THE USE OF CONSTRUCTED WETLANDS FOR THE TREATMENT OF URBAN RUNOFF

LIAN N L SCHOLES

Urban Pollution Research Centre
Middlesex University

Submitted in partial fulfilment of the requirements of
Middlesex University for the degree of Doctor of Philosophy

December 1999

ABSTRACT

In 1995, the Environment Agency for England and Wales developed urban runoff treatment wetlands at two selected locations in Outer London. The systems have been monitored for a wide range of parameters including heavy metals, suspended solids and BOD over a period of two years. Seven storm events were also monitored. The ability of micro-organisms, isolated from the rhizosphere of wetland plants collected at both systems, to tolerate and accumulate heavy metals has also been investigated.

This study has demonstrated that constructed wetland treatment systems are capable of reducing the pollutant loadings associated with urban runoff, and that such systems can be successfully established within urban areas. During dry weather, pollutant concentrations and loadings were typically low and associated removal efficiencies highly variable. However, during storm events, pollutant loadings increased and removal efficiencies improved, with mean removal efficiencies of 71% for Zn, 69% for Pb and 81% for Cr at the Dagenham wetland. An exception to this was for suspended solids which showed an overall increase of 99% during storm events. Several design and operational issues have been identified and addressed during the course of the monitoring programme, and recommendations for the improved design and operation of urban runoff treatment wetland systems have been made.

A range of micro-organisms, isolated from both wetland systems, were able to tolerate elevated Zn and Pb concentrations. Two strains (*Beauveria bassiana* and *Rhodotorula mucilaginosa*) were selected for further work. Both strains could accumulate Zn and Pb, with *B. bassiana* showing a high capacity to bind Pb (maximum concentration of 136mgPb/g cells dry weight). Comparison of the growth of *B. bassiana* at 4°C and 30°C suggested that processes of microbial metal accumulation may occur throughout the year in treatment wetlands. The presence of Pb inside hyphae of *B. bassiana*, associated with hyphae walls and in the surrounding medium was confirmed. This study has found that micro-organisms isolated from urban runoff treatment wetlands can tolerate and accumulate Zn and Pb, and the application of these results to wetland treatment processes is discussed.

CONTENTS

page

SECTION TITLES

TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisors Professor Brian Shutes, Professor Mike Revitt, Dr Diane Purchase and Dr Maxine Forshaw for all their valuable guidance and advice throughout my research. I greatly appreciate all their help, moral support and good humour, without which this thesis would never have been completed. I would also like to thank Dr Tony Brain (Kings College, London), Dr Alice Warely (St Thomas' Hospital, London) and Dr John Stephen (University of Tennessee, USA) for their time and help with the TEM analysis and identification of the isolates. I would also like to gratefully acknowledge the Engineering and Physical Sciences Research Council and the Environment Agency for their support of this project.

I would like to thank the many people in the Urban Pollution Research Centre, past and present, for all their help and support. In particular, I would like to thank Alan La Grue (to whom I leave all my teflon beakers) and Paul Kershaw for all their technical support and banter. I would also especially like to thank Michaela Kendall, Arvind Mungur, Giusi Merlo Pich, Huw Jones and Hoshi Sepai for listening to all my traumas and for many valuable discussions.

I would like to thank my best friends Al Chisholm and Maria Greaves for putting-up with me for all these years, for listening and laughing, and for always being there. I am very grateful to my family for all their love and support and promise now to cut my hair and get a job! I would especially like to thank Jeremy Askew for his non-stop encouragement, for telling me my work was important(!), and who now knows more about reed beds than is probably required for a independent financial advisor. And finally, to my mum and J's mum, who I remember with much love and admiration, and to whose memories this thesis is dedicated.

CHAPTER ONE GENERAL INTRODUCTION

1.1 Background

Urban growth and development, through the construction of buildings, roads and other impermeable surfaces, alters the natural hydrological cycle, changing peak flow characteristics and the volume and quality of runoff (Revitt *et al.,* 1999). In the UK, urban areas are generally designed so that surface water drains directly to the closest watercourse as quickly as possible to prevent flooding. However, this approach ignores the potential pollutant loads generated from urban runoff and their impacts on receiving waters.

The use of constructed wetland systems to treat urban runoff is an emerging technology in the UK, with preliminary studies reporting the efficient removal of a range of pollutants from the water column (Mungur *et al.,* 1998, Shutes *et al.,* 1997, Cooper *et al.,* 1996). However, this treatment procedure still involves some uncertainty as the conventional approach of designing a constructed wetland for treating wastewater of a characteristic quality and quantity is inappropriate for urban runoff as both the pollutant loads and hydrological responses are highly variable. There are currently no established design and performance criteria for constructed wetlands for the treatment of urban runoff. The Environment Agency for England and Wales (EA) has shown considerable interest in the use of urban runoff treatment wetlands, primarily as a treatment facility but also as an opportunity for enhancing the environment by creating a new habitat in urban areas.

