)_2.6H_2O$	0.010	0.010
VI	$FeCl_3.6H_2O$	0.17	0.84
	$Na_2EDTA^*.2H_2O$	0.28	1.40
VII	MOPS**	490	490

*= Ethylenediaminetetraacetic acid
**= 3-(N-morpholino)propanesulfonic acid

1 L of SIS medium is prepared from autoclaved deionised water and the following recipe:

- 10 ml of stock solution I
- 5 ml of stock solution II
- 5 ml of stock solution III
- 5 ml of stock solution IV
- 1 ml of stock solution V

- 5 ml of stock solution VI
- 1 ml of stock solution VII

The SIS medium is used for all dilutions of the treated landfill leachate.

3.4.3.3 Exposure procedure

Between 4-6 fronds (The smallest unit capable of reproduction an individual/single "leaf like" (OECD, 2002)) were aseptically transferred to a plastic Petri dish. Plastic Petri dishes were found to be a suitable container as the fronds had enough room to grow and the fronds did not stick to the sides of the dish. The Petri dishes were then transferred to the incubator. Placement of Petri dishes in the incubator was randomised for testing. A 7-day testing regime was adopted with regular checking of growth for any abnormalities in the growth pattern.

The incubator was located in a temperature-controlled room at 22°C with 7,200 lux of illumination coming from 2 florescent bulbs (Osram Active 3350, Germany). These bulbs were selected for their broad-spectrum range of light output. . For each sample a control was made up with the standard freshwater. Three replicates were carried out for each dilution and the control

3.4.3.4 Observations

At the start of the procedure, the numbers of fronds were counted and recorded on the Petri dish. The pH of the solution was checked at both the start and termination to verify it had remained between 6 and 8.

At termination of the test, visual changes in frond colour and structure were noted. Frond numbers were counted at the termination of the test. Inhibition of growth rate was calculated from the following equation:

$$\%I_r = \frac{(\mu_C - \mu_T)}{\mu_C} X100$$

Where:

— $\%I_r$: percent inhibition in average specific growth rate

— μ: number of fronds

— μ_C: mean value for the μ in the control

— μ_T: mean value for the μ in the treatment group

Due to time limitations dry weight and total frond area were not determined.

3.4.4 Microtox

This test uses the deep sea marine organism *Vibrio fischeri*. Toxicants interfere in the metabolic pathway in the reduction of FMNH2 and reduce the emission of light from the bacteria. The procedure adopted in this project is the ISO 11348-2 standard. The bacteria, instrument and reagents were supplied by SDIX (Segensworth East, UK). The bacteria were kept in a -21°C freezer until needed for testing. Test times in the initial experiment was 15mins but this was increased in the XAD experiments (Chapter X) to 30mins after a literature source suggested that for samples with low toxicity a longer experiment time might highlight the toxicity better (Vasseur *et al*., 1986).

3.4.4.1 Procedure

Due to the addition of reagents the highest concentration of treated landfill leachate was 81.9%. A cuvette containing 1.0 ml of 0.01% NaCl solution was placed into a refrigerated reagent well (Figure 3-1). After 5 mins cooling, the bacteria are collected from the freezer, mixed with the cooled 0.01% NaCl solution and placed back in the reagent well. A cuvette was placed in F3 and filled with 1.5ml of the dilutant solution (2% NaCl). Cuvettes are placed in row A and filled with 1.0 ml of the dilutant solution.

To a cuvette placed in C-1 2.5 ml of the sample to be assessed was dispensed. To the sample was added 250 µL of 22% NaCl solution was added and mixed. A 750 µL portion of the solution was discarded from this cuvette.

A 1:2 serial dilution was made by transferring 1.0 ml of the sample to the cuvette in A-5. The contents of the cuvette were mixed. A 1.0 ml portion of the diluted sample was transferred to A-4. This process was continued until A-2 where the contents were mixed and 1.0 ml of the contents were discarded. The contents of A-1 were used as a blank so no sample was added.

150 µL of bacteria solution contained in the reagent well was transferred to the cuvette in F-3 and mixed. A 100 µL portion of the contents of F-3 were transferred to the cuvettes in row B1-5 and the cuvette in D-1. After the transfer of the bacteria it was necessary to wait 15mins for the bacteria to reach room temperature.

The instrument (Figure 3-1) was blanked and then the light emissions of the bacteria in the cuvettes of row B and D-1 were measured. Quickly the contents of the sample cuvettes (row A and C-1) were pipetted to appropriate cuvette e.g. A-1 to B-1 and C-1 to D-1.

The experiment was run for 15 or 30mins and the light emissions were measured again. These light measurements were subtracted from the initial light measurements by the Microtox software and a EC_{50} calculated. The effect vs concentration was plotted and used to calculate an EC_{50}. Three replicates was carried out for each dilution.

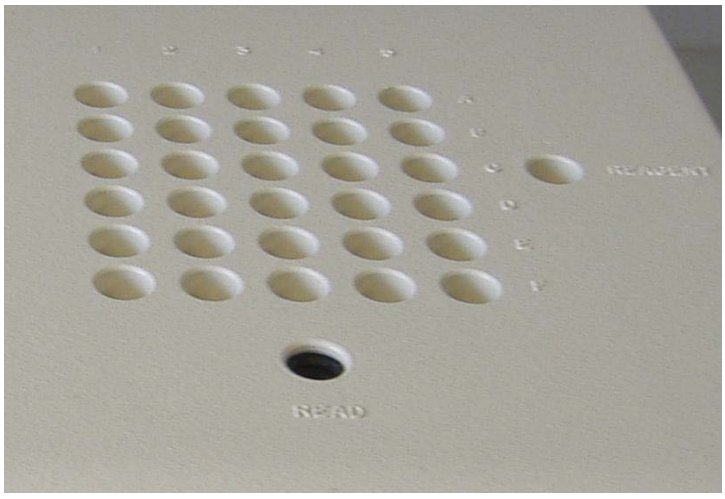

Figure 3-1: Image of the Microtox multiwell plate

3.4.5 Toxi-ChromoPlate™

This test comes in a prepackaged kit with all reagents and bacteria contained. This kit was keep at -20°C and defrosted when required. The test measures the capacity of toxicants to inhibit the *de novo* synthesis of an inducible enzyme - β-galactosidase in a pre-stressed *E. coli* strain. The sensitivity of the test is increased by lyophilizing the bacteria. By exposing and incubating, the bacteria's ability to hydrolyse a chromogenic compound is measured. A toxicant interferes with the ability for the bacteria to repair the enzyme involved in hydrolyse of the chromogenic compound and it is the interference of this repairing function that's measured in the test.

3.4.5.1 Procedure

A positive and a negative blank is used in this testing procedure. The positive blank is achieved using $HgCl_2$ and acts as a reference for the toxicity. By variation of the concentration of $HgCl_2$, a gradient of colour change is seen. The highest colour density indicates no toxicity and no colour 100% toxicity (Figure 3-2).

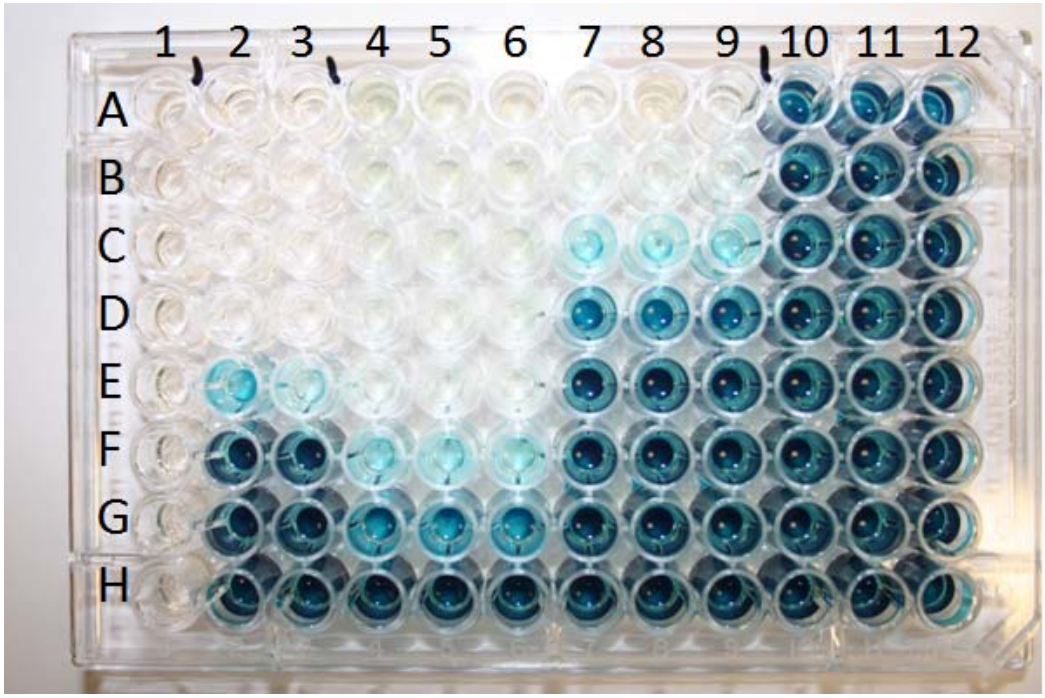

Figure 3-2: Toxi-Chromo multiwell plate with letter and number position highlighted

The filling of the multiwell plate follows this procedure (note column 12 was not used in testing):

1. 200 µL of sample was pipetted to wells 3A-11A, followed by 200 µL of standard toxicant and mixed.
2. 100 µL of diluent was added to added to the rest of the wells 3B-11B to 3H-11H.
3. A serial two-fold dilutionof each sample (and the standard toxicant) was performed by transferring 100 µL from Well A of each column into the next well (B) and continuing so by serial transferring 100 µL until Well G.
4. Row H has no toxin and functions as a blank.
5. The blank column (1) was filled with 100 µL mixture of enzyme β-galactosidase and co-factors required for the recovery of the bacteria from their stressed state.
6. Rehydration of the bacteria was performed by mixing lyophilized bacteria with a rehydration solution (composition unknown) and leaving at room temperature for 15 mins.
7. The toxicity test was performed by transferring 1 ml of rehydrated bacteria to a bottle vial containing the mixture of enzyme β-galactosidase and co-factors.
8. 100 µL of the bacteria mix in part 7 was transferred to all wells except those in row 1.
9. The multiwell plate was incubated at 37°C for 90mins
10. For colour development 100 µL of a chromogenic substrate was dispensed to all wells. The multiwell plate was incubated for a further 30 mins.

Colour development (optical density) was quantified spectrophotometry by using a 96 well micro-plate reader set to 615 nm (Spectra Max Plus 384, Molecular Devices, Sunnyvale California, USA). Toxicity was calculated by the equation:

$$\% \text{ Toxicity} = \frac{1 - OD_T}{OD_C} \text{ X } 100$$

Where:

— OD_T: is optical density of treated cells

OD_C: is optical density of control cells

3.5 Statistical treatment of toxicity testing results

Toxicity is normally presented as an effective or lethal concentration ($E(L)C_{50}$) where 50% of the samples effect was observed in the bioassays population. Due to the complex chemical composition of treated landfill leachate, the concentration can only be expressed as a % of the actual sample concentration. All statistics have been calculated with Statistica with details of individual tests given in the relevant methodology chapter.

To calculate a suitable $E(L)C_{50\ a}$ non-linear regression analysis of the data was performed using Statistica (StatSoft, Bedford, U.K.). The multiple regressions performed by Statisitca involve the formation of a trend line through the multiple data points on a graph (Figure 3-3). This process is carried out internally within the Statisitca software and is not displayed to the user.

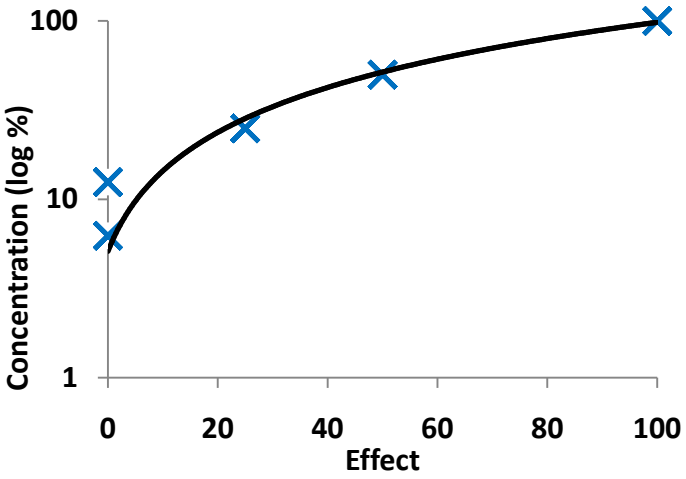

Figure 3-3: An example of the graph formed in multiple regression analysis. Each point represents a mean of observations. The y-axis is a logarithmic scale.

To produce an EC value the effect is set as the independent variable and the concentration is set as the dependent variable. The 50% effect on the species is calculated within the software when the user asks for a prediction on the 50% on the independent as this is used to calculate the E(L)C_{50} as a %. An example of multiple regression analysis is shown below (Table 3-3). Each dilution set of 3/4 replicates was averaged and used to calculate an EC_{50}.

Table 3-3: Multiple regression analysis for Arpley *T. platyurus* Unfiltered pH 3

	Predicting Values for (Arpley unstacked T) variable: Dependent Include condition: v3='Unfiltered pH 3'		
Variable	B-Weight	Value	B-Weight * Value
Independent	-0.889365	50.00000	-44.4683
Intercept			99.9822
Predicted			55.5140
-95.0%CL			50.5182
+95.0%CL			60.5098

4 Overview of selected landfill leachate chemistry and toxicity

4.1 Rationale for work

Landfill is still the primary destination of municipal solid waste in the U.K. though this is changing as more waste is redirected for recycling and incineration (Defra, 2009). The objective of the EU Landfill Directive 1999 (CoE, 1999) is to protect the environment from the adverse effects of landfilling e.g. methane release and pollutant contamination of the water table (Fatta *et al*., 1999). With the introduction of technical management requirements for the operation of landfills e.g. using geosynthetic linings the chance of leachate seepage to the outside environment is limited. The co-disposal of hazardous, non-hazardous and inert wastes was stopped with the introduction of the Directive. Liquid wastes disposal in landfills was also halted. The Directive required that hazardous and municipal (including non-hazardous) waste be separated and disposed of at different sites. Incremental targets for the diversion of biodegradable waste from landfill were introduced with the Directive i.e. reduce biodegradable waste going to landfill to 75% of 1995 figures by 2010.

Even though the co-disposal of wastes has ceased in the UK, municipal waste can still contain a number of different waste types. Slack *et al*., (2005) carried out a study of the types of waste that were being disposed of as municipal waste in the U.K. The types of waste identified included: household papers; gardening products including pesticides and herbicides; paint and inks; household cleaning products such as bleach; pharmaceuticals; and waste cooking oils. These types of chemicals and their degradation products can cause environmental damage through a number of mechanisms e.g. toxic, corrosive, flammable, reactive, carcinogenic, teratogenic, mutagenic and ecotoxic, among other hazards, and can also be bioaccumulative and/or persistent (Slack *et al*., 2005).

4.2 Introduction

The chemical composition of landfill leachate is highly variable. Age, size, location, and waste type landfilled are some of the main influences on the composition of landfill leachate (Wang *et al*., 2003). A number of general parameters are used to describe and compare landfill leachate. These general parameters describe the bulk qualities of landfill leachate and allow a general impression of the leachate's age, strength and biodegradability. The parameters most often encountered are: chemical oxygen demand (COD); biological oxygen demand (BOD); ammoniacal-nitrogen; and BOD/COD ratio (Table 4-1).

Table 4-1: Differences in leachate composition of young and older leachates (Kurniawan *et al*., 2006b).

Parameter	<5 years old leachate	>5 years old leachate
COD	30,000-60,000	5,000-20,000
BOD	4,000-13,000	<2,000
NH_4-N	500-2000	3000-5000
BOD/COD	0.4-0.7	<0.1

These bulk parameters only offer a very general view of landfill leachates. To fully understand the chemistry of a leachate more information is needed than just bulk chemical characterisitcs. Landfill leachates tend to have a very high content of inorganic ions e.g. chloride, potassium, sodium and calcium. These salts can vary in concentration from mg/L to g/L. These concentrations have an effect on the nature of other ions and compounds within the leachate (Kjeldsen *et al*., 2002).

Depending on the type and age of the landfill, varying amount of xenobiotic organic compounds will be present which are not fully considered in the bulk parameters. These xenobiotic organics concentrations can vary from ng/L to mg/L concentrations. Many of the compounds can cause severe problems to biological life e.g. endocrine disruptors. A full

understanding of the concentration and their toxicity will help tailor the correct treatment strategy for making landfill leachate effluent safe to the environment.

4.2.1 Marston Vale leachate treatment plant

The Marston Vale leachate treatment plant (MVP) is situated on the Stewartby landfill site, Bedfordshire. The plant and landfill are both operated by Waste Recycling Group (WRG). MVP treats the Stewartby landfill leachate plus 17 other landfill leachates generated from nearby landfill sites operated by WRG. Landfill leachates from the 17 landfills have a variable strength.

The plant was commissioned in 2002 by Birse Process Engineering Ltd, U.K. MVP is designed to treat up to 400 m^3 d^{-1} of leachate (Gibbs, 2007). Due to the variability in imported leachate strength from the various sites it is necessary to blend the leachates in a pair of reception tanks. This blending process stops a toxic shock reaction of adding a leachate with an abnormally high chemical strength to the activated sludge. A separate tank with a probe monitors the dissolved oxygen (DO) concentration of the blended leachate. If the measured DO concentration is below 1 mg/L a signal is sent to the main computer to initiate a plant shut down in order to stop potential damage to the activated sludge population (Toddington *et al.*, 2005). Unfortunately this measurement of toxicity has not operated since 2007 as it was found to cause too many false alarms.

The blended leachate is pumped from the reception tanks through a heat exchanger to a dedicated 500 m^3 d^{-1} aerated activated sludge tank. Powdered activated carbon (PAC) is added to the aeration tank. PAC is added in order to adsorb toxic organic compounds as which stops toxic shock of the activated sludge by organic toxic shock (Lee and Lim, 2005). The PAC also helps assist in the settling of solids from the aeration tank. The dedicated aeration tank has a weir system where the overflow is diverted to a clarifying tank. In the clarifying tank the solids are allowed to settle at the bottom. The clarifying tank also operates

a weir system with the overflow being directed to a dissolved air flocculation tank. Solid removal is vital in order to recycle activated sludge to create an effluent that meets discharge standards. Finally the leachate is passed through a sand filter before discharge to sewers operated by Anglian Water.

To achieve an effluent suitable for discharge the chemical conditions of the leachate treatment plant need to be tightly controlled. The leachate needs to be maintained at alkaline pH and this is achieved by the addition of 47% caustic soda (Gibbs, 2007). The addition of caustic soda is automated through the use of probes in the aeration tank. The activated sludge is fed by the addition of phosphoric acid as phosphate is often deficient in landfill leachate. This treatment plant is able to remove 70% of COD and 100% of NH_4-N from the influent before discharge to sewers for further treatment (Toddington *et al*., 2005).

MVP was selected for this project due to the advanced chemical and biological treatment regime adopted when compared to the other sites in the UK. Stewartby landfill is also of interest because it has received hazardous waste in the past which might have implications for the toxicity of the landfill leachate e.g. high concentrations chlorinated compounds in the waste.

4.2.2 Arpley landfill leachate treatment plant

Arpley landfill in Merseyside began operating in 1988 and is one of the largest landfills in the UK (Robinson *et al*., 2003b). At present it receives 800,000 tonnes of municipal waste a year from around Merseyside. This site covers 202 Ha acres, with 80 Ha of former landfill being converted to a nature reserve.

WRG took over the Arpley landfill site from Cheshire County Council in 1999. At the takeover, the site had 8m leachate head but with consent for just 1m. After six months of operation by WRG the Environment Agency imposed a fine of £19,000 for this head of leachate. To remedy the situation WRG tankered away 140,000 litres of leachate for

treatment and invested over £3 million for leachate collection and treatment. Treatability trials were carried out by WRG and it was initially envisaged that the nearby sewage treatment works (STW) could be used to treat the effluent prior to discharge. With this in mind a £100,000 pipeline was bored under the Mersey River. Due to not meeting discharge consents effluents from the leachate treatment plant could not be discharged through the STW due to the chemical composition of the treated landfill leachate. Instead, the effluent had to be discharged to the River Mersey.

Chemical analysis of the leachate revealed the presence of poly chlorinated biphenyls (PCB) at concentrations of <1 mg L^{-1} (Robinson *et al*., 2003b). Through a detailed investigation, Enviros were able to demonstrate that the total amount of PCB that had been disposed of in the landfill was 100g and that the effluent being discharged to the Mersey contained a lower concentration of PCB than was already present in the Mersey (Robinson *et al*., 2003b). This is a small amount of a toxic substance in the landfill is unlikely to be a large risk to the environment.

The treatment plant cost £2 million when it was finally completed in 2001 and is capable of treating up to 450 m^3/d of leachate. Due to the amount of leachate needing treatment three sequencing batch reactors (SBR) were commissioned at the site. The SBR units run on a 20-hour on 4-hour empty routine. During the emptying stage most solids are retained in the unit. The 3 SBR are roofed in order to maintain treatment efficiency during the winter.

After these treatments the leachate is pumped into an extensive reed bed system. This four phase reed bed system is used to polish the leachate and can treat 450 m^3/d. After this process the leachate is pumped to a dissolved air flocculation device to remove suspended solids. This device operates using pumped air to create flocs of suspended solids that are then mechanically scooped off the surface. This treatment system is on average able to remove

100% of NH_4-N and 80% of COD (Robinson *et al*., 2003b). Treated landfill leachate is discharged into the River Mersey.

Arpley landfill, like Stewartby, has received hazardous waste in the past. The treatment strategy adopted at Arpley is a simpler design than MVP. The Enviros SBR with reed bed polishing stage became very popular in the 1990's due to it simple design and fully computerised control and operation. Arpley was selected to make a comparison between the simpler SBR method and the more complicated continuous reactor of MVP.

4.2.3 Buckden, Cambridgeshire

Buckden landfill leachate treatment plant was commissioned in 1995 by Enviros Consulting Ltd, U.K (formerly Aspinwall and Company). Buckden leachate treatment plant was installed due to the release of isoproturon and mecoprop into the River Ouse that resulted from a lack of leachate containment. In 1994 a large fin drain collection and interception system was installed to capture the leachate. Primary treatment is performed in twin SBR that are designed to treat up to 100 m^3/day (Robinson *et al*., 2003a). Secondary treatment is performed by reed bed followed by ozonation followed by a 2^{nd} larger reed bed. Ozonation is used to ring-opening cyclic organic compounds e.g. the phenyl group of isoproturon. The effluent is finally discharged to the River Great Ouse. This treatment system is on average able to remove 100% of NH_4-N and >60% of COD from the influent (Robinson and Barr, 1999).

Initially the Environment Agency was so concerned about the quality of the Buckden effluent that they insisted that its toxicity should be assessed with *Oncorhynchus mykiss* (rainbow trout) – at a cost of £1,100 a month, due to fresh fish needed every 4 days. The high cost was due to the need for two tanks containing 12 fish each with the fish being replaced every four days. Over a six month period no toxic effects were observed on the trout swimming in the effluent. Since the trout test was so expensive it has been replaced with the Microtox testing

system for screening of effluent quality which is more labour efficient and costs the operator less. GC-MS tests of the effluent have revealed the presence of one toxic compound in the effluent, bromal-8,9-tribromacetaldehyde. This species is not present in the influent or the effluent from the first reed bed so its production is being linked with the ozonation process (Robinson *et al*., 2003a).

Due to the presence of isoproturon and mecoprop in the leachates the usual SBR treatment strategy of Enviros was supplemented with an ozonation plant. At present the ozonation plant is set to the lowest output level due to the activated sludge being able to remove the isoproturon and mecoprop.

The leachate from Buckden has a long history of toxicity testing due to the presence of isoproturon and mecoprop in the landfills. These two features made it an interesting site for investigation. Buckden landfill is also due to expand in the next 5 years so an assessment of the present treatment strategies' effectiveness was considered an informative process for WRG (Farrow, 2008 personal communication).

4.3 Aim and objective

Aim: To characterise the MVP, Buckden and Arpley landfill leachates.

Objectives:

I. Demonstrate the changes in quality of the leachates before and after treatment. Compare and contrast the changes in the chemical composition of raw and treated leachates.
II. Present organic and inorganic chemical data from the database maintained by WRG on raw and treated landfill leachates from Arpley, MVP and Buckden.
III. Compare the leachate quality data for Arpley, MVP and Buckdem with reports on leachates from the U.K and rest of the world.

IV. Compare the chemistry of these leachates so that relationships with toxicity can be hypothesized.

4.4 Chemical composition of raw and treated leachate analysis

4.4.1 Arpley raw and treated landfill leachate

Chemical data from the WRG Access database and arranged into a pivot table in Excel 2007. Sampling varied between daily for COD, nitrate and nitrite and monthly for the other parameters. COD, nitrate, chloride and nitrite were determined with Hach cell tests at WRG's on-site labs. The other parameters were determined at Severn Trent laboratories, Coventry.

The available chemical data for Arpley starts on the 13.12.2006 and ends on the 06.07.2009 (Table 4-2). The changes in the chemistry of leachate between the raw and treated leachate is demonstrated. The concentrations of ammoniacal-nitrogen, alkalinity, BOD, calcium, and COD all fall rapidly during the treatment of leachate. This change was expected as biological treatment targets ammoniacal-nitrogen, BOD and COD.

The concentration of sodium increases by 2,600 mg/l during the treatment of landfill leachate. Sulphate concentration almost doubles during the treatment process which is linked to the metabolism of sulphide to sulphate. Magnesium concentration rises by an average of 26 mg/L between the raw and treated leachates. The concentration of Cl^- remained unchanged with the treatment.

Biological nitrification of ammoniacal-nitrogen with oxygen is the standard method for the elimination of ammoniacal-nitrogen in landfill leachate. This process converts ammoniacal-nitrogen to nitrite, a highly toxic substance to most biological organisms. Therefore nitrite needs to be converted to nitrate, a much safer compound, during treatment of landfill leachate. This process of ammoniacal-nitrogen removal is seen in the changes of concentration from 1,296 mg/L in the influent to 0.7 mg/L in the final effluent. This reduction

in ammoniacal-nitrogen was accompanied by an equally large rise in the concentration of nitrate in the effluent, from 3mg/L to 1,436 mg/L in the effluent.

Activated sludge uses phosphate and phosphorus as a metabolic nutrient and was the limiting nutrient for proper functioning of biological treatment of landfill leachate (Ozturk *et al*., 2003). The influent concentration 0.81 mg/L of phosphate which is increased to 7.4 mg/L in the effluent. Phosphorus concentration increases between the influent and the effluent from 1.5 to 7.8 mg/L. These increases are mostly likely due to the addition of phosphoric acid as a feed for the activated sludge.

Heavy metals in the treated leachates are concentrations below the toxic level. This low concentration was to be expected as it has been previously shown that heavy metals are attenuated in the methanogenic phase of landfilling (Kjeldsen *et al*., 2002). The concentration of manganese decreases during the treatment process from 0.8 to 0.1275 mg/L. Manganese was probably being lost in the sludge from the biological process due to oxidation and precipitation.

Suspended solids are removed during the treatment process via flocculation and the reed bed system. These solids can carry toxicants on their surface which in turn can be ingested by filter feeders (Norberg-King *et al*., 1991) The suspended solid content of the Arpley leachate was reduced from 107 mg/L to 8.41 mg/L in the effluent. Standard deviation of 5.4 mg/L around the mean which demonstrates treatment was able to consistently reduce the suspended solid content of landfill leachate.

Table 4-2: Arpley influent and effluent concentrations of COD, BOD, inorganic ions and heavy metals. Red list chemicals highlighted in red (Source: WRG chemical database).

Determinant	Influent Avg(mg/L)	Max	Min	Stdev	CEV (%)	Effluent Avg (mg/L)	Max	Min	Stdev	CEV (%)
Alkalinity as CaCO3	2,341.35	4,960.00	351.00	1117.12	47.71	7,100.00*	7,100.00	7100.00	1.00	0.00
Ammoniacal nitrogen	1296.0	3350	8.1	482.2	37.2	0.7	100	0.01	4.1	500.6
Arsenic (Dissolved)	0.03	0.03	0.02	0.01	28.28	-	-	-	-	-
Barium (Dissolved)	0.05	0.08	0.02	0.04	84.85	-	-	-	-	-
Beryllium (Dissolved)	0.01	0.01	0.01	0.00	0.00	-	-	-	-	-
BOD (5 Day)	3090.96	16500.00	20.00	3111.38	100.66	3.43	11.50	2.00	1.91	55.78
Boron (Dissolved)	9.95	24.90	1.03	3.50	35.23	-	-	-	-	-
Cadmium (Dissolved)	0.01*	0.01	0.01	0.00	0.00	0.01*	0.01	0.01	0.00	0.00
Calcium (Dissolved)	69.01	152.00	22.00	37.90	54.92	150.00*	150.00	150.00	0.00	0.00
Chloride	2,662.41	3,400.00	2,070.00	379.57	14.26	2,406.59	4,250.00	747.00	611.17	25.40
Chromium (Dissolved)	0.17	0.60	0.10	0.10	57.70	0.20	0.20	0.20	0.00	0.00
Cobalt (Dissolved)	0.02*	0.02	0.02	0.00	0.00	-	-	-	-	-
Conductivity (ms cm^{-1})	20,010.52	34,900.00	806.00	7551.72	37.74	18,500.00*	18,500.00	18,500.00	0.00	0.00
Copper (Dissolved)	0.01	0.01	0.01	0.00	0.00	0.20*	0.20	0.20	0.00	0.00
Lead (Dissolved)	0.01	0.03	0.01	0.01	55.79	0.01*	0.01	0.01	0.00	0.00
Magnesium (Dissolved)	84.10	168.00	47.00	24.23	28.81	50.00*	50.00	50.00	0.00	0.00
Manganese (Dissolved)	0.17	0.38	0.07	0.09	53.17	0.80*	0.80	0.80	0.00	0.00
Mercury (Dissolved)	0.00*	0.00	0.00	0.00	0.00	0.00*	0.00	0.00	0.00	0.00
Molybdenum (Dissolved)	0.01*	0.01	0.01	0.00	0.00	-	-	-	-	-
Nitrate	2.7	39.2	0.2	5.9	201.2	1279.6	2210.0	0.2	373.1	29.2
Nitrite	1.15	80.3	0.01	7.6	660.9	0.24	3.58	0.01	0.36	
Phosphate (Total) as P	0.81	1.44	0.18	0.89	109.99	7.41	19.50	0.63	3.81	51.44
Phosphorus (Dissolved)	1.50	2.40	0.60	1.27	84.85	7.79	10.70	5.74	2.59	33.24
Potassium (Dissolved)	927.42	1,310.00	113.00	254.15	27.40	720.00	720.00	720.00	0.00	0.00
Selenium (Dissolved)	0.18	0.18	0.17	0.01	4.04	-	-	-	-	-
Sulphate as SO4 (Dissolved)	250.39	641.00	134.00	118.44	47.30	116.00*	116.00	116.00	0.00	0.00
Tellurium (Dissolved)	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-
Zinc (Dissolved)	0.24	0.58	0.08	0.14	59.23	1.40*	1.40	1.40	0.00	0.00

Avg= Average; Stdev= Standard deviation; CEV= Coefficient of variation; - = outside of detection limits; *= only 1 measurement in database

An extensive chemical determination of many organic compounds was routinely carried out by WRG as part of their consent from the Environment Agency (Table 4-5). Determination of organic compounds was carried out by GC-MS by Severn Trent Laboratories, Coventry. These determinations include the List I (Red List pollutants). The Red List was introduced in 1989 and is a group of 24 chemicals (Table 4-3) that pose a particularly high hazard to the environment due to their toxicity, bioaccumulative nature and concentration in the environment (highlighted as red in the tables) (Agg and Zabel, 1990). These chemicals on the red list need to be reduced from the environment due to the high hazard posed by them to the health.

The extensive range of compound concentrations determined are all present in very small amounts with many of them being below detection limits. The compounds that did appear within the detection limits were present in very small concentrations which had very little variation in the concentration over the sampling period. This data demonstrates that all of the Red List substances are being discharged in levels below that of the *D. magna* EC_{50}.

Table 4-3: The List I (Red List) chemicals as identified by the Environment Agency and the 48 hr (unless stated) *Daphnia magna* EC_{50} concentration from Pesticides Database (PAN, 2010).

Parameter	*D. magna* EC_{50} (µg/L)	Parameter	*D. magna* EC_{50} (µg/L)
Cadmium	13.2 (Max conc)	Aldrin	28.0
Mercury	20,000	Isodrin	1,000
Lindane	1.4	Endrin	41.0
Pentachlorophenol	2.71 (24 hr)	1,2-dichloroethane	324,000
DDT	0.1	Perchloroethylene	7,500
Endosulfan sulphate	2,120	Tributyl tin acetate	3.3
Carbon tetrachloride	7,700	Trichloroethylene	59.0
Chloroform	29,000	Trichlorobenzene	2,359
Hexachlorobenzene	0.85	Dichlorvos	0.26
Hexachlorobutadiene	500	Dieldrin	79.5
Dieldrin	79.5	Malathion	2.1
Simazine	3,500	Atrazine	39,000

A second group of organic compounds known as 'List II' are considered less toxic than the List I chemicals (Table 4-4). Due to the lower hazard posed to the environment these chemicals need only have their effluent concentration reduced. Mecoprop is a herbicide that is linked to environmental harm but is present in an average concentration of 0.02 µg/L which is far below the *Daphnia magna* EC_{50} of 100 mg/L. PCB concentrations is very low in the effluent and of no concern to the environment which is in agreement with Robinson *et al* (2003).

Table 4-4: The List II chemicals as identified by the Environment Agency

2,4-dichlorophenol	Dichlorvos	Dimethoate	Endosulfan	Fenitrothion	2,4-D
Arsenic	Atrazine	Azinphos-methyl	Biphenyl	Benzene	4-chloro-3-methyl
Vanadium	Zinc	Chloronitrotoluenes	Nickel	Phenol	2-chlorophenol
Copper	Boron	Lead	Iron	Mothproofers	Demeton
Arsenic	Bentazone	Chromium	Linuron	Mecoprop	Naphthalene
Omethoate	Simazine	Trifluralin	Xylenes	Toluene	Trichloroethanes

Table 4-5: Arpley "red list" organic compound average concentrations in effluent from 27.11.2006 to 29.10.2010. 'Red List' substances highlighted red in tables (Source: WRG chemical database).

Determinant	Avg (µg/L)	Max	Min	Stdev	CoV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
1,1,1,2-Tetrachloroethane*	1.00	1.00	1.00	0.00	0.00	2,6-Dichlorobenzonitrile*	0.02	0.02	0.02	0.00	0.00
1,1,1-Trichloroethane*	1.00	1.00	1.00	0.00	0.00	2-Chlorotoluene*	1.00	1.00	1.00	0.00	0.00
1,1,2,2-Tetrachloroethane*	1.00	1.00	1.00	0.00	0.00	4,6-Dinitro-2-methylphenol*	0.02	0.02	0.02	0.00	0.00
1,1,2-Trichloroethane*	1.00	1.00	1.00	0.00	0.00	4-Chlorotoluene*	1.00	1.00	1.00	0.00	0.00
1,1-Dichloroethane*	1.00	1.00	1.00	0.00	0.00	4-CPA*	0.02	0.02	0.02	0.00	0.00
1,1-Dichloroethene*	1.00	1.00	1.00	0.00	0.00	Aldrin*	0.02	0.02	0.02	0.00	0.00
1,1-Dichloropropene*	1.00	1.00	1.00	0.00	0.00	alpha - Lindane*	0.10	0.10	0.10	0.00	0.00
1,2,3,4-Tetrachlorobenzene*	0.02	0.02	0.02	0.00	0.00	Ametryn	0.03	0.04	0.02	0.01	43.30
1,2,3-Trichlorobenzene	2.01	5.00	0.01	2.73	136.25	Atrazine	0.04	0.06	0.03	0.02	43.30
1,2,3-Trichloropropane*	1.00	1.00	1.00	0.00	0.00	Azinphos-ethyl	0.01	0.02	0.01	0.01	43.30
1,2,4-Trichlorobenzene*	5.00	5.00	5.00	0.00	0.00	Azinphos-methyl	0.01	0.02	0.01	0.01	43.30
1,2,4-Trimethylbenzene*	1.00	1.00	1.00	0.00	0.00	Benazolin*	0.02	0.02	0.02	0.00	0.00
1,2-Dibromo-3-chloropropane*	5.00	5.00	5.00	0.00	0.00	Bentazone*	0.02	0.02	0.02	0.00	0.00
1,2-Dibromoethane*	1.00	1.00	1.00	0.00	0.00	Benzene*	1.00	1.00	1.00	0.00	0.00
1,2-Dichlorobenzene*	5.00	5.00	5.00	0.00	0.00	Bromobenzene*	1.00	1.00	1.00	0.00	0.00
1,2-Dichloroethane*	1.00	1.00	1.00	0.00	0.00	Bromochloromethane*	1.00	1.00	1.00	0.00	0.00
1,2-Dichloropropane*	1.00	1.00	1.00	0.00	0.00	Bromodichloromethane*	1.00	1.00	1.00	0.00	0.00
1,3,5-Trichlorobenzene*	0.02	0.02	0.02	0.00	0.00	Bromoform*	1.00	1.00	1.00	0.00	0.00
1,3,5-Trimethylbenzene*	1.00	1.00	1.00	0.00	0.00	Bromomethane*	5.00	5.00	5.00	0.00	0.00
1,3-Dichlorobenzene*	1.00	1.00	1.00	0.00	0.00	Bromoxynil*	0.02	0.02	0.02	0.00	0.00
1,3-Dichloropropane*	1.00	1.00	1.00	0.00	0.00	Carbon Tetrachloride*	1.00	1.00	1.00	0.00	0.00
1,4-Dichlorobenzene*	1.00	1.00	1.00	0.00	0.00	Carbophenothion	0.01	0.02	0.01	0.01	43.30
2-(1-Methylpropyl)-4,6-dinitrophenol*	0.02	0.02	0.02	0.00	0.00	Chlordane*	0.01	0.01	0.01	0.00	0.00
2,2-Dichloropropane*	1.00	1.00	1.00	0.00	0.00	Chlorfenvinphos	0.04	0.06	0.03	0.02	43.30
2,3,6-TBA*	0.02	0.02	0.02	0.00	0.00	Chlorobenzene*	1.00	1.00	1.00	0.00	0.00
2,4,5-T*	0.02	0.02	0.02	0.00	0.00	Chloroethane*	5.00	5.00	5.00	0.00	0.00
2,4,5-Trichlorophenol*	0.02	0.02	0.02	0.00	0.00	Chloroform*	5.00	5.00	5.00	0.00	0.00
2,4-D*	0.02	0.02	0.02	0.00	0.00	Chloromethane*	1.00	1.00	1.00	0.00	0.00

Determinant	Avg (µg/L)	Max	Min	Stdev	CoV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
2,4-DB*	0.02	0.02	0.02	0.00	0.00	Chlorpyralid	0.02	0.02	0.02	0.00	0.00
2,4-Dinitrophenol*	0.02	0.02	0.02	0.00	0.00	Chlorpyriphos*	0.02	0.02	0.02	0.00	0.00
Chlorpyriphos-ethyl	0.03	0.04	0.02	0.01	47.14	HCH-alpha*	0.10	0.10	0.10	0.00	0.00
Chlorpyriphos-methyl	0.01	0.02	0.01	0.01	43.30	HCH-beta*	0.02	0.02	0.02	0.00	0.00
Chlorthalonil*	0.01	0.01	0.01	0.00	0.00	HCH-delta*	0.01	0.01	0.01	0.00	0.00
cis-1,2-dichloroethene*	1.00	1.00	1.00	0.00	0.00	HCH-gamma*	0.02	0.02	0.02	0.00	0.00
cis-1,3-dichloropropene*	1.00	1.00	1.00	0.00	0.00	Heptachlor*	0.01	0.01	0.01	0.00	0.00
cis-chlordane*	0.02	0.02	0.02	0.00	0.00	Heptachlorepoxide*	0.02	0.02	0.02	0.00	0.00
cis-permethrin*	0.01	0.01	0.01	0.00	0.00	Hexachlorobenzene*	0.02	0.02	0.02	0.00	0.00
delta-Lindane*	0.01	0.01	0.01	0.00	0.00	Hexachlorobutadiene*	5.00	5.00	5.00	0.00	0.00
Diazinon	0.01	0.02	0.01	0.01	43.30	Ioxynil*	0.02	0.02	0.02	0.00	0.00
Dibromochloromethane*	1.00	1.00	1.00	0.00	0.00	Isodrin*	0.02	0.02	0.02	0.00	0.00
Dibromomethane*	1.00	1.00	1.00	0.00	0.00	iso-propyl benzene*	1.00	1.00	1.00	0.00	0.00
Dibutyl Tin	0.04	0.05	0.02	0.02	60.61	m & p xylene*	1.00	1.00	1.00	0.00	0.00
Dicamba*	0.02	0.02	0.02	0.00	0.00	Malathion	0.03	0.04	0.02	0.01	43.30
Dichlofop*	0.02	0.02	0.02	0.00	0.00	MCPA*	0.02	0.02	0.02	0.00	0.00
Dichlorodifluoromethane*	1.00	1.00	1.00	0.00	0.00	MCPB*	0.02	0.02	0.02	0.00	0.00
Dichloroprop*	0.02	0.02	0.02	0.00	0.00	Mecoprop*	0.02	0.02	0.02	0.00	0.00
Dichlorprop*	0.02	0.02	0.02	0.00	0.00	Methacriphos	0.01	0.02	0.01	0.01	43.30
Dichlorvos	0.01	0.02	0.01	0.01	43.30	Methoxychlor*	0.01	0.01	0.01	0.00	0.00
Dieldrin*	0.01	0.01	0.01	0.00	0.00	Mevinphos	0.03	0.04	0.02	0.01	43.30
Dimethoate	0.03	0.04	0.02	0.01	43.30	Napthalene*	5.00	5.00	5.00	0.00	0.00
Endosulfan A/B	0.02	0.04	0.02	0.01	25.50	n-butylbenzene*	1.00	1.00	1.00	0.00	0.00
Endosulfan I*	0.01	0.01	0.01	0.00	0.00	o,p-DDD*	0.01	0.01	0.01	0.00	0.00
Endosulfan II*	0.01	0.01	0.01	0.00	0.00	o,p-DDE*	0.02	0.02	0.02	0.00	0.00
Endosulfan Sulphate*	0.01	0.01	0.01	0.00	0.00	o,p-DDT*	0.01	0.01	0.01	0.00	0.00
Endrin*	0.01	0.01	0.01	0.00	0.00	o-xylene*	1.00	1.00	1.00	0.00	0.00
Endrin Ketone*	0.02	0.02	0.02	0.00	0.00	p,p-DDD*	0.01	0.01	0.01	0.00	0.00
Ethion	0.01	0.02	0.01	0.01	43.30	p,p-DDE*	0.01	0.01	0.01	0.00	0.00
Ethylbenzene*	1.00	1.00	1.00	0.00	0.00	p,p-DDT*	0.02	0.02	0.02	0.00	0.00
Etrimphos	0.01	0.02	0.01	0.01	43.30	Parathion-ethyl	0.01	0.02	0.01	0.01	43.30
Fenitrothion	0.026	0.04	0.02	0.01	43.30	Parathion-methyl	0.03	0.04	0.02	0.01	43.30

Determinant	Avg (µg/L)	Max	Min	Stdev	CoV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
Fenoprop*	0.02	0.02	0.02	0.00	0.00	PCB 28*	0.05	0.05	0.05	0.00	0.00
Fenthion	0.03	0.04	0.02	0.01	43.30	PCB 52*	0.05	0.05	0.05	0.00	0.00
Flamprop*	0.02	0.02	0.02	0.00	0.00	PCB101*	0.05	0.05	0.05	0.00	0.00
Flamprop - Isopropyl*	0.02	0.02	0.02	0.00	0.00	PCB118*	0.05	0.05	0.05	0.00	0.00
PCB138*	0.05	0.05	0.05	0.00	0.00	Simizine	0.021	0.03	0.011	0.02	66.6
PCB153*	0.05	0.05	0.05	0.00	0.00	Triallate*	0.02	0.02	0.02	0.00	0.00
PCB180*	0.05	0.05	0.05	0.00	0.00	Triazaphos	0.01	0.04	0.00	0.01	60.16
Pendimethalin*	0.02	0.02	0.02	0.00	0.00	Tributyl Tin	0.02	0.10	0.02	0.02	64.45
Pentachlorobenzene*	0.02	0.02	0.02	0.00	0.00	Trichlopyr*	0.02	0.02	0.02	0.00	0.00
Pentachlorophenol (PCP)	0.04	0.05	0.02	0.02	60.61	Trichloroethene*	5.00	5.00	5.00	0.00	0.00
Phenoxy Acetic Acid*	0.02	0.02	0.02	0.00	0.00	Trichlorofluoromethane*	1.00	1.00	1.00	0.00	0.00
Phenoxy Butyric Acid*	0.02	0.02	0.02	0.00	0.00	Trietazine	0.03	0.04	0.02	0.01	43.30
Phenoxy Propionic Acid*	0.02	0.02	0.02	0.00	0.00	Trifluralin*	0.02	0.02	0.02	0.00	0.00
Phosalone	0.01	0.02	0.01	0.01	43.30	Triphenyltin	0.28	0.50	0.05	0.32	115.71
Phosphamidon	0.03	0.04	0.02	0.01	43.30	Vinyl Chloride*	1.00	1.00	1.00	0.00	0.00
						Triadimefon*	0.02	0.02	0.02	0.00	0.00

Avg= Average; Stdev= Standard deviation; CoV= Coefficient of variation; *= only 1 measurement in database

4.4.2 MVP raw and treated landfill leachate

The chemical database for MVP is more limited than the Arpley database (Table 4-6). This is due to the discharge consents for Anglian Water sewers accepting treated leachate and conducting further treatment on the leachate compared with Arpley were the treated leachate is discharged straight to the River Mersey. The treatment plant influent is high in ammoniacal-nitrogen, alkalinity, BOD and COD. Variance of the influent chemicals is mostly low with only BOD varying widely by ≥81.6%.Treatment of the influent significantly reduce the concentration of these compounds. Reduction of BOD and COD indicates that a significant proportion of the organic content is biodegradable. The concentration of chloride is reduced by treatment from 3,572to 2,859 mg/L but this reduction is probably not due to the treatment process as chloride is a conservative ion e.g. concentration unaltered by biological treatment. The effluent ammoniacal-nitrogen data contains a probable anomaly in the 219 value as no similar value is present in the database.

Table 4-6: MVP influent and effluent average concentrations of COD and inorganic ions (Source: WRG chemical database).

Determinant	Influent Avg (mg/L)	Max	Min	Stdev	CoV	Effluent Avg (mg/L)	Max	Min	StDev	CoV
Alkalinity as $CaCO_3$	4,598.8	5,100	2,822	598.00	13.0	1,453.0	2,052	259.8	225.76	15.4
Ammoniacal-nitrogen	647.3	1,200.00	220	231.82	35.8	3.4	219	0.03	16.38	480.3
BOD (5 Day)	343.4	1,100	140	280.41	81.6	3.2	18	1	2.38	73.7
Chloride	3,572.1	5,510	348	668.34	18.7	2,859.3	5,700	709.5	726.65	25.4
COD	2,544.5	4,408	317	551.12	21.6	795.6	1,570	20	184.52	23.1
Conductivity (s cm^{-1})	17,574.9	26	6	4.92	28.0	1,5829.	33,800	1,290	2,857.00	18.0
Phosphate (Total) as P	0.1	0.1	0.1	0	0	-	-	-	-	-
Phosphate (Total) as PO_4	2.0	25.6	0.01	2.17	108	-	-	-	-	-
Calcium*	-	-	-	-	-	174.5	231	119	35.06	13.2
Magnesium*	-	-	-	-	-	116.0	143	96	15.32	20.1
Potassium*	-	-	-	-	-	840.7	934	702	78.90	9.38

*= Determined by David Thomas; Avg= Average; Stdev= Standard deviation; CEV= Coefficient of variation; - = outside of detection limits; *= only 1 measurement in database

More organic compounds are determined for MVP effluents than for Arpley effluents (Table 4-7). This extensive dataset was carried out for Anglian Water as they collect the effluents in the sewer system and treat for a second time. Determination of organic compounds was carried out with GC-MS by Severn Trent Laboratories, Coventry. The Red List (List I) shows that these compounds are present in the effluent at very low concentrations and no individual pollutant is in the toxic range of *D. magna* (compare Table 4-3 with Table 4-7). The highest concentration was for 4-Bromofluorobenzene with 91.9 µg/L. Many of the compounds concentration was out of the range of detection. Concentrations of nitro and chloro-benzene/phenol compounds in the effluents are very low and of no concern to the environment (Halfon and Reggiani, 1986). The herbicide mecoprop is present in slightly higher concentrations in the effluent of MVP vs Arpley. PCB concentrations in the effluent were very low at 0.1 µg/L with little variation.