Constructed wetland treatment systems are also known to be an ideal environment for a wide range of micro-organisms, and their microbial diversity and numbers have been reported as being comparable to those of natural wetland systems (Duncan and Groffman, 1994). In recent years, particularly within industry, there has been considerable interest in metal sorption by a range of biomass types as a cost-effective process for removing metals from wastewater (Zhang *et al.,* 1998). However, the metal removal capability of micro-organisms in constructed wetland treatment systems has not been investigated.

It is against this background that the Urban Pollution Research Centre (UPRC) at Middlesex University, in collaboration with the Environment Agency for England and Wales, Thames Region, undertook an investigation into the design and treatment performance of constructed wetlands receiving urban runoff. The results of this study are presented in this thesis. This work also complements and further develops previous studies carried out within the UPRC on the use of macrophytes for heavy metal control (Zhang 1990), and the use of constructed wetland systems to treat both highway runoff (Mungur, 1997) and airport runoff (Chong *et al.,* 1999). Current UPRC studies aim to gain an understanding of processes of removal occurring within wetland systems, and include investigations into the degradation of hydrocarbons using wetland lysimeters, and the role of biofilms in hydrocarbon remediation.

1.2 Aims of the research programme

The aims of this research programme, which were developed in collaboration with the Environment Agency for England and Wales, Thames Region, are:-

- to investigate the physical, chemical and biological processes which occur in constructed wetlands designed for the treatment of urban runoff, in order to obtain an understanding of the processes of pollutant degradation, uptake and bioaccumulation by different wetland components. Particular emphasis being placed on operational monitoring of the wetlands, with specific regard to water quality and bioaccumulation assessment programmes.

- to assess the water quality improvement performance achieved by two constructed wetlands, using both systems as research and development test beds to evaluate their potential use for further similar projects.

- to develop recommendations for the design and management of constructed wetlands for the treatment of urban runoff.

- to obtain a scientific understanding of the mechanisms and processes of removal and immobilisation of heavy metals by constructed wetland substrates, plant and microbial species, involving laboratory experiments to complement fieldwork.

1.3 Outline of thesis

This thesis is presented in six chapters including this Introduction. Chapter two presents a literature review of the use of wetlands as wastewater treatment systems, together with a review of the metal sorption ability of micro-organisms. In Chapter three, the two wetland systems monitored are described, and field methodology and laboratory experiments and analytical techniques are given. In Chapter four the results and statistical analysis of data collected at both treatment wetlands during dry weather and wet weather conditions over a two year monitoring programme are presented. In Chapter five, the results of a range of experiments carried out to determine the ability of micro-organisms isolated from both systems to accumulate Zn and Pb are presented and discussed. Chapter six summarises the findings of this research, and presents recommendations for further research.

1.4 Conferences

During the period of this research papers were presented at the following conferences.

- *Sixth International Conference on Wetland Systems for Water Pollution Control*, Aguas de Sao Pedro-SP, Brazil, 27 September-2 October 1998.

- *Fifth Symposium on the Biogeochemistry of Wetlands*, Royal Holloway College, University of London, 16-19 September 1997.

- *Fourth International Conference on Trace Metals in the Aquatic Environment*, Kuala Lumpur, Malaysia, 19-23 May 1997.

- *London Freshwater Group (Summer Meeting),* Middlesex University, London, 21 June 1996.

- *Seventh European Postgraduate Conference on Urban Runoff and Water Quality of Receiving Waters*, Elspeet, Holland, 6-10 May 1996.

1.5 Publications

The following papers were published. Those papers that have been peer reviewed are marked with an asterisk (*).

Scholes, L.N.L., Shutes, R.B.E., Revitt, D.M., Purchase, D. and Forshaw, M. (1999). The Removal of Urban Pollutants by Constructed Wetlands During Wet Weather. *Water Science and Technology,* 40, 333-340 (*).

Revitt, D.M., Shutes, R.B.E., Scholes, L.N.L. (1999). The Use of Constructed Wetlands for Reducing the Impacts of Urban Surface Runoff on Receiving Water Quality. In: *Impacts of Urban Growth on Surface Water and Ground Water Quality*. J. B. Ellis (ed), IAHS Press, Wallingford, 349-356 (*)

Scholes, L.N.L., Shutes, R.B.E., Revitt, D.M., Forshaw, M. and Purchase, D. (1998). The Treatment of Metals in Urban Runoff by Constructed Wetlands. *The Science of the Total Environment*, 214, 211-219 (*).

Shutes, R.B.E., Revitt, D.M., Mungur, A.S. and Scholes, L.N.L. (1997). Design of Wetland Systems for the Treatment of Urban Runoff. *Water Science and Technology*, 35, 19-25 (*).

Shutes, R.B.E., Revitt, D.M., Mungur, A.S. and Scholes, L.N.L. (1997). Design of Wetland Systems for the Treatment of Urban Runoff. *Water Quality International*, March/April 1997, 35-38.

Scholes, L.N.L. and Gallagher, P. (1996). The Public Perception and Wildlife Benefits of a Wetland System for the Treatment of Urban Runoff. *International Association on Water Quality, Specialist Group on the Use of Macrophytes for Water Pollution Control Newsletter,* No. 15, 6-7.

Scholes, L.N.L. (1996). The Sustainable Management of Urban Runoff. *Proceedings of the Erasmus Postgraduate Conference on Urban Runoff,* Elspeet, Holland, 6-10 May 1996.

Scholes, L.N.L., Shutes, R.B.E., Revitt, D.M., Forshaw, M. and Purchase, D. (1995). Constructed Wetlands and Sustainable Environmental Management in the UK. *International Association on Water Quality, Specialist Group on the Use of Macrophytes for Water Pollution Control Newsletter,* No. 13, 11-13.

CHAPTER 2 WETLAND WASTEWATER AND URBAN RUNOFF TREATMENT SYSTEMS

2.1 Wetlands as wastewater treatment systems

2.1.1 Natural and constructed wetland wastewater treatment systems

The criteria for defining a wetland appear to vary greatly both between authors and countries. However, wetlands are generally considered to be areas which are wet for long enough to alter the biological, chemical and physical properties of the soil due to flooding, and to exclude plant species that cannot grow in saturated soils, such as marshes, fens, bogs, swamps, saltmarshes and mangroves (Kadlec and Knight, 1996). Wetlands are highly productive habitats which receive, hold and recycle nutrients, and support a wide variety of vegetation and a diverse fauna (Smith, 1989). They perform many valuable functions including flood control, sediment stabilisation and groundwater recharge. In addition, they provide an important habitat for wildlife, in particular wildfowl (Merritt, 1994). However, over the last 20 years wetlands have been receiving increasing attention due to their ability to improve water quality (Hammer and Bastian, 1989). They provide effective treatment for many types of water pollution, effectively removing or converting large quantities of pollutants from point sources and non-point sources, including organic matter, suspended solids, heavy metals and excess nutrients, at a relatively low cost. This involves processes such as sedimentation, filtration, physical and chemical immobilisation, microbial interactions and uptake by vegetation.

Although both natural and constructed wetlands may act as efficient water treatment systems, there are several reasons why it is more advantageous to construct wetlands for wastewater treatment. Natural wetlands are now recognised as valuable resources which are under threat; California has lost 91% of its wetlands over the past 200 years; France has lost 67%, and Greece and Italy 63% and 66% respectively (Anderson, 1996). These losses are mainly due to the introduction of drainage procedures for agricultural purposes and the construction of roads and ports. Hence the protection of remaining sites is now an

issue of increasing public concern. Many natural wetlands are sites of national importance as wildlife habitats, for example, for migrating and resident birds. There may also be conflict over use if a natural wetland recharges groundwater which is a source of drinking water. The use of constructed wetlands avoids such potential conflicts over use (Ewel, 1987; Olson, 1992). Constructed wetlands are man-made, involving saturated substrates, emergent and submergent vegetation, animal life and water for human use and benefit (Hammer and Bastian, 1989). It is possible to exercise more control over constructed wetlands, such as the establishment of a well defined substrate composition, type of vegetation and flow pattern. In addition, constructed wetlands offer several further advantages including site selection, flexibility in sizing and control over hydraulic pathways and retention times. Hence, the use of constructed wetlands is the preferred option in the use of wetlands as wastewater treatment systems.

2.1.2 Pollutant removal processes

The removal of pollutants by constructed wetland treatment systems is the result of a complex combination of biological, chemical and physical processes. It is now realised that the representation of wetlands as simple sinks or biological filters does not adequately reflect the complex set of interactions involved in the removal of pollutants from the water column, and the role of wetlands as a wastewater treatment systems. Although the main removal processes are understood, there remains a need to understand how the processes of pollutant removal occur and interact in a wetland, and to what extent (Mackney, 1990). The major removal mechanisms are outlined in the following sections, and are summarised in Table 2.1.