Table 4-7: The average concentration of 'Red List'organic compounds in the MVP treated leachates from 27.11.2006 to 31.8.2010. 'Red List'substances highlighted red in tables (Source: WRG chemical database).

Determinant (µg/L)	Average (µg/L)	Max	Min	Stdev	COV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
1,1,1,2-Tetrachloroethane*	3.00	3.00	3.00	0.00	0.00	1,3,5-Trinitrobenzene	4.80	8.00	4.00	1.79	37.27
1,1,1-Trichloroethane*	3.00	3.00	3.00	0.00	0.00	1,3-Dichlorobenzene	4.80	8.00	4.00	1.79	37.27
1,1,2,2-Tetrachloroethane*	3.00	3.00	3.00	0.00	0.00	1,3-Dichloropropane*	3.00	3.00	3.00	0.00	0.00
1,1,2-Trichloroethane*	3.00	3.00	3.00	0.00	0.00	1,3-Dinitrobenzene	4.80	8.00	4.00	1.79	37.27
1,1-Dichloroethane*	3.00	3.00	3.00	0.00	0.00	1,4-Dichlorobenzene	4.80	8.00	4.00	1.79	37.27
1,1-Dichloroethene*	3.00	3.00	3.00	0.00	0.00	1,4-Naphthoquinone	4.80	8.00	4.00	1.79	37.27
1,1-Dichloropropene*	3.00	3.00	3.00	0.00	0.00	1-Naphthylamine	4.80	8.00	4.00	1.79	37.27
1,2,3,4,6,7,8-heptachlorodibenzodioxin*	0.00	0.00	0.00	0.00	0.00	2-(1-Methylpropyl)-4,6-dinitrophenol	4.80	8.00	4.00	1.79	37.27
1,2,3,4,6,7,8-heptachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00	2,2-Dichloropropane*	3.00	3.00	3.00	0.00	0.00
1,2,3,4,7,8,9-heptachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00	2,3,4,6,7,8-hexachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00
1,2,3,4,7,8-hexachlorodibenzodioxin*	0.00	0.00	0.00	0.00	0.00	2,3,4,6-Tetrachlorophenol	4.80	8.00	4.00	1.79	37.27
1,2,3,4,7,8-hexachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00	2,3,4,7,8-pentachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00
1,2,3,6,7,8-hexachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00	2,3,7,8,-tetrachlorodibenzodioxin*	0.00	0.00	0.00	0.00	0.00
1,2,3,6,7,8-hexchlorodibenzodioxin*	0.00	0.00	0.00	0.00	0.00	2,3,7,8-tetrachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00
1,2,3,7,8,9-hexachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00	2,4,5-Trichlorophenol	4.80	8.00	4.00	1.79	37.27
1,2,3,7,8,9-hexachlorodibenzo-p-dioxin*	0.00	0.00	0.00	0.00	0.00	2,4,6-Trichlorophenol	4.80	8.00	4.00	1.79	37.27
1,2,3,7,8-pentachlorodibenzofuran*	0.00	0.00	0.00	0.00	0.00	2,4-D	0.03	0.05	0.01	0.03	94.28
1,2,3,7,8-pentachlorodibenzo-p-dioxin*	0.00	0.00	0.00	0.00	0.00	2,4-Dichlorophenol	4.67	8.00	4.00	1.63	34.99
1,2,3-Trichlorobenzene	2.50	3.00	0.00	1.22	48.99	2,4-Dinitrotoluene	4.80	8.00	4.00	1.79	37.27
1,2,3-Trichloropropane	3.00	3.00	3.00	0.00	0.00	2,6-Dichlorophenol	4.80	8.00	4.00	1.79	37.27
1,2,4,5-Tetrachlorobenzene	4.80	8.00	4.00	1.79	37.27	2,6-Dinitrotoluene	4.80	8.00	4.00	1.79	37.27
1,2,4-Trichlorobenzene	0.15	4.00	0.00	0.67	445.33	2-Acetylaminofluorene	4.80	8.00	4.00	1.79	37.27
1,2,4-Trimethylbenzene*	3.00	3.00	3.00	0.00	0.00	2-Chloronaphthalene	4.80	8.00	4.00	1.79	37.27
1,2-Dibromo-3-chloropropane*	3.00	3.00	3.00	0.00	0.00	2-Chlorophenol	4.67	8.00	4.00	1.63	34.99

Determinant (µg/L)	Average (µg/L)	Max	Min	Stdev	COV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
1,2-Dibromoethane*	3.00	3.00	3.00	0.00	0.00	2-Chlorotoluene*	3.00	3.00	3.00	0.00	0.00
1,2-Dichlorobenzene	4.80	8.00	4.00	1.79	37.27	2-Methyl-4,6-dinitrophenol	4.80	8.00	4.00	1.79	37.27
1,2-Dichloroethane	0.23	3.00	0.10	0.59	259.41	2-Methylnaphthalene	4.80	8.00	4.00	1.79	37.27
1,2-Dichloropropane*	3.00	3.00	3.00	0.00	0.00	2-Methylphenol	4.80	8.00	4.00	1.79	37.27
1,3,5-Trichlorobenzene*	4.00	4.00	4.00	0.00	0.00	2-Naphthylamine	4.80	8.00	4.00	1.79	37.27
1,3,5-Trimethylbenzene*	3.00	3.00	3.00	0.00	0.00	2-Nitroaniline	6.00	8.00	4.00	2.83	47.14
2-Nitroaniline*	4.00	4.00	4.00	0.00	0.00	Benzo-ghi-perylene	4.02	8.00	0.10	2.50	62.20
2-Picoline	4.80	8.00	4.00	1.79	37.27	Benzo-k-fluoranthene	4.02	8.00	0.10	2.50	62.20
3- & 4-Chlorophenol	4.80	8.00	4.00	1.79	37.27	Benzyl alcohol	4.80	8.00	4.00	1.79	37.27
3- & 4-Methylphenol	4.80	8.00	4.00	1.79	37.27	Biphenyl	0.01	0.01	0.01	0.00	0.00
3,3-Dichlorobenzidine	4.80	8.00	4.00	1.79	37.27	bis(2-chloroethoxy)methane	4.80	8.00	4.00	1.79	37.27
3,3-Dimethylbenzidine	4.80	8.00	4.00	1.79	37.27	bis(2-chloroethyl) ether	4.80	8.00	4.00	1.79	37.27
3-Methylcholanthrene	4.80	8.00	4.00	1.79	37.27	bis(2-Chloroisopropyl) ether	4.80	8.00	4.00	1.79	37.27
3-Nitroaniline	4.80	8.00	4.00	1.79	37.27	bis(2-Ethylhexyl)phthalate	4.67	8.00	4.00	1.63	34.99
4-Aminobiphenyl	4.80	8.00	4.00	1.79	37.27	Bisphenol A*	0.01	0.01	0.01	0.00	0.00
4-Bromofluorobenzene (Recovered)	91.89	105.10	87.40	4.39	4.77	Bromobenzene*	3.00	3.00	3.00	0.00	0.00
4-Bromophenyl phenyl ether	4.80	8.00	4.00	1.79	37.27	Bromochloromethane*	3.00	3.00	3.00	0.00	0.00
4-Chloro-3-methylphenol	4.67	8.00	4.00	1.63	34.99	Bromodichloromethane*	3.00	3.00	3.00	0.00	0.00
4-Chloroaniline	4.80	8.00	4.00	1.79	37.27	Bromoform*	3.00	3.00	3.00	0.00	0.00
4-Chlorophenyl phenylether	4.80	8.00	4.00	1.79	37.27	Bromomethane*	3.00	3.00	3.00	0.00	0.00
4-Chlorotoluene	3.00	3.00	3.00	0.00	0.00	Bromophos*	0.00	0.00	0.00	0.00	0.00
4-Nitrophenol	4.80	8.00	4.00	1.79	37.27	Butylbenzyl Phthalate	4.80	8.00	4.00	1.79	37.27
7,12-Dimethylbenz(a)anthracene	4.80	8.00	4.00	1.79	37.27	Carbazole	4.80	8.00	4.00	1.79	37.27
Acenaphthene	4.02	8.00	0.10	2.50	62.20	Carbon Tetrachloride	6.39	10.00	1.00	3.23	50.53
Acenaphthylene	4.02	8.00	0.10	2.50	62.20	Carbophenothion*	0.00	0.00	0.00	0.00	0.00
Acetophenone	4.80	8.00	4.00	1.79	37.27	Chlordane-alpha*	2.00	2.00	2.00	0.00	0.00
Aldrin	0.00	0.00	0.00	0.00	0	Chlordane-gamma*	2.00	2.00	2.00	0.00	0.00
Amitraz*	0.01	0.01	0.01	0.00	0.00	Chlorfenvinphos*	0.00	0.00	0.00	0.00	0.00
Aniline	4.80	8.00	4.00	1.79	37.27	Chlorobenzene*	3.00	3.00	3.00	0.00	0.00
Anthracene	4.02	8.00	0.10	2.50	62.20	Chlorobenzilate	4.80	8.00	4.00	1.79	37.27

Determinant (µg/L)	Average (µg/L)	Max	Min	Stdev	COV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
Atrazine	0.11	1.08	0.00	0.14	130.86	Chloroethane*	3.00	3.00	3.00	0.00	0.00
Azinphos-ethyl	0.50	2.00	0.00	1.00	200.00	Chloroform*	3.00	3.00	3.00	0.00	0.00
Azinphos-methyl	0.09	0.75	0.00	0.16	179.27	Chloromethane*	3.00	3.00	3.00	0.00	0.00
Azobenzene	4.80	8.00	4.00	1.79	37.27	Chloronitrotoluene*	0.01	0.01	0.01	0.00	0.00
Bentazone*	0.05	0.05	0.05	0.00	0.00	Chlorpyriphos-ethyl*	0.00	0.00	0.00	0.00	0.00
Benzene*	1.00	1.00	1.00	0.00	0.00	Chlorpyriphos-methyl*	0.00	0.00	0.00	0.00	0.00
Benzidine	4.80	8.00	4.00	1.79	37.27	Chlortoluron*	0.06	0.06	0.06	0.00	0.00
Benzo-a-anthracene	4.02	8.00	0.10	2.50	62.20	Chrysene	4.02	8.00	0.10	2.50	62.20
Benzo-a-pyrene	4.02	8.00	0.10	2.50	62.20	cis-1,2-dichloroethene*	3.00	3.00	3.00	0.00	0.00
Benzo-b-fluoranthene	4.02	8.00	0.10	2.50	62.20	cis-1,3-dichloropropene*	3.00	3.00	3.00	0.00	0.00
cis-permethrin*	2.00	2.00	2.00	0.00	0.00	Ethyl methanesulfonate	4.80	8.00	4.00	1.79	37.27
Cyfluthrin*	0.01	0.01	0.01	0.00	0.00	Ethylbenzene*	3.00	3.00	3.00	0.00	0.00
Cypermethrin*	4.00	4.00	4.00	0.00	0.00	Fenitrothion	0.09	0.75	0.00	0.16	182.78
Cyromazine*	0.01	0.01	0.01	0.00	0.00	Fenthion*	0.00	0.00	0.00	0.00	0.00
DDT (all isomers) *	0.00	0.00	0.00	0.00	0.00	Flucofuron*	0.01	0.01	0.01	0.00	0.00
Deltamethrin	0.01	0.01	0.01	0.00	0.00	Flumethrin*	0.01	0.01	0.01	0.00	0.00
Diallate	4.80	8.00	4.00	1.79	37.27	Fluorene	4.02	8.00	0.10	2.50	62.20
Diazinon*	0.00	0.00	0.00	0.00	0.00	HCH-alpha*	2.00	2.00	2.00	0.00	0.00
Dibenz-ah-anthracene	4.02	8.00	0.10	2.50	62.20	HCH-beta	71.00	100.00	2.00	40.63	57.23
Dibenzofuran	4.80	8.00	4.00	1.79	37.27	HCH-gamma	6.29	40.00	1.00	5.20	82.73
Dibromochloromethane*	3.00	3.00	3.00	0.00	0.00	Heptachlor Epoxide*	2.00	2.00	2.00	0.00	0.00
Dibromofluoromethane (Recovered)	100.75	104.90	96.40	2.30	2.28	Hexachlorobenzene	0.18	8.00	0.00	0.94	515.48
Dibromomethane*	3.00	3.00	3.00	0.00	0.00	Hexachlorobutadiene	3.50	4.00	3.00	0.55	15.65
Dichlobenil*	2.00	2.00	2.00	0.00	0.00	Hexachlorocyclpentadiene*	4.00	4.00	4.00	0.00	0.00
Dichlorodifluoromethane*	3.00	3.00	3.00	0.00	0.00	Hexachloroethane	4.80	8.00	4.00	1.79	37.27
Dichloromethane*	10.00	10.00	10.00	0.00	0.00	Hexachloropropene	4.80	8.00	4.00	1.79	37.27
Dichlorvos	0.13	3.00	0.00	0.34	265.18	High-cis Cypermethrin*	0.01	0.01	0.01	0.00	0.00
Dieldrin*	0.00	0.00	0.00	0.00	0.00	Indeno-123-cd-pyrene	4.02	8.00	0.10	2.50	62.20
Diethyl phthalate	4.80	8.00	4.00	1.79	37.27	Isodrin	4.67	8.00	4.00	1.63	34.99
Dimethoate*	0.00	0.00	0.00	0.00	0.00	Isophorone	4.80	8.00	4.00	1.79	37.27
Dimethyl phthalate	4.80	8.00	4.00	1.79	37.27	iso-propyl benzene*	3.00	3.00	3.00	0.00	0.00
di-n-Butyl phthalate	4.80	8.00	4.00	1.79	37.27	Isoproturon*	0.05	0.05	0.05	0.00	0.00

Determinant (µg/L)	Average (µg/L)	Max	Min	Stdev	COV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
di-n-octyl phthalate	4.80	8.00	4.00	1.79	37.27	Isosafrole	4.80	8.00	4.00	1.79	37.27
Dioxalthion	4.78	8.70	0.00	4.39	91.78	Kepone	4.80	8.00	4.00	1.79	37.27
Diphenylamine	4.80	8.00	4.00	1.79	37.27	Linuron*	0.05	0.05	0.05	0.00	0.00
Disulphoton*	0.00	0.00	0.00	0.00	0.00	m & p xylene	0.46	3.00	0.10	0.93	201.31
Diuron*	0.05	0.05	0.05	0.00	0.00	Malathion	0.09	0.75	0.00	0.16	180.52
Endosulfan Sulphate*	2.00	2.00	2.00	0.00	0.00	MCPA*	0.05	0.05	0.05	0.00	0.00
Endosulfan-beta*	2.00	2.00	2.00	0.00	0.00	Mecoprop	0.30	2.97	0.04	0.51	171.54
Endosulphan-alpha	0.02	0.12	0.00	0.02	92.84	Mevinphos	0.00	0.02	0.00	0.01	218.17
Endrin*	0.00	0.00	0.00	0.00	0.00	MTBE*	3.00	3.00	3.00	0.00	0.00
Ethion	0.00	0.01	0.00	0.01	136.93	n-butylbenzene*	3.00	3.00	3.00	0.00	0.00
Nitrobenzene	12.20	41.00	4.00	16.19	132.73	Pentachlorobenzene	4.80	8.00	4.00	1.79	37.27
N-Nitrosodiethylamine	4.80	8.00	4.00	1.79	37.27	Pentachloroethane	4.80	8.00	4.00	1.79	37.27
N-Nitrosodimethylamine	4.80	8.00	4.00	1.79	37.27	Pentachloronitrobenzene	4.80	8.00	4.00	1.79	37.27
N-Nitroso-di-N-butylamine	4.80	8.00	4.00	1.79	37.27	Pentachlorophenol (PCP)	0.76	9.00	0.05	1.64	215.08
N-Nitrosomethylethylamine	4.80	8.00	4.00	1.79	37.27	Phenanthrene	4.10	8.00	0.60	2.35	57.20
N-Nitrosopiperidine	4.80	8.00	4.00	1.79	37.27	Phenol	4.80	8.00	4.00	1.79	37.27
N-Nitrosopyrrolidine	4.80	8.00	4.00	1.79	37.27	Phorate*	0.00	0.00	0.00	0.00	0.00
Nonyl Phenol	0.01	0.01	0.01	0.00	0.00	Phosalone	0.04	0.11	0.00	0.06	138.07
Nonyl Phenol Ethoxylate	0.01	0.01	0.01	0.00	0.00	Pirimiphos-methyl*	0.00	0.00	0.00	0.00	0.00
n-propylbenzene*	3.00	3.00	3.00	0.00	0.00	p-iso propyl toluene*	3.00	3.00	3.00	0.00	0.00
o,p-DDE	8.60	40.00	1.00	7.77	90.37	Propetamphos	0.08	0.26	0.00	0.12	155.72
o,p-DDT	8.01	40.00	1.00	7.10	88.65	Propyzamide	4.80	8.00	4.00	1.79	37.27
o,p-TDE	8.05	40.00	1.00	7.06	87.67	Pyrene	4.02	8.00	0.10	2.50	62.20
octachlorodibenzo-4-dioxin*	0.01	0.01	0.01	0.00	0.00	Pyridine	4.80	8.00	4.00	1.79	37.27
o-toluidine	4.80	8.00	4.00	1.79	37.27	Simazine	0.09	0.75	0.00	0.11	128.98
o-xylene	0.44	3.00	0.10	0.93	213.95	Styrene*	3.00	3.00	3.00	0.00	0.00
p,p-DDT	11.63	400.00	1.00	34.13	293.41	TAME (tert-amyl methyl ether) *	3.00	3.00	3.00	0.00	0.00
p,p-TDE	8.11	40.00	1.00	7.11	87.73	Tecnazene*	2.00	2.00	2.00	0.00	0.00
PAH Total (EPA16) *	1.10	1.10	1.10	0.00	0.00	Terbufos*	0.00	0.00	0.00	0.00	0.00
Parathion-methyl*	0.00	0.00	0.00	0.00	0.00	Tetrachloroethene*	3.00	3.00	3.00	0.00	0.00

Determinant (µg/L)	Average (µg/L)	Max	Min	Stdev	COV	Determinant	Average (µg/L)	Max	Min	Stdev	COV
PCB (7 Congeners Total) *	0.20	0.20	0.20	0.00	0.00	Tetrachloromethane	0.10	0.20	0.10	0.01	9.09
PCB 28	0.01	0.20	0.00	0.02	328.54	Toluene*	3.00	3.00	3.00	0.00	0.00
PCB 52	0.01	0.20	0.00	0.02	309.03	Toluene-d8 (Recovered)	97.25	105.30	91.20	3.97	4.09
PCB101	0.01	0.20	0.00	0.02	349.14	trans-1,2-dichloroethene*	3.00	3.00	3.00	0.00	0.00
PCB118	0.01	0.20	0.00	0.02	340.75	trans-1,3-dichloropropene*	3.00	3.00	3.00	0.00	0.00
PCB138	0.01	0.20	0.00	0.02	336.83	trans-permethrin*	2.00	2.00	2.00	0.00	0.00
PCB153	0.01	0.20	0.00	0.02	352.81	Triadimefon	0.17	0.34	0.00	0.17	96.45
PCB180	0.01	0.20	0.00	0.02	350.52	Triallate*	2.00	2.00	2.00	0.00	0.00
p-Dimethylaminoazobenzene)	4.80	8.00	4.00	1.79	37.27	Tributyl Tin*	0.02	0.02	0.02	0.00	0.00

Avg= Average; Stdev= Standard deviation; CEV= Coefficient of variation; - = outside of detection limits; *= only 1 measurement in database

4.4.3 Buckden raw and treated landfill leachate

Table 4-8: Buckden influent and treated leachate average concentrations of COD and inorganic ions (Source: WRG chemical database). All data in (mg/L) unless stated.

Determinant	Influent Min	Influent Mean	Influent Max	Influent COV (%)	Treated leachate Min	Treated leachate Mean	Treated leachate Max	Treated leachate CEV (%)
Alkalinity as CaCO3	833.0	3,452.6	12,000.0	67.8	180.0	558.4	1,110.0	43.7
Ammoniacal-nitrogen	1.0	706.8	3,000.0	77.0	0.1	1.7	20.0	141.0
BOD (5 Day)	32.5	185.2	1,400.0	104.1	1.0	7.0	88.0	170.7
Calcium (Dissolved)	82.0	164.2	354.0	32.4	150.0	186.8	250.0	14.1
Chloride	51.0	1,447.3	4,600.0	51.1	406.0	931.1	1,600.0	28.5
COD	150.0	1,802.4	17,000.0	136.1	71.0	368.7	845.0	35.5
Conductivity	1,700.0	13,262.3	39,000.0	65.3	3,500.0	9,474.5	65,000.0	83.1
Isoproturon	0.1	1.1	3.7	119.2	0.1	0.7	6.3	173.9
Manganese (Dissolved)	0.0	0.2	0.5	56.5	0.0	0.2	0.6	64.3
Mecoprop	0.3	44.4	119.0	68.1	0.1	0.3	1.8	134.7
Nitrate as N	1.0	5.3	24.0	100.7	33.0	442.4	1,400.0	41.9
Nitrite as N	0.1	0.7	20.0	395.8	0.1	8.4	220.0	340.6
pH	7.0	7.8	8.7	3.2	7.2	8.0	8.7	4.2
Potassium (Dissolved)	0.6	331.3	1,100.0	77.1	0.1	263.6	370.0	27.3
Sodium (Dissolved)	643.0	977.7	2,400.0	34.4	685.0	1,232.8	1,800.0	24.1
Sulphate as SO4 (Dissolved)	12.0	133.4	609.0		190.0	205.0	220.0	10.3
Suspended Solids	5.0	135.8	720.0	76.8	1.0	14.6	76.0	96.9

Avg= Average; Stdev= Standard deviation; CEV= Coefficient of variation; - = outside of detection limits; *= only 1 measurement in database

Chemical data for raw and treated landfill leachate demonstrate that the Buckden raw and treated leachates than those from Arpley and MVP (Table 4-8). The Buckden database is more complete than that held for MVP which is due to the tighter discharge consent imposed by the Environment Agency for the release of these effluents into the River Ouse. Metabolism of ammoniacal-nitrogen is efficient in the twin SBR setup at Buckden with the concentration reduced from 706 to 1.7 mg/L and a corresponding rise in the concentration of nitrate from 5 to 443 mg/L. The mean COD concentration of 1,802 mg/L in Buckden influents is lower than the >2,500 mg/L from Arpley and MVP. Similarly, the concentration of potassium and chloride, 263 and 931 mg/L respectively, are much lower than the concentrations from MVP and Arpley. These ions have been identified previously as posing a significant risk to the aquatic environment (Mount *et al*., 1997). Concentrations of calcium and sodium are similar between all three sites which indicate that these ions are unlikely to be the product of waste decomposition and more likely the product of environmental factors e.g. soil mineral composition.

4.5 Comparison with previously reported data

Chemical composition data for landfill leachate from the U.K. and around the world are presented in Table 4-9. Treatment of landfill leachate is able to remove >40% of the influent COD concentration. This significant removal indicates that a large concentration of COD from the landfill is biodegradable. The remaining COD concentration is refractory to biological treatment processes and is likely humic and fulvic acids (Huo *et al*., 2009).

Ammoniacal-nitrogen in landfills is produced by the breakdown of proteins in the landfill. Formation of ammoniacal-nitrogen is also possible from breakdown of ammonium containing products e.g. food preservers, plastics and fertilisers (Pivato and Gaspari, 2006). Ammoniacal-nitrogen is a very toxic compound in the non-ionised form with removal a key target of any treatment operation. The Environment Agency impose a limit of 1.8 mg/L for

ammoniacal-nitrogen discharge to the River Ouse which is a sensitive waterway (Robinson and Barr, 1999). From the data ammoniacal-nitrogen is being removed to levels of≤1 mg/L which meets the Environment Agency's limit.

The concentration of sodium at Arpley and MVP was 3,872 and 4,411 mg/L respectively. Examination of the previously reported concentrations of sodium from the U.K. is generally much lower at≤2,000 mg/L than the MVP and Arpley concentrations. Only one of the reports has a similar sodium concentration. From the international reports sodium concentration is in all cases is lower than the concentrations from Arpley and MVP which is possibly due to the addition of caustic soda for pH control.

Potassium concentration varies greatly between each of the sites reported. The MVP and Arpley samples average K concentrations were 840 and 905 mg/L respectively. The reported concentrations of K range from a low of 190 to a high of 925 in the UK samples. Internationally the concentration of K ranges from 546 mg/L in Italy to 1,799 mg/L in Taiwan. Potassium is a key constituent of most fertilisers and so the high levels of this ion in many of the samples can probably be linked to the disposal of gardening products e.g. weed killer (Slack *et al*., 2004).

Average calcium concentrations in MVP and Arpley samples were 174 and 68 mg/L respectively. Previously reported concentrations in the UK are similar to these levels though the concentration at Arpley does seem slightly low. MVP and Arpley are very similar to the international reports of Ca concentrations in landfill leachate. Similarly, the Mg concentration of Arpley and MVP are similar to the previously reported concentrations.

Chloride is considered a conservative ion, concentration is unaffected by biological treatment, in landfill leachate (Fatta *et al*., 1999). The average concentration of chloride at Arpley and MVP was 2,618 and 3,565 mg/L respectively. Of the international studies only

the sample from Greece has a higher concentration of Cl^- . Generally, the concentration of Cl^- in the MVP samples was twice as high as the other reported samples.

The concentration of $CaCO_3$ has been shown by the report of Robinson and Barr (1999) to be reduced by biological treatment. The alkalinity concentration of MVP of Arpley was 1,684 and 2,443 mg/L respectively. The concentration in treated landfill leachates from the UK was lower though the concentration was similar to that of MVP. Unfortunately the international reports lack data on the alkalinity concentration in treated leachates.

Sulphide is toxic at concentrations of 5 mg/L so the conversion to the less toxic sulphate is a key treatment strategy for reducing the risk posed by landfill leachate to the environment (Kjeldsen *et al*., 2002). Sulphate concentration is not reported in the UK references. Only 1 measurement was reported by Robinson and Barr (1999) for Buckden and is reproduced correctly here. The MVP and Arpley average sulphate concentrations were low when compared to the reports from international reports. A very high concentration of 2,237 mg/L was recorded in the Italian sample (Pivato and Gaspari, 2006).

Biological treatment of landfill leachate effectively reduces the COD concentration of the samples by $\geq$42%. The concentrations of Ca^{2+}, Na^+, Mg^{2+}, K^+, Cl^- are reduced slightly during treatment. The concentration of $CaCO_3$ drops significantly with biological treatment. Conversely, the concentration of SO_4^{2-} rises rapidly with treatment as sulphide is converted to sulphate (Devare and Bahadir, 1994).

Table 4-9: Average chemical composition of both raw and biologically treated landfill leachates. All data is in mg/L.

Location	UT/T*	COD	Total NH_3	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	K^+	$CaCO_3$	SO_4^{2-}	Reference
Trecatti, U.K.	UT	~1,000	541	750	249	206	1,070	469	4,540	-	(Robinson and Barr, 1999)
	T	299	0.5	608	262	193	991	379	608	-	
Buckden, U.K.	UT	600	405	1100	141	70	1,830	190	2,560	~50	(Robinson and Barr, 1999)
	T	350	1	-	171	-	1,740	194	1,270	-	
Fiskerton, U.K.	UT	1,640	414	620	180	236	897	571	4,430	-	(Robinson and Barr, 1999)
	T	378	0.08	582	153	141	912	528	720	-	
Summerston, U.K.	UT	2,140	1,120	1530	109	154	1,970	844	5,780	-	(Robinson and Barr, 1999)
	T	1,020	0.5	1700	406	179	1,970	925	95	-	
Deep Moor, U.K.	UT	1,070	167	621	200	134	634	329	2,565	-	(Robinson and Barr, 1999)
	T	154	0.6	496	161	113	533	260	550	-	
Florida, U.S.A.	T	1850	~1,300	1,495	-	-	-	555	6,213	-	(Ward *et al*., 2002)
Taiwan	UT	4,340**	-	3,524**	133.7**	163**	-	1,799**	-	-	(Fan *et al*., 2006)
Hong Kong	T	1,670**	1,640**	1,190**	-	63**	-	632**	-	-	(Chu *et al*., 1994)
Germany	UT	63,700	-	-	290**	270**	-	-	-		(Al-Yaqout

Location	UT/T*	COD	Total NH_3	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	K^+	$CaCO_3$	SO_4^{2-}	Reference
		**									and Hamoda, 2003)
Germany	UT	2,975	-	989	170	109	1,243	953	-	50	(Devare and Bahadir, 1994)
	T	61	-	828	136	109	944	660	-	567	
India	UT	27,200	2,675	545	-	-	-	1,590	2,645	-	(Mor *et al*., 2006)
Greece	UT	5,086	1,216	1,984	57	-	4,149	1,676	2,685	356	(Fatta *et al*., 1999)
Italy	UT	2,140	1,194	654	172	105	1,950	594	7,400	847	(Pivato and Gaspari, 2006)
	UT	1,393	854	1,070	118	110	1,879	630	4,400	2,237	
	UT	1,832	770	644	204	150	1,099	546	7,050	205	

* UT = Raw, T = Treated; ** Highest concentration;

4.6 Conclusions

- The effectiveness of Arpley and MVP landfill leachate treatment has been demonstrated in the reduction of the COD, BOD, nitrite, phosphate and ammoniacal-nitrogen concentrations.
- The presence of Red list toxic organic compounds in the effluents is very low and not of immediate concern in terms of risk of to the environment if discharged.
- Many other organic compounds are present in concentrations below the detector limits of the GC-MS instrument used.

5 Long term toxicity responses and a toxicity identification evaluation procedure of treated landfill leachates

5.1 Rationale for work

The toxicity and genotoxicity of municipal (non-hazardous) landfill leachates is as high as the toxicity of industrial (hazardous) landfill leachates (Schrab *et al.*, 1993, Kjeldsen *et al.*, 2002). This is counterintuitive though it can be explained by the concentration of ammoniacal-nitrogen in both types of waste. Ammoniacal-nitrogen is generated from the breakdown of amines which are found in many organic compounds that will be present in both types of landfill. This degradation product is highly toxic to most organisms.

Municipal waste contains many of the same compounds as industrial wastes (Slack *et al.*, 2004). Chemical assessment of landfill leachate can never detect all possible toxicants due to the complex matrix of chemical interactions (Yatribi and Nejmeddine, 2000). To overcome this limitation whole effluent toxicity (WET) testing with micro bioassays aims to give a holistic view of the toxicity of complex chemical solutions like landfill leachate. In this chapter the compiled WET testing results of Marston Vale leachate treatment plant (MVP) and Arpley treatment plant's effluents are presented to demonstrate the toxicity levels over the course of one year.

An initial screening operation for determining toxicity levels in treated landfill leachate is needed. The toxicity identification evaluation (TIE) procedure acts as a screening operation for investigating the toxicity levels in industrial effluents. This procedure allows researchers to identify the classes of substances that are the cause of toxicity in samples. Classes of substance that can be identified with this procedure include xenobiotic organic substances, heavy metals, and ammonia. Previous reports on the composition of landfill leachate had

identified these substances as being present. By identifying the classes of substances responsible for toxicity a number of research routes was opened for exploration.

This work is to act as an initial determinant of the levels of toxicity found in treated landfill leachate samples from three landfill leachate treatment plants operated by the project sponsor Waste Recycling Group (WRG).

5.2 Introduction

Leachate generation is principally by the percolation of water through waste deposited in a landfill. The percolating water becomes contaminated through contact with the decomposing waste material. Ammoniacal-nitrogen, readily biodegradable substances e.g. volatile fatty acids, heavy metals, and hundreds of toxic trace organic substances, and many inorganic salts have been identified within landfill leachate (Kjeldsen *et al*., 2002). Leachate treatment reduces its hazard to the environment and is designed to meet local discharge standards.

Globally, biological treatment is the favoured strategy for the treatment of landfill leachate with further secondary treatment by ozonation and membrane filtration becoming more common (Surmacz-Gorska *et al*., 2004). Biological treatment is especially effective at removing ammoniacal-nitrogen and reducing biological oxygen demand (BOD) (Robinson *et al*., 2003a). Although leachate treatment can consistently achieve the discharge requirements imposed by environmental regulators, concerns remain over residual substances discharged to the environment and could include toxins. For example, although the concentration of organic substances is reduced by biological treatment it is not wholly removed and effluent COD concentrations of >500 mg/L are common (Robinson *et al*., 2003b). Toxicity of the COD fraction of treated landfill leachate has not previously been reported and it is thought to be non-toxic as it is primarily made of up of humic substances (Kang *et al*., 2002) and previously reported to limit the toxicity of some pesticides (McDonald *et al*., 2004). There are concerns regarding the heavy metal binding capacity of humic substances and to what

extent it can limit toxicity in landfill leachate (Barker and Stuckey, 1999, Robinson *et al*., 2003a). Due to the variability in the composition of landfill leachate the concentrations of organic pollutants can vary widely (Robinson *et al.*, 2003a, Cotman and Gotvajn, 2010). This variation can cause concern with regulators and result in operators being required to reduce the COD concentration.

Due to the complex nature of landfill leachate traditional chromatographic-mass spectrometry analysis would be time consuming, costly, and may not reveal all of the micropollutants present or provide a measure of their potential harm to the environment. Focusing on particular toxins for analysis is not suitable for identifying possible interactions between toxins. This analysis is also not able to determine changes in the toxicity of effluents over time. An alternative approach is to use bioassays for assessing the toxicity of the potentially complex mixture of residual hazards present in treated leachate (Toussaint *et al*., 1995). Acute toxicity assessment is able to detect a larger range of possible hazards associated with landfill leachate than traditional chemical identification. Assessment using organisms that represent the different trophic levels of aquatic environments is necessary, with a battery comprising a number of species recommended (Bernard *et al*., 1996, Thomas *et al*., 2009).

5.2.1 Toxicity identification procedure

Acute WET testing can identify whether toxins are present within a sample of landfill leachate but cannot reveal the exact cause. The TIE Phase 1 procedure alters the bioavailability of the components found in wastewater samples by chemical and physical manipulations. These manipulations render a particular group of toxicants not bioavailable. These manipulations can reveal the types of substances that produce adverse responses in organisms (Figure 1) (Norberg-King *et al*., 1991).

The TIE procedure was developed by the United States Environmental Protection Agency (USEPA) to help industry identify the causes of toxicity in their effluents (Norberg-King *et*

al., 1991). By identifying the causes of toxicity in effluents, strategies can be introduced into treatment for the removal of these toxins. TIE procedures have been used to assess the toxicity of paper mill effluents (Wang *et al.*, 2008, Reyes *et al.*, 2009), metal plating industry (Kim *et al*., 2008), dye industry (Chan *et al*., 2003), sewage treatment (Svenson *et al.*, 2000, Babín *et al.*, 2001, Hongxia *et al.*, 2004) and agricultural runoff (Werner *et al.*, 2000, Anderson *et al.*, 2002).

The TIE procedure involves 3 phases which work to identify the causes of toxicity. In the TIE Phase 1 a series of chemical and physical manipulations are used to identify the classes that are the cause of toxicity (Norberg-King *et al*., 1991). After the class of substance has been identified the TIE Phase 2 is used to determine the chemical identity (Durham *et al*., 1993). Depending on the chemical class that has been identified a suitable chemical isolation technique is used e.g. for an organic pollutant solid phase extraction (SPE) is utilised to extract the substance and GC-MS used to determine the concentration (Fernández *et al*., 2004, Reyes *et al*., 2009). If Phase 1 and 2 have identified the likely causes of toxicity Phase 3 uses a hypothesis led approach to confirm the substance(s) causing toxicity in a sample. For example, by spiking a sample with S^{2-}, the eluent from a SPE cartridge was shown that S^{2-} was the reason for toxicity in marine sediments collected close to Hong Kong Island (Kwok *et al*., 2005).

The Phase 1 process aims to reveal whether the cause of toxicity is attributable to individual toxins or is caused by a group of toxins. The TIE procedure acts as a screening exercise to identify the classes of substances that could be the cause of toxicity, it does not definitively identify the exact substance causing toxicity.

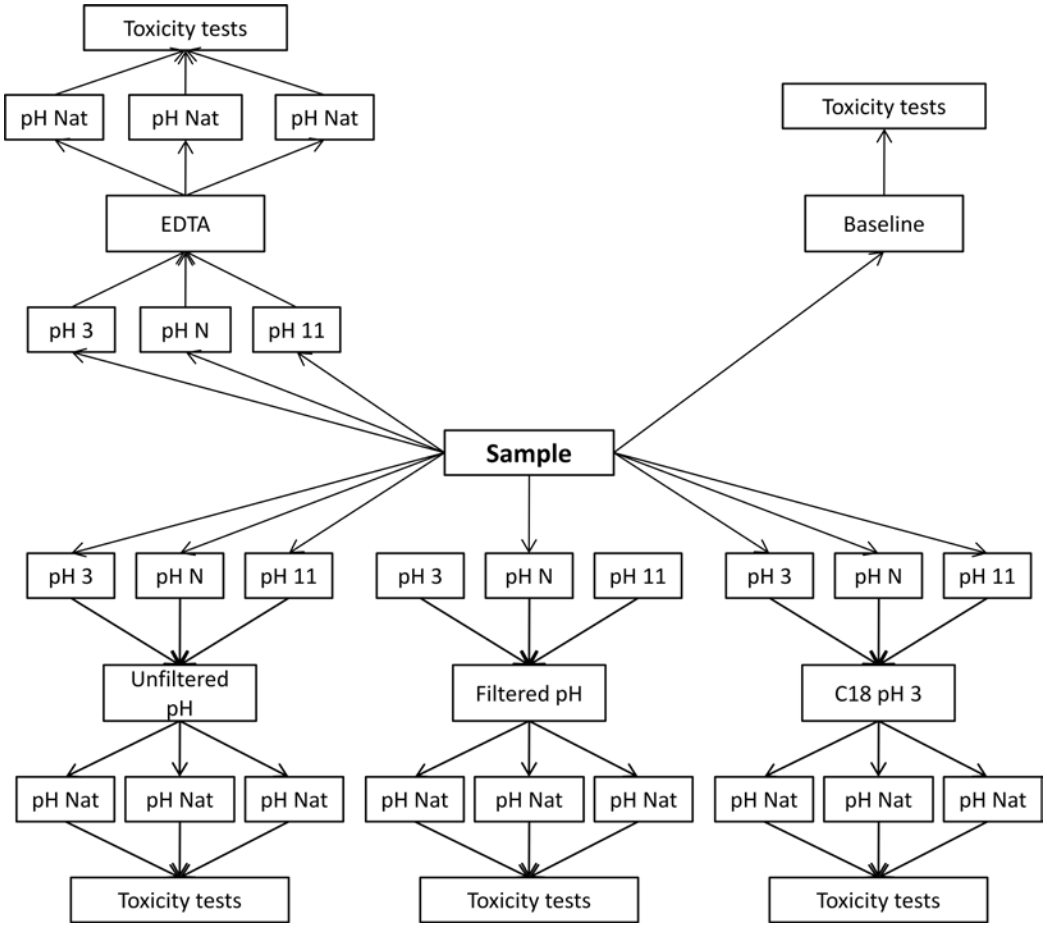

Figure 5-1: The TIE procedure for treated landfill leachate samples. Nat=Baseline pH (Norberg-King *et al*., 1991).

The baseline test for toxicity is the first test performed in the TIE procedure. Its purpose is to act as a benchmark by which the other manipulations are compared. This comparison indicates whether the TIE manipulations have had any effect on toxicity, whether positive or negative. The chemical or physical nature of any toxicity can then be deduced by comparison to this test.

Sample pH plays a major role in the chemistry of wastewater samples. The pH is the major method for developing information on the nature of toxicity in the TIE procedure. Alteration of pH can affect the solubility, polarity, volatility, stability and speciation of substances responsible for toxicity (Norberg-King *et al*., 1991).

Acids and bases are strongly affected by alteration of pH. Solution pH affects the speciation equilibrium. For example, at pH 4 an acid such as phenol is at 50:50 ratio between the nonionic and ionised form, whereas at pH 3 the same acid's ratio shifts to 10% ionic and 90% nonionic. The same principle holds for bases, notably ammoniacal-nitrogen where at pH 9.25 the ratio of ionised to nonionic is 50:50 but at pH 10 the ratio is 90:10 (Atkins *et al*., 2009). As ammoniacal-nitrogen is over hundred times more toxic to organisms than ammonium at pH 7.5 (EPA, 1999), pH changes can cause a huge change in toxicity of a sample containing ammoniacal-nitrogen, especially at pH <7.5. The solubility of substances also changes between the ionised and nonionic forms (Mount *et al*., 1997). Nonionic forms tend to be less polar than the ionised forms of substances. This makes the nonionic forms easier to remove from solution e.g. nonionic substances can be removed easier with powdered activated carbon (Weinberg and Narkis, 1987). This characteristic is exploited in the filtration and the SPE stage of the TIE procedure.

Solution pH affects the forms metal ions can take. In water, many heavy metal ions can exhibit many forms that depend on the pH of the solution (Figure 5-2). As the figure shows the speciation of Fe in water can be considerably altered by the alteration of pH. The alteration of pH can change the oxidation state of cations. Due to changes in solubility the bioavailability of the metal ion and thus its toxicity will be altered due to changes in chemical state (Isidori *et al*., 2003). Heavy metal concentrations in landfills vary widely and depend on such variables as pH, organic content and colloidal matter (Kjeldsen *et al*., 2002). Heavy metal concentrations in methanogenic leachates tend to be much lower than the amounts that are present in waste landfilled (Robinson, 2005). Reasons for the low concentrations have been attributed to the precipitation with carbonate and sulphides (Christensen *et al*., 2000) and adsorption with humic and fulvic acids (Stevenson, 1994).

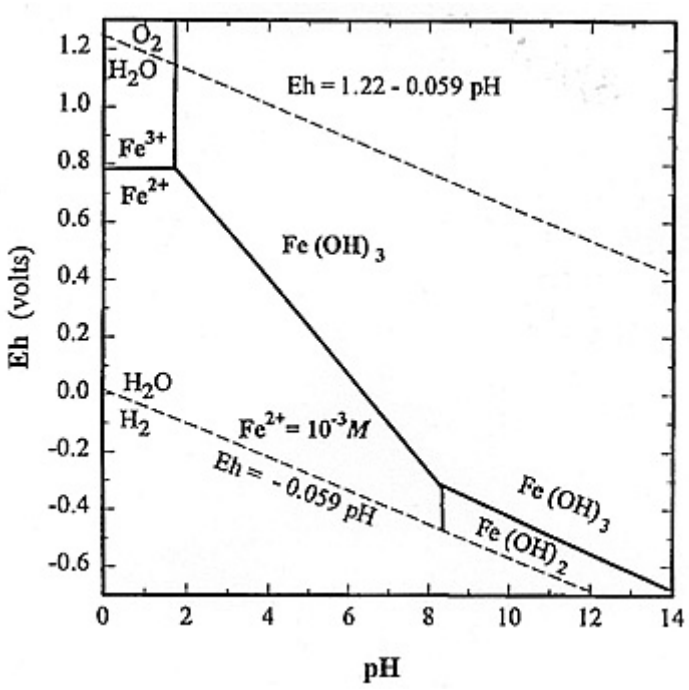

Figure 5-2: Phase diagram showing the speciation of Fe in water at pH values of 0-14.

Norberg-King *et al*, (1991) showed toxicity remained constant in aqueous solutions that had been pH altered and returned to the natural pH. This is explained as the forward reaction being very fast but the reverse reaction is very slow. The pH alteration test acts as a blank to further tests carried out in the TIE procedure. The TIE procedure requires the pH of the sample to be altered from the natural pH to either 3 or 11. The pH is returned to the natural (original) state once all the other TIE manipulations have been completed. In practice the TIE procedure manipulations took approximately 4-6 hours. It was necessary to regularly check the pH of samples altered and add required acid or base if necessary.

Toxins can become adsorbed to the surface of solid particles remaining in treated landfill leachate. Filtration of samples can remove these adsorbed toxins. Adsorption of toxins can reduce their bioavailability to organisms that live in water thus reducing the sample toxicity. Filter feeders can ingest these polluted solid particles and bioaccumulate the toxins over time (Norberg-King *et al*., 1991). The nature of the solid particles can also affect the binding

capacity between the particle and toxin. Surface area, particle charge, effluent matrix and substance polarity/charge all play a role in the adsorption of toxins to solid particles (Ma *et al*., 2002, Stronkhorst *et al*., 2003, Yang *et al*., 2006, Van Hoecke *et al*., 2008, Yang and Xing, 2009). These characteristics of solid particles are greatly affected by pH alterations.

During the filtration stage pH alteration is also carried out. pH can make certain classes of substances insoluble in water e.g. heavy metals. When combined with pH alteration, filtration is able to remove these substances from the sample. pH alteration can also affect the bonding ability of organic acids and bases to the surfaces of solid particles (Gessner and Hasan, 1987). Filtration is able to remove these adsorbed particles. Toxicity can measure the driving of toxins from or onto the surface of solid particles by changes in solution pH.

Non-polar metal chelates and organic substances are targeted in the following phase of manipulations. The sample is passed through a SPE that is packed with Octadecyl (C_{18}) sorbent. This reversed phase chromatographic method elutes the polar phase first and it is the non-polar substances that are retained on the surface of the sorbent. Many toxic substances found in water are not considered water soluble and are non-polar even though they are found within the aqueous sample (Norberg-King *et al*., 1991). This allows them to be extracted using SPE columns were the solid phase can adsorb these slightly soluble substances to the surface. Octadecyl beads used in the solid phase extraction (SPE) columns can be damaged by solutions with pH with values >10. To protect the integrity of the SPE column in this manipulation the pH was altered to 9.

A separate TIE procedure is available for contaminated sediments is available from the USEPA (Ho and Burgess, 2007). The procedure follows a similar pathway to the freshwater TIE procedure i.e. Phase 1 evaluation of toxicity; Phase 2 identification of the causes of toxicity; Phase 3 confirmation of the causes of toxicity. In the Phase 2 of this procedure a chemical test for the identification of toxicity caused by major ions is suggested. According

to the authors, this was part of the original freshwater TIE procedure but removed in later revisions of the document. This procedure uses sodium thiosulphate as a chelating agent of cations and anions. Sodium thiosulphate can be used in parallel with EDTA to understand causes of toxicity by major ions. Thiosulphate reacts with chloride ions in the following reaction:

$$2HCl + Na_2S_2O_3 \rightarrow 2NaCl + SO_{2(g)} + S + H_2O$$

This reaction thus removes chloride ions from the sample. Chloride is present in leachate at concentrations >2,000 mg/L. Mount *et al*, (1997) identified chloride as a toxicant and due to its high concentration is a likely cause of the toxicity in treated leachate. Mount *et al*, (1997) identified potassium as having the highest toxicity in their series (see above). Addition of thiosulphate to a solution containing potassium follows the reaction:

$$2K^+ + SO_4^{2-} \rightarrow K_2SO_{4(s)}$$

The final test of the TIE procedure carried out was the addition of EDTA (ethylenediaminetetraacetic acid) to the sample. EDTA is a chelating agent of many cationic metals including Ba^{2+}, Pb^{2+}, Ni^{2+}, and Mn^{2+}. As chelates of metal ions and EDTA are pH sensitive this phase of testing was performed on unaltered pH samples. These complexes are relatively strong and the toxicity of metal ion reduced. EDTA also has the ability to complex group 1 and 2 metal ions (Atkins *et al*., 2009). There is an associated toxicity with EDTA and for this reason care is needed to be taken with additions of EDTA. A concentration of 0.092 g/L was used in this phase of testing as this had been shown to be effective for reducing the concentration of metal cations without causing toxicity (Isidori *et al*., 2003).

Isidori *et al*. (2003) reported that *D. magna* toxicity depended on the TIE manipulation used. At the 3 sites sampled the toxicity of the sample was decreased by lowering the pH to 3. For the Casone sites, the sample toxicity was increased by pH alteration to 11. The Uttaro sample toxicity was not reduced by pH 11 alteration. It was concluded from the data that *D. magna*

test was less sensitive to the landfill leachate samples than the other invertebrate species used in the battery i.e. *Brachionus calyciflorus* and *Thamnocephalus platyurus*.

Research in the literature has mainly concentrated on the toxicity of raw landfill leachate. The research that has been carried out on treated landfill leachate produced a mixed picture of effectiveness at treatment (Okamura *et al.*, 2005, Osaki *et al.*, 2006, Celere *et al.*, 2007, Bortolotto *et al.*, 2009). Okamura *et al*., (2005) identified that the dissolved organic carbon content was the cause of toxicity within the treated landfill leachate samples tested. Silva *et al*, (2004) was able to identify that the major ions were the the causes of toxicity in treated landfill leachates from a Brazilian landfill. Celere *et al*, (2007) was able to demonstrate that the high levels of zinc and lead in treated samples were the causes of toxicity.

The collected results of WET testing carried out in this project for Arpley and MVP are presented. For MVP and Arpley this data comprises monthly WET testing data over a year. As far as this author is aware this amount of data has not been presented in the literature. Within the literature there is a trend to collect 2 or 3 samples for toxicity determination. It is felt that this might not give a complete picture of the toxicity of landfill leachate. As part of the Environment Agency's discharge consent for Buckden landfill leachate treatment plant WRG were required to conduct WET testing with Microtox following the success of the fish testing. WRG have kindly supplied Microtox data for Buckden effluents for comparison between Arpley and MVP. The toxicity data for the three sites are presented in order to demonstrate that the toxicities displayed within in the project are representative and not exceptional samples.

5.2.2 Hypothesis

Null hypothesis: The causes of residual toxicity in treated landfill leachate are not determinable by manipulation of the solution chemistry and physical characteristics.

Alternative hypothesis: The causes of residual toxicity in treated landfill leachate are determinable by manipulation of the solution chemistry and physical characteristics.

5.2.3 Experiment aim and objective

Aim: Demonstrate the long term toxicity patterns of treated landfill leachate samples from MVP and Arpley and to identify the causes of residual toxicity with the use of a TIE Phase 1 procedure.

Objectives:

1. WET testing is a valuable tool for understanding the risk that wastewater effluents pose to the environment. Monthly WET testing between 2009 and 2010 for MVP effluents was collected and presented in order to highlight the levels of toxicity over a year long period. A less complete dataset for Arpley effluents is to be presented, as well as Microtox testing carried out for WRG with Buckden effluents.
2. Compare the sensitivities of each of *L. minor*, *D. magna* and Microtox to the effluents.
3. Collect treated leachate samples from Arpley, MVP, and Buckden leachate treatment plants and determine the levels of residual toxicity in these samples towards *Lemna minor, D. magna, T. platyurus, Vibrio fischeri (*Microtox™), *Escherichia coli* (Toxi-ChromoPlate™).
4. Perform a TIE procedure to identify the causes of any residual toxicity in treated landfill leachate samples.
5. Analyse the results to determine whether any of the manipulations produce significant changes in the toxicity towards the test species.

This experimental procedure is designed to screen toxicity from three different treated landfill leachates. The three sites selected have differences in treatment technologies (Chapter 3)

which may affect chemical composition of the effluent. To highlight the possible causes of toxicity in treated landfill leachate a toxicity identification evaluation (TIE) procedure was used. This procedure should lead to more research questions on the possible classes that are the cause of toxicity within treated landfill leachate. Research is needed as the literature has suggested that there are 4 possible causes of toxicity within treated landfill leachate i.e. residual ammoniacal-nitrogen, organic content, heavy metals, and major ion concentration.

5.3 Methods and materials

One sample was collected from MVP, Arpley and Buckden landfill leachate treatment plants. The samples were collected in the manner described in the Methodology chapter (Chapter 3). The baseline toxicity of each site's sample was determined immediately on arrival at Cranfield University.

For each of the TIE manipulations a blank was performed for comparison. The blank was made by performing the function of the TIE procedure on dilution water.

5.3.1 Toxicity identification procedure

To reveal pH-sensitive characteristics the sample's pH was altered to pH 3 with 1 mol and 0.1 mol HCl (Fisher Scientific Chemicals, UK) or to pH 11 with 1 mol and 0.1 mol NaOH (Fisher Scientific Chemicals, UK). Following pH adjustment the samples were brought back to their baseline pH value with addition of suitable quantities of acid and base. To remove solid particles the sample's pH was altered, filtered through 0.45 µm glass fibre filter papers (Munktell Filter AB, Sweden), and adjusted back to the baseline pH values. To remove the organic hydrophobic fraction the sample pH was altered (pH 9 instead of 11 to preserve the integrity of the C18 solid-phase extraction column), filtered, fractionated by 6 ml Chromabond C18 EC solid-phase extraction column (Fisher Scientific Chemicals, UK), and pH adjusted back to the baseline value.

5.3.2 Bioassays for assessing toxicity

In this phase of WET testing was carried out with *L. minor, D. magna, T. platyurus, V. fischeri (*Microtox™), *E. coli* (Toxi-ChromoPlate™). (Chapter 3).

5.3.3 Statistics

From the raw toxicity testing data E(L)C_{50} values were calculated by multiple regression analysis using STATISTICA (Statsoft, Bedford, U.K.). Full details are given in the Methodology chapter (Section 3.5)

This E(L)C_{50} data was used in an factorial analysis of variance (ANOVA) test. Unlike a student's T-Test which compares 2 means, ANOVA compares means between many results. ANOVA compares the mean toxicity E(L)C_{50} of results obtained to determine whether a significant difference is exists between groups of treatments. A residuals test was used to highlight fit with the model and any outliers removed from the model. A post-hoc Fisher LSD highlighted groups (letters on the graphs) of results that were statistically different to other groups.

5.4 Results and Discussion

5.4.1 Long term WET testing of MVP, Arpley and Buckden treated leachates

To demonstrate the variability or stability in the toxicity responses of the *Daphnia magna* and *Lemna minor* a monthly sampling routine was carried out on MVP treated leachates (Figure 5-3). The toxicity response of *D. magna* was lower than *L. minor*. EC_{50} was generally in the range of 50-70% with only the 28.05.10 sample higher. These toxicity levels are considered low especially when compared to a raw leachates EC_{50} of <3% (Isidori *et al*., 2003). By producing this work the general pattern and any variability in the toxicity can be highlighted.

L. minor was in all cases more sensitive to the treated leachates collected from MVP. There was considerable variability in the responses of *L. minor* towards the leachate samples. Variability in the responses was in most cases not significant. This demonstrates that there is

high degree of variability in the levels of response within fronds from the same culture. The highest toxicity recorded was with the 15.06.2010 sample were a toxicity of 23.8% was recorded.

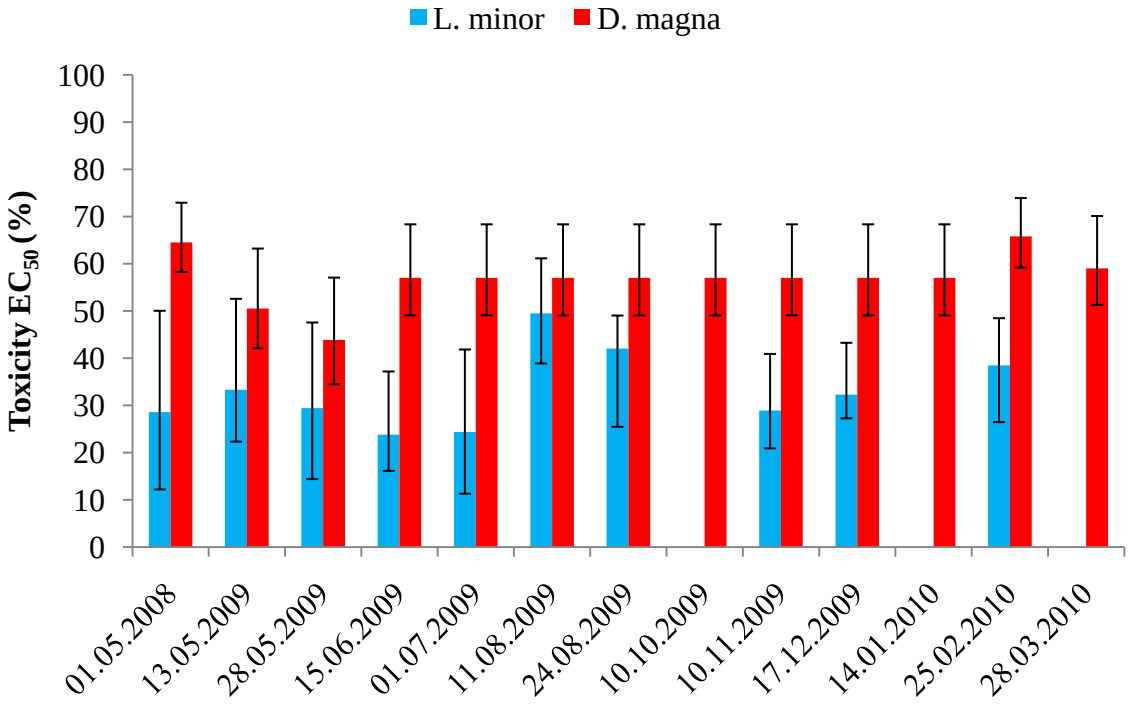

Figure 5-3: EC_{50} toxicity of MVP treated leachates towards *D. magna* (n=4) and *L. minor* (n=3). 95% confidence intervals shown as bars on the column.

Overall the Arpley leachate was slightly more toxic than the MVP leachate (Figure 5-4). The response of *L. minor* towards the Arpley samples was fairly consistent in most cases e.g. 20-30%. This is a more consistent response when compared to the MVP leachate response to *L. minor* which was more variable. *D. magna* responses show a degree of variability towards the samples. The toxicity varied between 40 and 60% over the sampling period. More samples could not be obtained from WRG for Arpley.

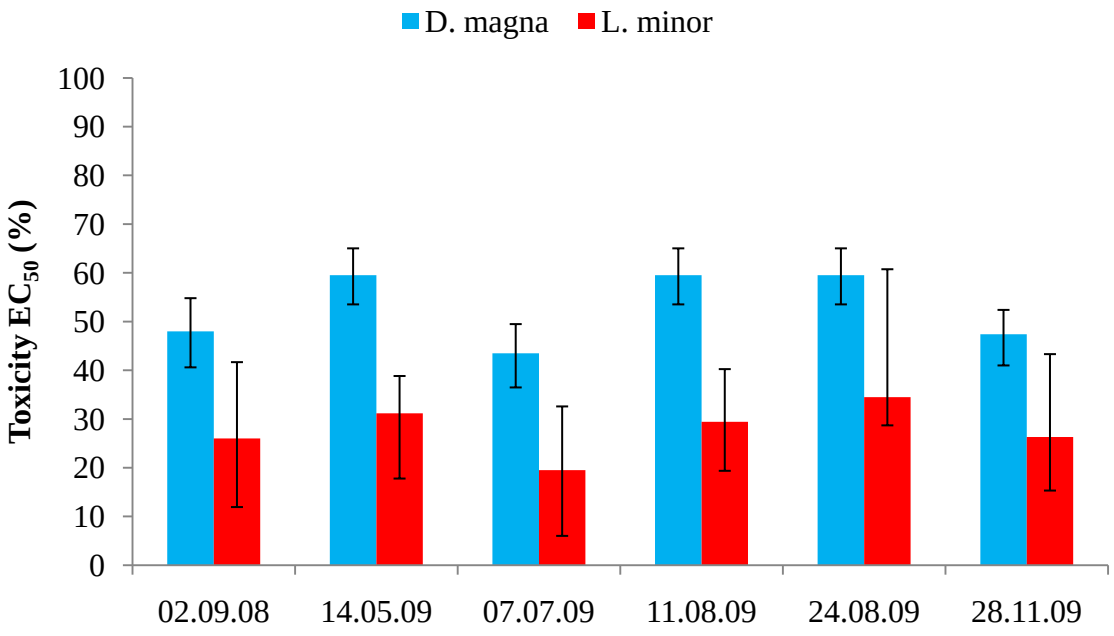

Figure 5-4: EC_{50} toxicity of MVP treated leachates towards *L. minor* (n=4) and *L. minor* (n=3). 95% confidence intervals shown as bars on the column.

Following the success of the chronic fish WET the Environment Agency required WRG to conduct toxicity assessments of Buckden treated leachates with Microtox (Figure 5-5). This Microtox testing was conducted by 'ALcontrol laboratories', UK. The toxicity responses show that the Buckden effluents caused small inhibition of light production (2-5%) within the test candidates. This response is within the error of detection range. Microtox has a low sensitivity towards landfill leachate (Section 2.4.1). Both *O. mykiss* (Robinson *et al*., 2003a) and Microtox tests displayed no toxicity towards the Buckden effluent and demonstrate that Buckden landfill is a lower strength leachate compared to MVP and Arpley.

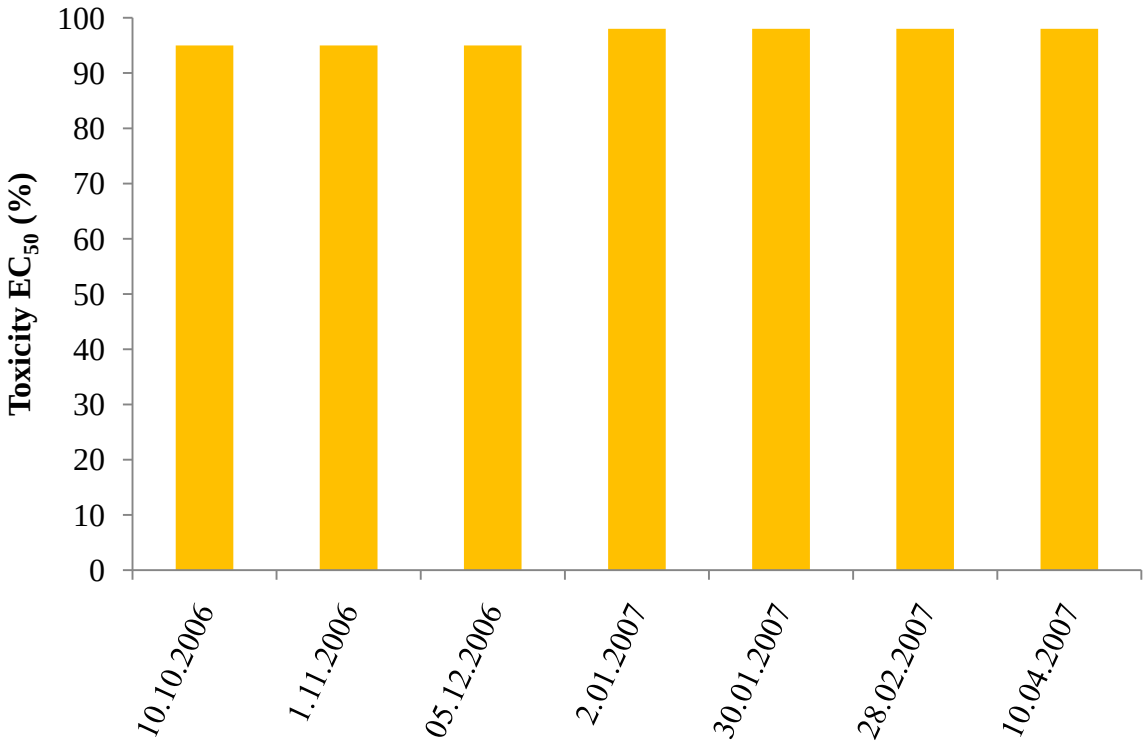

Figure 5-5: EC_{50} toxicity of Buckden treated leachates towards the Microtox test. No variance data was supplied by the laboratory.

A raw leachate generally causes a toxicity EC_{50} response of <0.5% (not presented in this thesis). Compared to these samples it is clear that treatment is effectively reducing toxicity. There remains an amount of residual toxicity in the leachates that needs to be explained. An exploration of residual toxicity of these leachates is necessary to determine whether this toxicity is a cause of concern i.e. is it a bioaccumulative organic compound or a mutagenic toxic response.

5.4.2 TIE procedure

The chemical analyses of MVP, Buckden and Arpley landfill effluents are presented in Table 5-1. The COD values vary considerably with Arpley having the highest COD concentration at 1,072 mg L^{-1}. The BOD values are similar between the three sites. The BOD:COD ratios are all low and this indicates that most biodegradable material has been removed in the treatment

of the leachate. The ammoniacal-nitrogen concentrations were below detection limits for the cell tests. The low ammoniacal-nitrogen concentrations illustrate the effectiveness of biological treatment for the nitrification of ammoniacal-nitrogen (Robinson and Barr, 1999, Robinson *et al*., 2003a). Phosphoric acid is added to the treatment works as a feed for the bacteria. The concentration of phosphate in the leachates collected varied greatly between 1 and 15.0 mg/L. The higher levels in the Buckden and Arpley samples, 7.1 and 15.0 mg/L respectively suggests that excess phosphoric acid is added during treatment and savings are possible by reducing the input of phosphoric acid.

The conductivity of the Arpley sample was the highest of the 3 sites at 21,600 µS/cm which implies a high concentration of salts within the sample. These high levels of ions are reflected in the Atomic Absorbance (AA) analysis. The mean sodium concentration was lowest at MVP at 4,775.8 mg/L. The mean sodium concentration was higher at Buckden and Arpley at 6,519.5 and 6,784.0 mg/L respectively. Chloride levels within the samples were lowest at MVP with a concentration of 1,987 mg/L and higher at Buckden at 2,550 mg/L and Arpley at 3,070 mg/l. Potassium levels were lowest at MVP with a concentration of 934.0 mg/L and higher at Buckden with 1,387.8 and higher still at Arpley at 1,461.3 mg/L. The magnesium concentration showed little variability between the three sites, with MVP having the lowest concentration at 113.4 and Buckden having the highest mean magnesium concentration at 126.2 mg/L. The mean calcium concentration was highest at MVP at 195.3 mg/L and lower at Arpley and Buckden, 141.0 and 152.6 mg/L respectively.

Table 5-1: Mean values of chemical parameters for treated Stewartby Buckden and Arpley landfill leachates. All values in mg/L unless stated (± standard deviation).

Chemical parameter	MVP	Buckden	Arpley
COD	628 (±38.3)	310 (±11.5)	1,072 (±126)
BOD	8.7 (±0.5)	8.9 (±0.2)	9.0 (±0.5)
BOD:COD ratio	0.01	0.02	0.009
Ammoniacal-nitrogen	<0.1 (±0.05)	<0.1 (±0.0)	<0.1 (±0.0)
PO_4-P	1 (±0.1)	7.1 (±0.1)	15.0 (±0.1)
Chloride	1,987	2,550	3076
Calcium	195.3	141.0	152.6
Sodium	4,775.8	6,519.5	6,784.0
Potassium	934.0	1,387.8	1,461.3
Magnesium	113.4	126.2	124.8
Conductivity (µS/cm)	16,000	17,800	21,600
Suspended solids	22.6	12.1	17.5
pH	6.72	8.10	8.33

The toxicity of the samples from the 3 landfill sites towards the 5 species tested is presented in Figures 5.6-5.9. Overall, the level of toxicity recorded in the 3 sites was low. This scheme of testing has highlighted the variability of treated leachate toxicity from different sites with toxicity following the progression Arpley>Stewartby>Buckden.

The level of toxicity is very similar to most of the treated leachate toxicities reported by Okamura *et al*. (2005) for *L. minor*. Okamura *et al*. (2005) found that only 2 treated landfill leachate samples out from 17 sites displayed any serious levels of toxicity i.e. EC_{50} >10%. The 15 other treated landfill leachate samples displayed a toxicity of 20% EC_{50}. A raw leachate in general will have a EC_{50} of <3% (Isidori *et al*., 2003) which is many times larger than the levels reported here where the EC_{50} ranged between 11-100% (an EC_{50} of 100% equals no toxicity). This implies that the treatment being carried out on each of the sites is significantly reducing toxicity (in a range finding test a raw leachate sample collected from MVP had an EC_{50}=<0.5% towards *D. magna*).

The *L. minor* test produced a significantly higher response to the baseline samples from the 3 sites tested when compared to the other bioassays. Both the zooplankton based tests (*D. magna* and *T. platyurus*) produced similar levels of response to the samples tested indicating that only one of the tests was needed in the battery. *E. coli's* baseline response to the 3 sites tested was similar in all cases unlike in the other tests carried out where there was a marked difference between Buckden and the other 2 sites. The Microtox based test did not produce a response to any of the 3 sites samples used in this testing period.

The baseline toxicity towards *D. magna* was variable in the three samples collected (see Figure 2). This indicates that some toxicity was caused by a pH sensitive species that was related to the solid phase. Due to adsorption onto the active surface during the SPE procedure this is most likely an organic species (Okamura *et al*., 2005). In most cases the responses of *D. magna* to the TIE procedure was no significant change. This was the case with the MVP and Arpley samples where no changes in the toxicity with application of the TIE procedure. Buckden's baseline toxicity was lower than the other samples toxicity with an EC_{50} of 72%. The TIE procedure did reduce the toxicity of the Buckden samples toxicity from the baseline. There remained a significant fraction (~40%) of residual toxicity altered with the TIE procedure.

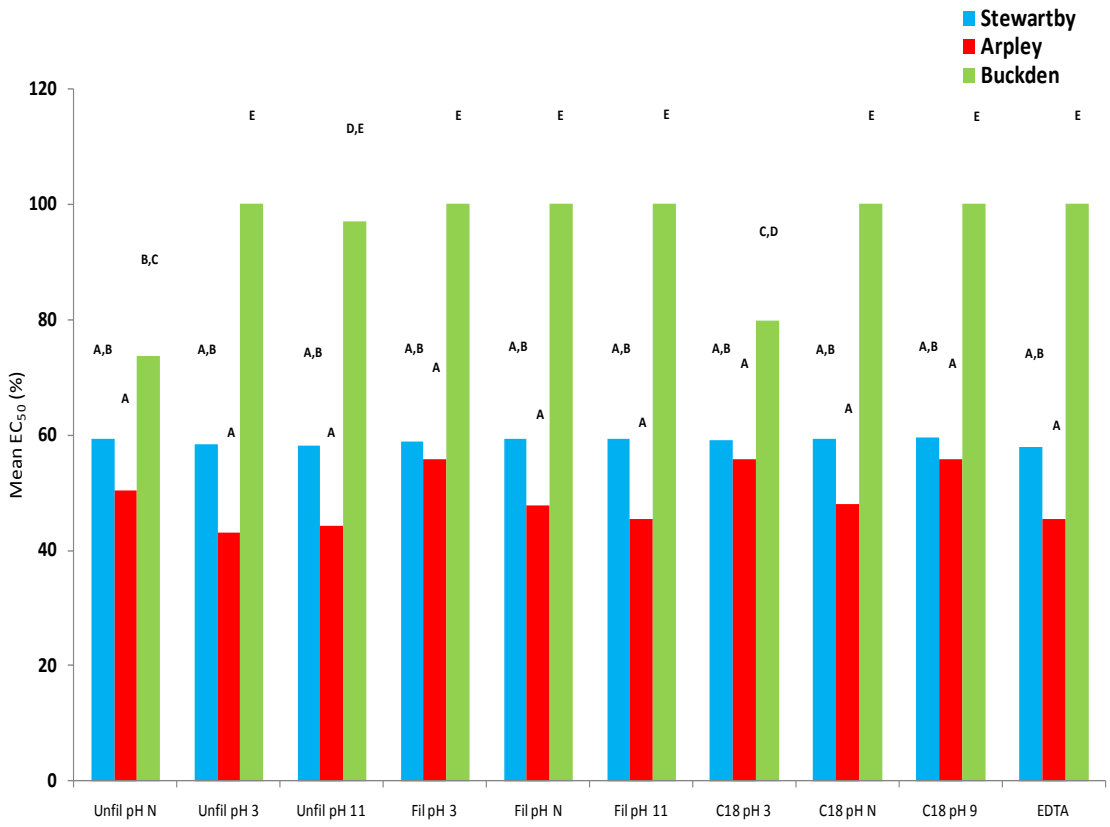

Figure 5-6: *D. magna* average EC50 (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different.

L. minor displayed a higher sensitivity to the TIE manipulations than the other four species tested (Figure 5-7). Manipulation of the MVP sample pH resulted in statistically significant a toxicity reduction indicates that a partial amount of toxicity in this sample towards *L. minor* is pH sensitive. These responses were not reduced with filtration or C18 extraction which leaves an ionic species the most likely candidate for the cause of toxicity. The application of the TIE procedure on the Arpley sample resulted in no significant changes in toxicity recorded. The Buckden sample recorded a similar type of response as the Arpley sample.

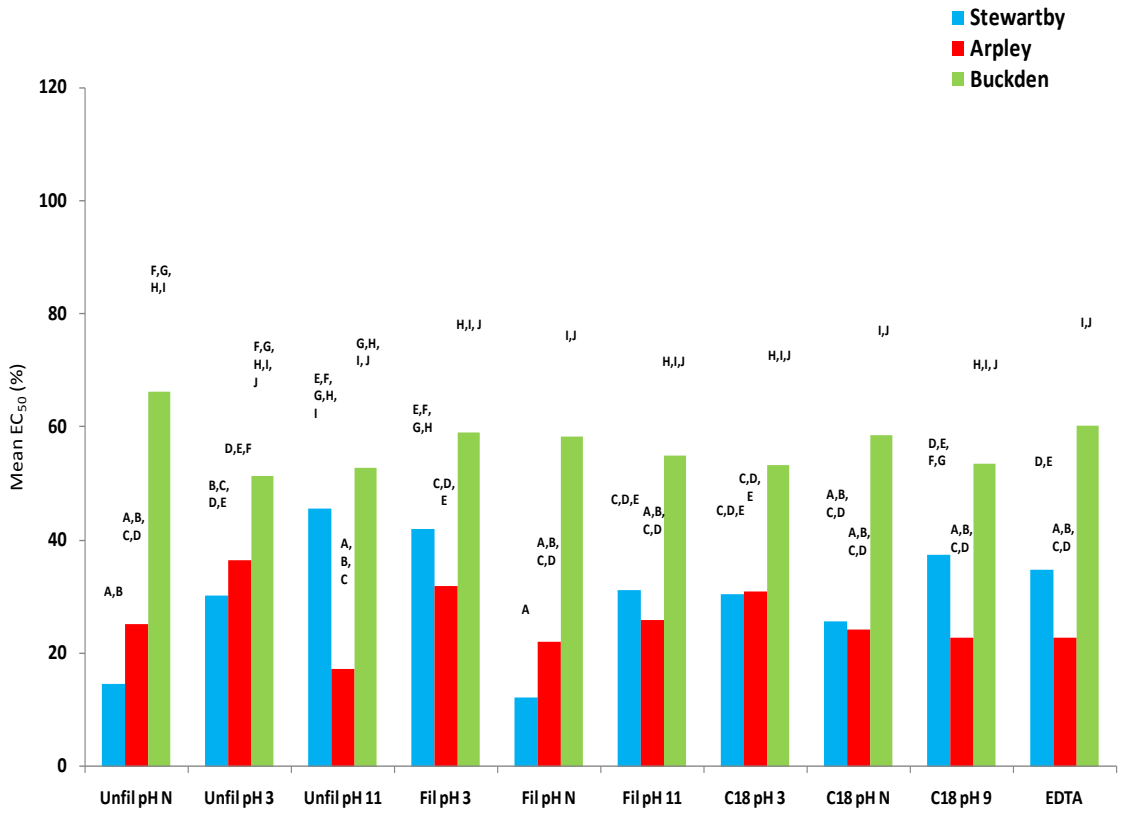

Figure 5-7: *L. minor* average EC_{50} (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different.

The *E. coli* based test recorded an actual increase in toxicity in the Arpley sample with filtration and pH change (Figure 5-8). This increase with filtration suggests that solid particles are able to negate toxicity in some cases though toxicity still remained in the samples. Solid particles have been previously reported as a strong influence on toxicity especially when the pH is altered as there binding sites are sensitive to pH (Northcott and Jones, 2000). The application of the TIE procedure had the effect of nullifying the toxicity of the Stewartby and Buckden samples in the following cases: unfiltered pH 11, filtered pH 3, C18 pH N and C18 pH 9. Conversely the application of the TIE procedure increased the

toxicity of the Arpley in the following cases: filtered pH 3, filtered pH 11, C18 pH N and C18 pH 9. Toxicity of the Arpley sample was reduced with the addition of EDTA.

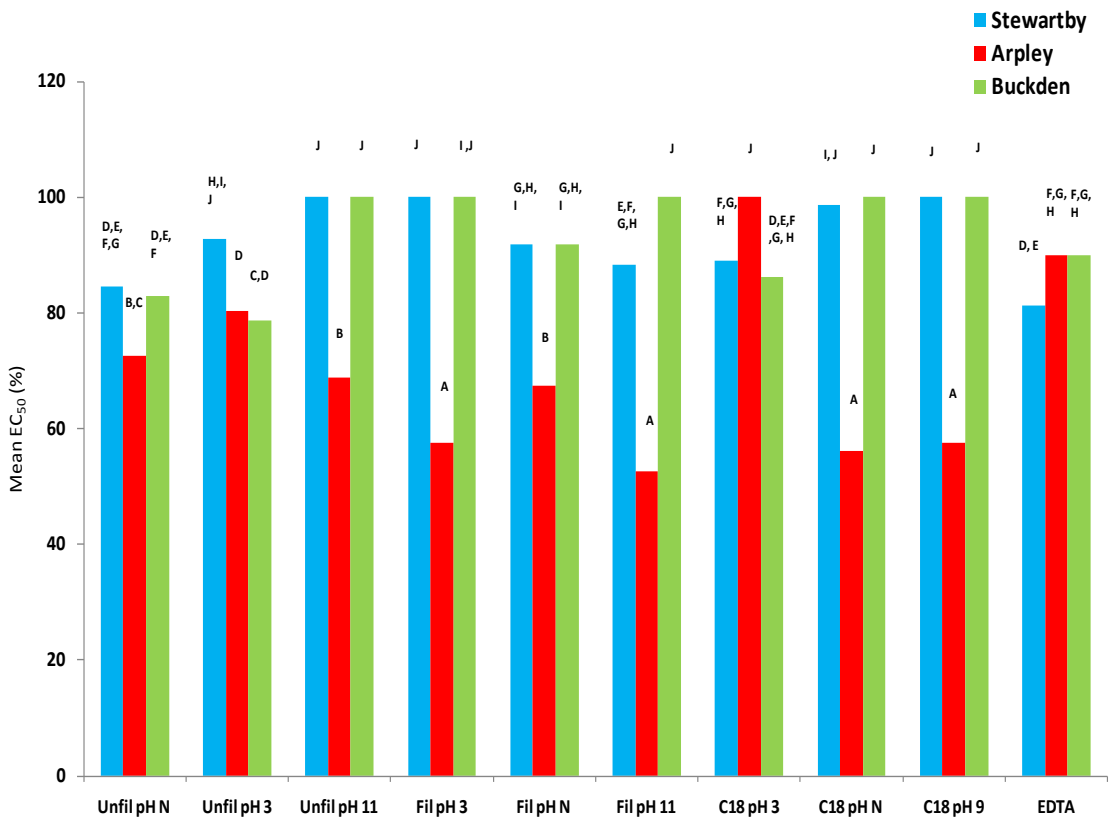

Figure 5-8: *E. coli* average EC_{50} (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different.

Similar responses were recorded in the *T. platyurus* as were found in the *D. magna* tests (See Figure 5). *T. platyurus* proved to be slightly more sensitive to the Stewartby and Arpley samples than the *D. magna* test but was significantly less sensitive to the Buckden sample. Isidori *et al.* (2003) reported that *T. platyurus* was more sensitive to landfill leachate than *D. magna* and the results from this testing confirm this. These small reductions were statistically significant to the baseline unfiltered pH N (baseline toxicity). While there is still a decrease in the toxicity there remains 40-50% toxicity in the Stewartby and Arpley samples

that is not explained by the TIE procedure. The EDTA test with the Arpley sample resulted in a significant reduction of toxicity. In all other tests there were significant reductions in toxicity. Manipulation of the Stewartby sample saw significant reductions in: unfiltered pH 3; unfiltered pH 11, filtered pH 3; filtered pH N; C18 pH N; C18 pH 9.

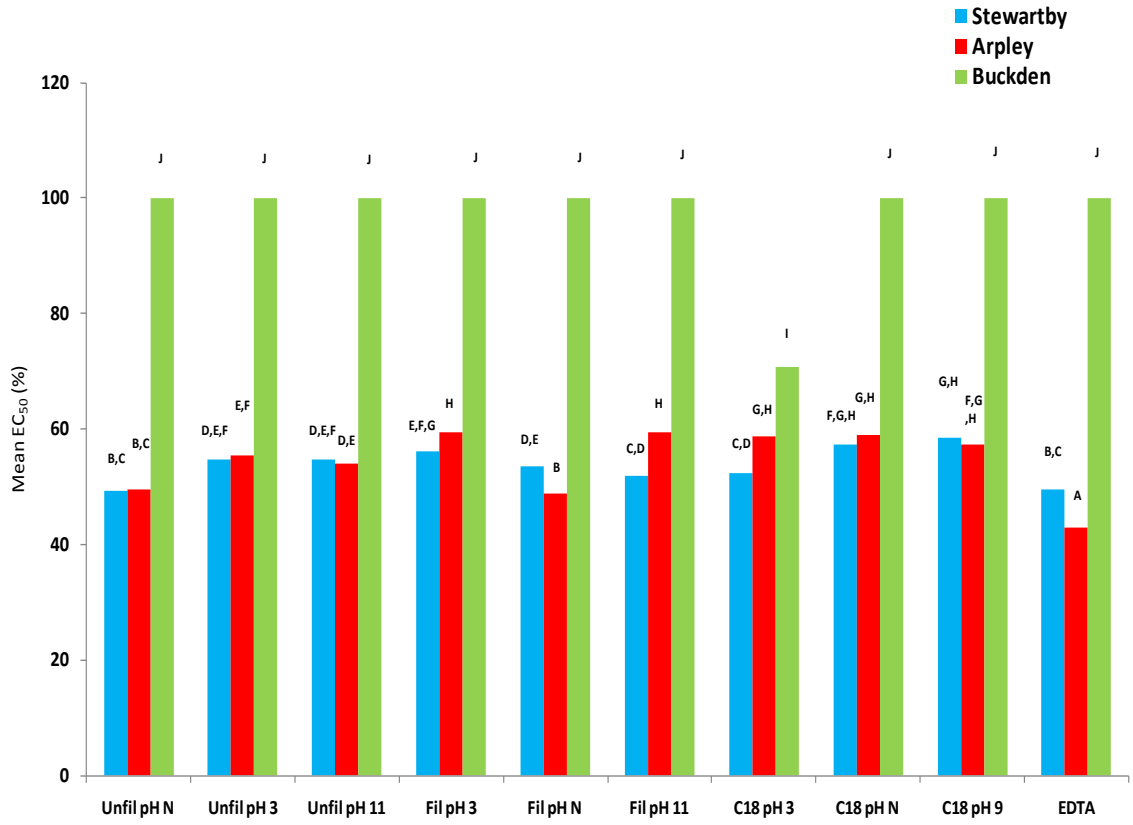

Figure 5-9: *T. platyurus* average EC_{50} (as % effluent) responses to treated landfill leachates from 3 sites. 95% confidence intervals are shown as bars. Each letter represents a group of means that is not statistically different.

Chemical analyses of the physico-chemical characteristics of the leachate samples showed differences between all three sites. There was a large difference in the COD concentration between each of the sites with a difference of 762 mg/L between the lowest and highest. These differences in composition can be attributed to landfill age, leachate treatment, and types of waste landfilled e.g. 1,094mg/L for a industrial landfill leachate compared to 378

mg/L for a MSW landfill leachate (Bertazzoli and Pelegrini, 2002). Each of the sites uses a form of biological treatment. The chemical analysis has showed that the treatment is reducing the ammoniacal-nitrogen and BOD concentration down to acceptable discharge levels. This reduction is seen in the EC_{50} of a raw leachate being <0.5% compared to the ~60% in the testing carried out here.

The causes of residual toxicity in treated leachate were not clearly identified from the application of the TIE procedure. A considerable degree of variability was noted in the performance of the TIE procedure with no consistent pattern evident. What was clear was that each bioassay test displayed a different response to the same samples. This difference indicates that each test has a 'spectrum' of sensitivity towards different components of treated landfill leachate (Okamura *et al*., 2005).

No response was recorded in the Microtox tests carried out with the treated leachate samples. In a TIE procedure the Microtox test was shown to be the least sensitive of the 5 biotests used in the procedure (Isidori *et al*., 2003). In the literature review of this project, Slooffs analysis (Table 2-11) showed that from the results previously reported the Microtox test was the least sensitive to the contaminants found in landfill leachate samples.

A limitation of the TIE procedure is that only the EDTA based test can have an effect on the concentration of major ions such as Mg^{2+}, and Ca^{2+}. These ions are present in high concentrations (Table 5-1). The analyses carried out showed that in most cases MVP had the lowest concentration of these ions and Arpley had the highest. The lower mean values of MVP leachate can be attributed to the blending of 4 sites leachates at the plant (Gibbs, 2007). These ions have been implicated as a cause of toxicity in treated landfill leachate (Bortolotto *et al*., 2009). It seems a real possibility that these major ions, particularly chloride, are the cause of toxicity within these treated landfill leachate samples.

Small modifications of toxicity were noted with application of the TIE procedure but no definite pattern was observable. High sensitivity of *L. minor* has been noted with previous work on this species (Mackenzie *et al*., 2003). The higher response recorded in the *L. minor* tests is thought to be linked to the higher sensitivity towards the major ion content of these samples. This leaves a question on the effect that chronic exposure of treated landfill leachate has on the aquatic environment. A chronic test using *Asellus aquaticus* (freshwater crustacean) has been performed using raw leachate where it was found that a 30 mg/L of COD discharge was considered a safe discharge limit (Bloor *et al*., 2006). This type of testing offers greater confidence in predictions on the hazard posed by the discharge of effluents to aquatic environments. Due to time constraints it was not possible to carry out such a testing program. Future research into this area could provide valuable data on the interactions between treated leachates and freshwater species.

The SPE phase of this work did not work as expected due to COD reductions of <200 mg/L. Only small non-significant changes in toxicity was recorded which makes conclusions on the role of COD in toxicity uncertain. The role of recalcitrant COD in the toxicity of these samples is a key area for investigation in this project. For this reason a further set of experiments is needed in order to identify whether the COD fraction is the cause of toxicity within treated landfill leachate samples.

The main limitation of this work was that only one sample could be collected, analysed and tested with such a complete battery of tests. The testing battery was very expensive and time constrained to execute due to the need to complete toxicity tests within a 72 hour period. Conclusions on the solution chemistry and levels of toxicity are difficult with only one sample tested. To overcome this limitation a regular sampling routine was introduced on the finishing of this work so that the long term toxicity of the samples could be more accurately assessed. The long term testing demonstrated that toxicity remained relatively consistent over

the course of a year at both MVP and Arpley. Toxicity levels remaining constant demonstrate that the samples collected in the TIE were not abnormal. Treatment of landfill leachate is thus able to produce a leachate with low toxicity that is safe for discharge to the environment.

Application of the TIE procedure therefore suggests that residual toxicity in treated leachate may not be the result of recalcitrant organic substances or heavy metals. This suggests that major ions, which are unaffected by biological treatment and the TIE procedure manipulations, could account for the levels of residual toxicity in treated landfill leachate in the samples collected. For example, chloride toxicity is well documented with the USEPA advising a final chloride standard concentration in fresh water to not exceed 852 mg/L (Gregory and Sindt, 2008). The toxicity of chloride has been shown to be reduced by the presence of other major cations (Mount *et al.*, 1997). Mount *et al* (1997) carried out to work determine the effects of raised major ion concentrations in receiving waters caused by industrial effluents. Effluents from the oil industry known as produced waters have elevated concentrations of major ions. These waters are reported to have a range of toxicities towards *D. magna* e.g. EC_{50} range from 100% to 3%. The authors found that toxicity could be explained by K, Mg, HCO_3, SO_4 and Cl^- in their modelling and experimental work. Levels of Cl^- found in landfill leachate in this study might explain the toxicity that was recorded. A lack of response in the marine species *V. fischeri* and responses in the other freshwater species would make sense as *V. fischeri* have cell wall mechanisms for dealing with high chloride concentrations. Similar low levels of response in comparison to other tests used in the battery were also reported by Isidori *et al*. (2003).

5.5 Conclusions

The year-long WET testing of Arpley and MVP demonstrate that the toxicity of these leachates is generally low by comparison to untreated leachate (EC_{50} <3%). The toxicity

response of *L. minor* is higher and more variable than *D. magna* test. Although comparatively low, the causes of the residual toxicity found should be investigated to understand whether this can be removed by further treatment. Toxicity levels from the two sites effluents remain relatively consistent throughout the testing period. It is clear that the *L. minor* based test is more sensitive to the effluents than the *D. magna* test. This is in agreement with the findings from the literature review on the sensitivities of each of the tests. The Buckden effluent caused no significant response in the Microtox tests carried out on behalf of WRG.

Overall the levels of toxicity were low in the samples collected. These low levels show the effectiveness of the treatment strategies adopted at each of the sites. With the added effect of dilution upon discharge these effluents would pose little hazard to the aquatic ecosystem.

The TIE procedure did make some significant modifications to toxicity in the samples but due to significant variation about the mean in most of the TIE manipulations were insignificant. This makes firm conclusions on the nature of toxicity in treated landfill leachate very difficult. This could be due to the low levels of toxicity present not being altered by the TIE procedure. Chloride and other main ions are the main suspects for the causes of the majority of toxicity within the treated landfill samples due to the lack of changes in toxicity with application of the TIE procedure. Application of the TIE procedure was a success in that it identified a number of new avenues to explore in developing an understanding on the causes of toxicity within treated landfill leachate samples.

L. minor was seen to be particularly sensitive to the effluent samples from all sites. As plant species are keystones to the health of aquatic environments there needs to be more research into limiting any hazard to their health. The invertebrate based tests showed similar responses to each of the site's effluents. It can be concluded that only one of these species would be needed for a testing battery of effluent toxicity which can be decided by the user based on price and time to gain results. *V. fischeri* and the genetically modified *E. coli* tests both

showed very low responses to the effluent samples. This test could have a role to play in a continuous monitoring procedure as the testing turnaround is fast and problems can be identified quickly.

One of the major limitations of the procedure was the cost and the amount of time needed for preparation by one person. This testing procedure is not suitable for one person to run and at minimum 2 trained people should be carrying out this work. The cost of this work also makes further sampling and application of the procedure prohibitive.

Further work is needed to identify the causes of the low level residual toxicity that has been recorded. The cause of toxicity is almost certainly due to major ions within the samples. Treatment of these ions might not be economically possible but could be possible by a dilution effect of receiving waters.

6 Reduction of recalcitrant COD with a selection of XAD resins

6.1 Findings from previous work

The role of recalcitrant organic substances in residual toxicity was the initial focus of this project. The Environment Agency was scrutinizing the levels of chemical oxygen demand (COD) in effluents from landfill leachate treatment plants operated by the project sponsors Waste Recycling Group (WRG). WRG were concerned that the imposition of a lower discharge consent for COD would require new and expensive equipment e.g. ozonation systems to achieve lower concentrations of COD in their effluents. For this reason WRG required experimental evidence on whether the COD fraction of treated landfill leachate was safe for discharge or whether further treatment was needed to reduce the effluent COD concentration.

The TIE procedure was unsuccessful in determining whether the residual toxicity detected in treated landfill leachate was caused by recalcitrant organic compounds. This failure was due to no changes in the toxicity responses for some treatments and then a sudden increase in toxicity during the pH 3 solid phase extraction procedure. For this reason further work was needed to develop the understanding on the possibility of the COD fraction posing a toxicological risk to the environment. Focus on the COD fraction required a dedicated COD removal strategy. WET testing following the removal of COD fractions would enable a picture to be constructed on the role of recalcitrant COD plays in residual toxicity of treated landfill leachate.

6.2 Introduction

6.2.1 Background

Percolation of water through landfilled waste produces a leachate rich in solids and soluble compounds. The chemistry of the leachate produced is influenced by the nature of the waste

landfilled and its stage of decomposition. It is estimated that about 66% of the material in municipal landfills is biodegradable (Williams, 2005). Decomposition of waste in landfills goes through a number of stages where the composition of the waste radically changes which in turn changes the composition of the leachate produced. These degradation stages can follow biological, chemical and physical routes.

The first stage of decomposition of landfilled waste is aerobic hydrolysis of polymeric organic compounds. Aerobic bacteria hydrolyse chemical bonds in polymers with water until the supply of oxygen is depleted (Guerrero *et al*., 1999). During this stage carbon dioxide is produced which dissolves in water to produce carbonic acid which in turn lowers the pH of the leachate (Williams, 2005). In modern landfills, the waste is compacted so as to limit the amount of oxygen as the ongoing chemical reactions produces a large amount of heat and can cause internal fires. It is during this phase that humification (genesis of humic and fulvic acids) begins with the creation of the building blocks of humic substances (Huo *et al*., 2009).

Following the depletion of oxygen anaerobic conditions set in. Anaerobic hydrolysis and fermentation of carbohydrates, proteins and lipids is the next stage in decomposition of landfilled waste. During this stage organic acids are released due to the actions of acetogenic bacteria within the landfill. It is during this phase of decomposition that ammoniacal-nitrogen is released in high concentrations by deamination of proteins (Kjeldsen *et al*., 2002). Degradation of cellulose and hemicellulose during this stage alters the organic content of waste to lower molecular weight organic acids (Barlaz *et al*., 1989). This phase is characterised by a high ratio of biological oxygen demand to COD e.g. a ratio of 0.7 is typical in landfill leachate (Fan *et al*., 2006).

Acetogenenic bacteria degrade the larger organic molecules to acetic acid which results in a large fall in the leachate pH i.e. pH of 4 are normal (Table 6-1) (Kjeldsen *et al*., 2002). Lower pH values increase the solubility of heavy metal cations and their concentration within

leachate is increased (Baun and Christensen, 2004a). Using size separation techniques to determine the association of metal ions it was shown that the metal cations were associated exclusively with <0.45 µm colloidal matter and organic molecules (Jensen *et al*., 1999)

The three previous stages of decomposition was relatively rapid whereas the fourth stage, methanogenesis, can take up to 90 years to complete (Lo, 1996). This long stage is termed the methanogenic phase and is characterised by the final degradation of organic compounds to methane and carbon dioxide. This characterised by a significant reduction in the COD and the biological oxygen demand (BOD) concentration (Table 6-1). Due to the depletion of organic acids in this phase the pH of the leachate begins to rise to ~ 8.0.

Table 6-1: Average changes in COD, BOD and pH between the acetogenic phase and methanogenic phase. (Kjeldsen *et al*., 2002).

Parameter	Average acetogenic phase (mg/L)	Average methanogenic phase (mg/L)
COD	22,000	3,000
BOD	13,000	180
pH	4.0	8.0

Kjeldesen *et al*. (2002) identified four fractions in the composition of landfill leachate that are influenced by the age of the landfill:

1. Dissolved organic matter, measureable as COD. This fraction is mostly humic acids, fulvic acids and humin.
2. Xenobiotic organic compounds whose origins are both household and industrial waste e.g. solvents and pharmaceuticals.
3. Inorganic ions e.g. NH_4^+, PO_4^{3-}, Ca^{2+}, Mg^{2+}, Na^+, SO_4.
4. Heavy metals e.g. Cd^{2+}, Zn^{2+}, Cu^{2+}.

Biological treatment is the favoured method of rendering landfill leachate safe before discharge. Biological treatment is effective at removing ammoniacal-nitrogen and in reducing the BOD to levels acceptable for discharge (Robinson and Barr, 1999). A large reduction in the BOD:COD ratio is normal for biological treated leachates (Robinson *et al*., 1992). This method of treatment is able to effectively reduce the readily biodegradable organics (Kjeldsen *et al*., 2002). The remaining leachate contains a significant concentration of organic compounds commonly measured as the chemical oxygen demand (COD). Typical concentrations in the UK range between 100-1,500 mg/L in treated landfill leachates (Robinson and Barr, 1999). This COD fraction is resistant to routine biological treatment but can be reduced by the advanced oxidation processes e.g. ozonation (Kurniawan *et al*., 2006b). As well as the COD the leachate contains a variety of major ions such as Cl, Mg, K, Na, HCO_3^-.