2.1.2.1 Physical processes

Sedimentation is the removal of particulate matter from the water column due to gravitational settling. The process is most effective on sediments larger than 10μm with specific gravities greater than 1.0 and begins to occur when the flow is <0.2m/s (Merritt, 1994). The removal of suspended solids is an important wastewater treatment process for several reasons. Suspended solids cause turbidity in the water column which

attenuates light penetration, thereby reducing photosynthesis. If suspended solids are rich in organic matter, high levels of biochemical oxygen demand result, decreasing dissolved oxygen content. High levels of suspended solids can also cause river and stream beds to silt-up (Hemond and Benoit, 1988). However, perhaps the most important reason for the removal of suspended solids from the water column is due to the association between particulate matter and a range of pollutants such as heavy metals, nutrients, pesticides and bacteria (Duncan, 1995). Conditions for sedimentation are enhanced in wetlands as dense stands of reeds, or gravel substrate, reduce flow velocity (Reinelt and Horner, 1995), turbulence and also inhibit formation of wind generated waves (Kadlec and Knight, 1996).

Table 2. 1 Principal pollutant removal mechanisms in constructed wetland wastewater treatment systems.

Removal processes	Pollutants removed
Physical	
Sedimentation	suspended solids and associated pollutants
Filtration (litter layer)	suspended solids and associated pollutants
Adsorption	heavy metals, phosphates, pesticides, organics
Volatilisation	ammonia
Chemical	
Specific adsorption	phosphates, heavy metals
Precipitation	phosphates, heavy metals
Biological	
Biological uptake	phosphates, nitrates, ammonia, heavy metals
Nitrification and denitrification	ammonia, nitrates
Microbially-mediated oxidation and reduction	heavy metals

It was initially believed that the enhanced pollutant removal efficiency of wetlands was partly due to the suspended solids being removed by the physical filtration of wastewater through dense stands of vegetation. However, studies have shown that due to a combination of plant stem diameter, particle diameter and water velocity, the collection efficiency of such a sieving mechanism is almost negligible (Kadlec and

Knight, 1996). Wetland vegetation does, however, improve the removal of suspended solids through the buffering of incoming wastewater which decreases flow rate, thus enhancing conditions for sedimentation and reducing risk of resuspension of already settled sediments (Brix, 1996; Wong and Geiger, 1997). In addition, the mechanical trapping of sediments occurs in the litter layer.

Adsorption involves the binding of dissolved or particulate substances onto suspended solids, vegetation and settled bottom sediment, and can be either chemical (known as specific adsorption; see Section 2.1.2.2) or physical. Physical adsorption occurs when a positively charged metal ion binds to a negatively charged site on the adsorption surface due to electrostatic forces (Harrison and de Mora, 1996). This bonded cation may be displaced by a different cation which has a greater electrostatic attraction for the binding site in a process known as cation exchange, and therefore adsorption is not always a permanent process. However, it is an important mechanism in removing a range of pollutants such as phosphorus, heavy metals, pesticides and organics from the water column. Once adsorbed to suspended solids pollutants can be removed from the water column by sedimentation. Further processes of transformation and/or degradation may then occur within the deposited sediment (see Sections 2.1.2.2 and 2.1.2.3).

Volatilisation is the conversion of a liquid or solid to a vapour. Volatilisation of ammonia can occur in wetlands. Ammonium compounds may be lost from the water column by this process following conversion to ammonia (Cooper *et al.,* 1996). This only occurs under alkaline conditions (pH >9), which do not usually occur in a wetland environment. However, algal blooms can temporarily elevate pH and therefore volatilisation may at times be an important wetland removal process.

2.1.2.2 Chemical processes

Specific adsorption is the process whereby adsorbed substances are held in place by chemical bonds, resulting in the formation of a complex which is more stable than that formed by physical adsorption. Phosphates and heavy metals specifically adsorb to hydrous Fe and Al oxides, clays and organic molecules, by processes such as ligand exchange and chelation. Further processes, such as adsorption of ionic hydrates to

suspended solids followed by sedimentation, results in the removal of a range of pollutants from the water column. The overall ability of a wetland to either physically or specifically adsorb pollutants is limited by the sorptive capacity of the suspended solids, sediments and vegetation present (Hemond and Benoit, 1988).

The precipitation of insoluble compounds from the water column, again coupled with sedimentation, is another important wetland removal process. Pollutants such as phosphates and heavy metals will form insoluble complexes. Phosphates may be removed from solution by precipitation of insoluble Fe, Al and Ca phosphate and as insoluble organics (Hemond and Benoit, 1988). Heavy metals precipitate out as hydroxides, carbonates, phosphates and other salts. Precipitation, followed by sedimentation, results in the transfer of pollutants from the water column to the sediment, where further transformation processes may occur.

2.1.2.3 Biological processes

Many wetland plants and micro-organisms can bioaccumulate a range of pollutants such as those containing phosphorus, nitrogen (Brix, 1996) and heavy metals (Zhang *et al.*, 1990). Emergent vegetation primarily takes up pollutants from the sediment, floating vegetation removes pollutants directly from the water column, and submerged, rooted vegetation takes up pollutants from both the sediment and the water column (Barko and Smart, 1980). Roots and rhizomes are the main storage zones in emergent and submergent macrophytes, with lesser amounts being stored in the leaves (Kadlec and Knight, 1996). For plant uptake to occur vegetation must be actively growing, and therefore climate may dictate the importance of bioaccumulation as a pollutant removal process. For example, in a tropical climate bioaccumulation may be a year round removal process reflecting the continuous growing season, whereas in temperate climates uptake by vegetation occurs only in spring and summer, with vegetation dying back in the autumn and winter. Studies have shown that prior to this winter die-back, much of the nutrients and heavy metals stored in stems and leaves are translocated to the roots and rhizomes, and are therefore not released to the water column. The stored nutrients are used for new growth the following season (Wetzel, 1993), whereas the heavy metals remain in the below-ground section until root/rhizome death, when they

are then incorporated into the sediment (Kadlec and Knight, 1996). Although plants do not bioaccumulate during autumn and winter months in a temperate climate, micro-organisms such as fungi, bacteria and actinomycetes, many of which are dependent on plants as a carbon source or as an attachment site, remain active in the wetland throughout the year and will continue to participate in pollutant uptake processes. However, there has been little research into assessing the seasonal microbial biomass of wetlands, and into quantifying the amount and type of pollutants that microbes can accumulate/transform within a wetland environment.

Nitrification is the microbially mediated oxidation of ammonia to nitrate, and is the main ammonia reducing mechanism in many wetland systems. It is a two step process, in which ammonia is firstly oxidised to nitrite followed by further oxidation to nitrate (Kadlec and Knight, 1996). The first step is mediated primarily by the bacteria *Nitrosomonas*, and the second by *Nitrobacter*. The processes release energy which is used by the bacteria to synthesise new cells. Nitrification only occurs in the presence of oxygen and Cooper *et al.* (1996) recommend that dissolved oxygen concentrations should be >1mg/l. The minimum temperature for nitrification to occur reflects the growth requirements of *Nitrosomonas* and *Nitrobacter* which do not grow below temperatures of 5°C and 4°C respectively. The optimal pH range for this process to occur is 7.5-8.6, although systems can be acclimatised to nitrify at a lower pH values (Cooper *et al.,* 1996).

Denitrification is the reduction of nitrate to nitrogen, a process which occurs under anoxic conditions. Several genera of bacteria are capable of nitrate reduction, the most common of which are *Bacillus*, *Enterobacter*, *Micrococcus*, *Pseudomonas* and *Spirillum* (Kadlec and Knight, 1996). Denitrification is a two step process, involving the reduction of nitrate to nitrite, followed by the reduction of nitrite to nitric oxide, nitrous oxide and nitrogen, with most being lost as free nitrogen gas (Cooper *et al.,* 1996). Because nitrogen is permanently lost from the wetland, this process can continue indefinitely without damaging the system. As with nitrification, optimal conditions for denitrification reflect the optimal growth conditions of the bacteria involved. Organic matter is required as a carbon source, the optimal pH range is 7-8 and denitrification occurs only very slowly, if at all, below 5^0C (Cooper *et al.,* 1996). Many of the genera involved in denitrification are facultative anaerobes, whereby they switch to aerobic

metabolism in the presence of free oxygen. Because the use of free oxygen yields more energy than the use of nitrate, these microbes will not denitrify in the presence of free oxygen, and therefore anoxic conditions are necessary for this process to occur.

Within wetland substrates there are both aerobic zones, which tend to be rich in organic matter, and anaerobic zones, which tend to be dominated by inorganic material. Both zones support a wide range of micro-organisms, of which metal-oxidising bacteria and sulphate-reducing bacteria, found in the aerobic and anaerobic zones respectively, form important components (Cooper *et al.,* 1996). The microbially-mediated oxidation of ferrous iron by bacteria such as *Thiobacillus ferooxidans* (Fortin *et al.,* 1995), followed by precipitation of ferric iron as ferric iron oxyhydroxide is well understood (Webb *et al.,* 1998), and the mechanism involved in the oxidation and subsequent precipitation of a range of other metals, such as Pb, Zn, Cu, Ni, Ag and Au, is thought to be similar (Cooper *et al.,* 1996).