6.2.2 Humic and fulvic acid description

The high residual COD concentration in treated landfill leachate is reportedly composed of humic substances (Huo *et al*., 2008). Humic substances are further subdivided into three fractions: humic acid, fulvic acid and humin. These fractions are classified based on their particular chemical characteristics e.g. solubility in water and acid. Humic acid is not soluble in aqueous solutions at <pH 2 whereas fulvic acids are soluble at all pH values (Stevenson, 1994). Humic and fulvic acids are macromolecular compounds (>1,500 Da) that are held together by covalent bonds. Humic and fulvic acids have a characteristic dark brown colour and earthy smell that is a result of their aromatic-polymeric formation (Aiken, 1985). Most information on humic and fulvic acids comes from studies on soils and marine sediment and this work has been shown to be applicable to the refractory compounds found in landfill leachate (Calace and Petronio, 1997).

Elemental analysis of humic and fulvic acids demonstrate significant differences in the composition of functional groups. The carbon content of fulvic and humic acids tends to be similar whereas the oxygen content is significantly higher in fulvic acids compared to humic acids e.g. 32.8-38.3% in humic acid and 39.7-49.8% in fulvic acid (Williams, 2005). This higher oxygen content is attributed to a higher proportion of COOH groups in fulvic acids. The idealised Buffle structure of fulvic acid demonstrates the high proportion of COOH and -OH in the structure (Figure 6-1).

Figure 6-1: Idealised Buffle structure of fulvic acid (Aiken, 1985)

The formation of humic substances in landfill leachate is not well understood though 4 formation pathways in soils have been proposed. All four formation processes proposed are believed to contribute to the formation of humic substances in municipal landfills (Christensen *et al*., 1998). Each of the 4 proposed pathways utilise a specific starting material that is likely present in landfilled waste.

The lignin theory proposes a condensation reaction between lignin and protein (Waksman, 1932) but is now considered outdated by many researchers (Stevenson, 1994). Condensation of lignin and protein produces humic acid and bypasses the formation of fulvic acid.

The 3 other theories on the formation of humic substances are considered to be more applicable to the formation of humic substances and are especially applicable to landfills

(Figure 6-2). Polymerisation of sugars and amines form a n-substituted glycosylamine via a Shiff base intermediary which is believed to be a better explanation of the origin of fulvic and humic acids (Maillard, 1913). Further reactions create an unstable n-substituted-1-amino-deoxy-2-ketose. Fragmentation of this molecule is possible and further polymerisation of these fragments occurs to form a brown coloured polymer.

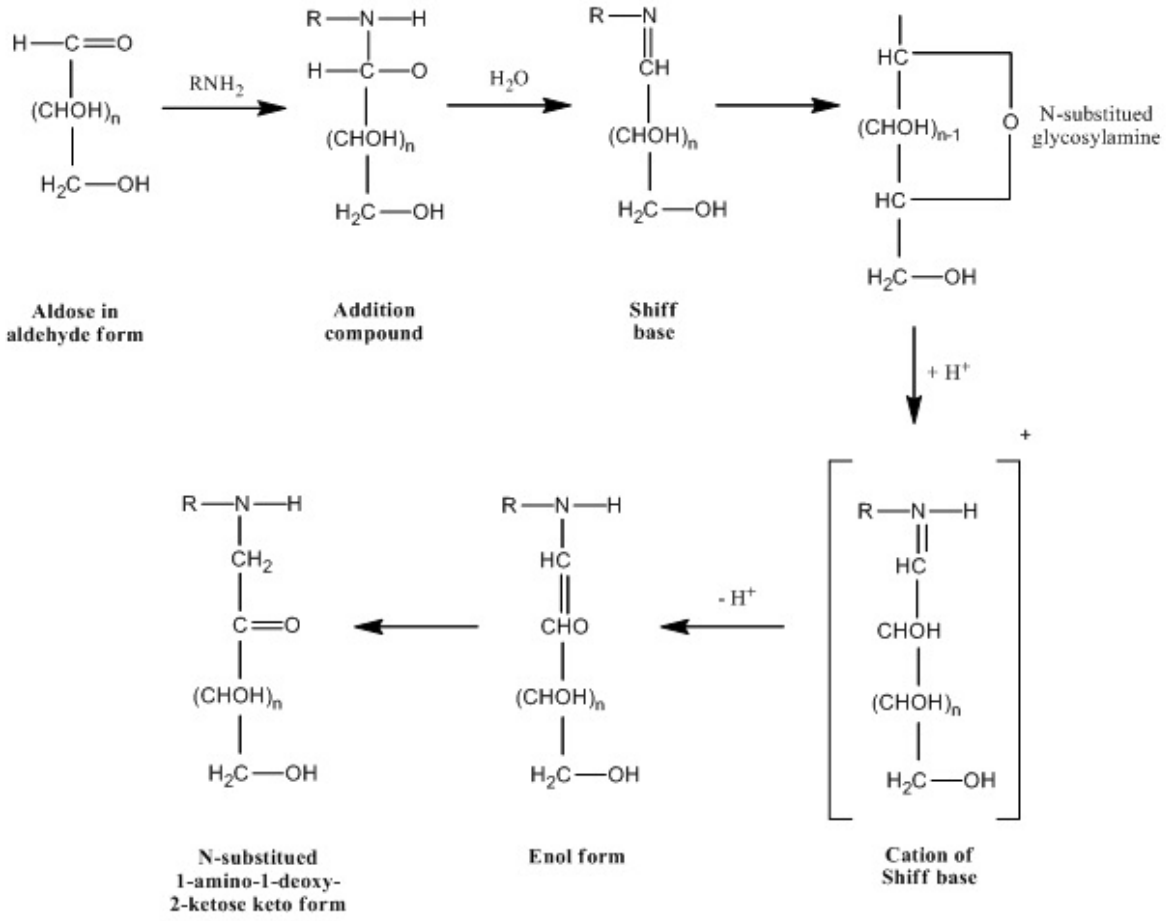

Figure 6-2: Sugar-amine condensation reaction to form n-substitued-1-amino-deoxy-2-ketose via an Amadori rearrangement (Stevenson, 1994).

The n-substituted-1-amino-deoxy-2-ketose is susceptible to a number of fragmentation pathways. All of these fragmentation products are highly reactive and in the presence of amino acids can go on to form a brown nitrogenous polymer (Stevenson, 1994).

Two other theories exist on the formation of humic substances from polyphenols and quinones. Polyphenols are a product of the decomposition of lignin by microorganisms. These polyphenols are readily oxidised by microbes to quinones. Combination of quinones in the presence of amino acids produces humic polymers (Figure 6-3). This process is the likely mechanism for the production of humic substances within landfills because the starting materials being present within the waste landfilled. This process explains the high proportion of aromatic humic substances in landfill leachate and their hydrophilicity (Senesi, 1996).

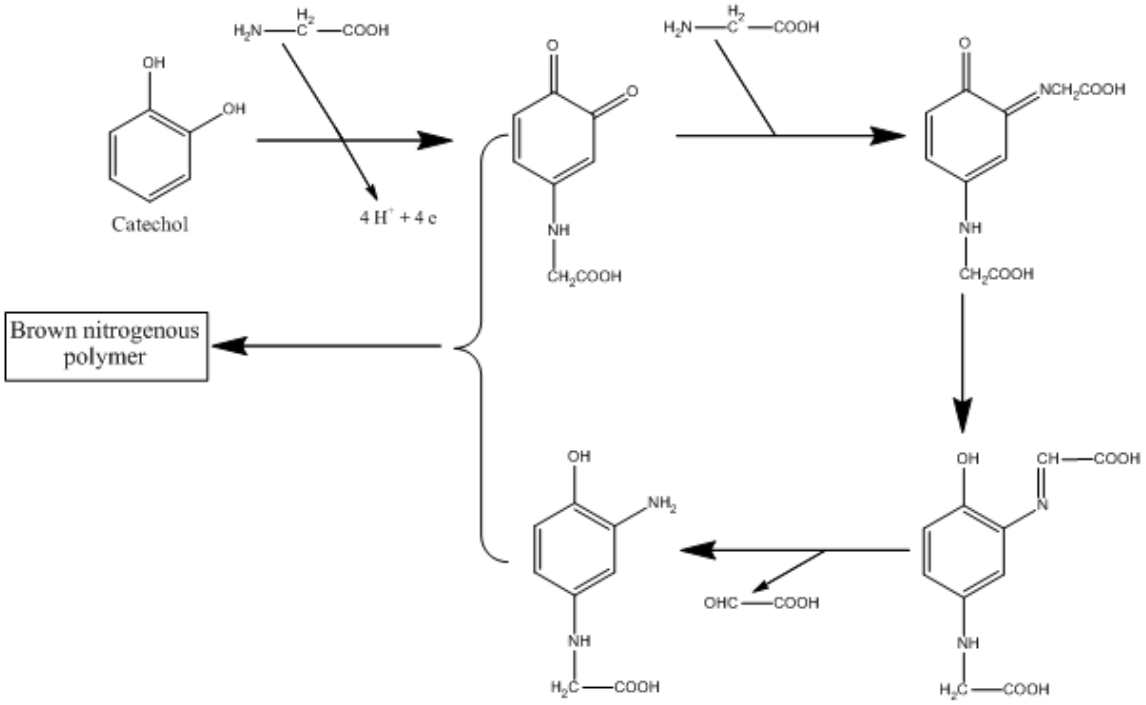

Figure 6-3: Combination of quinones and amino acids to form humic substances (Stevenson, 1994).

During the first 12 months of waste decomposition there is a reduction in the humic acid content of the leachate to a fulvic acid and transphilic substance (fraction of intermediate polarity isolated from XAD-4 resin e.g. 2-chlorophenol) dominated leachate. Using XAD-8

and XAD-4 to separate the COD fraction it was shown that a young landfill leachate (<5 months) was 12% humic acid (Figure 6-4) (Berthe *et al*., 2008).The humic acid content of a young leachate (<1 year) was reported as 0.5-5% of the COD content (Blakey *et al*., 1992). This initial concentration of humic acid comes from the waste landfilled decomposing and releasing the humic acid contained within the waste. After 12 months the proportion of fulvic acid and transphilic substances begins to rise and by 21 months after landfilling the humic acid content has risen >50% of the organic content of landfill leachate.

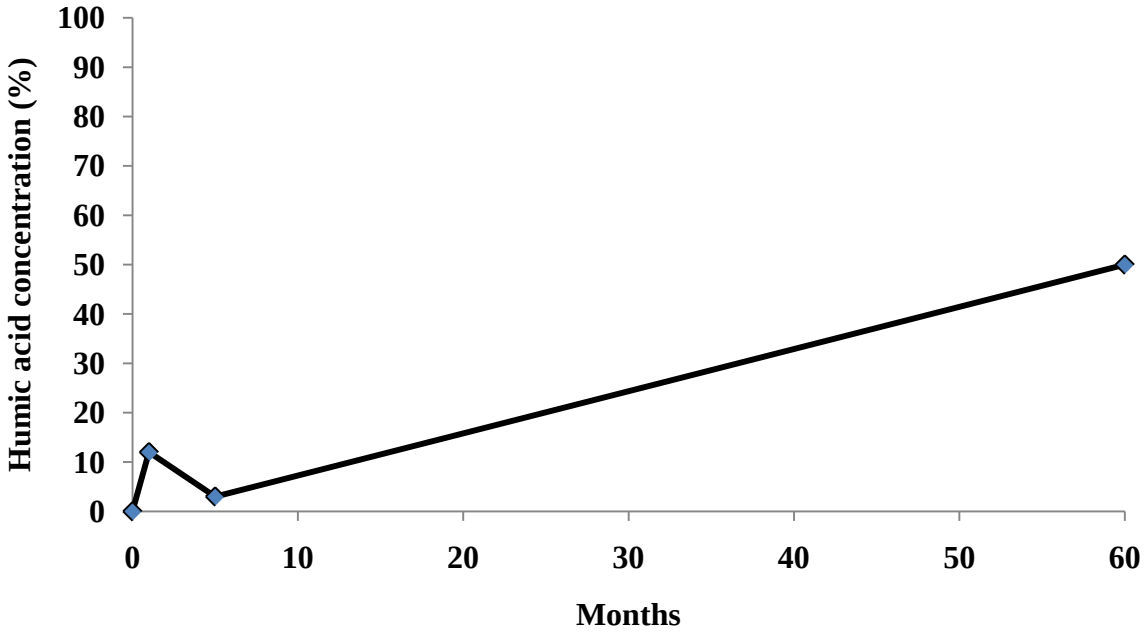

Figure 6-4: Changes in the humic acid content of the COD in landfill leachate based on previous reports (Blakey *et al*., 1992).

The characteristics of this fraction change from a fulvic dominated fraction in a young leachate to a humic acid dominated fraction in an older leachate. This is due to the polymerising of fulvic acids fragments into larger humic acid molecules (Huo *et al*., 2008). The molecular weight of the dissolved organic fraction increases as the landfill ages (Kang *et al*., 2002). The increase in size was explained by an increase in the humic to fulvic acid ratio.

Structure characterisation of the dissolved organic matter of landfill leachate has shown the structure of humic and fulvic acids changes with the age of the landfill. Analysis of different aged landfill leachates with fluorescence spectra showed the 'simpler' structural elements of fulvic acids were removed as the landfill aged with a greater ring like characteristic in the structure of the macro compounds due to an increase in humic acid (Xi *et al*., 2008). XAD-8 was used to fractionate leachates varying in age from 1-5-10 years (Zhang *et al*., 2008). Using UV spectroscopy the humic acid content of the landfill leachate was found to increase along with the concentration of aromatic molecules (Park *et al*., 1999). UV analysis demonstrated that >60% of the acid molecules were over 1.5 nm in size which would indicate a high proportion of humic acid molecules. Park *et al* (1999) went on to show that the FT-IR spectra produced from the XAD-8 fractionation was very similar to that of a purchased Aldrich humic acid mix except for a peak at 1,050 cm^{-1} though no explanation of which functional groups vibration is at 1,050 is given by the authors.

Present in many aspects of human and animal life due to natural production in soils and in presence in food means the toxicity of humic and fulvic acid is considered low (EMEA, 1999). Humic and fulvic acids have the ability to sequester smaller molecules into their structure due to their macromolecular structure (Christensen *et al*., 1998). This sequestering is due to the high number of Van der Waals and other intramolecular interactions such as H-bonding that are possible with the large and complex structure of humic and fulvic acids (Milne *et al*., 2003).

Humic and fulvic acids are classified as weak Bronsted acids. Loss of a proton from the carboxyl group allows cation capture to take place in the form of a salt (Stevenson, 1994). Single valent metal ions such as Na and K can form salts with this area of the structure. The formation of such bonds is through columbic and electrostatic attractions. This method is the process by which single valent metal ions are bound to humic and fulvic acids.

The binding of multivalent metal ions is mainly due to coordination between free electron pairs on the functional groups of humic and fulvic acid and empty orbitals on the metal ions (Stevenson, 1994). Atmospheric levels of Cd^{2+} and Pb^{2+} have risen and resulted in the introduction of these toxic heavy metals into the soils. Due to the presence of humic substances their toxicity has been negated by the formation of heavy metal-humic complexes (Heinrichs and Mayer, 1980). This exchange of cations is the method by which plants are able to obtain and maintain micronutrient levels in soils so there is a possible mechanism for the introduction of toxic heavy metals into organisms instead of nutrients (Evans, 1989). Humic substances in landfill leachate might introduce toxic materials into the aquatic environment via this mechanism or by ingestion by filter feeders.

Humic and fulvic acids have a high buffering capacity in solutions which is vital for the growth of plants. The buffering capacity limits the forms that many metal ions can take in solution. For example Al^{3+} becomes highly toxic below pH 5.5 but due to the buffering capacity of humic and fulvic acids this toxicity is limited in soils (Dobranskyte *et al*., 2006). Humic and fulvic acids could be responsible for toxicity due to their ability to chelate toxic metal ions e.g. Cd^{2+} and Pb^{2+}, at neutral pH and release these toxins with a drop in pH (Shuman, 1998). Humic acid have a protective ability in reducing the toxicity of Ni towards *Daphnia pulex* by complexation of the metal ion (Kozlova *et al*., 2009).

These macro-molecules are able to bind hydrophobic contaminants within the structure of the molecule (Christensen *et al*., 1998). Humic and fulvic acid have the ability to reduce the bioaccumulation of benzo[k]fluoranthene in organisms which reduces the overall hazard of this substance (Chen *et al*., 2008). This attenuation process operates through the COO^- functional groups that are so common on the humic and fulvic molecules. Attenuation of xenobiotics in landfills has also been reported (Christensen *et al*., 2001). The authors note that there are other processes such as dilution and bacterial redox reactions taking place to

remove the xenobiotics from the landfill. Attenuation tends to take place a short distance from the source of the pollution (Frommer *et al*., 2006). These xenobiotic substances and their degradation products are present within the untreated leachate (Baun *et al*., 2004). Baun *et al* (2004) reported a total 55 xenobiotic substances and 10 degradation products were recorded in the leachates from ten Danish landfills. These 10 substances were present in µg/L concentrations. Only after a 122 times pre-concentration of the sample did one sample cause a toxic response in an Ames test. These results show that natural attenuation via organic macromolecules can take place within landfills that reduce the concentration of harmful xenobiotic compounds in the leachate.

Due to the complex structure of humic substances, the effect on toxicity can be synergistic or antagonistic. It is difficult to make predictions on toxicity due to the effects of humic and fulvic acids on complex systems. From the presented evidence humic and fulvic acids could be a vital component within leachate to limit damage to the environment. For this reason, toxicity assessment with organisms that display a range of chemical and physical sensitivities is required in order to make quantitative conclusions on the effects of toxicity of this fraction.

6.2.3 Use of XAD to remove COD from leachate – basic approach

XAD-7 is acrylic in nature and a slightly polar resin. This resin is often used to adsorb the acidic elements of the COD fraction. XAD-7 was used instead of XAD-8 as both resins operate by the same mechanism of organic molecule adsorption. XAD-4 is made from a styrenedivinylbenzene and has a much smaller pore size than XAD-7. XAD-4 is non-polar nature and is used to adsorb non-polar molecules in landfill leachates.

Previously reported work on fractionation of the organic content of raw landfill leachate using XAD resins showed that it is possible to remove≥80% of available COD with XAD -8 and XAD-4 (Li *et al*., 2008). This work highlights the possibility for XAD resins to reduce the COD concentration and allow effective conclusions on the possibility of its role in

toxicity. No work has previously been carried out using XAD resins to assess the effect on toxicity by reducing the COD fractions concentration in treated landfill leachate. To determine the size and polarity of dissolved organic matter (DOM) in treated landfill leachate XAD-7 and 4 used to fractionate the leachate (Bu *et al*., 2010). The authors reported that 62% of the DOM was acidic in nature and <10% of the DOM was non-acidic in nature.

Due to the high concentration of humic substances within treated landfill leachate, a possible role in toxicity and a shortage of information from the TIE work, a dedicated investigation of the toxicity of this fraction was needed. The objective of this work was to remove various fractions of COD from a treated leachate sample and assess toxicity response towards *Lemna minor* and *Daphnia magna*.

A rapid batch procedure for the isolation and removal of humic and fulvic acids with XAD-8 resin has been developed (Van Zomeren and Comans, 2007). Due to Amberlite ceasing production of XAD-8 a replacement resin has been selected which shares similar characteristics, XAD-7HP (Wagland, 2008). This rapid batch procedure offers an advantage over the other technique in that it requires a much shorter experimental time i.e. 1-4h as opposed to the 40h of the previous system (Aiken, 1985).

The procedure starts with filtration of the sample to remove solids that might be present. This is followed by acidification to pH 1-2 with nitric acid for 24 hours (Figure 6-5). Centrifugation of the sample leaves a pellet of humic acid and a supernatant of fulvic and hydrophilic acids. The supernatant is decanted and fractionated with the XAD resins that remove the targeted fraction of COD. The toxicity of the fractionated samples was tested using *L. minor* and *D. magna*.

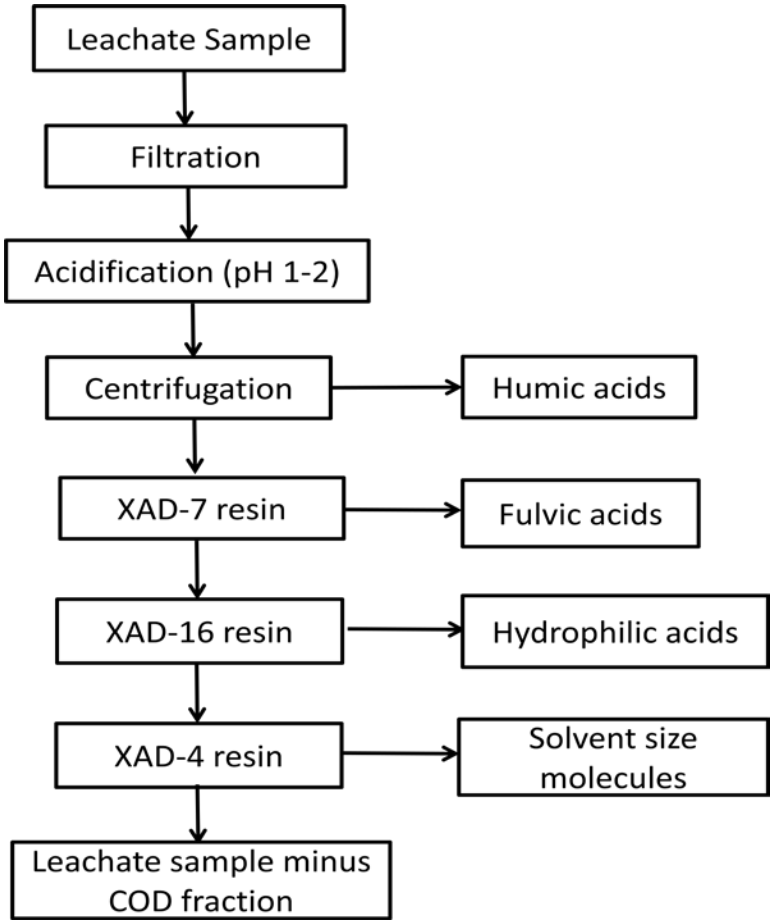

Figure 6-5: Flow diagram of the steps for the rapid removal of humic and fulvic acids from treated landfill leachate samples.

For this series of experiments, 3 XAD resins were selected based on the size and polarity of organic molecules they will adsorb to their surface. XAD-7HP was selected due to its large pore size and its known ability to adsorb macromolecules such as plant extracts and enzymes with molecular weight >1000 Da which are of equivalent size to fulvic acids (Figure 6-1)

XAD-4 was selected for its ability to remove the smallest organic chemicals with a molecular weight of 50-200 Da e.g. organic solvents (toluene) and metabolic by-products (isoprene) (Figure 6-6). This resin removes non-polar molecules.

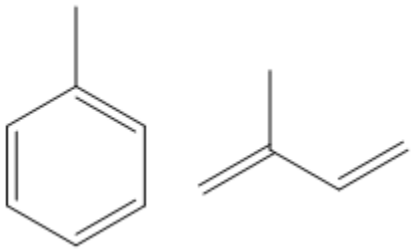

Figure 6-6: XAD-4 is designed to target solvent size molecules such as toluene and isoprene (left to right).

XAD-16N was selected for its ability to remove molecules <200-800 Da e.g. antibiotics and pesticides. This resin should remove any xenobiotics not removed during biological treatment and breakage of humic and fulvic acids due to acid hydrolysis in the centrifugation stage of the rapid batch procedure (Figure 6-7).

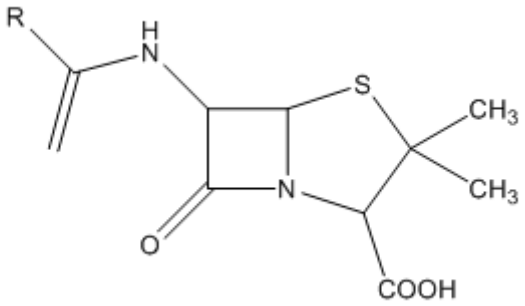

Figure 6-7: XAD-16 is designed to remove antibiotic sized molecules e.g. penicillin**.**

6.3 Hypothesis

Null hypothesis: The COD fraction of treated landfill leachate is not responsible for residual toxicity.

Alternative hypothesis: The COD fraction of treated landfill leachate is responsible for residual toxicity.

6.4 Aims and objectives

Aim: Develop an understanding of the role of the COD fraction of residual toxicity in treated landfill leachate.

Objectives:

I. Reduce the humic acid concentration with centrifugation and fulvic acid concentration with XAD-7 extraction. Following each procedure determine the toxicity of the samples.

II. Repeat I and add XAD-4 extraction to remove non-polar organic compounds. Determine whether the addition of a 2nd resin altered the toxicity of the sample.

III. Repeat II and add XAD-16 extraction which is designed to remove hydrophobic organic compounds a small to medium molecular weight compounds. Determine whether the addition of a 2nd resin altered the toxicity of the sample.

6.5 Materials and methods

This phase of experimenting used a rapid batch procedure for the removal of COD (Van Zomeren and Comans, 2007). The procedure was altered to decrease the time required for the removal of COD with the XAD resins. In this procedure the contact time of sample was reduced to ~10mins as opposed to the 1 hour advised in the procedure of Van Zomeren and Comans (2007). This was made possible by use of a glass column filled with XAD instead of beakers used by Van Zomeren and Comans (2007).

Over a period of 6 months, six samples from the Marston Vale leachate treatment plant (MVP) and five samples from Arpley were collected for this phase of experiments. The samples were collected in the method outlined in the Methodology chapter.

WET testing in this phase of testing was carried with *Daphnia magna* and *Lemna minor*. Both procedures are outlined in detail in the Methodology chapter.

6.5.1 XAD resins

Three types of XAD resin were used in this series of experiments: XAD-7; XAD-16 and XAD-4. The experimental procedures adopted are highlight in below (Figure 6-8). It was found that XAD resins needed to be added to the experimental procedure following evaluation of the data. Following extraction with XAD-7 there remained a significant quantity of COD in the leachate and the toxicity remained unaltered. This left a degree of uncertainty whether the nature of the toxicity was altered following the XAD-7 extraction. A XAD-4 resin was introduced to the procedure following the results of the XAD-7 procedure. This resin was introduced as it was thought that the remaining COD fraction would be made up of small organic molecules e.g. Figure 6-6. Unfortunately, this resin only removed a small proportion of the COD fraction. Thus it was necessary to introduce a third resin, XAD-16, into the procedure to remove molecules not adsorbed by XAD-7 and 4.

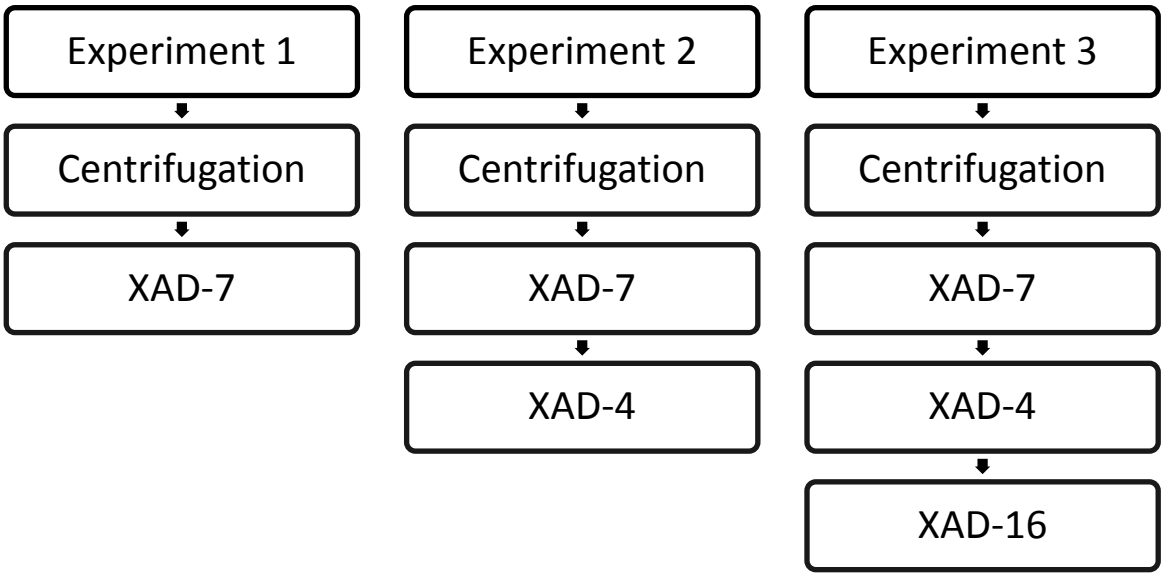

Figure 6-8: Experimental process adopted for the reduction of the COD fraction in treated landfill leachate from MVP and Arpley

The resins were all obtained from Sigma-Aldrich (Sigma-Aldrich, UK). Soxhlet extraction was necessary to remove impurities from the XAD resins. The XAD resin was soaked in 5% NaOH overnight (Fisher Scientific Chemicals, UK). After soaking, the XAD was packed into cellulose thimbles that are placed into the soxhlet extractor.

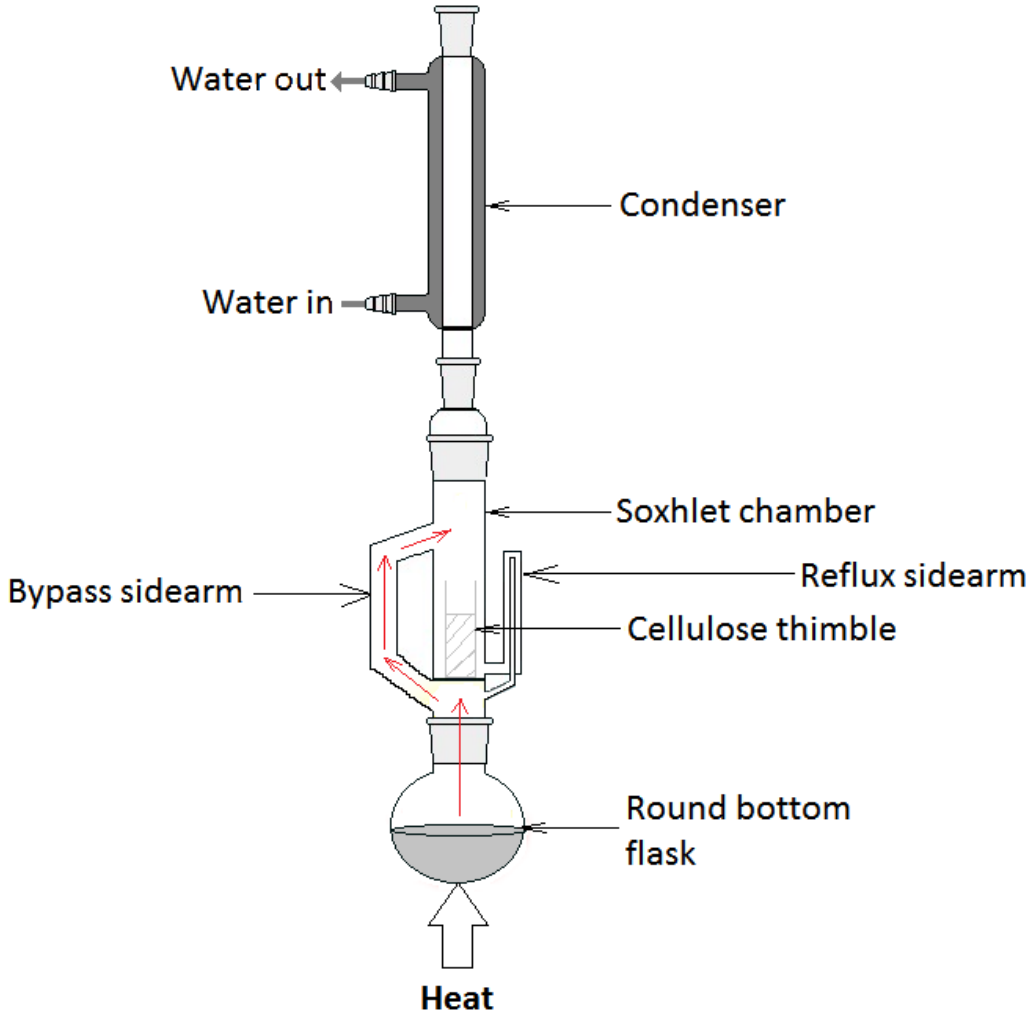

Figure 6-9: Soxhlet extraction apparatus used for cleaning XAD resin.

The procedure began with a methanol extraction. The solvent was heated in a round bottom flask and the vapours rise through the bypass sidearm until cooled by the condenser (see Figure 6-9). The cooled solvent collects in the soxhlet extractor which effectively washes the XAD resin. The solvent is able to remove contaminants from the solid XAD due to the

limited solubility of the contaminants in the solvents used. This continues until the reflux sidearm is filled with solvent which causes the solvent to empty out of the bottom of soxhlet extractor and the whole process cycles until stopped. Methanol is used for the first 48 hours. After 48 hours the methanol is swapped for acetonitrile for a further 48 hours. Following this aecetonitrile is changed for methanol for a further 48 hours (Goslan *et al*., 2002).

Soxhlet extraction was performed using 99.5% pure methanol and 99% pure acetonitrile (Fisher Scientific Chemicals, UK). Cellulose extraction thimbles, 41mm x 123mm, were packed with XAD resin (Fisher Scientific Chemicals, UK). Refluxing was carried out for 48 hours. After refluxing the XAD resin was removed from the soxhlet extractor and washed with alternating 10 L aliquots of 0.1 M HCl, 0.1 M NaoH, DI water. This was carried out to remove any remaining traces of solvent. After 48 hours of washing a 50 ml aliquot of washed DI water was analysed for its total organic carbon content (TOC) (Shimadzu TOC-5000A, Japan). If any TOC was recorded washing was restarted and continued until the TOC value was <0.001 mg/L.

6.5.2 COD removal procedure

Sample pH was determined immediately after arrival at Cranfield University. The treated landfill leachate was acidified to pH 2 with HNO_3 for 24 hours before COD removal commenced. HNO_3 was selected as the nitrate concentration in the treated landfill leachates was already high and there is little toxicity associated with nitrate (Kim *et al*., 2008).

The acidified leachate sample was centrifuged at 10,000 rpm for 10 minutes (MSE Falcon 6/300, U.K.). A pellet of humic acids was formed at the bottom of the centrifuge tube. The supernatant was decanted from the tube and this supernatant was used in the next step.

After cleaning, each XAD resin was packed into a glass column with dimensions 2.5 x 50 cm (volume of 246 ml) (Econo-Column Chromatography Columns, Biorad, U.K.). The acidified treated landfill leachate was injected to the column with a peristaltic pump. The treated

landfill leachate was passed through the resin and the leachate collected from the column end. The sample was neutralised back to the natural pH with addition of NaOH (Fisher Scientific Chemicals, UK). The COD concentration was determined using the method outlined in the Methodology (Chapter 3). Samples used in WET testing were always at the natural pH value. The point at which samples were collected is referred to in results section. Three repeats were carried out for each of the treatments. A significant difference between each of the treatments was determined with a student's T-test.

6.6 Results and discussion

In total five samples were collected from the Arpley and six from the MVP leachate treatment plants. The physico-chemical characteristics of the samples collected are presented in (Table 6-2). The mean COD concentration remained relatively constant in the summer month samples from MVP. There was a noticeable drop, ~200 mg/L, in the COD concentration in the winter months. The COD concentration was more variable in the Arpley samples with a range 558-1,100 mg/L. Like the MVP samples, there was significant drop in concentration of COD in the sample collected in the winter months.

The BOD concentrations displayed little variation in the samples collected from MVP. The concentration varied between 6 to 8 mg/L. The BOD concentration from Arpley remained constant throughout the entire process at 8 mg/L. Ammonical nitrogen is the compound that has received the greatest concern over its presence in landfill leachate. Both types of biological treatment employed at each of the plants were able to reduce the concentration to a level undetectable by the cell tests used in this project i.e. <0.1 mg/L.

The major ion concentration is a candidate source of toxicity in treated landfill leachates. For this reason the concentrations of Ca^{2+}, Na^{+}, Mg^{2+}, K^{+} were determined using Atomic Absorption spectroscopy (Chapter 3). The mean Mg concentration in the MVP samples was between 96-125 mg/L. There was a slight reduction in the concentration between the summer

samples and the winter samples. The Arpley Mg mean concentration was higher in most cases than the MVP concentration. There was no variation between the summer and winter samples in these samples.

The K^+ concentrations in the MVP samples had a range of 702 to 968mg/L. The mean K^+ concentration in the Arpley samples were considerably higher and ranged from 968 to 1,498 mg/L. Sulphate concentrations in the MVP concentration varied over a range of 124-224 mg/L. The concentration variation showed no pattern with the other cation concentrations. The mean Arpley sulphate concentration was lower and showed considerable stability over the sampling period. The mean sulphate concentration ranged from 81-99 mg/L in the samples collected. The concentration of Na in the MVP samples ranged from 3,804 to 4,775 mg/L. The Na concentration in the Arpley samples was considerably higher and varied 4,495 to 5,917 mg/L. There was no sign of seasonal variation in the concentration of Na in either source. These ions did not follow a similar dilution pattern as the COD concentration in the winter and summer months.

Table 6-2: Chemical parameter data of treated landfill leachate samples collected from MVP and Arpley (A)

Parameter (mg/L)	MVP 13.05.09	MVP 23.05.09	MVP 11.08.09	MVP 24.08.09	MVP 10.11.09	MVP 17.12.09	A 11.08.09	A 24.08.09	A 2.09.09	A 24.09.09	A 28.11.09
COD	855	850	850	928	644	607	790	1,000	706	923	558
BOD	8	7	8	6	8	8	8	8	8	8	8
NH_3-N	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mg^{2+}	113	128	125	96	109	101	108	134	126	104	122
K^{+}	934	786	702	794	837	863	968	1,146	1,498	1,071	983
Cl^{-}	3,994	3,570	4,371	4,000	3,311	3,449	2,337	2,744	2,003	2,245	2,554
SO_4^{2-}	132	124	224	126	184	153	89	93	99	81	99
HCO_3^{-}	1053	945	1,269	1,893	1,183	1,680	2,491	1,662	1,368	1,839	2,104
Na^{+}	4,775	4,652	4,128	3,804	4,106	4,598	5,917	4,495	5,891	5,434	5,826
Ca^{2+}	195	231	152	144	176	119	142	134	253	179	78

6.6.1 Tests using XAD-7

The COD removal efficiency of the various stages was assessed and the removal calculated (Figure 6-10). The centrifugation stage removed between 18-28% of the COD from the original sample. The XAD-7 extraction reduced the COD concentration between 43-62%. These results suggest that the ~20% of the samples are humic acids and between 20 and 40% of the samples are fulvic acids (Figure 6-5). The COD concentration of the samples indicates that the landfill leachate was <10 years old in terms of ratio of humic to fulvic acids in the COD fraction.

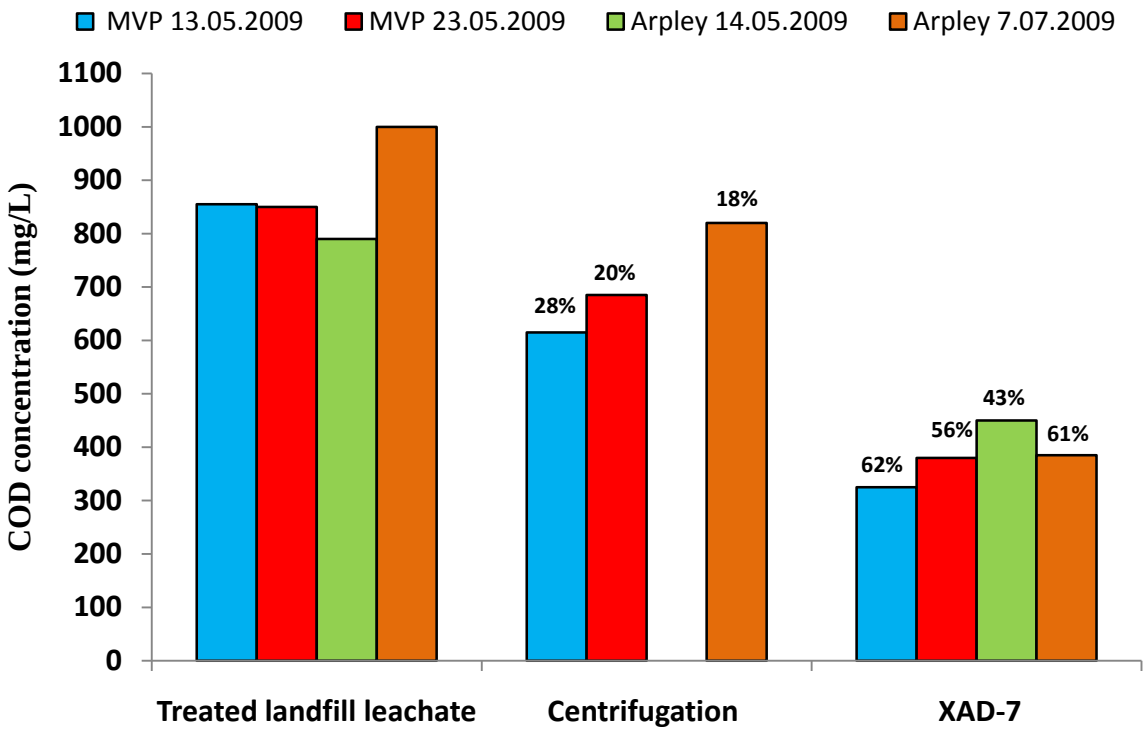

Figure 6-10: The mean COD concentration (mg/L) at the start and after application of each stage of the rapid batch procedure (n=3). The percentages above each bar represent the percentage removal between the original and the application.

The toxicity of the MVP treated landfill leachate samples towards *D. magna* varied on the two sampling occasions (Figure 6-11). EC_{50} toxicity was 50% and 43% on the two sampling

occasions which was higher than the 59% recorded in the TIE procedure (Figure 6-13). After centrifugation the toxicity increased slightly but this rise was not significant. Following XAD-7 extraction the 13.05.09 sample recorded a reduction in the toxicity to 55%. Conversely, the sample collected on the 23.05.09 there was an increase in the toxicity from 43% in the original sample to 37% in the XAD-7 extracted. These changes in toxicity means are not significant ($p < 0.05$).

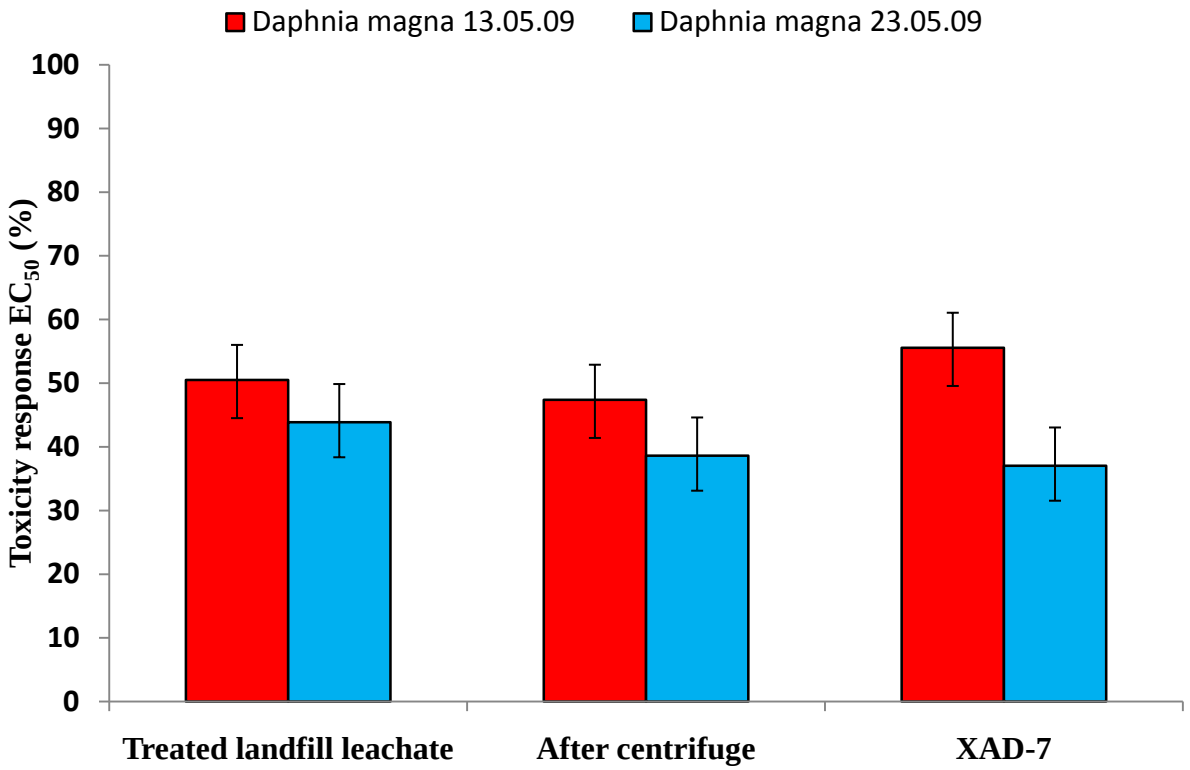

Figure 6-11: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure with samples collected from MVP (n=4). 95% confidence intervals shown as bars.

The toxicity responses of *L. minor* displayed a high degree of variability in the responses (Figure 6-12). The sample collected on the 13.05.09 recorded a toxicity of 29% whereas the 23.05.09 sample recorded a lower toxicity of 43% though there is no statistical difference. After centrifugation the toxicity of the 13.05.09 sample decreased but the toxicity of the

23.05.09 sample increased slightly. The result of extraction with XAD-7 was an increase in the toxicity from the original samples. Due to the high variability inherent of the *L .minor* test these alterations in toxicity were not statistically significant. These changes in toxicity means are not significant ($p < 0.05$).

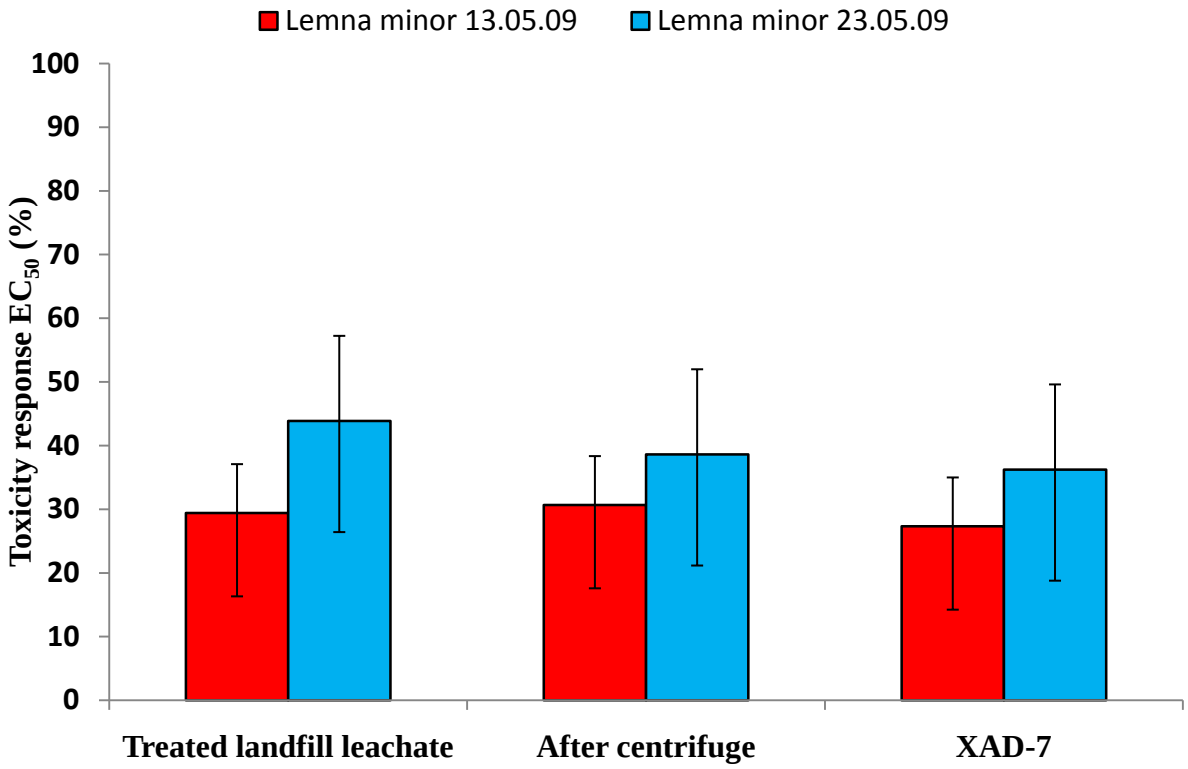

Figure 6-12: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure with samples collected from MVP (n=3). 95% confidence intervals shown as bars.