The reduction of sulphate occurs under anaerobic conditions, resulting in the production of H_2S. The most common micro-organisms involved in the process are the bacteria *Desulfotomaculum* and *Desulfovibrio* (Fortin *et al.,* 1995). H_2S ionises to give S^{2-} which readily reacts with dissolved metals, precipitating them as insoluble sulphides. It is suggested that the precipitation of metals as sulphides rather than as oxhydroxides is more desirable, as the precipitation of metal oxyhydroxides increases acidity (Webb *et al.,* 1998; Eger, 1994), which may cause the resolubilisation of precipitated metals, whereas the reduction of sulphate results in an increase in alkalinity (White *et al.,* 1997; Eger, 1994). Another advantage is that, since sulphates are precipitated within the sediment, they are less susceptible to resuspension (Cooper *et al.,* 1996).

2.1.3 Current use of constructed wetland treatment systems

Interest in constructed wetland treatment systems began in Germany in the 1970's following work by Kikuth and Seidel, and was further developed in the 1980's in several European countries, namely Denmark, France, Belgium, Austria, the Netherlands, Sweden and the UK (Cooper *et al.,* 1996; Berezowsky, 1995). At this time the UK Water Industry became interested in reed bed systems for the treatment of domestic sewage from small

villages and constructed its first system for this purpose in 1985. There are now more than 400 systems in operation in the UK alone (Cooper and Green, 1995). The majority of these systems are sub-surface flow systems (Section 2.3.1) for the secondary and tertiary treatment of domestic sewage, and therefore it is these studies which have generated the most reliable amount of data on constructed wetland treatment performance. In general, the experience gained from wetland systems has shown that BOD removal is 80-90%, total N removal is 30% and total-P removal is 30-40% (Cooper, 1993). Constructed wetlands are also popular in the USA; a database set up by the US EPA in 1991 listed over 200 wetland treatment systems (Brown, 1994). The USA experience differs from that in Europe in that the majority of these sites are large surface flow wetlands designed to treat municipal wastewater from towns and cities (Cooper *et al.,* 1996; Wood, 1994). Using results from the EPA wetland database, Knight *et al.* (1993) calculated overall removal efficiencies of 50-90% for BOD, 40-94% for total suspended solids, 30-98% for total nitrogen and 20-90% for total phosphorus. Constructed wetland treatment systems are also currently being used in many countries, including Australia, China, Egypt, Brazil, India, and South Africa.

The use of constructed wetlands to treat domestic wastewater is now considered a relatively mature technology (Hiley, 1994), and these systems are increasingly being employed for the treatment of a range of industrial effluents. However, many wetland treatment systems have had variable success, indicating that further research is required before both the pollutant removal mechanisms and treatment capabilities of constructed wetlands are fully understood (Mackney, 1990). The European Guidelines 1990, which have been revised by Cooper *et al.* (1996), the Construction Industry Research and Information Association (CIRIA) report 'Design and Management of Constructed Wetlands for the Treatment of Wastewater' (CIRIA, 1997) and the 'The Design and Performance of Vegetated Treatment Systems for Highway Runoff - Review and Feasibility Study' (Halcrow, 1996) provide recommendations for designing constructed wetlands in temperate climates. However, there are no published guidelines for systems in tropical climates.

The variation in treatment efficiency is possibly due to the early enthusiasm for wetlands, which where seen as sustainable, low cost alternatives to conventional systems, offering robustness, and simplicity in design and operation. This led to a large number of systems being constructed, without any scientific basis to their design. Reports in the literature agree that pollutant removal efficiencies in wetlands are site specific, and performance varies with parameters such as wetland hydrology, type of pre-treatment, soil type (surface flow wetlands) or media type (sub-surface flow wetlands), type and nature of plant cover and hydraulic loading rate (Gersberg *et al.,* 1984; Tomljanovich and Perez, 1989; Kadlec and Knight, 1996). Constructed wetlands must be designed as natural integrated ecosystems, since many of the components are interdependent. For example, wetland plants depend on microbial activity (which they support with organic matter) for nutrient regeneration, resulting in their availability from organic detrital and soil sources. Most evaluations of wetlands have been based on input/output analyses, with little attention being paid to the physical, biological and chemical processes occurring within the wetland. As a result, there is very little quantitative or qualitative information on the rates of these processes and the factors that affect them, making the optimisation of treatment performance difficult at the current time.

Considerable research is now being carried out into the wetland treatment of a wide variety of wastewater types. Constructed wetlands can be used for the treatment of intermittent, episodic or continuous high or low volume flows of wastewater from a variety of sources including mine wastewater, landfill leachate, dairy waste, pulp mill effluent, and agricultural, urban, highway and airport runoff (Lan *et al.,* 1992; Martin *et al.,* 1998; Karpiscak *et al.,* 1998, Tettleton *et al.,* 1993; Higgins *et al.,* 1993; Revitt *et al.,* 1999; Ellis *et al.,* 1994, Chong *et al.,* 1999). Wherever wastewater treatment contains biologically or chemically active substances, it is appropriate to undertake treatment using constructed wetlands, either on their own or in combination with conventional systems (CIRIA, 1997).

2.1.4 Comparison of conventional systems and constructed wetland systems for the treatment of wastewaters

The fundamental difference between conventional and wetland wastewater treatment systems is that in conventional systems, wastewater is treated in highly managed, energy intensive environments, whereas in wetland systems treatment occurs at a comparatively slow rate in essentially unmanaged environments (Wood, 1991). The consequences of this are that conventional systems require a more sophisticated infrastructure with associated equipment but less land than wetland systems. Conventional systems are subject to greater operational control and less environmental influence than wetland processes. Also with conventional systems, discrete biological and chemical processes can be specifically controlled and regulated, which is only partly possible in constructed wetlands where cleaning processes are integrated both temporally and spacially (Brix, 1993). Constructed wetland wastewater treatment systems offer several direct advantages such as low maintenance requirements, enabling them to be sited in isolated areas, and low technology, so that highly skilled and trained operators are not required. Constructed wetlands are robust systems, usually more flexible and less susceptible to variations in loadings than conventional treatment works (Mungur, 1997). Construction costs are not as low as originally believed but the low energy requirements of wetland treatment systems result in significant operational savings. Constructed wetland treatment systems also offer indirect benefits such as providing the wildlife and recreational benefits commonly associated with natural wetland systems. Furthermore, they are an example of sustainable environmental management fulfilling the requirements of Agenda 21, which was developed at the United Nations Conference on Environment and Development (UNCED, 1993) (Scholes *et al.,* 1995).

2.2 Nature and Impact of Urban Runoff

2.2.1 Characteristics of urban runoff

The development of buildings, roads and other infrastructure using impermeable materials results in the loss of the natural water retention properties which are provided by soils and vegetation (Merritt, 1994). Such urbanisation alters the natural hydrological cycle, changing peak flow characteristics and the volume and quality of the runoff (Revitt *et al.*, 1999). Traditionally, development sites have been engineered so that surface water is drained directly to the closest watercourse as quickly as possible. Such a system ignores the potential pollution loads generated from rainfall runoff and their impacts on receiving waters (Mungur *et al.*, 1994). Concern about the quality and management of urban runoff increased in the 1970's following several studies in the USA on the water quality of urban runoff which showed that higher pollutant levels are associated with more intensive development. Construction erosion can generate high levels of suspended solids and urban runoff pollutant levels can be comparable to secondary treated wastewater effluent (Livingston, 1989).

One of the most significant effects on the water quality in watercourses receiving input from surface water outfalls is due to the 'first flush' phenomenon, often associated with urban catchments. During this process, anoxic sediments which have built up within sewers, especially after dry periods, are discharged and can have a serious effect on receiving waters. Surveys of several rivers in East London, carried out by the National Rivers Authority (now the Environment Agency for England and Wales), have shown that the most important factor suppressing the river system is the water quality derived from surface water outlets (Scholes *et al.*, 1995). The flow from these outlets consists primarily of urban runoff from impervious surfaces and can result in a restriction of aquatic macroinvertebrate fauna to pollution tolerant families. In Florida, studies have shown that the 'first flush', corresponding to the first 2.5 cm of runoff, carries 90% of the pollutant load in a storm event (Livingston, 1989). The traditional pollution dilution approach involving urban rivers also creates excessive flows in the receiving watercourse which may cause erosion and flooding further downstream. Effects are increased by large quantities entering at a specific point causing disturbance of the substrates and a

consequent resuspension and remobilisation of settled sediments (Bascombe, 1991). Hence, this form of control treatment involving pollution dilution for urban runoff is now being discouraged.