Two samples were collected from Arpley landfill leachate treatment plant and fractionated with the rapid batch procedure (Figure 6-13). The EC_{50} toxicity recorded in the 14.05.09 sample towards *D. magna* was 59% and the toxicity of the 7.07.09 sample was 43%. Following centrifugation there was no recorded drop in toxicity of the samples. Extraction with XAD-7 resulted in the toxicity of both of the samples remaining unchanged. These changes in toxicity means are not significant ($p < 0.05$).

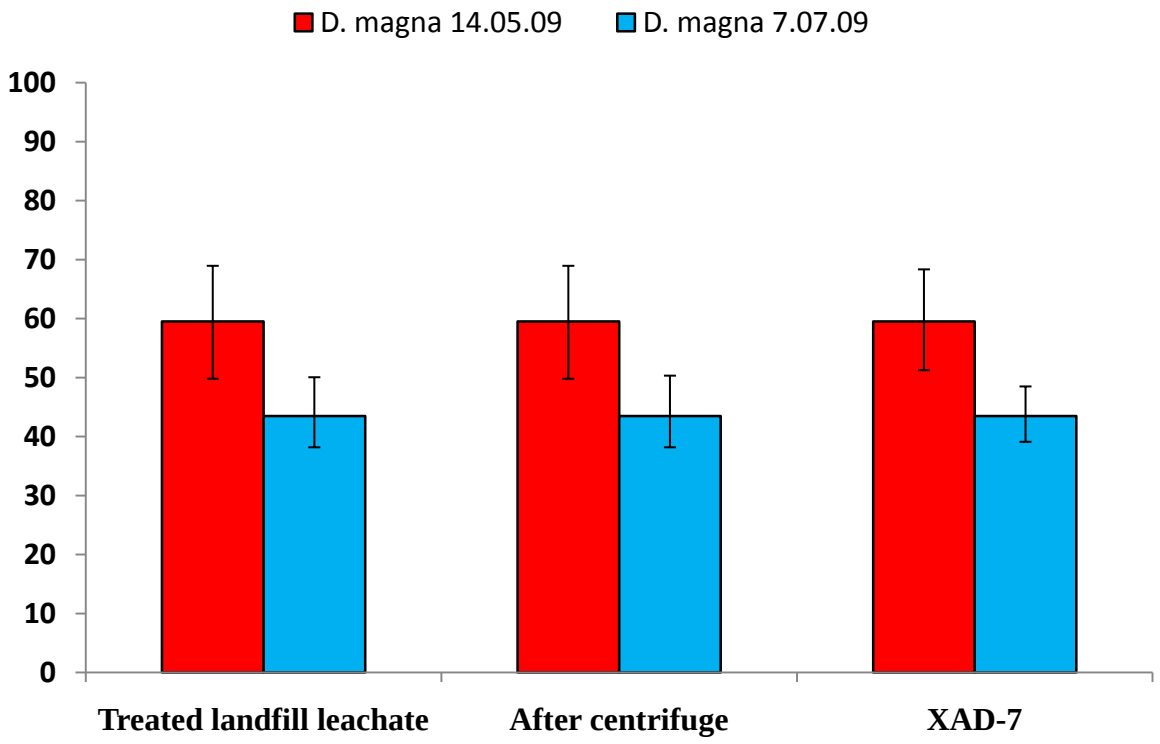

Figure 6-13: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure with samples collected from Arpley (n=4). 95% confidence intervals shown as bars.

The baseline toxicity of the samples towards *L. minor* was higher than towards the baseline *D. magna* (Figure 6-14). The 7.07.09 sample recorded a toxicity of 19%, the highest in this phase of the project and the 14.05.09 sample recorded a toxicity of 31%. Following centrifugation both samples displayed small changes in toxicity. The XAD-7 stage was able to reduce the toxicity of both of the samples. In the 7.07.09 sample the toxicity was reduced to 23%. In the 14.05.09 sample the toxicity was reduced to 39%. These reductions in the toxicity were not statistically significant due to the large error in the test. In the 100% concentrations, all of the *L. minor* fronds displayed chlorosis. These changes in toxicity means are not significant ($p < 0.05$).

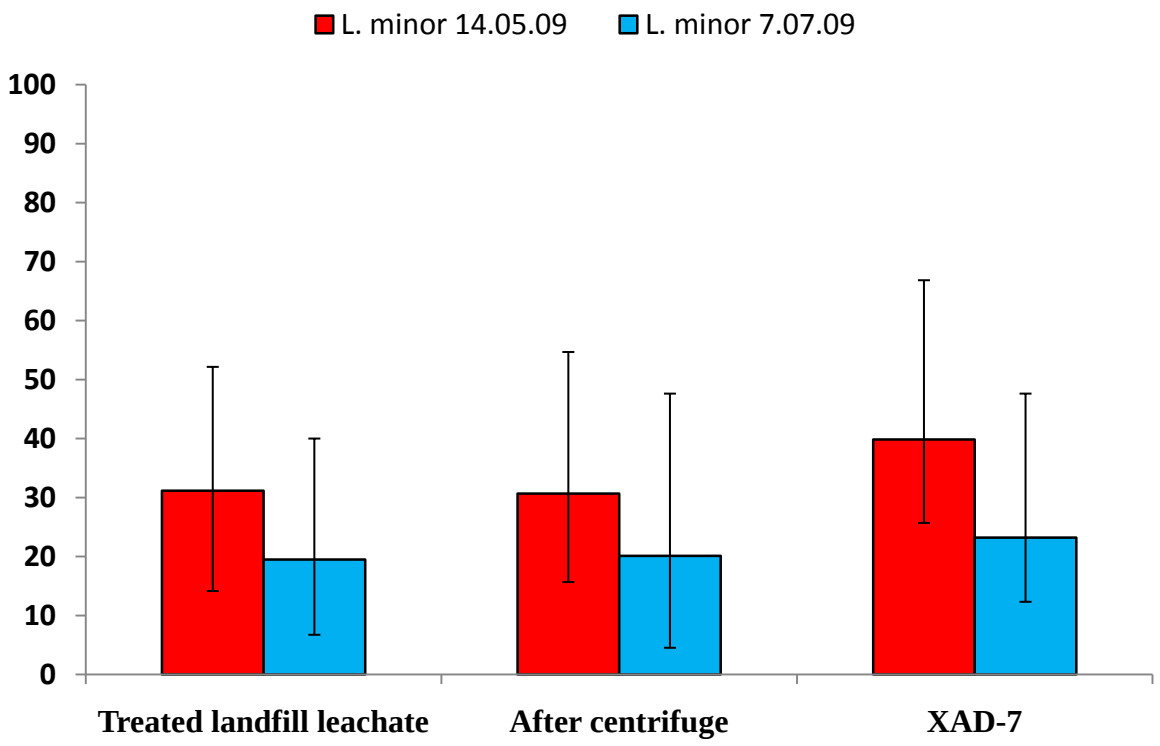

Figure 6-14: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure with samples collected from Arpley (n=3). 95% confidence intervals shown as bars.

6.6.2 Tests using XAD-7 and 4 in sequence

COD removal was determined after each of the XAD extractions (Figure 6-15). The XAD-7 extractions produced very similar results between the 4 samples with the range of 49-55% COD removal. Following the XAD-4 extraction, the COD removal was increased slightly by ≥17%. Overall, the largest COD removals were recorded in the samples collected from MVP.

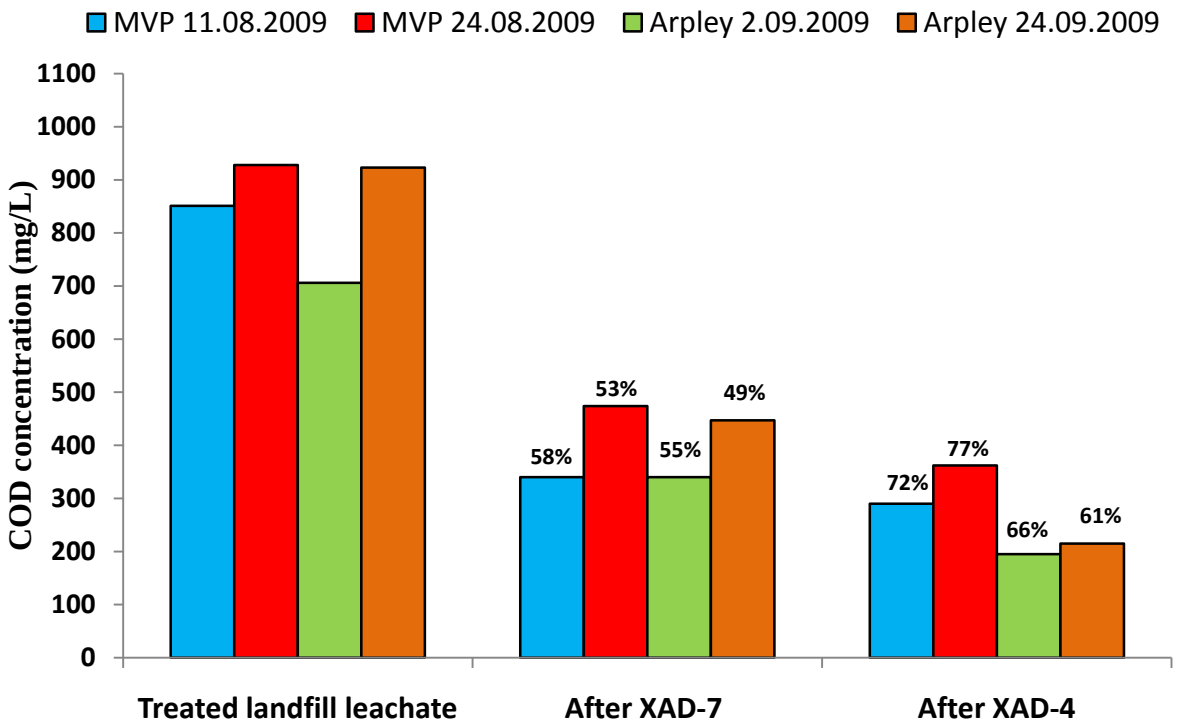

Figure 6-15: The mean COD concentration (mg/L) at the start and after application of each stage of the rapid batch procedure (n=3). The percentages above each bar represent the percentage difference between the original and the application.

In the second phase of testing a second resin, XAD-4, was added to the procedure in order to further reduce the concentration of COD specifically the non-polar compounds. The two MVP samples from the 11.08.09 and 24.08.09 produced an EC_{50} toxicity of 59% towards *D. magna* (Figure 6-16). Following centrifugation and extraction with XAD-7 there was no change in the toxicity of either of the samples. Similarly, further extraction with XAD-4 resulted in no change in toxicity. There was no significant difference between the results as all results were identical.

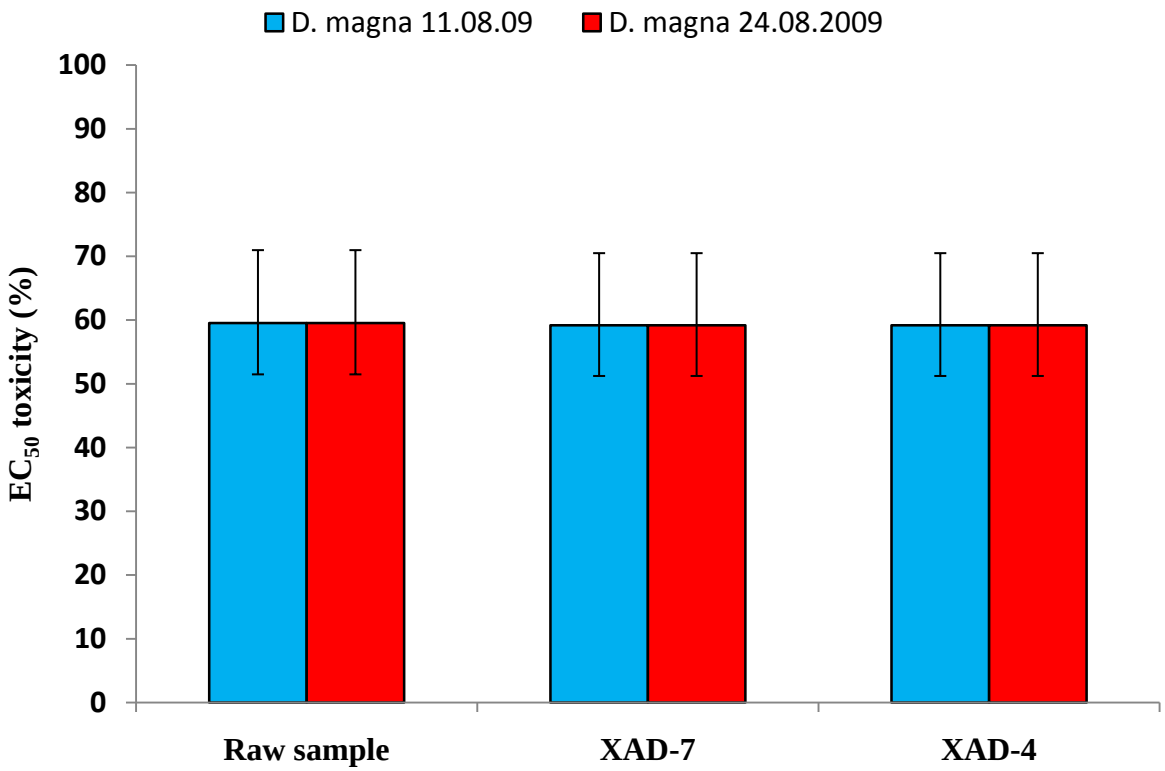

Figure 6-16: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from MVP (n=4). 95% confidence intervals shown as bars.

The *L. minor* test recorded an initial toxicity of 48% for the 11.08.09 sample and a toxicity of 42% for the 24.08.09 sample (Figure 6-17). Following centrifugation and extraction with XAD-7, both of the tests recorded slight reductions in toxicity though these are not significant changes. The extraction with the XAD-4 resin reduced the toxicity from the original samples 48% to 53% in the 11.08.09 sample after extraction with two resins. The toxicity of the 24.08.09 sample was reduced from 42% to 44% with the XAD-4 resins These changes in toxicity means are not significant ($p < 0.05$).

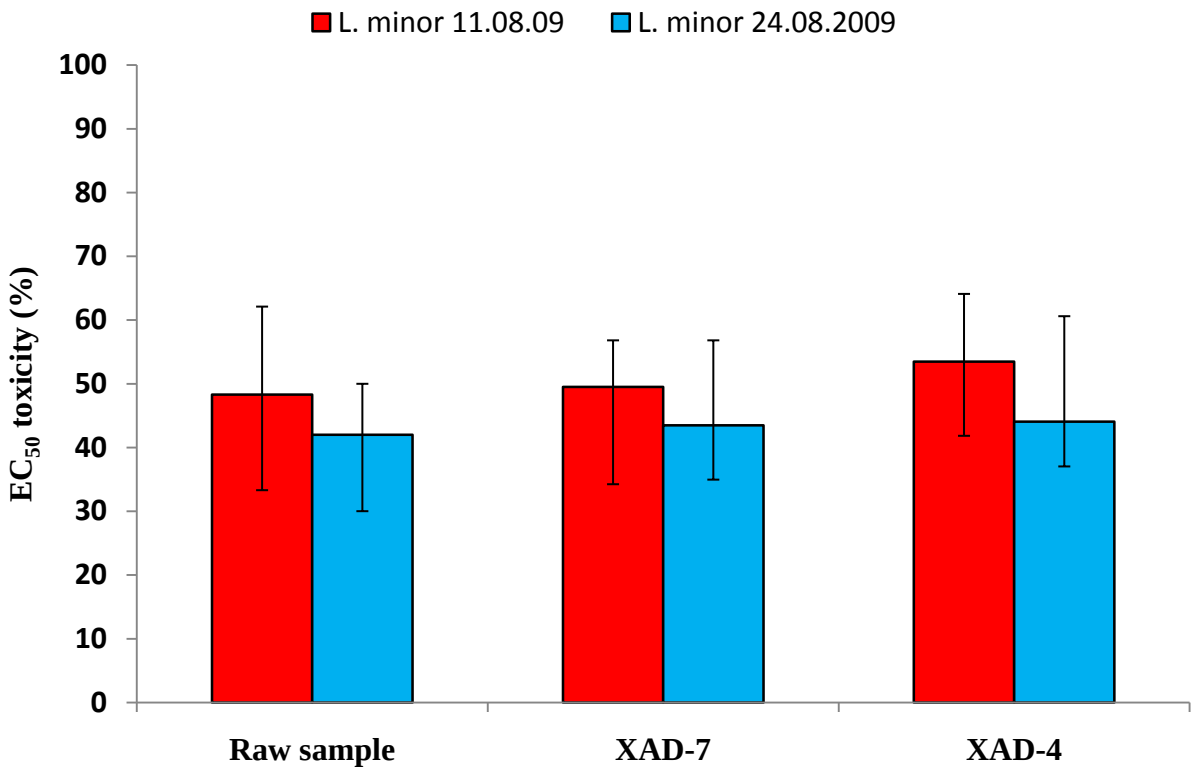

Figure 6-17: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from MVP (n=3). 95% confidence intervals shown as bars.

The samples collected from Arpley recorded an initial toxicity of 59.5% in both the samples towards *D. magna* (Figure 6-18). Following centrifugation and the extraction with XAD-7 there was no change in the toxicity compared to baseline toxicity. No change in the original toxicity was recorded following extraction with the XAD-4 resin. These changes in toxicity means are not significant ($p < 0.05$).

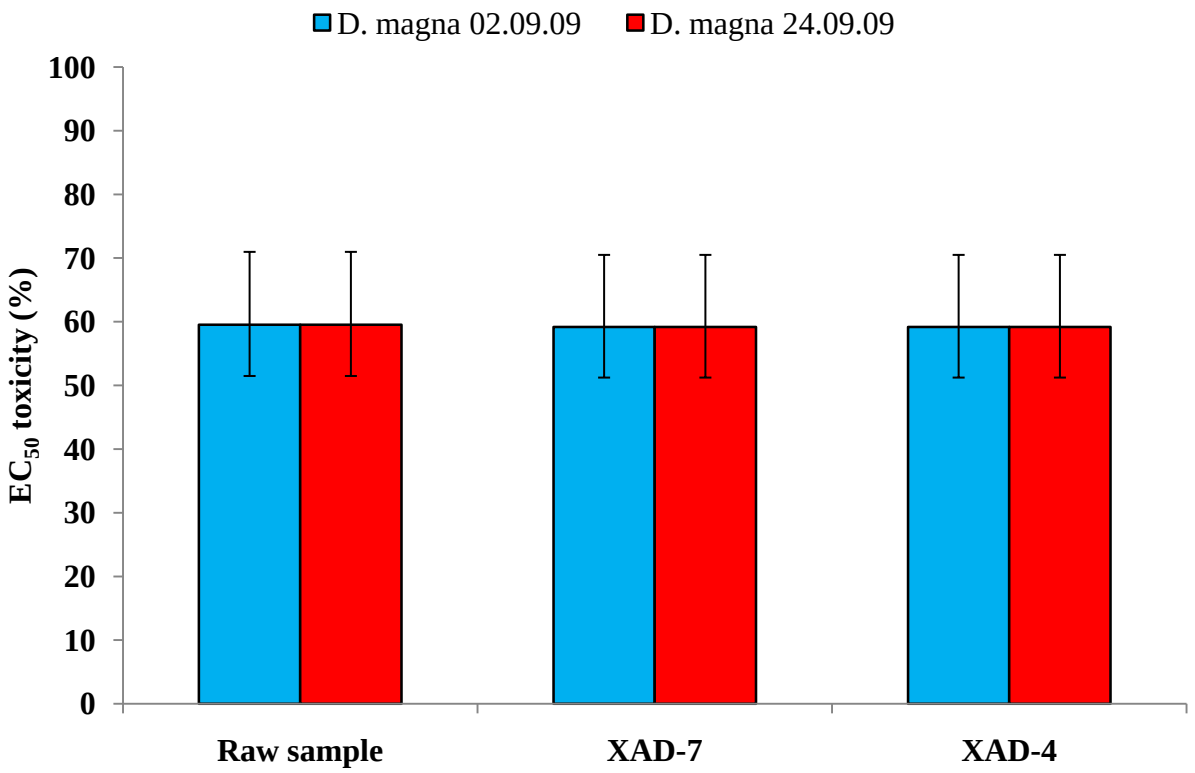

Figure 6-18: Average changes in toxicity response of *D. magna* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from Arpley (n=4). 95% confidence intervals shown as bars.

The 2.09.09 sample collected from Arpley recorded a toxicity of 29% towards *L. minor* and the 24.09.09 sample recorded a toxicity of 34% (Figure 6-19). Following centrifugation and extraction with XAD-7 the toxicity of the 2.09.09 sample had increased to 27% and the toxicity of the 24.09.09 had decreased to 37%. The XAD-4 extraction increased the samples toxicity fractionally to 27%. The XAD-4 process caused the toxicity of the 24.09.09 sample to remain unchanged at 37%. Like in the previous XAD-7 stage of testing these small changes in toxicity were not statistically significant. In the 100% concentrations, all of the *L. minor* fronds displayed chlorosis. These changes in toxicity means are not significant ($p < 0.05$).

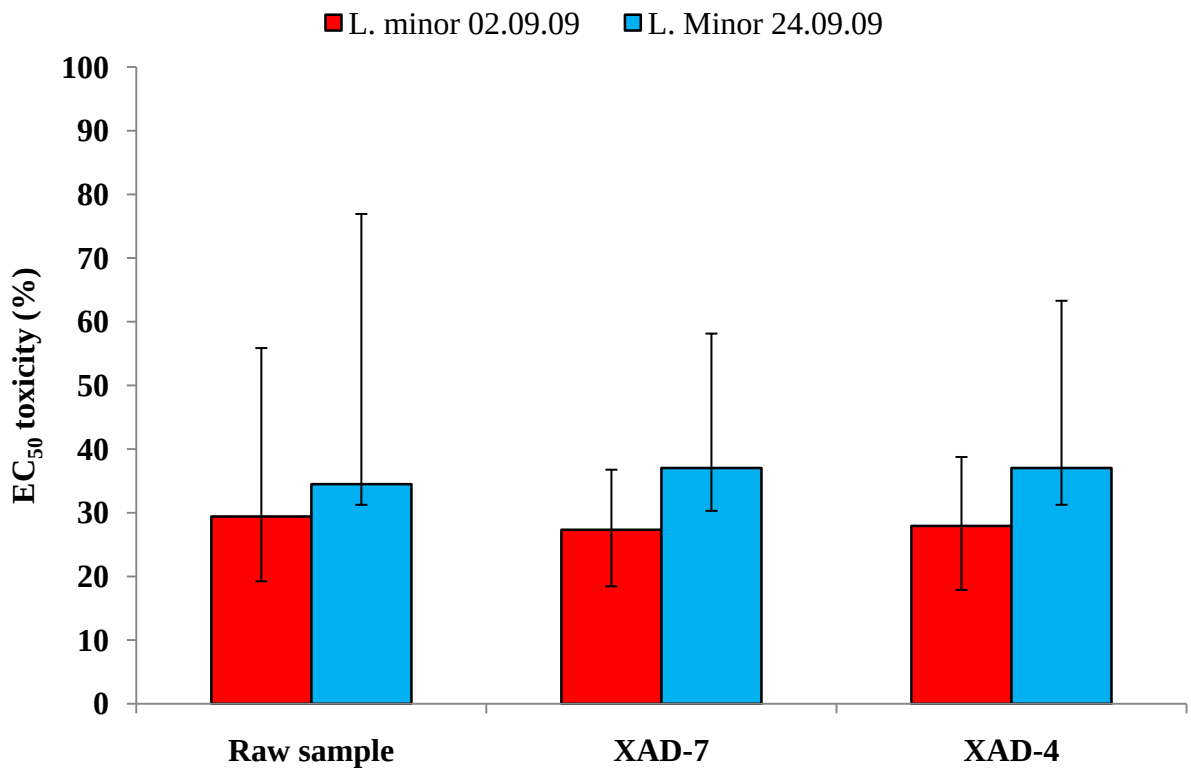

Figure 6-19: Average changes in toxicity response of *L. minor* following COD reductions after the rapid batch procedure plus XAD-4 with samples collected from Arpley (n=3). 95% confidence intervals shown as bars.

6.6.3 Tests using XAD-7, 4 and 16 in sequence

The mean COD concentration reduction was determined at each stage of the rapid batch COD removal process with XAD-7, 4 and 16 in sequence. The mean COD concentration was reduced by 41-55% with the XAD-7 extraction stage. The application of the XAD-4 resin reduced the mean COD concentration further by 52-67%. The application of the XAD-16 reduced the mean COD concentration by 82-90% of the original mean COD concentration.

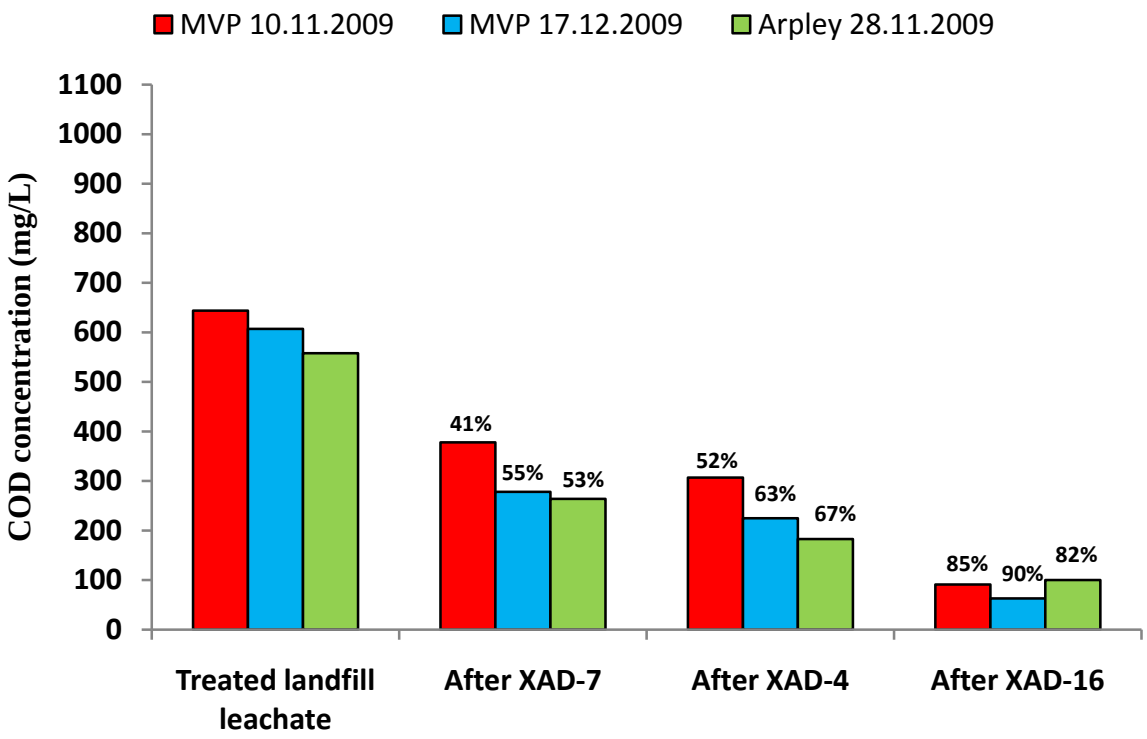

Figure 6-20: The mean COD concentration (mg/L) at the start and after application of each stage of the rapid batch procedure (n=3). The percentages above each bar represent the COD percentage reduction between the original and the application.

In order to reduce the concentration of COD even further a third XAD resin was added to the extraction procedure (Figure 6-21). The toxicity of MVP samples towards to *D. magna* was 59% in both of the samples tested. The 28.11.09 sample from Arpley had a slightly higher toxicity at 47% TU. Following the application of the centrifugation and the XAD removal the toxicity of the MVP samples remained unchanged at 59%. Following the application of the rapid batch procedure and extraction with XAD-16 the toxicity of the Arpley sample was reduced to 59%. These changes in toxicity means are not significant ($p < 0.05$).

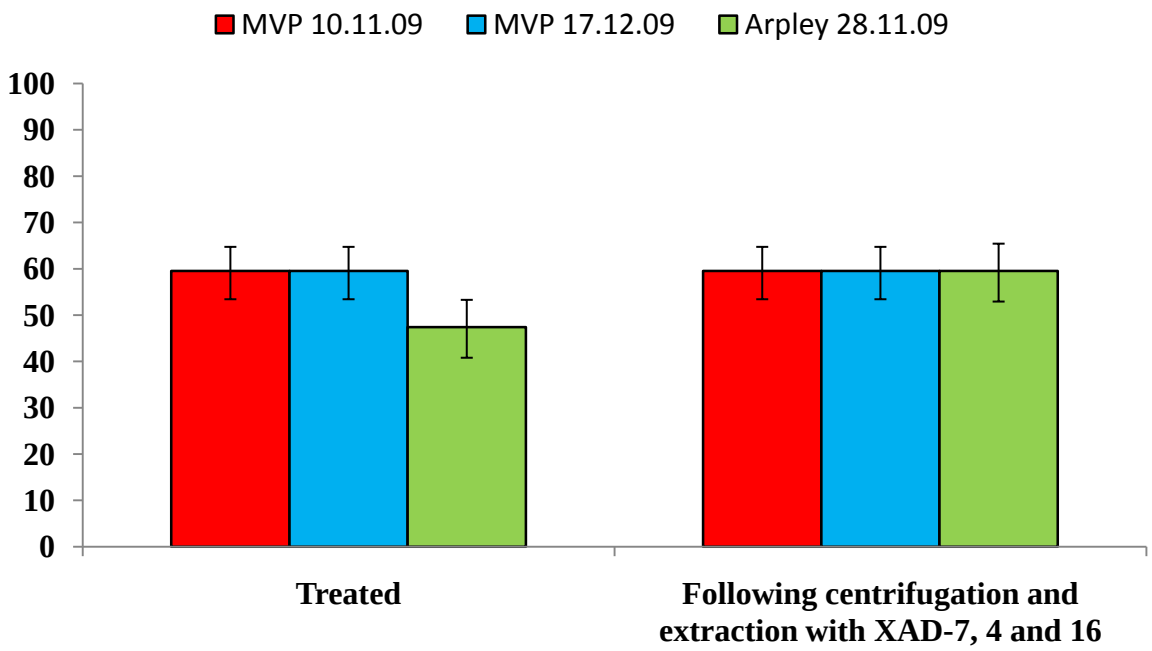

Figure 6-21: Effect of rapid batch removal process with XAD 4, 7 and 16 on treated landfill leachate toxicity displayed towards *D. magna* (n=4). Samples collected from MVP and Arpley. Error bars represent 95% confidence intervals.

The EC_{50} toxicity of the 10.11.09 and 17.12.09 samples from MVP towards *L. minor* was 28%for the 10.11.09 sample and 32% for the 17.12.09 sample (Figure 6-22). The 28.11.09 sample from Arpley landfill recorded an initial toxicity of 26%. Following the extraction procedure the toxicity of the samples was reduced to 30% in the 10.11.09 sample and the 17.12.09 samples toxicity had increased slightly 31%. The toxicity of the 28.11.09 Arpley sample decreased with the application of the extraction procedure to 35%. Chlorosis of the test fronds was recorded in the Arpley 100% concentration test plates. These changes are not significant reductions or increases of toxicity. These changes in toxicity means are not significant ($p < 0.05$).

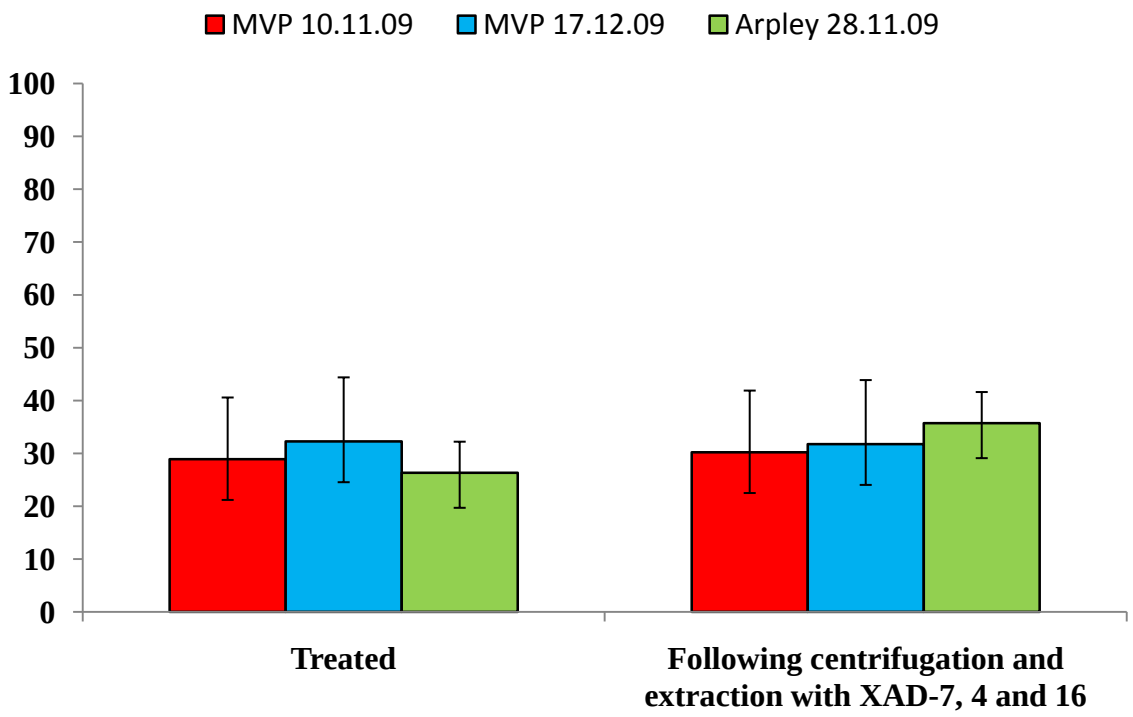

Figure 6-22: Effect of rapid batch removal process with XAD 4, 7 and 16 on treated landfill leachate toxicity displayed towards *L. minor* (n=3). Samples collected from MVP and Arpley. Error bars represent 95% confidence intervals.

Discussion

The COD content of treated landfill leachate is refractory to biological treatment. This is expected as humic and fulvic acids are formed from the decomposition of organic compounds by bacteria. Their presence in landfill leachate demonstrates their recalcitrance (Kang *et al*., 2002). This work has shown that a high proportion of the COD of treated leachate can be removed with centrifugation and extraction with XAD resins (see Figure 6-10).

There was considerable variation in the COD concentration of the samples collected from Arpley. Variation in COD concentration has been seen in various reports on leachate treatment (Robinson *et al*., 2003a, Robinson *et al*., 2003b, Žgajnar Gotvajn *et al*., 2009). Less variation was found in the samples collected from MVP. This lack of variation is attributed to

the blending of leachates from other sites with the leachate collected from Stewartby landfill (Gibbs, 2007).

The original rapid batch procedure (acidification-centrifugation-XAD-7 extraction) for the removal of humic and fulvic acids from the treated leachate samples demonstrated that ~20% of the COD fraction is humic acid and ~40% is fulvic acid. Huo *et al* (2009) reported that treated landfill leachate COD was ~70%– which seems to be in agreement with these results. Fulvic acid tends to be the dominant species found in younger landfills with the concentration of humic acid increasing as the landfill ages (Figure 6-4). Recirculation of leachate in landfills has been shown to increase the concentration of humic acid (Rodríguez *et al*., 2004). Recirculation is currently attracting a great deal of interest from landfill operators as a method for increasing the rate of decomposition of waste contained within landfills. This could have implications e.g. an increasingly more stable humic acid dominated COD composition which has the ability to sequester pollutants in the macrostructure.

Addition of XAD-4 to the rapid batch procedure resulted in a 10% increase in COD removal. XAD-4 was introduced to the procedure as it was thought there was a considerable quantity of solvent sized transphilic organic molecules remaining in the leachate (Do Nascimento Filho *et al*., 2001). This resin has previously been successful at fractionating the COD content of landfill leachate (Rodríguez *et al*., 2004, Bu *et al*., 2010). This testing demonstrated that there was only a small amount of these molecules remaining in the treated landfill leachate. Rodriguez *et al* (2004) reported that the XAD-4 resin was able to reduce the COD concentration by 48% from a raw leachate whereas XAD-8 (very similar to XAD-7) was able to only remove 8% of the COD concentration. The transphilic concentration in the samples was low which probably due to methanogenic conditions within the landfill decomposing most degradable organic molecules and the biological treatment of the leachates. Addition of

the XAD-4 to the procedure was useful in that it demonstrated the low concentrations of these small sized molecules in the landfill leachate.

XAD-16 resin is designed to remove small to medium sized polar organic molecules e.g. antibiotics (Soylak and Elci, 1997). In this work the resin was able to increase the COD removal efficiency by between 15-30% over that of XAD-7 and 4. This increase in removal of COD over that of XAD-7, suggests that either the XAD-7 contact time was too short to adsorb all the fulvic acid and the XAD-16 was adsorbing any remaining fulvic acid or possibly there was considerable amounts of smaller fulvic acid molecules e.g. precursors for fulvic acid in the leachates (Figure 6-23) (Huo *et al*., 2009). XAD-16 has been previously been reported to effectively remove humic substances from seawater (Lepane, 1999). Lepane (1999) was able to remove up to 70% of the humic substances present in seawater. Fulvic acid fragments will share similar functional groups to the structure shown. Phthalate esters have been reported to be adsorbed onto the surface of XAD-16 (Steele and Hardy, 2009).

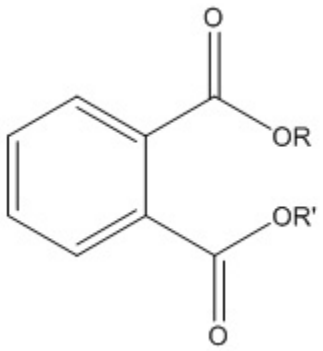

Figure 6-23: Generic phthalate ester structure.

Toxicity of the treated leachates varied over the course of these experiments though the toxicity remained low. The level of response was similar to the levels presented in the TIE work (Chapter 5). *D. magna* response was in most cases very similar to those reported in Chapter 4. Response of *L. minor* was again higher than *D. magna* and was similar to the responses reported in the TIE work (Chapter 5). These levels of response were also at the

levels reported previously (Mackenzie *et al*., 2003). Variations in the toxicity response of *L. minor* from the same culture have been reported previously (Kiss *et al*., 2001). This in agreement with the findings of this work with considerable variations in the response recorded.

The COD was significantly reduced by the XAD resins and results in no significant change in EC_{50} toxicity. From the literature review presented at start of this chapter it is clear that the causes of toxicity were not associated with the COD fractions removed in this procedure (assumed to be humic and fulvic acids). It remains a possibility that the remaining 10% of COD that could not be removed is the cause of residual toxicity. This subtractive approach had reached its limits where removing more COD could lead to the removal of other fractions unintentionally. There are possibilities of utilising membranes and advanced oxidation processes to remove the remaining organics but these are expensive to buy and operate. Further work could concentrate on chemical determinations of the XAD adsorbed fractions and the remaining 10% of organics. Facilities such GC-MS are available at Cranfield and would be a perfect project for an MSc thesis.

The lack of change in toxicity of the samples using these resins again points to a possible relationship between toxicity and elevated major ion concentrations present in treated landfill leachate (Table 6-2). These resins have no ability to retain ions so any major ions that were present within the solution should remain in the eluted sample from the XAD packed column. This would explain the why there was no change in the toxicity of the samples following the XAD fractionation.

The *D. magna* based test had very small errors between each of the groups used. Their reactions to the samples tended to be very uniform and this is reflected in the size of the error bars. The *L. minor* based test on the other hand showed considerable variation between the test groups that makes firm conclusions on changes in toxicity very difficult. The *L. minor*

test being so sensitive to the chemical composition of treated landfill leachate makes it an useful addition to any battery of tests but this high level of variability in testing makes the small changes in toxicity associated with treated landfill leachate very difficult detect with confidence. To overcome this a less sensitive test is needed so that they can be used as a comparative tool e.g. if both give a high response to a sample then the operator knows there is something wrong with the effluent.

The modified version of the rapid batch procedure reported by Van Zomeren *et al*. (2007) used in these experiments involved a <10 min adsorption time between the sample and the XAD resin. A contact time of 20 h did not make a great deal of difference to the overall adsorption ability of the XAD-8 and 4 resins (Li *et al*., 2008). In this work it was shown that a very short contact time between the resin and the sample worked effectively at removing the COD content from the samples. Two possible explanations could explain the final 10% of COD:

i) It is molecules of the size that should have been removed with XAD resins but were missed due to a short contact time with the XAD resins or

ii) It is made of molecules of a size that falls outside of XAD pore sizes e.g. too small for XAD-4 or too big for XAD-7. The most likely answer is that molecules failed to adsorb to the surfaces of the XAD resins used as the retention time was not long enough. This would suggest that they are similar to the molecules previously removed by the resins but were did not have long enough to become adsorbed to the surface of the resins and are thus non-toxic.

Humic acid is characterised by a dark brown colour and fulvic acid a lighter brown (Aiken, 1985). The leachates collected from Arpley landfill tended to be a darker brown whereas the leachates collected from MVP tended to be a lighter colour. The darker colour of Arpley leachates is in agreement with the age of the landfill i.e. 1993 and the treatment plant receiving leachates from only 1 site. Stewartby landfill has been operating since 1978 but due

to the high strength of the leachates other weaker leachates are tankered in and blended to weaken the leachates to make biological treatment possible.

Figure 6-24: Left image; from left to right, Arpley treated landfill leachate, Arpley leachate following centrifugation, Arpley leachate following XAD-7 extraction. Right image; from left to right, MVP treated leachate, following centrifugation and XAD-7extraction, following XAD-16 extraction, following XAD-4 extraction.

6.7 Limitations and implications of this approach

Only ~10% of the COD fraction remains after three types of XAD extraction. Residual toxicity could be the result of the remaining 10%. This is unlikely as there were no significant movements in toxicity. This would imply that the current treatment technology is suitable for reducing toxic organics and leaving an organic concentration that is high but non-toxic. There remains considerable residual toxicity that cannot be explained by COD, heavy metal and ammonia levels. The presence of inorganic ions in treated landfill leachate is the final fraction that canexplain residual toxicity. The atomic absorption spectroscopy analysis indicates these substances are present in elevated concentrations. Work on the toxicity of these ions was the next area of investigation for this project.

6.8 Conclusions

- The COD fraction was successfully fractionated with 3 types of XAD resin. Each resin was able to remove a fraction of the recalcitrant COD content of treated landfill leachate.
- Arpley treated landfill leachate COD concentration varied considerably over the course of the experiments. MVP leachate COD concentration had a much smaller variation. This variation is attributable to the treatment strategy adopted.
- The concentration of humic acid in landfill leachate is estimated at 15-20% of the COD concentration of treated landfill leachate
- The fulvic acid concentration is between 40 and 80% of the COD concentration of treated landfill leachate.
- COD is not responsible for the toxicity found in landfill leachate. The removal of COD from the treated landfill leachate samples had little or no effect on the toxicity of the samples.
- *D. magna* displayed relatively stable toxicity in the MVP samples. The MVP toxicity was on average EC_{50} of 59%. The toxicity of Arpley samples was more variable but remained overall low at EC_{50} of 45%.
- *L .minor* toxicity was variable between samples from MVP and Arpley. The variability in the effect displayed towards the same sample was very high. This variability is seen in the large errors of the test

7 Major ion toxicity and synthetic leachates

7.1 Findings from previous work

The toxicity identification evaluation (TIE) procedure is designed to identify classes of compounds responsible for acute toxicity in aqueous samples. The initial screening experiment performed in this project was a TIE Phase 1 procedure (see Chapter 5). No discernible pattern of acute toxicity changes was seen in the results from three treated leachate samples tested. This lack of pattern suggested that the acute toxicity could not be attributed to the organic fraction (COD), ammoniacal-nitrogen or heavy metals. A lack of change in the toxicity responses for many of the manipulations could be due to major ions such as chloride, magnesium and carbonate. These ions are less pH sensitive than organic compounds and heavy metals. Major ions are present in treated landfill leachate at elevated concentrations above that of freshwater (Goodfellow *et al*., 2000). This type of phenomenon of elevated major ion concentration not being highlighted by the TIE procedure has been reported previously (McCulloch *et al*., 1993). McCulloch *et al*, (1993) reported that toxicity was due to the concentration of major ions but the TIE procedure adopted could not isolate the causes.

The project's original hypothesis was that recalcitrant COD could be the cause of toxicity in treated landfill leachate. Due to the problems with the SPE stage of the TIE procedure more work was needed to determine whether the original hypothesis was supported. A series of XAD resins were prepared and used to reduce the COD concentration of samples from MVP and Arpley (Chapter 6). This work removed approximately 90% of the original COD but without reducing in the toxicity was recorded in the COD-reduced samples.

Heavy metals have received a high level of interest in the literature due to their high toxicity in low concentrations (Schrab *et al*., 1993). Bioaccumulation of heavy metals is another

problem associated with the discharge of these cations to the environment. The heavy metals concentrate in the tissues of organisms and lead to lethal concentrations in the organisms even though non-lethal concentrations were discharged in the first place (Bryan and Langston, 1992).

Heavy metal concentration in landfill leachate is a well studied area with many publications on the subject. The general conclusion from this body of work is that heavy metals are immobilised by adsorption onto organic colloidal matter or precipitation as inorganic salts of carbonate and sulphide. These processes in effect neutralise the toxic hazard posed by heavy metals in landfills (Kjeldsen *et al*., 2002). The EDTA stage of the TIE procedure is designed to precipitate the divalent metal cations. Results from this stage indicated that no metal toxicity could be detected (Chapter 4). Heavy metal toxicity should therefore will not be considered any further in this chapter.

Previous accounts on landfill leachate had identified ammoniacal-nitrogen as the chief cause of toxicity in landfill leachates (Clement and Merlin, 1995). Chemical analysis performed in this work demonstrated that treatment was reducing the concentration of ammoniacal-nitrogen to <0.1 mg/L. Ammoniacal-nitrogen is not the cause of toxicity due to the lack of toxicity reduction with the pH 3 tests in the TIE procedure (Isidori *et al*., 2003). If COD (or substances associated with it) , heavy metals (as influenced by EDTA) or the ammoniacal-nitrogen fractions of landfill leachate are not the causes of the relatively low levels of residual toxicity in treated landfill leachate then that leaves the major ion fraction in treated landfill leachate as the likely cause.

Major ions are the ionic components of natural fresh waters and are present at greater than trace levels (Mount *et al*., 1997). The types of ions that make up this fraction of waters are necessary to maintain the ecological health of these waters. In the WET tests used so far in this project the dilution water used had additions of major ions to stop osmotic shock reaction

(OECD, 2002). Many species have specific requirements for the ionic makeup of these solutions and increases in the concentrations of these ions can cause the death of organisms (Table 3-2).

The major ions of most interest in this project are:

$$Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}, HCO_3^{-}, SO_4^{2-}$$

7.1.1 General knowledge of major ion toxicity

Total dissolved solids (TDS) are defined as the sum of organic and inorganic compounds within a sample (APHA, 2006). The measurement also includes any solids that can pass through a 2µm mesh sieve. TDS are routinely quoted in the literature as an indication of the quality of water reported (Weber-Scannell and Duffy, 2007). In the previous chapter the organic fraction of the TDS of landfill leachate was shown to be non-toxic which leaves the inorganic fraction as the probable cause of toxicity.

Bulk chemistry parameters are routinely encountered in the literature when dealing with aqueous samples. Salinity describes the 'saltiness' and is calculated from the individual the concentrations of Na, Cl, Mg $CaSO_4$ and HCO_3. Aqueous conductivity refers to the ability to transport electrical charge through a solution and acts as a rough measure of the ionic composition of a sample. Conductivity is often reported in the literature as a description of the inorganic salt content of an aqueous sample. Conductivity only describes the bulk characteristics and not the concentration or ratios ions in the sample. TDS, salinity and conductivity are not suitable parameters for making predictions on toxicity. This is due the chemical composition being more complicated than these bulk measurements portray.

Contradictory studies exist for determining toxicity towards *Ceriodaphnia dubia* as a function of salinity. A water from an irrigation ditch had an LC_{50} corresponding to a conductivity of 3,500-4,000 µS/cm (Dickerson *et al*., 1996) but with a water from a coal mine the LC_{50} corresponded to 2,135 µS/cm (Merricks *et al*., 2007). This is a 50% reduction in the

conductivity that does not result in a 50% reduction in the EC_{50} toxicity. Analysis of rising salinity levels in Australian waterways demonstrated that as levels increased >1,000 mg/L there was an increasingly detrimental effect on the variety of species in freshwater systems (Nielsen *et al*., 2003a). The authors note that species are pushed out of ecosystems by rising salinity levels due to death or lose of prey. This emigration of species results in considerable loss of biodiversity as more salt tolerant species come to dominate the affected area. Conductivity levels of 10 µS/cm resulted in complete population mortality of two species of mayfly and one species of midge (Hassell *et al*., 2006).

An imbalance in the ratio of anion to cations of major ions in freshwaters is a potential cause of toxicity (Goodfellow *et al*., 2000). Ion imbalance is a condition when the concentration of ion(s) in an effluent exceeds normal ranges and is a potential cause of toxicity. Ion imbalance toxicity is the result of two possible mechanisms: ion deficiency and ion excess (Douglas *et al*., 1996). Ion deficiency describes a solution where there is deficiency in the concentrations of either of cations and anions or an excess in the concentration of cation/anion (Grewal, 2010). This process can happen when a ion is released during an extraction process such as mining. Ion excess, the most common form of imbalance toxicity, is where there is an excess cation or anion concentration that causes osmotic shock to an organisms cell walls and the metabolic functioning of the organism (Pinho *et al*., 2007).

A proof of ion imbalance in a solution is possible. Firstly an accurate determination of the concentration of major ions in the sample followed by a determination of toxicity. Depending on which ions are present in too higher concentration the imbalance can be corrected with addition of missing ions (Douglas and Horne, 1997). Conversely if the ion imbalance is not corrected then the toxicity remains between tests (Schiff, 1992).

Many organisms have a small ion tolerance range and salinity. A Toxicity Identification Evaluation procedure carried in Trinidad demonstrated that ion imbalance in produced waters

(water used to remove oil from wells) was a significant cause of whole effluent toxicity as well as fine droplets of oil (Elias-Samlalsingh and Agard, 2004).The TIE procedure was unable to demonstrate that the major ions were the cause of toxicity and further testing was needed to come to this conclusion. Construction of synthetic solutions demonstrated that a high level of magnesium was the cause of toxicity in such waters.

Plants are especially sensitive to ion imbalance toxicity (Singh and Bhati, 2008). Plants are vital food source in ecosystems and effects to plants can have knock on effects in the ecosystem. In aquatic tests an imbalance of ions caused the Na^+, K^+-ATPase activity of *Acartia tonsa* to be decreased which resulted in death of the test candidates (Pinho *et al*., 2007). Increased levels of salinity are responsible for the stunting of chick pea, wheat and barley growth of shoots and a reduction in the biomass which is having a large effect on the farming potential of Australia (Grewal, 2010).

In general Na^+ can be considered non-toxic to freshwater species though it can have an effect on ion deficiency toxicity (Goodfellow *et al*., 2000) though the toxicity of Cl^- is much higher than its counterpart Na^+ (Mount *et al*., 1997). Chloride toxicity EC_{50} towards *D. magna* becomes significant at values of ≥1,800 mg/L whereas Na^+ toxicity is much higher at ≥6,000 mg/L (Mount *et al*., 1997). A contradictory study determined the toxicity of Na^+ at a much lower concentration of 420.6 mg/L (Khangarot and Ray, 1989). In the two case studies on synthetic solutions reported by Goodfellow *et al* (2000) it was the high concentration of Cl^- (7,310 and 2,710) that was the cause of toxicity towards *C. dubia*. Mount *et al* (1997) reported similar effects for Ca^{2+} as that of Na^+. Any toxicity associated with Na^+ and Ca^{2+} could be attributed to the counter ion e.g. Cl^-. Problems can exist with very high levels of Ca^{2+} i.e. >3,000 mg/L though this is an unlikely case with landfill leachates where Na^+ is present in far higher concentrations (Fatta *et al*., 1999). Sulphate EC_{50} between species varies considerably e.g. *C. dubia* toxicity was 2,078 mg/L and 14,134 mg/L for *Sphaerium simile*

(Soucek and Kennedy, 2005). *D. magna* have been shown to have decreased reproduction and survival levels in solutions with salinity levels >4% (Smolders *et al*., 2005). These toxicity results demonstrate the detrimental effects that increased salinity levels have on the health of freshwater organisms.

Potassium was identified by Mount *et al* (1997) as posing the greatest toxicity towards the *D. magna* and *C. dubia*. There is a shortage of reports linking toxicity of potassium to *D. magna.* The toxic concentration of K^+ was experimentally determined at 141 mg/L (Khangarot and Ray, 1989). Research has concentrated on the toxicity of salts such as KCl and $K_2Cr_2O_7$ or the moderating effect of potassium on heavy metal toxicity. Reports on the toxicity have shown that potassium blocks the workings of the gill of *Dreissena polymorphia* (zebra mussel) (Fisher *et al*., 1991). Similarly, potassium has been reported to affect the functioning of *Oncorhynchus mykiss* (rainbow trout) gills (Tkatcheva *et al*., 2007). A similar effect is expected at similar concentrations for species such as *D. magna* which also use gills for breathing.

The toxicity of K^+, Cl^- and SO_4^{2-}, can be lowered in the presence of more than one cation e.g. Na^+ and Ca^{2+} (Mount *et al*., 1997). Calcium also has the ability to reduce the toxicity of the heavy metal Zn^{2+} in sewage liquors (Fjällborg *et al*., 2005). In making synthetic seawater the addition of a sea salt i.e. not just Na as the cation, was found to be less toxic than the addition in the same concentrations of NaCl (Kefford *et al*., 2004). This is particularly important considering the concentration of Na^+ and to a lesser extent the concentration of Ca^{2+}. The toxicity of SO_4^{2-} towards *D. magna* was shown to be significantly increased by a rise in the water hardness levels (Davies and Hall, 2007). The researchers also reported that the toxicity of Mg^{2+} was decreased as the ratio of Ca:Mg was increased. In landfill leachate there is more than one cation there is likely decrease in the overall toxicity.

The ability for these species to become acclimatised to increased concentrations of major ions can occur after chronic exposure to elevated levels of major ions. Over the course of several generations *C. dubi*a was shown to become acclimatised to the effects of sulphate toxicity so that the $LC_{50\ rose}$ from 500 to 1,000 mg/L (Soucek and Kennedy, 2005).

The physical chemistry of a water samples plays a role in the magnitude of ion toxicity. In particular the pH and temperature modify toxicity greatly (Lloyd, 1987). This requires that to effectively reproduce landfill leachate characteristics in synthetic solutions the pH and temperature need to be replicated or as close as possible. Predictions on the levels of toxicity are almost impossible to predict just from knowledge of the concentrations of major ions.

7.1.2 Knowledge of major ion sources in landfill leachate

In landfills the origins of these major ions can be attributed to the types of waste that have been landfilled. Nicholson *et al*, (1983) identified the likely origins of the ions within the landfill. Ca^{2+} and SO_4^{2-} were most likely from gypsum which is a component of concrete (Nicholson *et al*., 1983). Cement is a major component of wastes within landfills (Peters, 1998).

At low pH values (acidogenic phase), the concentrations of sulphate, calcium and magnesium are at their maximum (Table 7-1). During the methanogenic phase the organic acids are metabolised and the pH begins to rise from ~3 to neutral. The rise in pH causes the magnesium and calcium ions to become immobilised due to sorption and precipitation. Bacteria metabolise sulphate to sulphide within the landfill (Kjeldsen *et al*., 2002). Sulphide is a highly toxic substance that can cause damage to local ecosystems.

Chloride concentration in the initial stage of landfilling starts low <50 mg $(mm\ leachate)^{-1}$ $(kg\ dry\ waste)^{-1}$ (Blight *et al*., 1999). During the 1st 100 days after landfilling the concentration of chloride rises rapidly to levels > 200 mg $mm\ leachate^{-}\ kg^{-1}$ though this can be lower depending on the landfill cell filling technique used. After ~100 days, the

concentration of chloride plateau's and in the following 765 days the concentration only increased slightly at the 4 sites sampled (Blight *et al*., 1999). A rise in the chloride concentration as the landfill ages was confirmed in a similar set of determinations at 4 other landfills (Trabelsi *et al*., 2000). The total chloride content within an average North American landfill was calculated at between 0.18-0.20% (Rowe, 1995). No figures are available for the changes in the chloride concentration of landfill leachate but are likely to follow the same pattern as the landfill.

Unfortunately there is a lack of information on major ion toxicity in landfill leachate. Local regulators may require that further treatment is carried out for removing elevated levels of major ions in treated leachate samples (Robinson, 2007). The authors felt that this should not be a problem for most of their SBR plant commissions.

Table 7-1: Average concentration of ions between acidogenic and methanogenic phases (Kjeldsen *et al*., 2002).

Ion	Acidogenic phase (mg/L)	Methanogenic phase (mg/L)
Sulphate	500	80
Calcium	1200	60
Magnesium	470	180

7.1.3 Mount predictor for major ion toxicity

The United States Environment Protection Agency (USEPA) published a major paper on the relationship between ion concentration and toxicity in freshwaters (Mount *et al*., 1997). The study was driven by a concern that industrial effluents rich in major ions were leading to elevated salinity levels in freshwater systems and impacting on the overall health of ecosystems. This work aimed to provide a useful set of tools to industrial operators,

particularly oil producers, to help understand the hazard posed by major ion-rich effluents and reduce the impact their effluents have on the environment. Having a validated model that is simple to use is indispensible to operators as it offers a method for monitoring toxicity without needing to conduct species tests; inputting the numbers into a spread sheet to obtain a predicted toxicity for an effluent. The authors highlighted the problem that can exist in taking bulk measurements such as conductivity and 'predicting' the acute toxicity. The problems arise due to the major ions in these types of effluents being present with other compounds whether organics or heavy metals (Tietge *et al*., 1997).

Many industrial processes lead to elevated levels of ions in their discharges. Effluents from coal mining, the oil industry, wastewater treatment, municipal and industrial landfill leachate treatment, and paper mills have all been shown to have high total conductivities (Clement and Bouvet, 1993, Martínez-Jerónimo *et al*., 2005, Merricks *et al*., 2007). A conductivity of greater than 2,000 µS/cm or a TDS of >10% would be considered high and potentially toxic to *D. magna* (Goodfellow *et al*., 2000).

The work of Mount *et al* (1997) is relevant to watercourses that have or are becoming saline. Salinisation of freshwater courses is beginning to be a major problem in the protection of environments around the world (George *et al*., 1997). Due to industrial processes the release of Na^+ and Cl^- into freshwaters is an increasing threat to their health. Sources of Na^+ and Cl^- can be attributed to landfills, salt spreading on roads and agriculture (Panno *et al*., 2006). This is a very interesting as the treated leachate from MVP is discharged into the sewers and this treated leachate could be posing a significant threat to the environment as the effluent from the sewage works will contain significant quantities of Na^+ and Cl^-.

Contrary to the expected result, dilution to non-toxic levels does not always occur in water courses receiving industrial effluents high in Na^+ and Cl^- (Kennedy *et al*., 2004). Differences in the mixing potential of water courses can cause effluent mixtures to remain concentrated.

This implies that there is a potential hazard associated with discharge of treated landfill leachate into sensitive watercourses. For example: Buckden landfill is set to increase its intake of waste over the next 10 years, this will lead to an increase in the amount of leachate generated and treated. This treated leachate will have a higher major ion concentration than at present due to the larger amount of waste landfilled. This treated landfill leachate is to be discharged to the River Great Ouse which is considered a high quality watercourse (Robinson *et al*., 2003a). Consideration of the increased major ion concentration will need to be made so damage is not caused to this watercourse.

Mount *et al*, (1997) used 2 species of Daphnids: *Ceriodaphnia dubia* and *Daphnia magna* plus fathead minnows (fish) *Pimephales promelas* to determine the toxicity of ions, pairs of ions and combinations of 1 anion-2 cations or 1 cation-2 anions. The cations and anions the authors concentrated on were:

$$Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}, HCO_3^{-}.$$

In total 2,900 ion solutions were produced and the toxicity determined. The authors used Paradox 3.1 (Borland International, USA) to produce a toxicity model on the data obtained. This was done by performing multiple logistic analyses on the experimental toxicity data. Logistic regression analysis in this case worked as the responses for the test display binary reactions to pollutants i.e. alive or dead. Regression analysis allowed a prediction on the expected survival at a given concentration of major ions. The equation the authors used was of the form:

$$\text{logit}(P) = \ln[P/(1\text{-}P)]$$

$$= \beta_o + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_n X_n$$

Where P = proportion surviving , β = regression coefficent, X = ion concentration, and n = total number of significant terms in the model.

It became apparent to the authors that a number of transformations on the data were needed. In the final model a new variable was added to the equation, the NumCat variable. This variable was required as the original models were unable to accurately predict the toxicity of Cl^- towards *C. dubia* when there were two cations present in the solution. The authors note that this result was reproducible with actual experiments. The NumCat constant is defined:

"Equal to the number of cations representing at least 10% of the total number concentrations of cations and present at greater than 100 mg/L"

Each of the species used in this testing produced a unique set of regressions and NumCat variables. The authors showed that the toxicity of each of these ions followed a scale of toxicity: $K^+ > HCO_3 \approx Mg > Cl > SO_4$ and that Ca and Na posed little risk to the three species used. According to the authors, the single ion model worked very well at predicting toxicity. As more ions were added the model began to break down. From these findings, the authors were able to develop a theoretical equation that was able to predict the toxicity for 3 cations. The correlations between predicted and experimental toxicity for Cl^- was very good at concentrations <6,000 mg/L. The authors noted in their conclusions that concentrating on pairs of ions does not give a complete picture of how a water collected from an industrial effluent would perform in similar set of tests. It was expected that a more complete picture with multiple cations would be found in the future but unfortunately there has been no reports from the authors on this more complete equation.

The model developed has proved to be successful at predicting the acute toxicity of high salinity samples towards *C. dubia* and *P. promelas* (Dickerson *et al*., 1996). Using 6 different produced waters (waters from the oil industry) and synthetic waters Tietge *et al*, (1997) tested the model and found accurate predictions for *C. dubia* but predicted poorly for *D. magna*.

The accuracy of prediction varied significantly for the 6 samples from under to over predicting toxicity towards *D. magna*. The authors checked there were no technical problems with the *D. magna* test and that there was no drift in the predictor values. This left them with the conclusion that the model does not predict well for *D. magna*.

7.1.4 Synthetic solutions

Landfill leachate is a complex mixture of inorganic and organic compounds. From the previous work it looks increasingly likely that the cause of relatively low-level residual toxicity in treated landfill leachates from MVP and Arpley can be accounted for by the concentration of major ions. The Mount model purports to be able to predict major toxicity towards *D. magna* though this prediction is not as accurate as that for *C. dubia*. Problems with the model over and under predicting for *D. magna* have been mentioned by Tietge *et al* (1997).

Synthetic leachates have been previously used in modelling landfill leachates. For example: the growth of bacteria on sump collection pipes (Rowe *et al*., 2002); the leaching of heavy metals (Hooper *et al*., 1998); the degradation characteristics of wastes landfilled (Rowe *et al*., 2008); and the fate of xenobiotic organics in landfills (Behnisch *et al*., 2001).

Synthetic solutions have been used successfully in the USA for determining toxicity of produced waters (Mount *et al*., 1997, Tietge *et al*., 1997, Goodfellow *et al*., 2000). These solutions have the advantage that they only contain the substances of interest though this could also be considered a disadvantage in that some substances are not present that might influence the chemistry. Previously researchers have used spiking to detect toxicity of organic compounds (Okamura *et al*., 2005). As far as the author is aware there has been no work carried out trying to discover the causes of toxicity in municipal leachate samples by producing synthetic leachates.

7.2 Hypothesis

Null hypothesis: Modelling and making up synthetic leachates based on the concentrations found in treated leachate samples does not explain residual toxicity recorded.

Alternative hypothesis: Modelling and making synthetic leachates based on the concentrations found in treated leachate samples does explain residual toxicity recorded.

7.3 Objective

Aim: Carry out a two pronged approach to understanding the role of major ions in residual toxicity of treated landfill leachate i.e. a modelling approach and an additive experimental approach.

Objectives:

I. Chemical analysis of the major ion composition of previously collected samples from Arpley and MVP was needed in order to understand the chemistry of the samples. Using the model developed by Mount *et al* (1997) toxicity predictions was made based on the major ion determinations. These predictions will then be compared to the toxicity that has been previously recorded. A discussion on the suitability of this model for use my landfill operators as a risk prevention tool.

II. A number of synthetic leachates made based on the average major ion concentration of treated landfill leachate samples from MVP and Arpley. These synthetic leachates will be used in an attempt to recreate the toxicity responses of *D. magna* and *L. minor* towards treated landfill leachate samples from MVP and Arpley.

III. Calculate and determine whether there is an imbalance in the concentrations of ions within the treated leachates previously collected. A discussion of the effects of ion imbalance and possible remedies will be made and the likelihood of this being the cause of residual toxicity.

7.4 Materials and methods

7.4.1 Mount model

An equation based on the regression constants and the NumCat variables reported by Mount *et al* (1997) was used to make a spreadsheet calculator in consultation with a statistician (Pat Bellamy, Cranfield University). Statistica (Bedford, U.K.) was used to predict the 48 hr EC_{50} toxicity for *D. magna* based on the major ion concentrations of treated landfill leachate from MVP and Arpley. *D. magna* was selected as a test candidate by the authors due to its presence in most watercourses and used in the literature as almost standard toxicity test. The equation took the form:

$$P = 5.83((x_{Mg}V_{Mg})(x_{K}V_{K}Numcat_{K})(x_{HCO3}V_{HCO3})(x_{Cl}V_{Cl}NumCat_{Cl})(x_{SO4}V_{SO4}NumCat_{SO4})$$

Where: x = concentration; V= regression coefficient.

The probability of survival (P) is calculated with the formula:

$$P = \frac{\exp^{P}}{1+\exp^{p}}$$

Mount *et al* (1997) reported that regression and NumCat coefficients were needed in the model in order to match predictions to experimental results. The regression and NumCat coefficients are presented in Table 7-2.

Table 7-2: Regression and NumCat coefficients used in the Mount model (Mount *et al*., 1997).

Ion	Regression coefficient	NumCat coefficient
Mg^{2+}	-0.00510	Not needed
K^{+}	-0.0185	0.00677
HCO_3^-	-0.00397	Not needed
Cl^-	-0.00395	0.00146
SO_4^{2-}	-0.00255	0.00132

7.4.2 Synthetic leachate

Synthetic leachate was constructed according to the concentrations of the average major ion concentrations of Arpley and MVP leachates. The average concentration for Arpley was calculated from a database on the chemical analysis carried out by WRG; the average comes from a total of 31 measurements over 3 years. The MVP average was calculated from the 11 Atomic Absorption analyses (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}) carried out during this project (Chapter 6). The averages are presented in Table 7-3 and Table 7-4.

The first attempt to build a synthetic leachate involved use of a phosphate buffer and $CaCl_2$. When salts were added to the deionised water, the salts dissolved but within minutes a milky white precipitate appeared. Heat and stirring was applied to the solutions in an attempt to dissolve the precipitate but failed. Sonication was attempted to disperse the precipitate from the solution but this also failed. The milky white precipitate suggested a Ca species. After consultation with a list of K_{sp} it was found that Ca based salts have very low K_{sp} values e.g.

$CaPO_4$ K_{sp}= 10^{-23}. The solubility product constant (Ksp) is the constant for the equilibrium that between a solid ionic solute and its ions in an aqueous solution {Atkins, 2009 #231}.

From this information Ca^{2+} containing species were removed from the recipes for the formation of synthetic leachates. Instead they were replaced with other salts. In the second recipe to be attempted it was found that a different precipitate formed. This precipitate, $MgPO_4$, was a gel like substance that again would not clear with any method.

In the third attempt at building a synthetic leachate the solution was autoclaved to sterilise the solutions. The autoclaving process produced a heavy white precipitate that again would not dissolve.

Finally, a method was developed for producing a synthetic leachate. Ions were sourced from the salts contained in Table 7-3 and Table 7-4. This solution contained no Ca^{2+} or PO_4 as it was found these combined to form insoluble salts. It was felt that the omission of Ca^{2+} and PO_4 was acceptable in order to carry out the experiments because of their low direct toxicity. Ideally in future experiments a method for the inclusion of Ca^{2+} and PO_4 would be found. The solution was buffered at suitable pH with the addition of 1 ml of 3-(N-morpholino)propanesulfonic acid (MOPS). Sterilisation is a necessary step as *L. minor* feed solutions are ideal breading grounds for bacteria and the growth of bacteria strips the solution of valuable nutrients and gives a false result due to competition for nutrients. Because autoclaving caused a problem with the formation of precipitates the synthetic leachates were sterilised by ultrafiltration.

Assessment of toxicity was carried out using *D. magna* as outlined in the Methodology (Chapter 3). For *D. magna* three separate repeats were used to determine the toxicity of MVP and Arpley synthetic leachates. Unfortunately due to issues with culture infections only two repeats for *L. minor* with MVP and Arpley leachates could be carried out. The dilution series was altered in these experiments so as to offer a greater resolution in the precise point where

the EC_{50} is located (see below). The concentration series was changed due to a lack of resolution of the previous results. This lack of resolution gave what was thought to be a slightly higher toxicity than what the solutions toxicity in reality was. The following dilutions were used.

- The dilution series in the experiments dated 18.02.10 was 100, 80, 60, 50, 25 and 0%.
- The dilution series in the experiments dated 25.02.10 was 100, 90, 80, 70, 60 and 0%.

Table 7-3: Arpley synthetic leachate recipe

Ion (AM)	**Source of ion (M_r)**	**Average ion concentration in leachate (mg/L)**	**Calculation**	**Amount of source needed (g)**
Cl (35.453)	NaCl (58.44)	2630	See corrected NaCl below	See corrected NaCl below
Mg (24.305)	$MgSO_4.7H_2O$ (246.08)	83.8	$(0.0838 \times \frac{1}{24.305}) \times 246.08$	0.848
K (39.098)	KCl (74.56)	920	$(0.920 \times \frac{1}{39.098}) \times 74.56$	1.789
HCO_3 (61.000)	$NaHCO_3$ (84.01)	1576	$(1.576 \times \frac{1}{61.000}) \times 84.01$	2.17
Corrected NaCl		1761	2.630-(1.789-0.920)= 1.761 $(1.761 \times \frac{1}{35.453})) \times 58.44$	2.818

AM= Atomic mass; M_r= Molecular mass

Table 7-4: MVP synthetic leachate recipe

Ion (MW)	Source of ion (MW)	Average ion concentration in leachate (mg/L)	Calculation	Amount of source needed (g)
Cl (35.453)	NaCl (58.44)	3500	See corrected NaCl below	See corrected NaCl below
Mg (24.305)	$MgSO_4.7H_2O$ (246.08)	114	$(0.114 \times \frac{1}{24.305}) \times 246.08$	1.154
K (39.098)	KCl (74.56)	784	$(0.784 \times \frac{1}{39.098}) \times 74.56$	1.495
HCO_3 (61.000)	$NaHCO_3$ (84.01)	1100	$(1.100 \times \frac{1}{61.000}) \times 84.01$	1.51
Corrected NaCl		1761	3.5-(1.495-0.784)= 2.789 $(2.789 \times \frac{1}{35.453}) \times 58.44$	2.818

AM= Atomic mass; MW= Molecular mass

7.5 Results

7.5.1 Mount model predictions

The Mount model was used to predict the toxicity of Arpley and MVP average leachates (Table 7-5 to Table 7-15). Average concentrations of the major ions were obtained from the WRG database for Arpley. For MVP the average concentration was based on the chemical determinations made previously. From the average data a number of simulated dilutions were made in order to match the dilutions used in a real *D. magna* test (Section 3.5.1). Each of the concentrations of major ions was entered into the Mount model and a prediction made on

toxicity. Virtual dilutions of the solutions were made so that an EC_{50} for *D. magna* could be calculated based on the predictions from the model.

The average concentration of Mg^{2+} was very similar in both of the leachates collected. The other major ions demonstrate a greater difference between the 2 sites. Arpley has a higher average concentration of K^+, HCO_3^-, SO_4^{2-} whereas MVP has a higher concentration of Cl^-. The Mount model predicted the same EC_{50} values for the Arpley and MVP (43.2% and 43.5% respectively).

Major ion analysis was carried out for each sample collected during this project. The Mount model was used to predict the toxicity of the samples towards *D. magna* (Table 7-5 to Table 7-15). The EC_{50} toxicity had a small range of 7.9%. The highest predicted toxicity was an Arpley sample 11.08.09 which had a predicted EC_{50} of 38.6%. The lowest predicted EC_{50} of 46.5% was with a MVP sample 23.05.10.

7.5.1.1 MVP Mount model predictions

Table 7-5: The calculated EC_{50} for MVP leachate collected on the 13.05.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival (%)	Recorded survival (%)
100	113	934	1053	3994	132	0	
50	56.5	467	526.5	1997	66	12.9	
25	28.25	233.5	263.25	998.5	33	80.8	
12.5	14.125	116.75	131.625	499.25	16.5	95.6	
6.25	7.0625	58.375	65.8125	249.625	8.25	98.1	
						EC_{50} = 44.32	**EC_{50} = 50.5**

Table 7-6: The calculated EC_{50} for MVP leachate collected on the 28.05.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival	Recorded survival (%)
100	128	786	945	3570	124	0	
50	64	393	472.5	1785	62	23.56	
25	32	196.5	236.25	892.5	31	86	
12.5	16	98.25	118.125	446.25	15.5	96.5	
6.25	8	49.125	59.0625	223.125	7.75	98.3	
						EC_{50} =46.5%	**EC_{50} = 43.6**

Table 7-7: The calculated EC_{50} for MVP leachate collected on the 11.08.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival	Recorded survival (%)
100	125	702	1269	4371	224	0	
50	62.5	351	634.5	2185.5	112	11.8	
25	31.25	175.5	317.25	1092.75	56	80.1	
12.5	15.625	87.75	158.625	546.375	28	95.7	
6.25	7.8125	43.875	79.3125	273.1875	14	98.1	
						EC_{50} =43.2%	**EC_{50} = 57.0**

Table 7-8: The calculated EC_{50} for MVP leachate collected on the 24.08.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival	Recorded survival (%)
100	96	794	1893	4000	126	0	
50	48	397	946.5	2000	63	3.6	
25	24	198.5	473.25	1000	31.5	68.9	
12.5	12	99.25	236.625	500	15.75	94.3	
6.25	6	49.625	118.3125	250	7.875	97.9	
						EC_{50} =40.17	**EC_{50} = 57.0**

Table 7-9: The calculated EC_{50} for MVP leachate collected on the 10.11.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival	Recorded survival (%)
100	109	837	1183	3311	184	0	
50	54.5	418.5	591.5	1655.5	92	16.9	
25	27.25	209.25	295.75	827.75	46	83.3	
12.5	13.625	104.625	147.875	413.875	23	96.1	
6.25	6.8125	52.3125	73.9375	206.9375	11.5	98.2	
						EC_{50} =44.7%	**EC_{50} = 57.0**

Table 7-10: The calculated EC_{50} for MVP leachate collected on the 17.12.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival	Recorded survival (%)
100	101	863	1680	3449	153	0	
50	50.5	431.5	840	1724.5	76.5	6.33	
25	25.25	215.75	420	862.25	38.25	74.1	
12.5	12.625	107.875	210	431.125	19.125	94.9	
6.25	6.3125	53.9375	105	215.5625	9.5625	97.8	
						EC_{50} =41.3%	**EC_{50} = 57.0**

7.5.1.2 Arpley Mount model predictions

Table 7-11: The calculated EC_{50} for Arpley leachate collected on the 7.07.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival (%)	Recorded survival (%)
100	126	1498	1368	2003	99	0	
50	63	749	684	1001.5	49.5	4.9	
25	31.5	374.5	342	500.75	24.75	71.5	
12.5	15.75	187.25	171	250.375	12.375	94.6	
6.25	7.875	93.625	85.5	125.1875	6.1875	97.9	
						EC_{50} =40.7%	**EC_{50} = 43.2**

Table 7-12: The calculated EC_{50} for Arpley leachate collected on the 14.09.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival (%)	Recorded survival (%)
100	104	1071	1839	2245	81	0	
50	52	535.5	919.5	1122.5	40.5	5.1	
25	26	267.75	459.75	561.25	20.25	72	
12.5	13	133.875	229.875	280.625	10.125	94.8	
6.25	6.5	66.9375	114.9375	140.3125	5.0625	97.9	
						EC_{50} =40.9%	**EC_{50} = 59.5**

Table 7-13: The calculated EC_{50} for Arpley leachate collected on the 11.08.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival	Recorded survival (%)
100	108	968	2491	2337	89	0	
50	57.24	513.04	1320.23	1238.61	47.17	1	
25	27	242	622.75	584.25	22.25	59.8	
12.5	13.5	121	311.375	292.125	11.125	93.1	
6.25	6.75	60.5	155.6875	146.0625	5.5625	97.6	
						EC_{50} =38.56%	**EC_{50} = 59.2**

Table 7-14: The calculated EC_{50} for Arpley leachate collected on the 24.08.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival (%)	Recorded survival (%)
100	134	1146	1662	1876	93	0	
50	67	573	831	938	46.5	6.6	
25	33.5	286.5	415.5	469	23.25	74.7	
12.5	16.75	143.25	207.75	234.5	11.625	95	
6.25	8.375	71.625	103.875	117.25	5.8125	97.9	
						EC_{50} =41.5	**EC_{50} = 59.2**

Table 7-15: The calculated EC_{50} for Arpley leachate collected on the 28.11.09

Conc. (%)	Mg	K	HCO_3	Cl	SO_4	Predicted survival (%)	Recorded survival (%)
100	122	983	2104	2554	99	0	
50	61	491.5	1052	1277	49.5	3.1	
25	30.5	245.75	526	638.5	24.75	66.6	
12.5	15.25	122.875	263	319.25	12.375	94	
6.25	7.625	61.4375	131.5	159.625	6.1875	97.7	
						EC_{50} =39.8%	**EC_{50} = 47.3**

7.5.2 The toxicity of synthetic leachates

To establish whether the levels of toxicity recorded in the previously collected samples could be attributed to the major ion concentration a set of experiments using synthetic solutions made with the average major ion concentrations was made up. These synthetic leachates were then used to determine toxicity towards *D. magna* and *L. minor* (Figure 7-1 to Figure 7-3). All of the synthetic leachate experiments were conducted with the refined dilution series (section 7.4.2).

Results for the synthetic solutions based on the ion composition of Arpley leachates are presented in Figure 7-1. The response of *D. magna* towards Arpley synthetic solutions marked 1 and 2 was an EC_{50} of 71% in both 24 and 48 hours. In the synthetic solution 3 there was an increase in the EC_{50} toxicity to 66 and 64% in the 24 and 48 hour tests respectively.

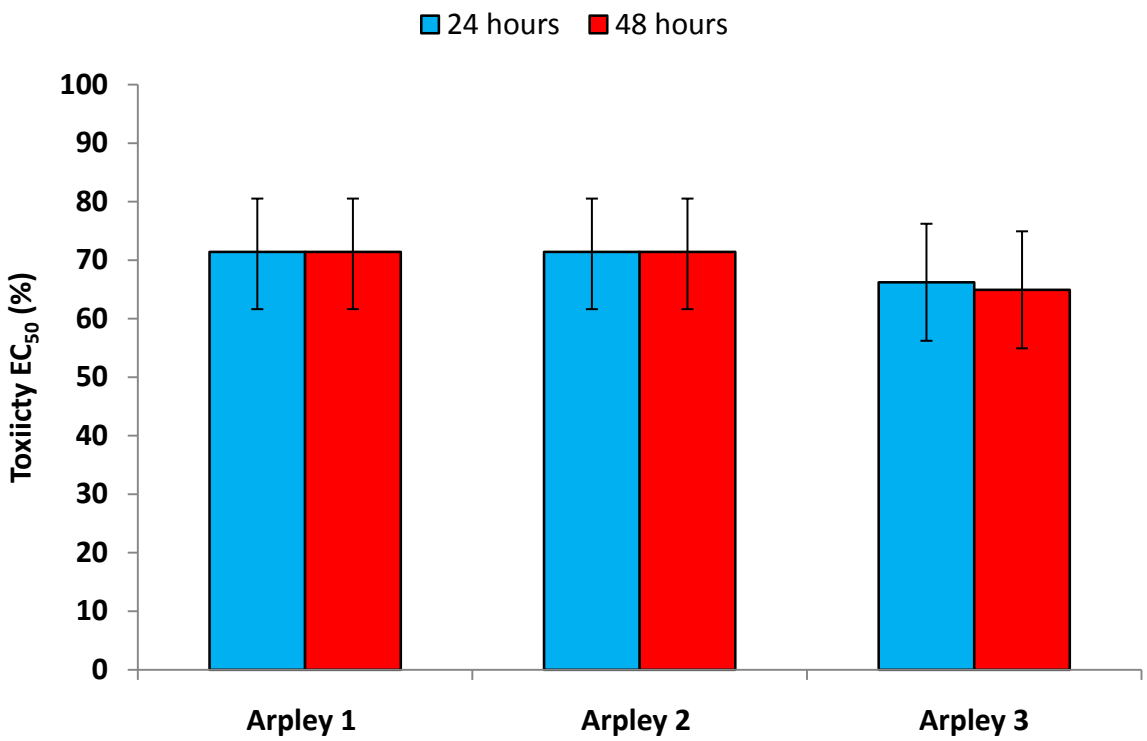

Figure 7-1: Response of *D. magna* towards three repeats of the synthetic leachate based on the average Arpley treated leachate major ion concentration. Error bars represent the 95% confidence intervals.

A similar set of experiments was performed with solutions made with major ion characteristics of the average MVP leachate (Figure 7-2). The synthetic solutions MVP 1-2 each produced an EC_{50} of 71% in both the 24 and 48 hour tests. The EC_{50} toxicity was recorded in MVP-3 as 77 and 72% in the 24 and 48 hour tests respectively. The difference in the toxicity between MVP 1-2 and 3 was not significant and so is probably a variation in the *D. magna* and not actual toxicity due to the overlap of error bars.

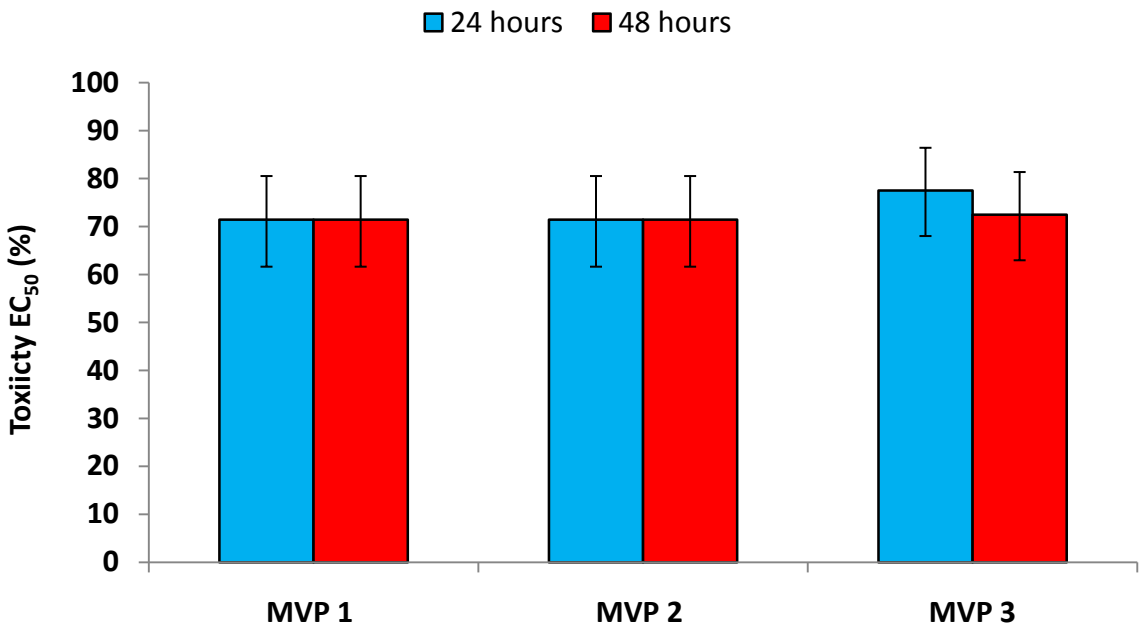

Figure 7-2: Response of *D. magna* towards three repeats of the synthetic leachate based on the average MVP treated leachate major ion concentration. Error bars represent the 95% confidence intervals.

L. minor was more sensitive to the Arpley and MVP synthetic leachates than *D. magna* (Figure 7-3). In both cases, the undiluted sample (100%) of synthetic leachate caused chlorosis in the fronds. Similar responses were noted in this test during the TIE and XAD phases. The synthetic MVP leachate produced an EC_{50} of 39%. The Arpley synthetic leachate produced a higher response with an EC_{50} response of 36% recorded.

The MVP and Arpley 26.04.10 solution testing was with a newly collected culture of *L. minor*. This was necessary after the previous stock had developed a bacterial infection. The fronds from the new stock were considerably smaller than the previous culture due to only being grown under laboratory conditions for 9 weeks. The response of the new culture of *L. minor* was lower in the second batch. The EC_{50} toxicity of the MVP lowered to 46% from 39%. The EC_{50} response of the Arpley sample was increased in the 2nd set from 37% to 31% in the second set of testing.

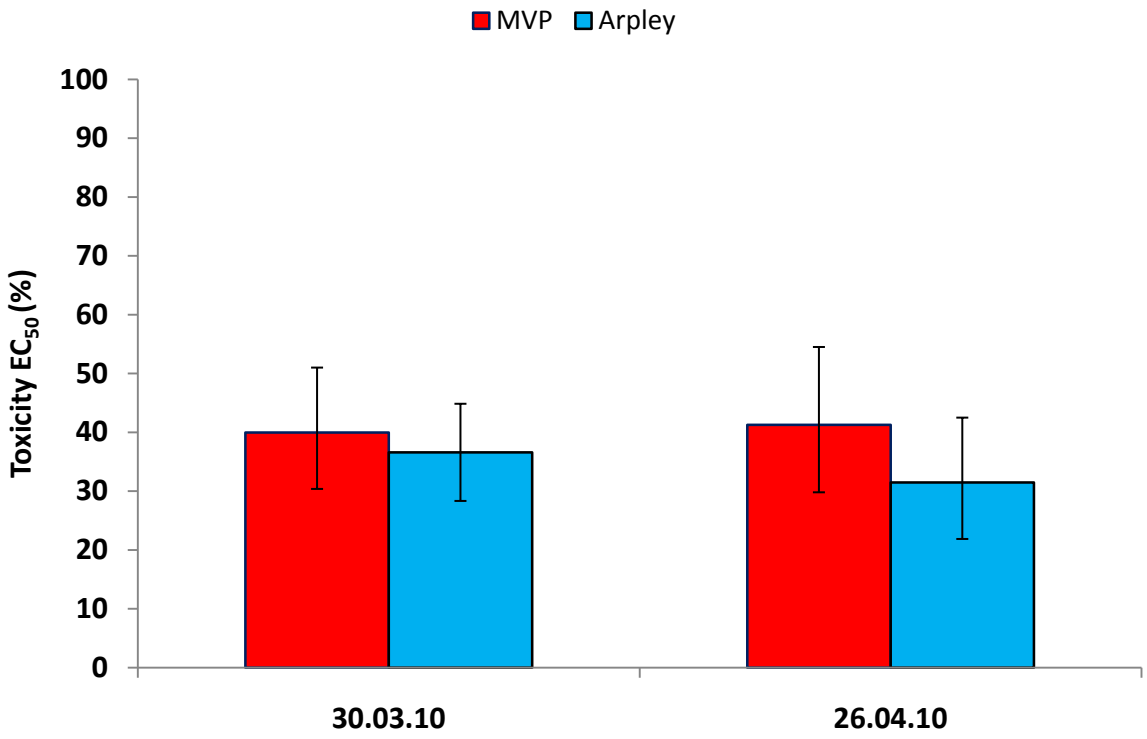

Figure 7-3: Response of *L. minor* to synthetic leachates based on average major ion concentrations from MVP and Arpley treated landfill leachates. Error bars represent the 95% confidence intervals.

7.6 Discussion

7.6.1 Mount model predictions

The Mount *et al* (1997) model was the first of the two approaches used to gain a better understanding of the role of major ions play in residual leachate toxicity. This model has the potential to be a useful tool for operators and regulators in understanding the levels of toxicity associated with effluent and helping identify methods to minimise the risk posed by major ion rich effluents. Using the model reported by Mount *et al* (1997), predictions were made of toxicity relating to the major ion concentrations in treated landfill leachate.

The model was unable to accurately predict the toxicity of treated landfill leachates collected from MVP and Arpley (Table 7-5 to Table 7-15). In all cases the model over predicted toxicity by ~20%, which is a significant over-prediction (see Table. This was a

disappointment as it was hoped that this model might explain the levels of toxicity present in treated landfill leachate and also be a useful tool for correlating the risk associated with major ion rich effluents.

Mount *et al*, (1997) noted that modelling *D. magna* toxicity was more difficult than modelling the more sensitive *C. dubia*. This was due to the *D. magna* having a higher senstitivy to the Cl^- anions. Tietge *et al*, (1997) reported that the Mount model was able to accurately predict the toxicity of produced waters towards *C. dubia* but unable to predict accurately for *D. magna*. The Mount model under and over predicted the toxicity of the produced waters when compared to the real samples. The underlying theory of the model is considered valid even with the over predictions. This model needs more work to make it work, possibly more work with the actual modelling. A validated model for understanding toxicity based on major ions is suitable for informing decisions on treatment for both the regulator and operators.

Unlike in the work of Tietge *et al* (1997) the model consistently over predicted the toxicity of treated landfill leachate samples from MVP and Arpley (Table 7-5 to Table 7-15). Over prediction suggests there is a chemical or physical component not included in the model that is reducing the toxicity of treated landfill leachate. Over prediction of toxicity is not necessarily detrimental as it makes an operator more careful over the composition of its effluents. This caution will cost more and would not be attractive to an operator struggling for funds. At present the model is not suitable for predicting *D. magna* toxicity associated with treated landfill leachate. This unsuitability for *D. magna* is made up for with the accuracy for predicting toxicity towards *C. dubia* and fathead minnows. There is a strong argument for introducing this model as a standard evaluation tool for operators of leachate treatment plants to gain a greater understanding of the effects these effluents have on the aquatic environment and help inform decisions on reducing the concentration of major ions in the effluents. This

model could be useful for predicting toxicity based on the concentration of major ions from a wide variety of industrial effluents. A warning that it only works for two species must be made clear to the user but as an evaluative tool it could be very useful in understanding the potential risks of toxicity.

Little data exists on the toxicity of individual major ions in the literature. The data in the literature is of salts and does not attempt to separate the ions toxicity from the salt's toxicity. An attempt to assign the individual toxicity of Cl^- in a chemically complex solution has been previously reported (Cooman *et al*., 2003) (Figure 7-4). Cooman *et al* (2003) used tannery wastewaters that are particularly high in Cl^-, SO_4^{2-}, Cr^{3+} and COD. This work attempted to determine whether major ion toxicity could be categorised as additive, partially additive or independent. They used the data supplied by Mount *et al* (1997) and made an assumption that the toxicity displayed in the data is caused by the Cl^- concentration of 11,300 mg/L and the ammoniacal-nitrogen concentration of 120 mg/L.

Using the work of Cooman *et al* (2003) predictions of Cl^- toxicity in treated landfill leachates can be made. The data points presented by Cooman *et al* (2003) were plotted and an equation for a best fit line generated (Figure 7-4). For the MVP leachate an EC_{50} toxicity of 82.9% is predicted and for the Arpley leachate an EC_{50} of 90.5%. These seem low considering the average EC_{50} of these sites is ~60% towards *D. magna*. According to the Mount model the remaining toxicity could be explained by the remaining major ions. The authors made a similar set of predictions of toxicity on the concentration of SO_4^{2-}. The concentrations of SO_4^{2-} in Arpley and MVP samples was 50-200 mg/L which would mean SO_4^{2-} is present at non-toxic concentrations. Cooman *et al* (2003) concluded this direction is a dead end as they believe the chemical matrix is too complicated to break down into a simple additive or independent toxicity model. The view that there are too many active contaminants is also been commented on in treatment sewage (Yatribi and Nejmeddine, 2000). Predicting toxicity

based on the concentration of individual ions is not possible. This adds weight to the argument that the Mount model should be further developed for examination of major ion rich effluents as it offers a simple to use tool in understanding the risks posed.

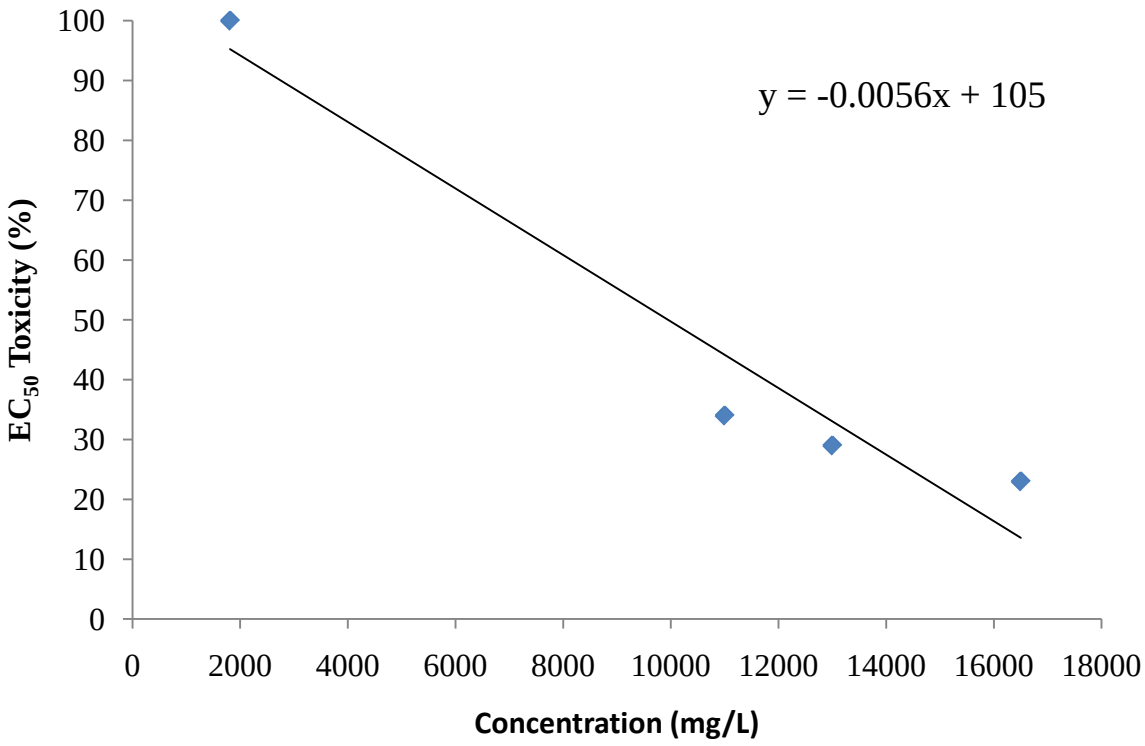

Figure 7-4: The relationship between chloride concentration and toxicity by Cooman *et al* (2003).

7.6.2 Synthetic leachates

In the synthetic experiments an attempt to replicate the major ion composition of the MVP and Arpley samples was made. A comparison between the mean synthetic EC_{50} results and the mean collected treated leachate EC_{50} results (which are derived from the long term toxicity testing results in Figure 5-3 and Figure 5-4) (Figure 7-5 to Figure 7-7). This was done in order to allow a comparison between the findings of the synthetic work and relating it back to the treated landfill leachates chemistry.

From the average of the combined results for *D. magna* responses to MVP samples it can be seen that there is a difference of 15% between the real and the synthetic leachate (Figure 7-5).

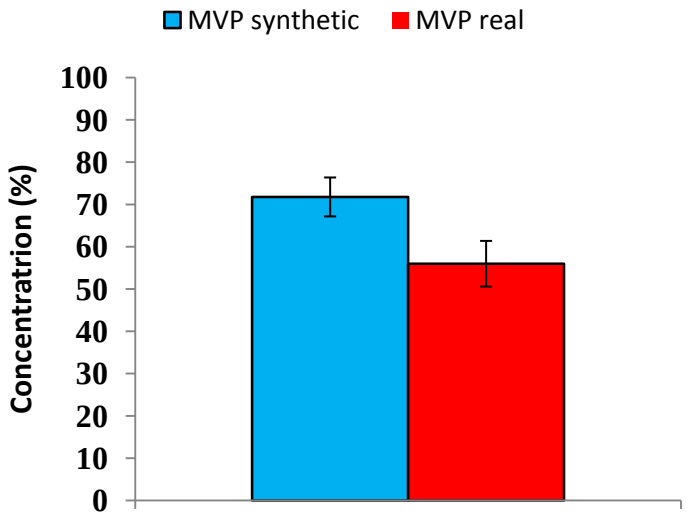

Figure 7-5: Comparison of average EC_{50} toxicity for *D. magna* towards MVP synthetic and real leachates. The standard deviation is shown as a bar on the column.

To test whether there was a significant difference between the results two hypotheses (see below) was produced for the comparison of real and synthetic EC_{50} toxicity for *D. magna*:

Null hypothesis: There is no significant difference in the EC_{50} toxicities of real and synthetic leachates from MVP

Alternative hypothesis: There is a significant difference in the EC_{50} toxicities of real and synthetic leachates from MVP

Calculations of significant difference was made with the alpha value set at 0.05 (Table 7-16).There were 13 degrees of freedom within the data and t = 9.64 for the data. Since the p value was < 0.05 the null hypothesis was rejected. This requires that the alternative hypothesis is supported therefore a significant difference between the real and synthetic

leachates exists. This difference is due to the comparison between the unmodified dilution series and the modified dilution series. It is likely that if the test was performed with similar dilution series there would be no statistical difference between the two toxicity results.

Table 7-16: Students t-test calculations to determine significance in difference between EC_{50} toxicities in MVP real (unmodified dilution series) and synthetic leachates towards *D. magna*.

Parameter	Synthetic leachate	Real leachate
Mean	71.77	56.90
Variance	0.36	29.37
Observations	3.00	13.00
df	13.00	
t Stat	9.64	
P(T<=t) two-tail	2.75E-07	
t Critical two-tail	2.160368652	

From the average of the combined results for *D. magna* responses to Arpley samples it can be seen that there is a difference of 16% between the real and the synthetic leachate (Figure 7-6).

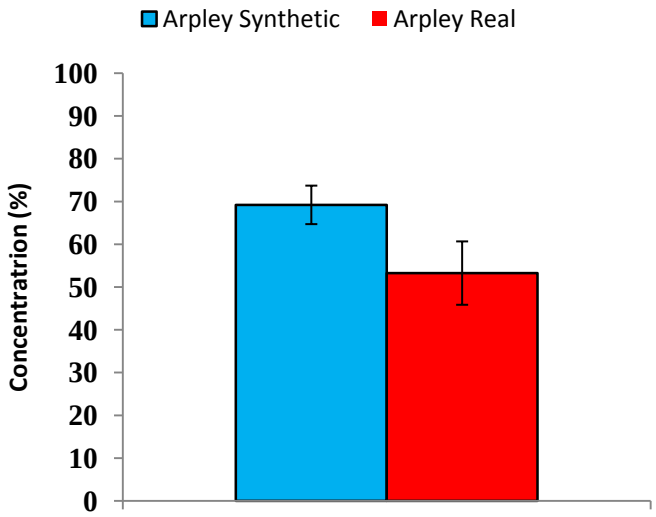

Figure 7-6: Comparison of average EC_{50} toxicity for *D. magna* towards Arpley synthetic and real leachates. The standard deviation is shown as a bar on the column.

Determination of significant difference was calculated with the same method used for MVP leachates and used the same hypotheses (Table 7-17). Since the $p < 0.05$ the null hypothesis is rejected. This requires that the alternative hypothesis is supported therefore a significant difference between the real and synthetic leachates exists. Like above this difference could be corrected by using similar dilution series.

Table 7-17: Student t-test calculations to determine significance in difference between EC_{50} toxicities in Arpley synthetic and real leachates (unmodified dilution series) towards *D. magna*.

Parameter	Synthetic leachate	Real leachate
Mean	69.25	52.91
Variance	14.17	54.95
Observations	3	6
df	7	
t Stat	4.39	
P(T<=t) two-tail	0.0032	
t Critical two-tail	2.36	

Determination of significant difference was calculated with the same method used for MVP leachates and used the same hypotheses (Table 7-18). Since the $p > 0.05$ the alternative hypothesis which is rejected. This requires for the null hypothesis is correct therefore a significant difference does not exist between the two treatments. From the presented data there is no difference and that treated landfill leachate toxicity can be recreated with the addition of major ions in the correct concentrations. This demonstrates that the levels of toxicity in treated landfill leachate are attributable to the concentrations of major ions. The cause of this is likely to the comparison of similar dilution series unlike when comparison of non-similar dilution series (Table 7-16 and Table 7-17) then a similar lack of significant difference between the results is expected.

Table 7-18: Students t-test calculations to determine significance in difference between EC_{50} toxicities in MVP synthetic and real leachates using the modified dilution series towards *D. magna*.

Parameter	Synthetic leachate	Real leachate
Mean	71.77	64.66
Variance	0.36	8
Observations	3	3
df	1	
t Stat	3.50	
P(T<=t) two-tail	0.18	
t Critical two-tail	12.71	

From the average of the combined results for *L. minor* responses to MVP samples it can be seen that there is a difference of 6% between the real and the synthetic leachate (Figure 7-7).

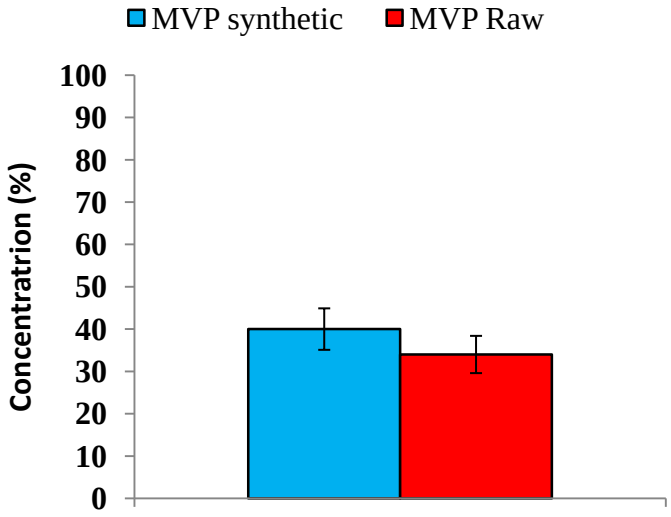

Figure 7-7: Comparison of average EC_{50} toxicity for *L. minor* towards MVP synthetic and real leachates. The standard deviation is shown as a bar on the column.

Calculations of significant difference was made Alpha value being set at 0.05 (Table 7-19).There were 10 degrees of freedom within the data and t = 2.86 for the data. Since the p < 0.05 the null hypothesis is rejected. This requires that the alternative hypothesis is correct therefore a significant difference between the real and synthetic leachates exists. Similar to

the work with *D. magna* this is due to the comparison between different dilution series and would be corrected if the dilution series had been similar.

Table 7-19: Students t-test calculations to determine significance in difference between EC_{50} toxicities in MVP real (unmodified dilution series) and synthetic leachates towards *L. minor*.

Parameter	Synthetic leachate	Real leachate
Mean	40.62	33.067
Variance	0.82	65.78
Observations	2	10
df	10	
t Stat	2.86	
P(T<=t) two-tail	0.017	
t Critical two-tail	2.23	

From the average of the combined results for *L. minor* responses to Arpley samples it can be seen that there is a difference of 6% between the real and the synthetic leachate (Figure 7-8).

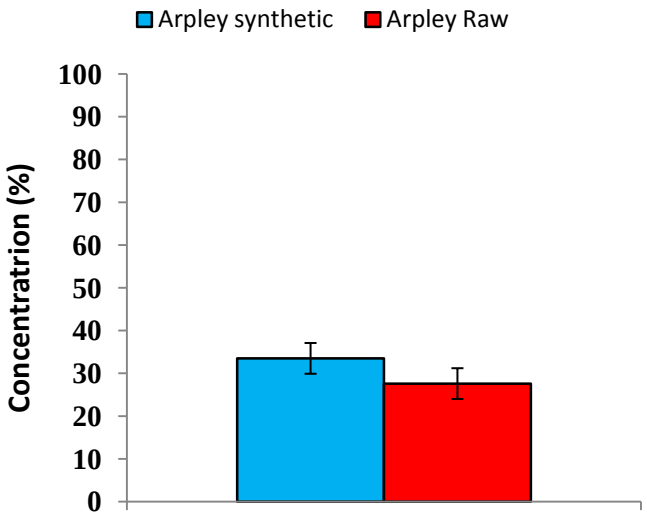

Figure 7-8: Comparison of average EC_{50} toxicity for *L. minor* towards Arpley synthetic and real leachates. The standard deviation is shown as a bar on the column.

Determination of significant difference was calculated with the same method used for Arpley leachates and using the same hypotheses (Table 7-20). Since $p > 0.05$ the alternative

hypothesis is rejected. This requires for the null hypothesis was correct therefore a significant difference does not exist between the two treatments. This result is due to the high variability of the *L. minor* test. It was impossible to use a modified dilution series, like with *D. magna* (Table 7-18), as there was significant variation. This is seen in the large error associated with the test which would have made a modified dilution series invalid if one of the dilutions resulted in no response when the other 2 replicates produced a positive result.

Table 7-20: Students t-test calculations to determine significance in difference between EC_{50} toxicities in Arpley real (unmodified dilution series) and synthetic leachates towards *L. minor*.

Parameter	Synthetic leachate	Real leachate
Mean	33.95	27.81
Variance	13.00	26.59
Observations	2	6
df	3	
t Stat	1.86	
P(T<=t) two-tail	0.16	
t Critical two-tail	3.18	

This analysis of significant difference between synthetic and treated leachates has shown that with a modified dilution series there is no significant difference between the two. This simple test demonstrates that the causes of toxicity in treated landfill leachate can be explained by the concentration of major ions in solution. Being able to explain the residual toxicity of treated landfill leachate by the concentration of major ions is an important finding for the project sponsor as it confirms the hypothesis that the COD fraction was non-toxic and safe for discharge without further expensive treatment methods. More work is needed to match the synthetic leachates with the real leachates though it would be relatively straightforward to carry out.

7.6.3 Discussion of the two pronged approach to understanding the role of major ions in residual leachate toxicity

This work with major ions attempted to resolve the causes of toxicity in treated landfill leachate through two difference approaches to the standard manipulation + whole effluent testing. Modelling offered the chance to determine whether the hypothesis of toxicity relating major ions could be answered without needing to conduct experiments. The model was a valid approach though the results were not as accurate as expected. This lack of accuracy was likely due to selecting *D. magna* at the start of the PhD process. *C. dubia* was looked into as an alternative species to be tested following the finding of the Mount *et al* (1997) paper. Finances would not allow this extra species into the procedure even though it would have been a good inclusion. None the less this model was very useful as it showed guided the research to the likely ions and the levels that can cause toxicity towards *D. magna.*

Building synthetic leachates was able to explain toxicity when they were comparing like with like dilution series. A more reliable result could have been obtained if the dilution series had been altered earlier in the project but altering the dilution series could have detrimentally after the toxicity results. Synthetic solutions have been shown here to be effective at explaining toxicity based on the concentration of major ions in effluents. Introduction of more substances into the solutions would be the next step for this procedure e.g. nitrate.

Unfortunately synthetic solutions are unlikely to be useful for the raw leachates as there is large amount of organic chemicals and this would prove impossible to replicate even if all chemicals could be determined. Ultimately the model of Mount *et al* (1997) needs to be updated to model *D. magna* better so that operators and regulators can predict toxicity and then introduce methods for reducing the major ion composition of effluents.

7.6.4 Limitations of experimental approach

Not using Ca^{2+} and PO_4 in the synthetic work is a limitation of the approach adopted. This decision was made so that experiments could be conducted. If more time had been available then a method for the inclusion of these ions into the synthetic solutions would have been found. Mount *et al* (1997) mentions that the inclusion of Na and Ca in the solutions limits the toxicity of K^+, Cl^- and SO_4^{2-} . There was a close correlation of synthetic to real leachates (Table 7-18 and Table 7-20) with the synthetic leachates being slightly less toxic than the real samples. As Ca is only present in concentrations of <250 mg/L then the lack of inclusion might not have been as significant as if the concentration was 2,000 mg/L.

The COD fraction was not included in this work. Humic and fulvic acid mixtures are obtainable from Aldrich chemicals. These refractory organics are non-toxic and would represent the COD fraction in treated landfill leachate. This omission was due to a lack of funds and time left in the project. Addition of this fraction is an obvious inclusion in further work in determining whether the COD fraction actually decreases the toxicity of major ions or whether it plays no role.

Not using the daphnid *C. dubia* is an obvious limitation of the work when trying to validate the model proposed by Mount *et al* (1997). *C. dubia*, like *D. magna*, is common in most waterways of the world. *C. dubia* was shown by Mount and Tietge as showing the closest correlation to the model described by Mount *et al* (1997). The choice of *D. magna* was made at the very start of the project from the findings of the literature review. The literature review was focused on the assessment of landfill leachate toxicity. *C. dubia* was identified during the literature review but only four references were identified for using this species to determine landfill leachate toxicity. Conversely, assessment of landfill leachate toxicity with *D. magna* is much more common with 24 sources using *D. magna* to assess landfill leachate toxicity. This led to the decision that D. magna should be part of the battery. *T. platyurus* was included

in the battery (Figure 5-9) for the testing as Isidori *et al* (2003) reported the species to be more sensitive than *D. magna* towards landfill leachate. This was the first report of *T. platyurus* assessment of landfill leachate toxicity and it was felt that further testing should be carried out to determine whether it was more suitable for testing than *D. magna*. *T. platyurus* test was not more sensitive than *D. magna* in the TIE procedure. Both tests produced similar results to all three of the treated leachate samples. In further testing it was decided to maintain the same species that had been used in the TIE procedure for continuity.

For these reasons *C. dubia* was not included in the first two experiments. During the preparations for the synthetic leachate experiments the Mount *et al* (1997) paper was located. This led to questions as to whether to change the battery to include *C. dubia* and remove *L. minor* or to keep the original setup. As the original results were with *D. magna* and *L. minor* it was decided that should remain.

7.7 Conclusions

- The model reported by Mount *et al* (1997) over predicts the toxicity of treated landfill leachate by approximately 20%.
- Synthetic leachate experiments show that the majority of toxicity in treated landfill leachate can be explained by the concentration of major ions. This is demonstrated best when the altered dilution series is used to determine the toxicity of samples. The difference between real and synthetic solutions is not significant.
- Determination of every possible contaminant in landfill leachate is virtually impossible and understanding the matrix of interactions between all contaminants also impossible. For this reason it is probably a futile to calculate the fraction of toxicity associated with each major ion. Instead a holistic view that toxicity in landfill leachate is caused by the major ions is a better approach to determine the causes of toxicity.

8 Overall project discussion

8.1 Introduction

This chapter sets out to integrate the findings from each investigation in the project. The findings are placed in a wider context and the significance for the operator, the regulator and the environment discussed. Consideration of the limits of the experimental approach is presented and possible avenues for future work suggested.

8.2 General findings

This project's original research question was to determine whether the recalcitrant chemical oxygen demand (COD) in treated landfill leachate was a potential hazard to the environment. COD concentration in treated landfill leachate ranges from 200-1,000 mg/L in the samples collected in this project. The high concentration and an uncertainty that the COD fraction could be directly or indirectly toxic to aquatic life forms brought the COD concentration under scrutiny from the Environment Agency. Concern over the high concentration of COD at the Environment Agency could lead to a lowering of the effluent COD consent. This would involve the commissioning of new plant equipment to achieve this new discharge consent e.g. ozonation plant. New plant equipment is very expensive and WRG needed to obtain more experimental evidence on the toxicity of the COD fraction in treated landfill leachate in order to argue the case that further COD removal is not warranted to protect the aquatic environment.

Previous reports had characterised this fraction as being mostly humic and fulvic acids (Kang *et al.*, 2002, Huo *et al.*, 2008). Humic and fulvic acids can act as carriers for toxic xenobiotic substances and heavy metals (Van Zomeren and Comans, 2007). Therefore the COD fraction could be a carrier of toxic substances into the environment so a closer examination of its role in toxicity was necessary.

The following research questions were set at the start of the project:

- Is treated landfill leachate toxic to the environment?
- If treated landfill leachate is toxic what is the cause of this toxicity?
- Is the COD fraction in treated landfill a possible toxic hazard to aquatic life?
- Are more complex treatment methods needed in order to reduce the COD fraction of landfill leachate?

To answer these questions standard chemical analysis of treated landfill leachate was needed so that the bulk chemical characteristics are understood e.g. COD and biological oxygen demand (BOD). Whole effluent toxicity (WET) testing was used to determine the effect that the chemical matrix has on aquatic organisms. WET testing determines the hazard posed by effluents to a species. Using more than one species to form a battery of bioassays allows the researcher to build a holistic picture of the hazard posed to the environment and to reduce the chance of anomalous responses if only one species was used.

The literature review identified previous WET testing of landfill leachate and in the selection of the microbiotests most suitable for determining the toxicity of treated landfill leachate. A battery of 5 microbiotests was selected for the initial stages of this project. The tests were selected to build a detailed picture of the causes of toxicity in treated landfill leachate. A comparison between microbiotests was included in the battery as the literature review had identified many differences in the sensitivity between microbiotests towards landfill leachate. The literature review proved to be very useful in understanding the results of the experiments. A lot of evidence for the types of response found came about from findings of the literature review.

8.2.1 Overview of selected landfill leachate chemistry and toxicity

In the initial stages of this work focused on three treated landfill samples that represented different treatment strategies and the strengths of leachate. Analysis of the chemical database

maintained by the project sponsors demonstrated the differences between Arpley raw and treated landfill leachates. The raw leachates tended to have high levels of COD, ammonia, and BOD with treatment of the leachate reducing these levels significantly. A number of inorganic salts were shown to be virtually unaffected by the treatment process e.g. potassium, chloride, magnesium. The concentration of toxic organic substances e.g. isoproturon were present in very low concentrations and would have little effect on the acute toxicity.

Unfortunately the inorganic chemical database held for MVP leachates contained much less information. The database demonstrated that COD, BOD and ammoniacal-nitrogen were being significantly reduced. With treatment the BOD and ammoniacal-nitrogen are being reduced to levels that are reaching the discharge consents imposed by the Environment Agency. The small amount of information from the database showed that the concentration of inorganic ions was unaltered by the treatment process.

The average leachates from Buckden were weaker than their counterparts from Arpley and MVP. In particular the concentration of potassium and chlorine were considerably lower in the samples from Buckden when compared to the samples from Arpley and MVP. These two ions are the cause of residual toxicity and their lower concentrations in the leachates from Buckden are the probable reason for its lower toxicity compared to the other two sites leachates.

From the monthly whole effluent toxicity (WET) testing the toxic response of *Daphnia magna* demonstrated slight variability in toxicity responses of the two test species whereas the toxicity response of *L. minor* was much more variable. The *D. magna* EC_{50} response to MVP leachates was between 43-65% and the *L. minor* response was 23-49%. Fewer determinations were possible with Arpley leachate due to problems with obtaining samples. Arpley responses towards *D. magna* ranged between 43-59% and *L. minor* 19-31%. Making

conclusions for one site being more toxic than the other is not possible due to the bigger toxicity dataset for MVP compared to Arpley.

Both the results for *D. magna* and *L. minor* toxicity are average for a treated landfill leachate sample based on reports from the literature (Table 2-6 and Table 2-8 respectively).

From the data it is clear that leachate toxicity from MVP was consistent throughout the sampling period. In general the toxicity levels of Arpley treated leachate were more variable than MVP. There is no seasonal variation in the quality of the leachate which indicates that the treatment is effective even in winter when the cold weather can affect the work of the activated sludge. This work demonstrated that the toxicity levels seen in the TIE, XAD and synthetic work was consistent with average toxicities.

8.2.2 Toxicity identification evaluation discussion and conclusions

Identification of the types of substance causing toxicity in a sample is possible with the manipulation of physical and chemical characteristics (Norberg-King *et al*., 1991). The Toxicity Identification Evaluation (TIE) procedure is designed as a screening exercise to identify the classes of substance responsible for toxicity. Many toxicants' characteristics are sensitive to changes in solution pH. By alteration of pH, the chemistry of the sample can be manipulated which in turn effects the toxicity. Following these manipulations toxicity is measured and compared to the baseline to determine whether the manipulation has affected the response. This allows changes in toxicity to be linked to a particular manipulation which can be used to elucidate the identity of a toxicant. The procedure has proven successful in many types of wastewater samples (Norberg-King *et al.*, 1991, Tietge *et al.*, 1997, Svenson *et al.*, 2000, Fernández *et al.*, 2004).

On commencement of the TIE experiments no other reports for the application of a TIE procedure with treated landfill leachate could be found. One previous application of the TIE procedure with raw leachate had shown that it was possible to identify the classes of

substances responsible for toxicity (Isidori *et al*., 2003). Isidori *et al* (2003) reported considerable variability in sensitivity between the 5 test species however.

The procedure demonstrated that the toxicity of treated landfill leachate was low towards 4 of the 5 species tested i.e. >50% EC_{50}. Only *L. minor* recorded a high response towards the leachate samples from the 3 leachate treatment plants e.g. <40% EC_{50}. Other researcher's findings on the toxicity of treated landfill leachate are similar to the findings in this project (Figure 5-6 to Figure 5-9) (Marttinen *et al*., 2002, Okamura *et al*., 2005, Svensson *et al*., 2005, Osaki *et al*., 2006, Bortolotto *et al*., 2009). The correlation of results suggests that the cause of toxicity is similar. Low toxicity of treated landfill leachate is attributed to the effective removal of ammonia and biodegradable organic fraction in the treatment stage. These elements are the most toxic and their removal would effectively reduce the toxicity of the leachates.

The TIE procedure demonstrated a highly variable set of results with no obvious pattern emerging. Clearly each species had an individual response to the treated landfill leachate e.g. Microtox displayed no toxicity towards the treated landfill leachate whereas *L. minor* displayed an EC_{50} 20-40%. The responses tended to be consistent even with the pH manipulations. A lack of change in toxicity with changes in the pH indicates that the toxicants were not sensitive to pH changes. This consistency is in complete contrast to the findings of Isidori *et al* (2003) where the toxicity varied widely with changes in pH etc. This finding might indicate that the TIE procedure is not be suitable for samples with such a low level of toxicities.

The TIE procedure has a solid phase extraction (SPE) stage. This stage is designed to remove the organic fraction from the sample. SPE cartridges are suggested in the TIE procedure as they are quick and easy to use. With the samples from Arpley and Buckden there was an anomalous result in the SPE pH 3 tests with *D. magna* where toxicity varied widely between

treatments. Due to the lack of changes and anomalous result it was decided more testing would be needed in order to answer the original research question on the role of COD in toxicity.

No discernable pattern of responses were evident following the procedure. A lack of significant toxicity change with application of the TIE procedure suggests that the causes of toxicity are species not sensitive to pH change e.g. inorganic ions. The lack of toxicity response with the Microtox test suggested that the substance causing toxicity to the other species was a substance that Microtox was tolerant to. Microtox is based on a deep sea species which would suggest a high tolerance to ions such as Na^+, Cl^-, $Mg^{2+}$and K^+.

The TIE work demonstrated that toxicity in treated landfill leachate was low which is in accordance with other reports. The original research question on COD toxicity could not be answered with the TIE procedure. This required more research on COD removal and the effect on toxicity. Also the possible toxicity effect of major ions such as Cl^- and Na^+ also needed investigation due to the elevated concentration of these in landfill leachate.

8.2.3 XAD discussion and conclusions

The results from TIE procedure SPE phase were inconclusive and could not answer the project's original research question. To answer the research question an improved COD removal procedure was needed. A rapid batch procedure for the removal of the humic substance fraction of solid and aqueous samples had been successfully trialled previously (Van Zomeren and Comans, 2007). The principle behind the removal of the organic fraction was successfully proven when applied to solid waste samples (Wagland, 2008).

The addition of WET testing following the removal of the organic fraction has only been trialled once previously (Baun *et al*., 2000). This testing was carried out using raw leachates collected at distances from the active area of the landfill. The authors hypothesised that attenuation of xenobiotic substances takes place via a number of chemical pathways e.g.

redox reactions involving bacteria. Baun *et al* (2000) used XAD-2 resin to remove polar and non-polar organic substances from the leachate samples. XAD-2 is designed to remove aromatic substances, grease and some antibiotics. One of the samples produced a toxic response to the Ames test (Chapter 2). The authors concluded that XAD resins were suitable for the removal of xenobiotic substances from complex matrix leachates such as landfill leachate.

The COD removal work carried out for this project used 3 types of XAD resin: 7, 16 and 4. These resins were selected to remove a range molecule sizes and polarities. Toxicity was determined from the effluent from the XAD packed column. This procedure was able to remove ~90% of the COD present in treated landfill leachate. The results of each resin were of a similar order to removals achieved with advanced oxidation treatments of leachate (Kurniawan *et al*., 2006b). These are very costly in terms of equipment purchases and running costs though XAD is unlikely to figure in the removal of COD on a full scale but is ideal for laboratory scale removals of refractory organics.

Based on the removal percentages of XAD 7 and centrifugation the ratio of humic to fulvic acids was 1:2 in the treated leachate samples. This is lower than the expected as previous research had suggested a higher proportion of humic to fulvic acid e.g. 1:1 (Berthe *et al*., 2008). From this work it was apparent that 75% of the COD fraction was humic and fulvic acids which is similar to the 70% reported by Huo *et al* (2009). The higher proportion of humic acids demonstrates that these leachates from Arpley and MVP are classified as >5 years old. Aging of leachates is indicated by an increased humic acid concentration.

COD is removed during the biological treatment of leachates e.g. 2544.5 mg/L in the raw MVP leachate and 795.6 mg/L in the treated leachate. Ten percent of the COD fraction of treated leachate is transphilic substances i.e. removed with XAD-4. This demonstrates very little COD is smaller organic molecules i.e. <150 Da. Small molecules such as isoprene and

toluene are targeted with XAD-4. In non-stabilised leachates the proportion of transphilic substances is much higher e.g. 30% (Bu *et al*., 2010). Stabilised leachates are characterised by this level (~10%) of transphilic substances in the leachate (Berthe *et al*., 2008). It is likely because degradation inside the landfill and biological treatment target the smaller molecules as does the synthesis of humic and fulvic acids use smaller molecules in the building of the macro molecules.

Retention time between the resins and the leachate samples in this work was relatively rapid <10 min. This is an improvement on the 2 h of Rodriguez *et al* (2004) and 1 h of Van Zomeren *et al* (2007). The improvement in retention of time is the result of using two resins i.e. XAD-7 and 16 was needed to remove high levels of COD. The toxicity of XAD-treated samples was no different to the original treated leachate samples. This new work has shown that COD (or substances closely associated with it) are not the cause of residual toxicity in treated landfill leachate. This work could prove to be very significant for the landfill operators in their negotiations on discharge consents. By showing the toxicity of treated landfill leachate is not associated with the COD the Environment Agency need not concentrate on the COD content of effluents. The operators do not need to invest in advanced COD treatment equipment as the hazard posed by the COD fraction is very small.

8.2.4 Major ions and synthetic leachate discussion and conclusions

Results from the XAD removal of the COD fraction did not support the hypothesis that the COD fraction is the cause of residual toxicity in treated landfill leachate. Manipulations of samples in the TIE procedure and a lack of response in the Microtox test generated a second hypothesis. This second hypothesis was that an elevated concentration of ions such as Cl and Na was the causes of toxicity in treated landfill leachate.

A model for predicting major ion toxicity towards three freshwater species was developed by the USEPA (Mount *et al*., 1997). This paper demonstrated, with the aid of >2000 experiments, a model of toxicity based on seven ions common in freshwater:

$$Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}, HCO_3^{-}, SO_4^{2-}$$

The work of Mount *et al* (1997) showed that the toxicity of ions towards *D. magna* followed a sequence of increasing toxicity:

$$K^{+} > HCO_3^{-} \approx Mg^{2+} > Cl^{-} > SO_4^{2-}$$

Atomic absorption spectroscopy analysis of the previously collected treated leachate samples was carried out to determine the concentration of major ion cations. The anions were determined separately. These determinations demonstrated that the major ions were present in landfill leachate in elevated concentrations. The levels of these major ions in the treated leachate samples were clearly high enough to cause toxic responses e.g. Cl concentration ≥1,500 mg/L (Cooman *et al*., 2003).

The Mount *et al* (1997) model was used with the data obtained from the chemical analysis of the previously collected leachates. This equation consistently over predicted the EC_{50} toxicity of the treated leachate samples by ~20% for Arpley and MVP. Over prediction is less of a risk to the environment than under predicting toxicity which leads to an effluent that poses a significant risk being released to the environment. The equation had issues with predicting toxicity for *D. magna* (Mount *et al*., 1997). Mount *et al* (1997) reported that chloride was the cause of the model failing to predict accurately for *D. magna*.

This model was developed to help operators understand the impact of their effluents and reduce the concentration of toxicants. If the Mount model was updated and able to successfully predict toxicity of treated landfill leachate towards *D. magna* it would be a powerful tool to operators. This in turn aids communications between operators and the regulator. The ability to predict toxicity from a validated model based on the concentrations

of major ions allows strategies for dealing with these effluents to be developed. It is then up to the regulator whether the concentrations of major ions are acceptable or a strategy for dealing with major ions is needed.

The work of Mount *et al* (1997) demonstrated that the ideas on major ion toxicity generated from the results of the TIE work (Chapter 5) were sensible. Values for toxic concentrations of the various major ions had been calculated by the researchers which could then be compared to the determined concentrations found within the treated landfill leachates. The researchers also demonstrated that the additive approach to understanding toxicity in complex solutions was a valid approach to take.

To test whether major ions were the sole cause of toxicity a series of synthetic leachate solutions based on the major ion concentrations of Arpley and MVP leachates were made up by dissolving salts of the major ions in deionised water. A constructive approach to understanding toxicity was designed to address the issue of residual toxicity from another experimental angle as the reductive approach had reached its limit. The toxicity of these solutions was determined with *D. magna* and *L. minor*. Synthetic solutions have previously been used to understand the occurrences of problems in wastewater treatment e.g. landfill sump blockage (Rowe *et al*., 2002). They have the advantage of only containing the substances of interest with no interference from other materials. The tests involving synthetic solutions were carried out with a modified dilution series to obtain a more accurate EC_{50} value. These solutions were able to produce toxicities that were slightly lower than the toxicities of the real treated leachates. The gap between the average of the collected EC_{50} and synthetic leachates was approximately 15% with *D. magna* and 6% with *L. minor*. With a freshly collected treated leachate sample the dilution series was slightly altered so that the EC_{50} could be calculated with greater precision. The gap between the synthetic and the changed dilution series sample narrowed the difference to 9% for *D. magna*. An exact match

between the synthetic solutions and the real leachates could not be expected as these synthetic solutions are greatly simplified in terms of composition. There are missing ions e.g. Ca^{2+}, and there is the possibility that there are missing elements e.g. COD.

These experiments demonstrate that major ions are almost certainly the cause of toxicity in treated landfill leachate. This is a major finding as it shows experimentally that the toxicity of treated leachate is reproducible within a laboratory. There is little risk in discharging a major ion rich effluent to water courses as dilution of the effluent should remove the potential hazard of the elevated concentrations of the major ions. But there is a developing view that discharge to watercourses does not dilute the concentration of salts in Australian watercourses as what would be expected (Nielsen *et al*., 2003b). This occurs in areas of the watercourse where the flow rate slows down and there is a higher concentration up of major ions. Periods of flooding can help relieve this effect as the high flow rates during these periods flush out the salts areas of high concentration i.e. the flood waters increase the flow rate in slow area. Whilst these studies focus on Australia where water shortages are becoming more common these findings could have implications within British watercourses in areas where the flow rates slow e.g. bends in the river. Accumulation of major ions within areas of slow flow rate changes the environment which force out less saline tolerant species and depletes biodiversity. Filter feeders, such as *D. magna*, are particularly sensitive to elevated levels of major ions (Soucek, 2007). To what extent the effluent of treated landfill leachate damages the watercourses is outside the remit of this project but would be a good area for further research.

8.3 Overall achievement and contribution to science

1. Long term WET testing has shown that the individual responses towards treated leachates were relatively similar and show that the treatment processes are relatively

consistent throughout the year. The long term testing of leachate effluents demonstrates the trends in toxicity variability.

2. This is the first reported application of the TIE procedure with treated landfill leachate. From the results the use of the TIE Phase 1 procedure is most likely not suitable for determining the causes of toxicity of biologically treated landfill leachate samples even though the procedure has proved successful for other more toxic wastewater samples. This is likely due to most of the toxicity being unresponsive towards pH manipulation and the physical manipulations concentrating on xenobiotics and heavy metals.
3. A bacterium, an invertebrate and a higher organism were needed for a complete battery for determining landfill leachate toxicity. Due to the lack of response with the Microtox test it was not included in most of the XAD and major ion toxicity work. There is an argument that Microtox should be a standard test i.e. daily or weekly for routine monitoring of toxicity in effluents. Apart from Microtox there is a need for routine monitoring of effluents as there are going to be changes in the toxicity of the effluents over time and problems need to be dealt with quickly.
4. Removal of ≥90% of the COD concentration was achieved in the XAD testing. Even after this significant removal no change in the toxicity of the treated landfill leachate samples. Before this work was carried out little agreement on the role of organic substances in treated leachate toxicity existed. This work showed that the various organic fractions are not the cause of toxicity in treated leachate. This is the first report of COD removal followed by WET testing on treated landfill leachate.
5. The Mount model was applied to landfill leachate samples as this model was able to predict toxicity based on elevated concentrations of major ions. *D. magna* was unable to accurately predict toxicity but did consistently over predict toxicity based on the

concentration of major ions. Complex matrixes of toxicity interactions between individual ions make it virtually impossible to predict toxicity based on an additive toxicity model. The Mount model offers the best option in attempting to understand toxicity of solutions containing more than one major ion present in a toxic concentration.

6. Synthetic solutions containing the concentrations of major ions are able to recreate toxicity levels recorded in treated landfill leachate. This is the first application of synthetic leachate toxicity to understand landfill leachate toxicity. This method had proved successful with produced waters and mine effluents but this is the first time used with landfill leachate. There is scope for further work from this as there are possible side interactions to investigate e.g. the introduction of Ca^{2+}.

8.4 Limitations of this work

The experimental starting point for this project was the TIE procedure. Initially this was due to be a comparative TIE procedure between a raw and a treated leachate samples from each of the sites. Due to budgetary constraints only the treated leachate samples could be collected and examined. Analysis of the results from the TIE procedure took many months and this coupled with procurement issues delayed the project. In retrospect a simpler screening exercise involving one or two species would have been a better starting point. This type of exercise could have collected results for many other sites and allowed for more repeats.

This limitation in the number of tests carried out was quickly identified. A program of monthly WET testing was put in place to overcome reliance on individual samples. The monthly testing showed that the results obtained in the TIE work was representative of overall leachate toxicity. This long term testing program demonstrated that there was little change in the quality of the leachates through the seasons.

The cost of *D. magna* test kits made it prohibitive to carry out speculative experiments i.e. trying out different combinations of major ions. Each toxicity determination with *D. magna* cost £32. All purchases needed to be authorised and paid for by the project sponsor. The project sponsor was affected due to the downturn of the economy and was unenthusiastic in purchasing new equipment and sought economies wherever possible e.g. not testing raw leachates.

Culturing of *L. minor* proved to be problematic in the later stages due to an infection being introduced into the laboratory used for growing *L. minor* it was difficult to grow enough infection free stocks. Introduction of the infection is possibly due to the Microbiology Lab fridge being shared with wastewater students as space is at a premium in the building. Two new cultures of *L. minor* were introduced but the same infections overwhelmed the new growths. This meant that *L. minor* experiments were only carried out when enough infection free cultures was available. A number of experiments with Ca^{2+}-PO_4 and *L. minor* were planned in order to overcome the lack of the expensive *D. magna* test kits.

Determination of the EC_{50} using Statistica tended to slightly overestimate the value. Determining the toxicity manually (pen and graph paper) produced a slightly lower toxicity e.g. ~5%, than when using the straight line equation method that Statistica produces. This is a limitation of the software but the slight overestimation of the toxicity is accaeptable. This overestimate could have been overcome if specialist software such as ToxCalc (McKinleyville, California, USA) though this costs >£1,000 for a single license. This software would be needed for high toxicity samples such as raw leachate.

Ceriodaphnia dubia was shown by Mount *et al* (1997) and Tietge *et al* (1997) to match the toxicity prediction model closer than *D. magna*. *C. dubia* was identified in the literature review as a possible test but due to only one report in the literature using this test with landfill leachate it was not selected. The responses of *D. magna* was lower than the equation

predicted but the responses between the treated leachate and the synthetic leachate were much closer. Under ideal circumstances *C. dubia* would have been included in the test battery but the original decision, a lack of time and funds meant that it could not be included. A long term toxicity database for *C. dubia* for use with the Mount predictor could be a very valuable next step.

Synthetic solutions have a problem in that they only contain a subset of the actual mix of ions interesting a real leachate effluent or a substitute for a particular fraction. The limitation of the approach taken in this work was the lack of a COD fraction. COD controls the chemistry of landfill leachate and not having it in the synthetic solutions was a limitation and might explain the difference in toxicity between real and synthetic solutions. COD was not added to make the experiments simpler to run. The lack of Ca^{2+} in the solutions might have made the toxicity slightly higher.

If it was possible to change the direction or the choices made in the course of this project two areas would be altered:

I. Inclusion of *Pseudokirchneriella subcapitata* (green algae) into the battery of tests. This species was in the original plan for carrying out WET testing but was abandoned when there it was realised that there was not enough orbital shakers for testing. There is a test kit available but the cost is high and it was felt that *L. minor* provided equal benefit. This test has proved to be successful for assessing the toxicity of low strength raw and treated leachates (Marttinen *et al*., 2002). In partnership with *L. minor* and D. magna, these species could make an excellent battery of tests.

II. Make use of an ion exchange resin to remove ions. Thiosulphate failed to reduce the concentration of chloride as specified in the TIE phase 1 for marine sediments. Use of these resins would exchange cations or anions and give extra evidence for the role of major ions in treated leachate toxicity.

8.5 Recommendations for further work

1. The formation of a dataset similar to the work of Mount *et al* (1997) for *L. minor*. *L. minor* was the most sensitive of the species tested in this work and is a major food source for many species in the ecosystem. From this work it is clear that *L. minor* is more sensitive to elevated concentrations of major ions. Building a database for the effect of major ions on this species would be a valuable resource.
2. Develop a better understanding of whether discharging major ion rich effluents is safe for the environment. There is a possibility that dilution of these effluents does not affect the concentration of these ions in the watercourses. The effluent from Arpley is discharged to the River Mersey and the Buckden effluent (due to increase discharges with increase in landfill size) is discharged to the River Great Ouse. Increasing salinity levels in the River Ouse could be having a detrimental effect on the health of watercourses.
3. Fish testing is a well established means for toxicity testing effluents with higher organisms. Obtaining a license for toxicity testing with fish is difficult. Testing with fish could offer valuable insights into the effects that effluent on higher organisms of an ecosystem. Toxicity testing of these treated leachates with fish will offer a valuable insight into the overall impact that effluents have on a top species within an ecosystem. This type costs >£1,000 a month.
4. Chronic WET testing is an increasing area of interest in understanding the effect of discharges over a period >30 days. This is an area which would offer a valuable insight into the hazard posed by treated landfill leachate. This testing can show if there is a long term hazard posed by these effluents and whether treatment for the removal of major ions is needed.

5. *C. dubia* testing is a possible avenue for checking whether the Mount predictor real works for treated landfill leachate. WET testing landfill leachate (both raw and treated) with *C. dubia* is a gap in the overall scientific knowledge. Testing the results with the Mount predictor will show whether the predictor is suitable for landfill leachate testing.
6. The work of Mount *et al* (1997) was possible because they had access to a culture of *D. magna*. This would allow a long term supply of test candidates without recourse to a private company. A proper culturing system would probably cost less than £500 to put in place and save a great deal of money.

References

AGG, A. R. & ZABEL, T. F. 1990. Red-list substances: Selection and monitoring. *Journal of the Institution of Water and Environmental Management,* 4**,** 44-50.

AIKEN, G. R. 1985. Isolation and concentration techniques for aquatic humic substances. *Humic Substances in Soil, Sediment, and Water***,** 363-385.

AIVASIDIS, A., MELIDIS, P. & GEORGIOU, D. 2002. Use of a microbial sensor: A new approach to the measurement of inhibitory effects on the microbial activity of activated sludge. *Bioprocess and Biosystems Engineering,* 25**,** 29-33.

AL-YAQOUT, A. F. & HAMODA, M. F. 2003. Evaluation of landfill leachate in arid climate - A case study. *Environment International,* 29**,** 593-600.

ALKASSASBEH, J. Y. M., HENG, L. Y. & SURIF, S. 2009. Toxicity testing and the effect of landfill leachate in malaysia on behavior of common carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae). *American Journal of Environmental Sciences,* 5**,** 209-217.

AMAHDAR, L., ANOUAR, A., ABABOU, B., VERSCHAEVE, L. & HILALI, A. 2009. In Vitro genotoxicity of settat town landfill leachate, morocco. *Arhiv za Higijenu Rada i Toksikologiju,* 60**,** 179-184.

AMES, B. N., LEE, F. D. & DURSTON, W. E. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proceedings of the National Academy of Sciences of the United States of America,* 70**,** 782-786.

ANDERSON, B. S., DE VLAMING, V., LARSEN, K., DEANOVIC, L. S., BIROSIK, S., SMITH, D. J., HUNT, J. W., PHILLIPS, B. M. & TJEERDEMA, R. S. 2002. Causes of ambient toxicity in the calleguas creek watershed of Southern California. *Environmental Monitoring and Assessment,* 78**,** 131-151.

APHA 2006. *Standard Methods for the examination of water and wastewater 21st Edition* Washington, AWWA.

ASSMUTH, T. & PENTTILA, S. 1995. Characteristics, determinants and interpretations of acute lethality in daphnids exposed to complex waste leachates. *Aquatic Toxicology,* 31**,** 125-141.

ATKINS, P. & DE PAULA, J. 2006. *Physical chemistry,* Oxford, Oxford University Press.

ATKINS, P., OVERTON, T., ROURKE, T. & WELLER, M. 2009. *Shriver and Atkins' Inorganic Chemistry,* Oxford, OUP.

ATWATER, J. W., JASPER, S., MAVINIC, D. S. & KOCH, F. A. 1983. Experiments using daphnia to measure landfill leachate toxicity. *Water Research,* 17**,** 1855-1861.

BABÍN, M. M., GARCÍA, P., FERNÁNDEZ, C., ALONSO, C., CARBONELL, G. & TARAZONA, J. V. 2001. Toxicological characterisation of sludge from sewage treatment plants using toxicity identification evaluation protocols based on in vitro toxicity tests. *Toxicology in Vitro,* 15**,** 519-524.

BARKER, D. J. & STUCKEY, D. C. 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Research,* 33**,** 3063-3082.

BARLAZ, M. A., HAM, R. K. & SCHAEFER, D. M. Year. Mass balance analysis of decomposed refuse in laboratory scale lysimeters. *In,* 1989 Austin, TX, USA. Publ by ASCE, 342-349.

BAUN, A., JENSEN, S. D., BJERG, P. L., CHRISTENSEN, T. H. & NYHOLM, N. 2000. Toxicity of organic chemical pollution in groundwater downgradient of a Landfill (Grindsted, Denmark). *Environmental Science and Technology,* 34**,** 1647-1652.

BAUN, A., KLØFT, L., BJERG, P. L. & NYHOLM, N. 1999. Toxicity testing of organic chemicals in groundwater polluted with landfill leachate. *Environmental Toxicology and Chemistry,* 18**,** 2046-2053.

BAUN, A., LEDIN, A., REITZEL, L. A., BJERG, P. L. & CHRISTENSEN, T. H. 2004. Xenobiotic organic compounds in leachates from ten Danish MSW landfills - Chemical analysis and toxicity tests. *Water Research,* 38**,** 3845-3858.

BAUN, D. & CHRISTENSEN, T. 2004a. Speciation of heavy metals in landfill leachate: a review. *Waste management & research,* 22**,** 3.

BAUN, D. L. & CHRISTENSEN, T. H. 2004b. Speciation of heavy metals in landfill leachate: A review. *Waste Management and Research,* 22**,** 3-23.

BEG, M. U. & AL-MUZAINI, S. 1998. Genotoxicity assay of landfill leachates. *Environmental Toxicology and Water Quality,* 13**,** 127-131.

BEHNISCH, P. A., FUJII, K., SHIOZAKI, K., KAWAKAMI, I. & SAKAI, S. I. 2001. Estrogenic and dioxin-like potency in each step of a controlled landfill leachate treatment plant in Japan. *Chemosphere,* 43**,** 977-984.

BERNARD, C., GUIDO, P., COLIN, J. & ANNE, L. D. D. 1996. Estimation of the hazard of landfllls through toxicity testing of leachates. I. Determination of leachate toxicity with a battery of acute tests. *Chemosphere,* 33**,** 2303-2320.

BERTAZZOLI, R. & PELEGRINI, R. 2002. Photoelectrochemical discoloration and degradation of organic pollutants in aqueous solutions. *Descoloração e degradação de poluentes orgânicos em soluções aquosas através do processo fotoeletroquímico,* 25**,** 477-482.

BERTHE, C., REDON, E. & FEUILLADE, G. 2008. Fractionation of the organic matter contained in leachate resulting from two modes of landfilling: An indicator of waste degradation. *Journal of Hazardous Materials,* 154**,** 262-271.

BESSER, J. M., BRUMBAUGH, W. G., MAY, T. W. & SCHMITT, C. J. 2007. Biomonitoring of lead, zinc, and cadmium in streams draining lead-mining and non-mining areas, Southeast Missouri, USA. *Environmental Monitoring and Assessment,* 129**,** 227-241.

BLAKEY, N., COSSU, R., MARIS, P. & MOSEY, F. 1992. *Anaerobic lagoons and UASB reactors: Laboratory experiments,* Amsterdam, Elsevier Applied Science Publisher.

BLIGHT, G. E., FOURIE, A. B., SHAMROCK, J., MBANDE, C. & MORRIS, J. W. F. 1999. The effect of waste composition on leachate and gas quality: A study in South Africa. *Waste Management and Research,* 17**,** 124-140.

BLOOR, M. C., BANKS, C. J. & KRIVTSOV, V. 2006. Population dynamics in Asellus aquaticus as modified by chronic leachate stress. *Engineering Geology,* 85**,** 9-13.

BORTOLOTTO, T., BERTOLDO, J. B., SILVEIRA, F. Z. D., DEFAVERI, T. M., SILVANO, J. & PICH, C. T. 2009. Evaluation of the toxic and genotoxic potential of landfill leachates using bioassays. *Environmental Toxicology and Pharmacology,* 28**,** 288-293.

BOZKURT, S., MORENO, L. & NERETNIEKS, I. 1999. Long-term fate of organics in waste deposits and its effect on metal release. *Science of the Total Environment,* 228**,** 135-152.

BRYAN, G. W. & LANGSTON, W. J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries. *Environmental Pollution,* 76**,** 89-131.

BU, L., WANG, K., ZHAO, Q. L., WEI, L. L., ZHANG, J. & YANG, J. C. 2010. Characterization of dissolved organic matter during landfill leachate treatment by sequencing batch reactor, aeration corrosive cell-Fenton, and granular activated carbon in series. *Journal of Hazardous Materials,* 179**,** 1096-1105.

CABRERA, G. L. & RODRIGUEZ, D. M. G. 1999. Genotoxicity of leachates from a landfill using three bioassays. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis,* 426**,** 207-210.

CALACE, N. & PETRONIO, B. M. 1997. Characterization of high molecular weight organic compounds in landfill leachate: Humic substances. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering,* 32**,** 2229-2244.

CAMERON, R. D. & KOCH, F. A. 1980. Toxicity of landfill leachates. *Journal of the Water Pollution Control Federation,* 52**,** 760-769.

CANTLE, J. E. 1982. *Atomic Absorption Spectrometry (Techniques and instrumentation in analytical chemistry),* New York, Elsevier Science Ltd.

CARSON, R. 1962. *Silent Spring,* New York, Penguin.

CELERE, M. S., OLIVEIRA, A. D. S., TREVILATO, T. M. B. & SEGURA-MUÑOZ, S. I. 2007. Metals in landfill leachate in Ribeirão Preto, São Paulo State, Brazil, and its relevance for public health. *Metais presentes no chorume coletado no aterro sanitário de Ribeirão Preto, São Paulo, Brasil, e sua relevância para saúde pública,* 23**,** 939-947.

CHAN, C. M., LO, W., WONG, K. Y. & CHUNG, W. F. 1999. Monitoring the toxicity of phenolic chemicals to activated sludge using a novel optical scanning respirometer. *Chemosphere,* 39**,** 1421-1432.

CHAN, Y. K., WONG, C. K., HSIEH, D. P. H., NG, S. P., LAU, T. K. & WONG, P. K. 2003. Application of a toxicity identification evaluation for a sample of effluent discharged from a dyeing factory in Hong Kong. *Environmental Toxicology,* 18**,** 312-316.

CHEN, S., KE, R., ZHA, J., WANG, Z. & KHAN, S. U. 2008. Influence of humic acid on bioavailability and toxicity of benzo[k]fluoranthene to Japanese medaka. *Environmental Science and Technology,* 42**,** 9431-9436.

CHENG VOLLMER, A. & VAN DYK, T. K. 2004. Stress responsive bacteria: Biosensors as environmental monitors. *Advances in Microbial Physiology,* 49**,** 131-174.

CHEUNG, K. C., CHU, L. M. & WONG, M. H. 1993. Toxic effect of landfill leachate on microalgae. *Water, Air, and Soil Pollution,* 69**,** 337-349.

CHO, E. A., TAMEDA, K., HANASHIMA, M., YAMADA, T. & HIGUCHI, S. 2009. Toxicological evaluation of the chemical oxidation methods for landfill stabilization. *Waste Management,* 29**,** 1006-1011.

CHRISTENSEN, J. B., JENSEN, D. L., GRØN, C., FILIP, Z. & CHRISTENSEN, T. H. 1998. Characterization of the dissolved organic carbon in landfill leachate-polluted groundwater. *Water Research,* 32**,** 125-135.

CHRISTENSEN, T. H., BJERG, P. L. & KJELDSEN, P. 2000. Natural attenuation: A feasible approach to remediation of ground water pollution at landfills? *Ground Water Monitoring and Remediation,* 20**,** 69-77.

CHRISTENSEN, T. H., KJELDSEN, P., BJERG, P. L., JENSEN, D. L., CHRISTENSEN, J. B., BAUN, A., ALBRECHTSEN, H. J. & HERON, G. 2001. Biogeochemistry of landfill leachate plumes. *Applied Geochemistry,* 16**,** 659-718.

CHU, L. M., CHEUNG, K. C. & WONG, M. H. 1994. Variations in the chemical properties of landfill leachate. *Environmental Management,* 18**,** 105-117.

CLEMENT, B. & BOUVET, Y. 1993. Assessment of landfill leachate toxicity using the duckweed Lemna minor. *Science of the Total Environment***,** 2.

CLEMENT, B. & MERLIN, G. 1995. The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Science of the Total Environment,* 170**,** 71-79.

COE 1999. Directive 1999/31/EC on the Landfill of Waste. *In:* EUROPE, C. O. (ed.). Brussels.

COMMISSION, E. 2000. Commission Decision 2000/532/EC replacing Decision 94/3/EC establishing a list of wastes pursuant to Article 1.(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of Council Directive 91/689/EEC on hazardous waste.

COOMAN, K., GAJARDO, M., NIETO, J., BORNHARDT, C. & VIDAL, G. 2003. Tannery wastewater characterization and toxicity effects on Daphnia spp. *Environmental Toxicology,* 18**,** 45-51.

COTMAN, M. & GOTVAJN, A. Z. 2009. Comparison of different physico-chemical methods for the removal of toxicants from landfill leachate. *Journal of Hazardous Materials,* In Press.

COTMAN, M. & GOTVAJN, A. Z. 2010. Comparison of different physico-chemical methods for the removal of toxicants from landfill leachate. *Journal of Hazardous Materials,* Article in press.

COWGILL, U. M., MILAZZO, D. P. & LANDENBERGER, B. D. 1991. The sensitivity of Lemna gibba G-3 and four clones of Lemna minor to eight common chemicals using a 7-day test. *Research Journal of the Water Pollution Control Federation,* 63**,** 991-998.

DAVE, G. & NILSSON, E. 2005. Increased reproductive toxicity of landfill leachate after degradation was caused by nitrite. *Aquatic Toxicology,* 73**,** 11-30.

DAVIES, T. D. & HALL, K. J. 2007. Importance of calcium in modifying the acute toxicity of sodium sulphate to Hyalella azteca and Daphnia magna. *Environmental Toxicology and Chemistry,* 26**,** 1243-1247.

DE MAAGD, P. G. J. 2000. Bioaccumulation tests applied in whole effluent assessment: A review. *Environmental Toxicology and Chemistry,* 19**,** 25-35.

DE SCHAMPHELAERE, K. A. C., FORREZ, I., DIERCKENS, K., SORGELOOS, P. & JANSSEN, C. R. 2007. Chronic toxicity of dietary copper to Daphnia magna. *Aquatic Toxicology,* 81**,** 409-418.

DEFRA. 2009. *Environment Agency report on the Landfill Allowances and Trading Scheme 2007/08* [Online]. London. Available: http://www.environment-agency.gov.uk/business/topics/waste/38989.aspx [Accessed 01.03 2010].

DEVARE, M. & BAHADIR, M. 1994. Biological monitoring of landfill leachate using plants and luminescent bacteria. *Chemosphere,* 28**,** 261-271.

DICKERSON, K. K., HUBERT, W. A. & BERGMAN, H. L. 1996. Toxicity assessment of water from lakes and wetlands receiving irrigation drain water. *Environmental Toxicology and Chemistry,* 15**,** 1097-1101.

DO NASCIMENTO FILHO, I., VON MÜHLEN, C. & CARAMÃO, E. B. 2001. Study of organic compounds in landfill leachate by spe and GC/MSD. *Estudo de compostos orgânicos em lixiviado de aterros sanitários por efs e CG/EM,* 24**,** 554-556.

DOBRANSKYTE, A., JUGDAOHSINGH, R., MCCROHAN, C. R., STUCHLIK, E., POWELL, J. J. & WHITE, K. N. 2006. Effect of humic acid on water chemistry, bioavailability and toxicity of aluminium in the freshwater snail, Lymnaea stagnalis, at neutral pH. *Environmental Pollution,* 140**,** 340-347.

DOUGLAS, W. S., GRASSO, S. S., HUTTON, D. G. & SCHROEDER, K. R. 1996. Ionic imbalance as a source of toxicity in an estuarine effluent. *Archives of Environmental Contamination and Toxicology,* 31**,** 426-432.

DOUGLAS, W. S. & HORNE, M. T. 1997. The interactive effects of essential ions and salinity on the survival of Mysidopsis bahia in 96-h acute toxicity tests of effluents discharged to marine and estuarine receiving waters. *Environmental Toxicology and Chemistry,* 16**,** 1996-2001.

DURHAM, E. J., NORBERG-KING, T. J. & BURKHARD, L. P. 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures. *In:* EPA (ed.).

DUTKA, B. J., TEICHGRÄBER, K. & LIFSHITZ, R. 1995. A modified SOS-Chromotest procedure to test for genotoxicity and cytotoxicity in sediments directly without extraction. *Chemosphere,* 31**,** 3273-3289.

ELIAS-SAMLALSINGH, N. & AGARD, J. B. R. 2004. Application of toxicity identification evaluation procedures for characterizing produced water using the tropical mysid, Metamysidopsis insularis. *Environmental Toxicology and Chemistry,* 23**,** 1194-1203.

ELLIS, M. M. 1937. Erosion silt as a factor in aquatic environment. *Ecology,* 17**,** 29–42.

EMEA 1999. Humic acids and their sodium salts. London: The European agency for the evaluation of medicinal products.

ENGEBRECHT, J., SIMON, M. & SILVERMAN, M. 1985. Measuring gene expression with light. *Science,* 227**,** 1345-1347.

EPA 1999. Update of Ambient Water Quality Criteria for Ammonia. http://www.epa.gov/waterscience/criteria/ammonia/99update.pdf.

ERNST, W. R., HENNIGAR, P., DOE, K., WADE, S. & JULIEN, G. 1994. Characterization of the chemical constituents and toxicity to aquatic organisms of a municipal landfill leachate. *Water pollution research journal of Canada,* 29**,** 89-101.

ERSES, A. S. & ONAY, T. T. 2003. In situ heavy metal attenuation in landfills under methanogenic conditions. *Journal of Hazardous Materials,* 99**,** 159-175.

EUROSTAT. 2008. *Treatment of municipal waste* [Online]. Brussels. Available: http://epp.eurostat.ec.europa.eu [Accessed 01.11.08 2008].

EVANS, L. J. 1989. Chemistry of metal retention by soils: Several processes are explained. *ES and T Contents,* 23**,** 1046-1056.

FAN, H. J., SHU, H. Y., YANG, H. S. & CHEN, W. C. 2006. Characteristics of landfill leachates in central Taiwan. *Science of the Total Environment,* 361**,** 25-37.

FATTA, D., PAPADOPOULOS, A. & LOIZIDOU, M. 1999. A study on the landfill leachate and its impact on the groundwater quality of the greater area. *Environmental Geochemistry and Health,* 21**,** 175-190.

FERETTI, D., CERETTI, E., ZERBINI, I., LIMINA, R. M., DOMINICI, L., MORETTI, M., MONARCA, S. & DONATO, F. 2009. Assessment of the genotoxic effects of gaseous emissions and percolates from three municipal landfills using plant bioassays. *Valutazione dei rischi genotossici derivanti dalla produzione di biogas e percolati in tre discariche controllate mediante test su vegetali.,* 21**,** 153-159.

FERNÁNDEZ, C., ALONSO, C., PRO, J., BABÍN, M. M., GARCÍA, P., TARAZONA, J. V. & CARBONELL, G. 2004. Toxicity Identification Evaluation of Organic Pollutants Based on Solid-Phase Micro-Extraction and Gas Chromatography/Mass Spectrometry. *Bulletin of Environmental Contamination and Toxicology,* 72**,** 903-910.

FISHER, S. W., STROMBERG, P., BRUNER, K. A. & BOULET, L. 1991. Molluscicidal activity of potassium to the zebra mussel, Dreissena polymorphia: Toxicity and mode of action. *Aquatic Toxicology,* 20**,** 219-234.

FJÄLLBORG, B., AHLBERG, G., NILSSON, E. & DAVE, G. 2005. Identification of metal toxicity in sewage sludge leachate. *Environment International,* 31**,** 25-31.

FRITHSEN, J., NACCI, D., OVIATT, C., STROBEL, C. J. & WALSH, R. (eds.) 1988. *Using single-species and whole ecosystem tests to characterise the toxicity of a sewage treatment plant effluent*: American soceity for testing and materials.

FROMMER, H., TUXEN, N. & BJERG, P. L. 2006. Fringe-controlled natural attenuation of phenoxy acids in a landfill plume: Integration of field-scale processes by reactive transport modeling. *Environmental Science and Technology,* 40**,** 4732-4738.

FULLADOSA, E., MURAT, J. C., MARTÍNEZ, M. & VILLAESCUSA, I. 2004. Effect of pH on Arsenate and Arsenite Toxicity to Luminescent Bacteria (Vibrio fischeri). *Archives of Environmental Contamination and Toxicology,* 46**,** 176-182.

GEORGE, R., MCFARLANE, D. & NULSEN, B. 1997. Salinity threatens the viability of agriculture and ecosystems in Western Australia. *Hydrogeology Journal,* 5**,** 6-21.

GESSNER, P. K. & HASAN, M. M. 1987. Freundlich and Langmuir isotherms as models for the adsorption of toxicants on activated charcoal. *Journal of Pharmaceutical Sciences,* 76**,** 319-327.

GIBBS, L. 2007. The Operation Of Marston Vale Leachate Treatment Plant. *CIWM 2007.* Paignton, U.K.

GIBSON, R., SMITH, M. D., SPARY, C. J., TYLER, C. R. & HILL, E. M. 2005. Mixtures of estrogenic contaminants in bile of fish exposed to wastewater treatment works effluents. *Environmental Science and Technology,* 39**,** 2461-2471.

GOI, A., VERESSININA, Y. & TRAPIDO, M. 2010. Fenton process for landfill leachate treatment: Evaluation of biodegradability and toxicity. *Journal of Environmental Engineering,* 136**,** 46-53.

GOODFELLOW, W. L., AUSLEY, L. W., BURTON, D. T., DENTON, D. L., DORN, P. B., GROTHE, D. R., HEBER, M. A., NORBERG-KING, T. J. & RODGERS JR, J. H. 2000. Major ion toxicity in effluents: A review with permitting recommendations. *Environmental Toxicology and Chemistry,* 19**,** 175-182.

GOSLAN, E. H., FEARING, D. A., BANKS, J., WILSON, D., HILLS, P., CAMPBELL, A. T. & PARSONS, S. A. 2002. Seasonal variations in the disinfection by-product precursor profile of a reservoir water. *Journal of Water Supply: Research and Technology - AQUA,* 51**,** 475-482.

GOTVAJN, A. Z., TIŠLER, T. & ZAGORC-KONČAN, J. 2009. Comparison of different treatment strategies for industrial landfill leachate. *Journal of Hazardous Materials,* 162**,** 1446-1456.

GREGORY, L. & SINDT, P. E. 2008. Chloride and TDS Water Quality Standards Update. Available: http://www.iawpca.org/about/govt_affairs/Chloride%20and%20TDS%20Update.pdf.

GREWAL, H. S. 2010. Water uptake, water use efficiency, plant growth and ionic balance of wheat, barley, canola and chickpea plants on a sodic vertosol with variable subsoil NaCl salinity. *Agricultural Water Management,* 97**,** 148-156.

GUERRERO, L., OMIL, F., MÉNDEZ, R. & LEMA, J. M. 1999. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. *Water Research,* 33**,** 3281-3290.

GUTIERREZ, M., ETXEBARRIA, J. & DE LAS FUENTES, L. 2002. Evaluation of wastewater toxicity: Comparative study between Microtox® and activated sludge oxygen uptake inhibition. *Water Research,* 36**,** 919-924.

HALFON, E. & REGGIANI, M. G. 1986. On ranking chemicals for environmental hazard. *Environmental Science and Technology,* 20**,** 1173-1179.

HASSELL, K. L., KEFFORD, B. J. & NUGEGODA, D. 2006. Sub-lethal and chronic salinity tolerances of three freshwater insects: Cloeon sp. and Centroptilum sp. (Ephemeroptera: Baetidae) and Chironomus sp. (Diptera: Chironomidae). *Journal of Experimental Biology,* 209**,** 4024-4032.

HAYES, K. R., DOUGLAS, W. S., TERRELL, Y., FISCHER, J., LYONS, L. A. & BRIGGS, L. J. 1993. Predictive ability of the Daphnia magna IQ Toxicity Test(TM) for ten diverse water treatment additives. *Bulletin of Environmental Contamination and Toxicology,* 51**,** 909-913.

HEINRICHS, H. & MAYER, R. 1980. The role of forest vegetation in the biogeochemical cycle of heavy metals. *Journal of Environmental Quality,* 9**,** 111-118.

HELMA, C., MERSCH-SUNDERMANN, V., HOUK, V. S., GLASBRENNER, U., KLEIN, C., WENQUING, L., KASSIE, F., SCHULTE-HERMANN, R. & KNASMU?LLER, S. 1996. Comparative evaluation of four bacterial assays for the detection of genotoxic effects in the dissolved water phases of aqueous matrices. *Environmental Science and Technology,* 30**,** 897-907.

HO, K. & BURGESS, R. M. 2007. Sediment Toxicity Identifcation Evaluation (TIE) Phase I, II, III, guidance document *In:* USEPA (ed.). Washington

HONGXIA, Y., JING, C., YUXIA, C., HUIHUA, S., ZHONGHAI, D. & HONGJUN, J. 2004. Application of toxicity identification evaluation procedures on wastewaters and sludge from a municipal sewage treatment works with industrial inputs. *Ecotoxicology and Environmental Safety,* 57**,** 426-430.

HOOPER, K., ISKANDER, M., SIVIA, G., HUSSEIN, F., HSU, J., DEGUZMAN, M., ODION, Z., ILEJAY, Z., FRED, S. Y., PETREAS, M. & SIMMONS, B. 1998. Toxicity characteristic leaching procedure fails to extract oxoanion- forming elements that are extracted by municipal solid waste leachates. *Environmental Science and Technology,* 32**,** 3825-3830.

HORANE 1991. *Biological Waste Water Treatment,* London., John Wiley and Sons Ltd.

HUO, S., XI, B., YU, H., HE, L., FAN, S. & LIU, H. 2008. Characteristics of dissolved organic matter (DOM) in leachate with different landfill ages. *Journal of Environmental Sciences,* 20**,** 492-498.

HUO, S. L., XI, B. D., YU, H. C. & LIU, H. L. 2009. Dissolved organic matter in leachate from different treatment processes. *Water and Environment Journal,* 23**,** 15-22.

ISIDORI, M., LAVORGNA, M., NARDELLI, A. & PARRELLA, A. 2003. Toxicity identification evaluation of leachates from municipal solid waste landfills: A multispecies approach. *Chemosphere,* 52**,** 85-94.

ISO 1998. 11348-2 Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 2: Method using liquid-dried bacteria.

ISO 1999. 15522 Water quality—Determination of the inhibitory effect of water constituents on the growth of activated sludge microorganisms.

ISO 2004. Water quality: Freshwater algal growth inhibition test with unicellular green algae. ISO.

JENSEN, D., LEDIN, A. & CHRISTENSEN, T. 1999. Speciation of heavy metals in landfill-leachate polluted groundwater. *Water Research,* 33**,** 2642-2650.

JOHNSON, I., HUTCHINGS, M., BENSTEAD, R., THAIN, J. & WHITEHOUSE, P. 2004. Bioassay selection, experimental design and quality control/assurance for use in effluent assessment and control. *Ecotoxicology,* 13**,** 437-447.

JURKONIENE, S., MAKSIMOV, G., DARGINAVIC?IENE, J., SADAUSKAS, K., VITKUS, R. & MANUSADZ?IANAS, L. 2004. Leachate toxicity assessment by responses of algae Nitellopsis obtusa membrane ATPase and cell resting potential, and with Daphtoxkit F? magna test. *Environmental Toxicology,* 19**,** 403-408.

KANG, K. H., SHIN, H. S. & PARK, H. 2002. Characterization of humic substances present in landfill leachates with different landfill ages and its implications. *Water Research,* 36**,** 4023-4032.

KEFFORD, B. J., PALMER, C. G., PAKHOMOVA, L. & NUGEGODA, D. 2004. Comparing test systems to measure the salinity tolerance of freshwater invertebrates. *Water SA,* 30**,** 499-506.

KENNEDY, A. J., CHERRY, D. S. & CURRIE, R. J. 2004. Evaluation of ecologically relevant bioassays for a lotic system impacted by a coal-mine effluent, using Isonychia. *Environmental Monitoring and Assessment,* 95**,** 37-55.

KHANGAROT, B. S. & RAY, P. K. 1989. Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea Daphnia magna Straus. *Ecotoxicology and Environmental Safety,* 18**,** 109-120.

KIM, E., JUN, Y. R., JO, H. J., SHIM, S. B. & JUNG, J. 2008. Toxicity identification in metal plating effluent: Implications in establishing effluent discharge limits using bioassays in Korea. *Marine Pollution Bulletin,* 57**,** 637-644.

KISS, I., KOVÁTS, N. & SZALAY, T. 2001. Role of environmental factors on the reproducibility of Lemna test. *Acta Biologica Hungarica,* 52**,** 179-185.

KJELDSEN, P., BARLAZ, M. A., ROOKER, A. P., BAUN, A., LEDIN, A. & CHRISTENSEN, T. H. 2002. Present and long-term composition of MSW landfill leachate: A review. *Critical Reviews in Environmental Science and Technology,* 32**,** 297-336.

KOZLOVA, T., WOOD, C. M. & MCGEER, J. C. 2009. The effect of water chemistry on the acute toxicity of nickel to the cladoceran Daphnia pulex and the development of a biotic ligand model. *Aquatic Toxicology,* 91**,** 221-228.

KROSS, B. C. & CHERRYHOLMES, K. (eds.) 1993. *Toxicity screening of sanitary landfill leachates: a comparative evaluation with Microtox analyses, chemical, and other toxicity screening methods,* Weinheim, Germany: VCH Verlagsgesellschaft.

KUCZYNSKA, A., WOSKA, L. & NAMIESNIK, J. 2006. Integrated approach for evaluation of pollution level of underground water in the vicinity of a municipal landfill. *Fresenius Environmental Bulletin,* 15**,** 692-696.

KURNIAWAN, T. A., LO, W. H. & CHAN, G. Y. S. 2006a. Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate. *Journal of Hazardous Materials,* 129**,** 80-100.

KURNIAWAN, T. A., LO, W. H. & CHAN, G. Y. S. 2006b. Radicals-catalyzed oxidation reactions for degradation of recalcitrant compounds from landfill leachate. *Chemical Engineering Journal,* 125**,** 35-57.

KWOK, Y. C., HSIEH, D. P. H. & WONG, P. K. 2005. Toxicity identification evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. *Marine Pollution Bulletin,* 51**,** 1085-1091.

LABRA, M., BERNASCONI, M., GRASSI, F., DE MATTIA, F., SGORBATI, S., AIROLDI, R. & CITTERIO, S. 2007. Toxic and genotoxic effects of potassium dichromate in Pseudokirchneriella subcapitata detected by microscopy and AFLP marker analysis. *Aquatic Botany,* 86**,** 229-235.

LATIF, M. & LICEK, E. 2004. Toxicity assessment of wastewaters, river waters, and sediments in Austria using cost-effective microbiotests. *Environmental Toxicology,* 19**,** 302-309.

LEDIN, A., REITZEL, L. A. & BJERG, P. L. 2005. Quantitative determination of toluene, ethylbenzene, and xylene degradation products in contaminated groundwater by solid-phase extraction and in-vial derivatization. *International Journal of Environmental Analytical Chemistry,* 85**,** 1075-1087.

LEE, K. M. & LIM, P. E. 2005. Bioregeneration of powdered activated carbon in the treatment of alkyl-substituted phenolic compounds in simultaneous adsorption and biodegradation processes. *Chemosphere,* 58**,** 407-416.

LEPANE, V. 1999. Comparison of XAD resins for the isolation of humic substances from seawater. *Journal of Chromatography A,* 845**,** 329-335.

LI, H. J., ZHAO, Y. C. & ZHANG, W. H. 2008. Resin adsorption for post-treatment of landfill leachate after a biological technique. *Hunan Daxue Xuebao/Journal of Hunan University Natural Sciences,* 35**,** 70-73.

LLOYD, R. (ed.) 1987. *Special Tests in Aquatic Toxicity for Chemical Mixtures: Interactions and Modification of Response by Variation of Physicochemical Conditions,* New York: Scope.

LO, I. 1996. Characteristics and treatment of leachates from domestic landfills. *Environment International,* 22**,** 433-442.

MA, H., KIM, S. D., ALLEN, H. E. & CHA, D. K. 2002. Effect of copper binding by suspended particulate matter on toxicity. *Environmental Toxicology and Chemistry,* 21**,** 710-714.

MACKENZIE, S. M., WAITE, S., METCALFE, D. J. & JOYCE, C. B. 2003. Landfill leachate ecotoxicity experiments using Lemna minor. *Water, Air, and Soil Pollution: Focus,* 3**,** 171-179.

MAILLARD, L. C. 1913. Formation de matières humiques par action des polypeptides sur les sucres. *Comptes Rendus de l'Académie des sciences***,** 889.

MARTÍNEZ-JERÓNIMO, F., VILLASEÑOR, R., RÍOS, G. & ESPINOSA-CHAVEZ, F. 2005. Toxicity of the crude oil water-soluble fraction and kaolin-adsorbed crude oil on Daphnia magna (Crustacea: Anomopoda). *Archives of Environmental Contamination and Toxicology,* 48**,** 444-449.

MARTTINEN, S. K., KETTUNEN, R. H., SORMUNEN, K. M., SOIMASUO, R. M. & RINTALA, J. A. 2002. Screening of physical-chemical methods for removal of organic material, nitrogen and toxicity from low strength landfill leachates. *Chemosphere,* 46**,** 851-858.

MCBRIDE, J. R., DONALDSON, E. M. & DERKSEN, G. 1979. Toxicity of landfill leachates to underyearling rainbow trout. *Bulliten of Environmental contaimination,* 23**,** 806-813.

MCCANN, J., SPINGARN, N. E., KOBORI, J. & AMES, B. N. 1975. Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. *Proceedings of the National Academy of Sciences of the United States of America,* 72**,** 979-983.

MCCULLOCH, W. L., GOODFELLOW JR, W. L. & BLACK, J. A. Year. Characterization, identification and confirmation of total dissolved solids as effluent toxicants. *In:* GORSUCH JOSEPH, W., DWYER JAMES, F., INGERSOLL CHRISTOPHER, G. & LA POINT THOMAS, W., eds., 1993 Pittsburgh, PA, USA. Publ by ASTM, 213-227.

MCDONALD, S., BISHOP, A. G., PRENZLER, P. D. & ROBARDS, K. 2004. Analytical chemistry of freshwater humic substances. *Analytica Chimica Acta,* 527**,** 105-124.

MEIGHEN, E. A. 1993. Bacterial bioluminescence: Organization, regulation, and application of the lux genes. *FASEB Journal,* 7**,** 1016-1022.

MEIGHEN, E. A. & DUNLAP, P. V. 1993. Physiological, biochemical and genetic control of bacterial bioluminescence. *Advances in Microbial Physiology,* 34**,** 1-67.

MERRICKS, T. C., CHERRY, D. S., ZIPPER, C. E., CURRIE, R. J. & VALENTI, T. W. 2007. Coal-mine hollow fill and settling pond influences on headwater streams in Southern West Virginia, USA. *Environmental Monitoring and Assessment,* 129**,** 359-378.

METCALF & EDDY 2002. *Wastewater Engineering: Treatment and Reuse,* New York, McGraw-Hill Higher Education.

MILNE, C. J., KINNIBURGH, D. G., VAN RIEMSDIJK, W. H. & TIPPING, E. 2003. Generic NICA - Donnan model parameters for metal-ion binding by humic substances. *Environmental Science and Technology,* 37**,** 958-971.

MOR, S., RAVINDRA, K., DAHIYA, R. P. & CHANDRA, A. 2006. Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site. *Environmental Monitoring and Assessment,* 118**,** 435-456.

MOUNT, D. R., GULLEY, D. D., HOCKETT, J. R., GARRISON, T. D. & EVANS, J. M. 1997. Statistical models to predict the toxicity of major ions to Ceriodaphnia dubia, Daphnia magna and Pimephales promelas (fathead minnows). *Environmental Toxicology and Chemistry,* 16**,** 2009-2019.

NARITA, N., TAKAHASHI, M. & SHOJI, R. 2005. Rapid activated sludge respiration inhibition test performed by CO 2 producing rate using a carbon dioxide sensor. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering,* 40**,** 1987-1996.

NICHOLSON, R. V., CHERRY, J. A. & REARDON, E. J. 1983. Migration of contaminants in groundwater at a landfill: A case study 6. Hydrogeochemistry. *Journal of Hydrology,* 63**,** 131-176.

NIELSEN, D. L., BROCK, M. A., REES, G. N. & BALDWIN, D. S. 2003a. effect of salinity fluctuations on freshwater macroinvertebrates *Australian Journal of Botany***,** 655-666.

NIELSEN, D. L., BROCK, M. A., REES, G. N. & BALDWIN, D. S. 2003b. Effects of increasing salinity on freshwater ecosystems in Australia. *Australian Journal of Botany,* 51**,** 655-665.

NOHAVA, M., VOGEL, W. R. & GAUGITSCH, H. 1995. Evaluation of the luminescent bacteria bioassay for the estimation of the toxicological potential of effluent water samples - Comparison with data from chemical analyses. *Environment International,* 21**,** 33-37.

NORBERG-KING, T. J., DURHAN, E. J., ANKLEY, G. T. & ROBERT, E. 1991. Application of toxicity identification evaluation procedures to the ambient waters of the Colusa basin drain, California. *Environmental Toxicology and Chemistry,* 10**,** 891-900.

NORTHCOTT, G. L. & JONES, K. C. 2000. Experimental approaches and analytical techniques for determining organic compound bound residues in soil and sediment. *Environmental Pollution,* 108**,** 19-43.

OECD 1992. 203 guideline for testing chemicals; Fish, Acute Toxicity Test. Paris, France.

OECD 1993. 209 Guideline for the testing of chemicals 209. Activated sludge respiration inhibition test. Paris, France.

OECD 1998. 211 Daphnia magna reproduction test, OECD Guidelines for Testing of Chemicals. Paris, France.

OECD 2002. 221 OECD guidelines for the testing of chemicals, revised proposal for a new guideline., *Lemna* sp. Growth Inhibition Test. Paris, France.

OECD 2006. OECD 221. *Lemna sp*. growth inhibition test.

OKAMURA, H., KUCHII, M., KOSE, T., MOHRI, S., YAMADA, M. & ONO, Y. 2005. Phytotoxic effects of landfill leachate in Japan. *Tenth International Waste Management and Landfill Symposium.* Sardinia.

OMURA, M., INAMASU, T. & ISHINISHI, N. 1992. Mutagenic activity of the leachate of municipal solid waste landfill. *Mutation Research - Genetic Toxicology Testing and Biomonitoring of Environmental or Occupational Exposure,* 298**,** 125-129.

OSAKI, K., KASHIWADA, S., TATARAZAKO, N. & ONO, Y. 2006. Toxicity testing of leachate from waste landfills using medaka (oryzias latipes) for monitoring environmental safety. *Environmental Monitoring and Assessment,* 117**,** 73-84.

OZTURK, I., ALTINBAS, M., KOYUNCU, I., ARIKAN, O. & GOMEC-YANGIN, C. 2003. Advanced physico-chemical treatment experiences on young municipal landfill leachates. *Waste Management,* 23**,** 441-446.

PAN. 2010. *Chemical Toxicity Studies on Aquatic Organisms* [Online]. Available: http://www.pesticideinfo.org/Search_Chemicals.jsp#ChemSearch [Accessed 22.06.10 2010].

PANNO, S. V., HACKLEY, K. C., HWANG, H. H., GREENBERG, S. E., KRAPAC, I. G., LANDSBERGER, S. & O'KELLY, D. J. 2006. Characterization and identification of Na-Cl sources in ground water. *Ground Water,* 44**,** 176-187.

PARK, S., JOE, K. S., HAN, S. H. & KIM, H. S. 1999. Characteristics of dissolved organic carbon in the leachate from Moonam sanitary landfill. *Environmental Technology,* 20**,** 419-424.

PERSOONE, G. 1999. THAMNOTOXKIT F^{TM} – Crustacean toxicity screening test for freshwater. *Standard operational procedure. Belgium***,** p 3–21.

PERSOONE, J., DE COEN 2000. *New Microbiotests for Routine Toxicity Screening and Biomonitoring* Kluwer Academic / Plenum Publishers

PETERS, C. S. 1998. Investigative and Management Techniques for Cement Kiln Dust and Pulp and Paper Process Wastes. *Environmental Progress,* 17**,** 142-147.

PINHO, G. L. L., PEDROSO, M. S., RODRIGUES, S. C., SOUZA, S. S. D. & BIANCHINI, A. 2007. Physiological effects of copper in the euryhaline copepod Acartia tonsa: Waterborne versus waterborne plus dietborne exposure. *Aquatic Toxicology,* 84**,** 62-70.

PIVATO, A. & GASPARI, L. 2006. Acute toxicity test of leachates from traditional and sustainable landfills using luminescent bacteria. *Waste Management,* 26**,** 1148-1155.

PLOTKIN, S. & RAM, N. M. 1984. Multiple bioassays to assess the toxicity of a sanitary landfill leachate. *Archives of Environmental Contamination and Toxicology,* 13**,** 197-206.

REYES, F., CHAMORRO, S., YEBER, M. C. & VIDAL, G. 2009. Characterization of E1 Kraft mill effluent by toxicity identification evaluation methodology. *Water, Air, and Soil Pollution,* 199**,** 183-190.

ROBINSON, A. H. 2005. Landfill leachate treatment. *Membrane Technology***,** 6-12.

ROBINSON, A. H., CARVILLE, M. & WALSH, T. 2003a. Advanced leachate treatment at Buckden landfill, Huntingdon, U.K. *Journal of Environmental Engineering and Science,* 2**,** 255-264.

ROBINSON, H. D. 2007. *Leachate treatment Introduction* [Online]. Available: http://www.leachate.co.uk/html/treatment.html [Accessed 05.07.10].

ROBINSON, H. D. & BARR, M. J. 1999. Aerobic biological treatment of landfill leachates. *Waste Management and Research,* 17**,** 478-486.

ROBINSON, H. D., BARR, M. J. & LAST, S. D. 1992. Leachate collection, treatment and disposal. *Journal of the Institution of Water and Environmental Management,* 6**,** 321-332.

ROBINSON, H. D., FARROW, S., LAST, S. D. & JONES, D. 2003b. Remediation of Leachate Problems at Arpley Landfill Site. *Ninth International Waste Management and Landfill Symposium.* Sardinia

RODRÍGUEZ, J., CASTRILLÓN, L., MARAÑÓN, E., SASTRE, H. & FERNÁNDEZ, E. 2004. Removal of non-biodegradable organic matter from landfill leachates by adsorption. *Water Research,* 38**,** 3297-3303.

ROJICKOVA-PADRTOVA, R., MARSALEK, B. & HOLOUBEK, I. 1998. Evaluation of alternative and standard toxicity assays for screening of environmental samples: Selection of an optimal test battery. *Chemosphere,* 37**,** 495-507.

ROSAL, R., RODEA-PALOMARES, I., BOLTES, K., FERNÁNDEZ-PIÑAS, F., LEGANÉS, F. & PETRE, A. 2010. Ecotoxicological assessment of surfactants in the

aquatic environment: Combined toxicity of docusate sodium with chlorinated pollutants. *Chemosphere,* In Press.
ROWE, R. K. 1995. Leachate characteristics for MSW landfills. *Geotechnical research centre report.* London.
ROWE, R. K., ISLAM, M. Z. & HSUAN, Y. G. 2008. Leachate chemical composition effects on OIT depletion in an HDPE geomembrane. *Geosynthetics International,* 15**,** 136-151.
ROWE, R. K., VANGULCK, J. F. & MILLWARD, S. C. 2002. Biologically induced clogging of a granular medium permeated with synthetic leachate. *Journal of Environmental Engineering and Science,* 1**,** 135-156.
RUCK, J. G., MARTIN, M. & MABON, M. (eds.) 2000. *Evaluation of toxkits as methods for monitoring water quality in New Zealand. In New microbiotests for routine toxicity screening and biomonitoring,* New York, USA: Kluwer Academic/Plenum.
RUTHERFORD, L. A., MATTHEWS, S. L., DOE, K. G. & JULIEN, G. R. J. 2000. Aquatic toxicity and environmental impact of leachate discharges from a municipal landfill. *Water Quality Research Journal of Canada,* 35**,** 39-57.
SANG, N. & LI, G. 2004. Genotoxicity of municipal landfill leachate on root tips of Vicia faba. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis,* 560**,** 159-165.
SANG, N., LI, G. & XIN, X. 2006. Municipal landfill leachate induces cytogenetic damage in root tips of Hordeum vulgare. *Ecotoxicology and Environmental Safety,* 63**,** 469-473.
SCHIFF, K. C., D.J. REISH, J.W. ANDERSON, AND S.M. BAY (ed.) 1992. *A Comparative Evaluation of Produced Water Toxicity,* New York: Plenum Press.
SCHRAB, G. E., BROWN, K. W. & DONNELLY, K. C. 1993. Acute and genetic toxicity of municipal landfill leachate. *Water, Air, and Soil Pollution,* 69**,** 99-112.
SELIVANOVSKAYA, S. Y., LATYPOVA, V. Z., STEPANOVA, N. Y. & HUNG, Y. 2004. *Bioassay of industrial waste pollutants,* Boca Raton, FL., USA., CRC Press.
SENESI, N. 1996. Chemical and physio-chemical parameters for quality evaluation of humic substances produced udring composting. *In:* BERTOLDI, M. D. (ed.) *The Science of Composting.* New York: Springer.
SHUMAN, L. M. 1998. Effect of organic waste amendments on cadmium and lead in soil fractions of two soils. *Communications in Soil Science and Plant Analysis,* 29**,** 2939-2952.
SILVA, A. C., DEZOTTI, M. & SANT'ANNA JR, G. L. 2004. Treatment and detoxification of a sanitary landfill leachate. *Chemosphere,* 55**,** 207-214.
SINGH, G. & BHATI, M. 2008. Changing effluent chemistry affect survival, growth and physiological function of Acacia nilotica seedlings in northwestern region of India. *Environmentalist,* 28**,** 175-184.
SLACK, R. J., GRONOW, J. R. & VOULVOULIS, N. 2004. Hazardous components of household waste. *Critical Reviews in Environmental Science and Technology,* 34**,** 419-445.
SLACK, R. J., GRONOW, J. R. & VOULVOULIS, N. 2005. Household hazardous waste in municipal landfills: Contaminants in leachate. *Science of the Total Environment,* 337**,** 119-137.
SLOMCZYNSKA, B., WASOWSKI, J. & SLOMCZYNSKI, T. 2004. Effect of advanced oxidation processes on the toxicity of municipal landfill leachates. *Water Science and Technology,* 49**,** 273-277.
SLOOFF, W. 1983. Benthic macroinvertebrates and water quality assessment: Some toxicological considerations. *Aquatic Toxicology,* 4**,** 73-82.

SMOLDERS, R., BAILLIEUL, M. & BLUST, R. 2005. Relationship between the energy status of Daphnia magna and its sensitivity to environmental stress. *Aquatic Toxicology,* 73**,** 155-170.

SOUCEK, D. J. 2007. Sodium sulfate impacts feeding, specific dynamic action, and growth rate in the freshwater bivalve Corbicula fluminea. *Aquatic Toxicology,* 83**,** 315-322.

SOUCEK, D. J. & KENNEDY, A. J. 2005. Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. *Environmental Toxicology and Chemistry,* 24**,** 1204-1210.

SOYLAK, M. & ELCI, L. 1997. Preconcentration and separation of trace metal ions from sea water samples by sorption on amberlite XAD-16 after complexation with sodium diethyl dithiocarbamate. *International Journal of Environmental Analytical Chemistry,* 66**,** 51-59.

STEELE, H. L. & HARDY, J. K. 2009. Permeation sampling of phthalate esters in water. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering,* 44**,** 340-345.

STEVENSON, F. J. 1994. *Humus Chemistry: Genesis, Composition, Reactions,* New York, Wiley

STRONKHORST, J., SCHIPPER, C., BRILS, J., DUBBELDAM, M., POSTMA, J. & VAN DE HOEVEN, N. 2003. Using marine bioassays to classify the toxicity of Dutch harbor sediments. *Environmental Toxicology and Chemistry,* 22**,** 1535-1547.

STRYER, L., BERG, J. M. & TYMOCZKO, J. L. 2002. *Biocehmistry,* New York, W. H. Freeman.

SURMACZ-GORSKA, J., BODZEK, M. & HUNG, Y. 2004. *Handbook of Industrial and Hazardous Wastes Treatment,* Cleveland, CRC Press.

SVENSON, A., SANDÉN, B., DALHAMMAR, G., REMBERGER, M. & KAJ, L. 2000. Toxicity identification and evaluation of nitrification inhibitors in wastewaters. *Environmental Toxicology,* 15**,** 527-532.

SVENSSON, B. M., MATHIASSON, L., MÅRTENSSON, L. & BERGSTRÖM, S. 2005. Artemia salina as test organism for assessment of acute toxicity of leachate water from landfills. *Environmental Monitoring and Assessment,* 102**,** 309-321.

TAKIGAMI, H., MATSUI, S., MATSUDA, T. & SHIMIZU, Y. 2002. The Bacillus subtilis rec-assay: A powerful tool for the detection of genotoxic substances in the water environment. Prospect for assessing potential impact of pollutants from stabilized wastes. *Waste Management,* 22**,** 209-213.

TALORETE, T., LIMAM, A., KAWANO, M., JENHANI, A. B. R., GHRABI, A. & ISODA, H. 2008. Stress response of mammalian cells incubated with landfill leachate. *Environmental Toxicology and Chemistry,* 27**,** 1084-1092.

THOMAS, D. J. L., TYRREL, S. F., SMITH, R. & FARROW, S. 2009. Bioassays for the evaluation of landfill leachate toxicity. *Journal of Toxicology and Environmental Health - Part B: Critical Reviews,* 12**,** 83-105.

TIETGE, J. E., HOCKETT, J. R. & EVANS, J. M. 1997. Major ion toxicity of six produced waters to three freshwater species: Application of ion toxicity models and TIE procedures. *Environmental Toxicology and Chemistry,* 16**,** 2002-2008.

TKATCHEVA, V., HOLOPAINEN, I. J., HYVÄRINEN, H. & KUKKONEN, J. V. K. 2007. The responses of rainbow trout gills to high lithium and potassium concentrations in water. *Ecotoxicology and Environmental Safety,* 68**,** 419-425.

TODDINGTON, R., PANKHANIA, M. & CLARK, E. C. 2005. Advanced leachate treatment at the Stewartby landfill site. *Journal of the Chartered Institution of Water and Environmental Management,* 19**,** 264-271.

TONKES, D. B., LEVERETT 2005. *Bioassays and tiered approaches for monitoring surface water quality and effluents*, CRC Press Inc.

TOUSSAINT, M. W., SHEDD, T. R., VAN DER SCHALIE, W. H. & LEATHER, G. R. 1995. A comparison of standard acute toxicity tests with rapid-screening toxicity tests. *Environmental Toxicology and Chemistry,* 14**,** 907-915.

TRABELSI, I., HORIBE, H., TANAKA, N. & MATSUTO, T. 2000. Origin of low carbon/nitrogen ratios in leachate from old municipal solid waste landfills. *Waste Management and Research,* 18**,** 224-234.

TSAI, K. P. & CHEN, C. Y. 2007. An algal toxicity database of organic toxicants derived by a closed-system technique. *Environmental Toxicology and Chemistry,* 26**,** 1931-1939.

USEPA 1985. Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Washington, DC: USEPA.

USEPA 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to

freshwater organism. Ohio, USA: U.S. EPA.

VAN HOECKE, K., DE SCHAMPHELAERE, K. A. C., VAN DER MEEREN, P., LUCAS, S. & JANSSEN, C. R. 2008. Ecotoxicity of silica nanoparticles to the green alga Pseudokirchneriella subcapitata: Importance of surface area. *Environmental Toxicology and Chemistry,* 27**,** 1948-1957.

VAN STEVENINCK, R. F. M., VAN STEVENINCK, M. E. & FERNANDO, D. R. 1992. Heavy-metal (Zn, Cd) tolerance in selected clones of duck weed (Lemna minor). *Plant and Soil,* 146**,** 271-280.

VAN STRAALEN, N. M. 2003. Ecotoxicology Becomes stress Ecology. *Environmental Science and Technology,* 37**,** 324-330.

VAN ZOMEREN, A. & COMANS, R. N. J. 2007. Measurement of humic and fulvic acid concentrations and dissolution properties by a rapid ratch procedure. *Environmental Science and Technology,* 41**,** 6755-6761.

VASSEUR, P., BOIS, F., FERARD, J. F. & RAST, C. 1986. Influence of physicochemical parameters on the Microtox test response. *Toxicity Assessment,* 1**,** 283-300.

WAGLAND, S. 2008. *Development and Evaluation of a Rapid Enzymatic. Hydrolysis Test Method to Assess the Biodegradability of Organic Waste.* PhD, Cranfield University.

WAKSMAN, S. A. 1932. *Humus,* Baltimore, Williams and Wilkins.

WANG, F., SMITH, D. W. & EL-DIN, M. G. 2003. Application of advanced oxidation methods for landfill leachate treatment – A review. *Journal of Environmental Engineering and Science***,** 413-427.

WANG, X., SHEN, L., YU, H. & WANG, D. 2008. Toxicity evaluation in a paper recycling mill effluent by coupling bioindicator of aging with the toxicity identification evaluation method in nematode Caenorhabditis elegans. *Journal of Environmental Sciences,* 20**,** 1373-1380.

WARD, M. L., BITTON, G. & TOWNSEND, T. 2005. Heavy metal binding capacity (HMBC) of municipal solid waste landfill leachates. *Chemosphere,* 60**,** 206-215.

WARD, M. L., BITTON, G., TOWNSEND, T. & BOOTH, M. 2002. Determining toxicity of leachates from Florida municipal solid waste landfills using a battery-of-tests approach. *Environmental Toxicology,* 17**,** 258-266.

WEBB, N. A. & WOOD, C. M. 1998. Physiological analysis of the stress response associated with acute silver nitrate exposure in freshwater rainbow trout (Oncorhynchus mykiss). *Environmental Toxicology and Chemistry,* 17**,** 579-588.

WEBER-SCANNELL, P. K. & DUFFY, L. K. 2007. Effects of total dissolved solids on aquatic organisms: A review of literature and recommendation for salmonid species. *American Journal of Environmental Sciences,* 3**,** 1-6.

WEINBERG, H. & NARKIS, N. 1987. Physico-chemical treatments for the complete removal of non-ionic surfactants from effluents. *Environmental Pollution,* 45**,** 245-260.

WERNER, I., DEANOVIC, L. A., CONNOR, V., DE VLAMING, V., BAILEY, H. C. & HINTON, D. E. 2000. Insecticide-caused toxicity to Ceriodaphnia dubia (Cladocera) in the Sacramento-San Joaquin River Delta, California, USA. *Environmental Toxicology and Chemistry,* 19**,** 215-227.

WILLIAMS, P. T. 2005. *Waste Treatment and Disposal,* Oxford, U.K., WileyBlackwell.

WITTEKINDT, E. F., BIRGIT AND HANSEN, PETER-DIEDRICH 2000. Genotoxicity assay: umu-test (ISO/DIS 13829, 2000). *TECHNOTOX-Workshop.* Mol, Belgium

WOLSKA, L., KUCZYN?SKA, A. & NAMIES?NIK, J. 2006. Quality of groundwater contaminated by leachates from seven Polish landfills - Chemical and ecotoxicological classifications. *Toxicological and Environmental Chemistry,* 88**,** 501-513.

WONG, M. H. 1989. Toxicity test of landfill leachate using Sarotherodon mossambicus (freshwater fish). *Ecotoxicology and Environmental Safety,* 17**,** 149-156.

XI, B. D., WEI, Z. M., ZHAO, Y., LI, M. X., LIU, H. L., JIANG, Y. H., HE, X. S. & YANG, T. X. 2008. Study on fluorescence characteristic of dissolved organic matter from municipal solid waste landfill leachate. *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis,* 28**,** 2605-2608.

YANG, K. & XING, B. 2009. Sorption of phenanthrene by humic acid-coated nanosized TiO₂ and ZnO. *Environmental Science and Technology,* 43**,** 1845-1851.

YANG, W., GAN, J., HUNTER, W. & SPURLOCK, F. 2006. Effect of suspended solids on bioavailability of pyrethroid insecticides. *Environmental Toxicology and Chemistry,* 25**,** 1585-1591.

YATRIBI, A. & NEJMEDDINE, A. 2000. Heavy metals fractionnation and mobility in soil downstream of tannery wastewaters. *Fractionnement et mobilité des métaux lourds dans un sol recevant des eaux usées de tanneries,* 13**,** 203-212.

ŽGAJNAR GOTVAJN, A., DERCO, J., TIŠLER, T., COTMAN, M. & ZAGORC-KONČAN, J. 2009. Removal of organics from different types of landfill leachate by ozonation. 60**,** 597-603.

ŽGAJNAR GOTVAJN, A. & ZAGORC-KONČAN, J. 2009. Identification of inhibitory effects of industrial effluents on nitrification. *Water Science and Technology,* 59**,** 797-803.

ZHANG, J. Z., YANG, Q., XI, B. D., WEI, Z. M., HE, X. S., LI, M. X. & YANG, T. X. 2008. Study on spectral characteristic of dissolved organic matter fractions extracted from municipal solid waste landfill leachate. *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis,* 28**,** 2583-2587.

Appendix 1