2.2.2 Constituents of urban runoff

The quality of urban drainage waters tends to be highly variable even within a single catchment area. The quantity of pollutants derived from a single catchment is determined by a number of factors including land use, characteristics of the drainage system and catchment area, the nature and frequency of storms and the weather conditions between storms (Merritt, 1994). The principal pollutants in urban runoff are biochemical and chemical oxygen demand, suspended solids, heavy metals, hydrocarbons, de-icing salts, faecal coliforms and particulate pollution. These pollutants originate from a range of sources such as vehicle and tyre wear, losses from vehicle lubrication systems, vehicle exhaust emissions, road surface wear, atmospheric deposition, deicing materials, spillages and litter (Halcrow, 1996, Maltby *et al.,* 1995). Pollutants accumulate rapidly on impervious surfaces and are readily washed-off by rain into receiving waterbodies. In addition to monitoring these determinands, Brodie (1989) recommends that the minimum water quality analysis that should be performed for characterisation of urban runoff should also include pH, dissolved oxygen, ammonia, nitrates, total organic carbon and phosphates. All surface runoff generates a pollutant load, although runoff from roads and highways is of most concern. Highways may occupy only 5-8% of a catchment area but can contribute 50% of suspended solids, 16% total hydrocarbon and 35-75% of heavy metals (Ellis and Revitt, 1991). Winter gritting and road surface wear are the two most important sources of inpipe sediment (CIRIA, 1986). Highway runoff is an important source of potentially toxic contaminants in freshwaters (Hoffman and Quinn, 1987). The most frequently detected contaminants in a nation wide survey of urban runoff in the United States included copper, lead, zinc, chromium, cadmium and nickel, and the polyaromatic hydrocarbons phenanthrene, naphthalene, pyrene and fluoranthene (Cole *et al.,* 1984). Table 2.2 shows the ranges of concentrations reported by various authors for some of the principal pollutants in runoff from urban areas, suburban roads and motorways, in comparison with values for rainfall in rural areas.

Table 2. 2 Levels of pollutants in different runoff types and rainwater (µg/l).

	Rural rainfall (µg/l)[1]	Suburban roads (µg/l)[2]	Motorways (µg/l)[2]	Urban runoff (µg/l)[3]
BOD_5		11-40*	110-5700*	
Suspended solids		8-25*	12.2-32*	
Cu	0.04-5.4	10-120	50-690	5-40
Pb	2-9	10-150	340-2410	50-150
Zn	4.2-150	20-1900	170-3550	300-500
Cd	0.03-0.7			0.5-3.0

* = mg/l

[1] = taken from Kadlec and Knight, (1996)

[2] = taken from Mungur, (1997)

[3] = GromaireMertz *et al.*, (1999)

2.2.3 Use of constructed wetlands to treat urban runoff

The concept of treating urban runoff is still new, and most studies have focused on the development and use of detention basins for both flood control and the removal of pollutants (Ellis and Revitt, 1991). It is not possible to treat urban runoff using conventional treatment systems, as the highly variable pollutant load and volume makes it both too expensive and too difficult to deal with. However, natural wetlands world-wide are recognised as playing an important role in mitigating the effects of severe storm events. They can perform a variety of functions including storage of stormwater, reduction of flood flows and velocity, reduced erosion and increased sedimentation, and modification of pollutants (Livingston, 1989). This information in combination with the success of constructed wetlands in treating other forms of wastewater has greatly increased interest in the feasibility of using constructed wetland treatment systems as a 'natural' solution to the problems of urban runoff.

Research has indicated that, under prevailing conditions, both natural and constructed wetlands receiving urban runoff have attained high removal efficiencies. Ellis (1994), examining data from experimental reedbeds, found removal rates of up to 45% for nutrients, 83.1% for COD, 89.5% for BOD and 99.9% for faecal coliforms. A constructed wetland system receiving urban runoff from a 1200 ha catchment area was reported to

have achieved removal efficiencies of 64% for suspended solids, 48% for Cr, 31% for Cu, 88% for Pb, 20% for Ni and 33% for Zn (Meiorin, 1989). Schiffer (1989) examined heavy metal removal in a wetland receiving urban runoff and found 40% removal for Cr, 87.5% for Cu, 83.3% for Pb, 25% for Ni and 66.7% for Zn. A constructed wetland system treating stormwater from a catchment area of 900 ha was reported to provide removal rates of 95% for suspended solids, 75% for nitrates, 37% for ammonia and 90% total phosphates (Livingston, 1989). Mungur *et al.* (1998) reported removal rates of 99.3%, 97.4%, 97.1% and 99.2% for Cd, Cu, Pb and Zn respectively, during a storm event for a wetland system receiving runoff from both a housing development and a bypass.

However, the use of constructed wetlands to treat urban runoff involves a large degree of uncertainty, as there are limited data available on relatively new systems. It is now accepted that, due to the hydrological and pollutant load variability of urban runoff, the conventional approach of designing a constructed wetland based on an effluent of a characteristic quality and flow, as in the case of domestic wastewater treatment, is inappropriate. There are currently no established design and performance criteria for constructed wetlands for the treatment of urban runoff. Due to the variations in both the quantity and quality of urban runoff, it is recommended that wetland systems should be designed to accentuate the numerous pollutant removal mechanisms and hence maximise treatment. Information currently available is sufficient for system design to reduce targeted pollutants but inadequate to optimise design and operation for consistent compliance. However, as the number of systems increases and more data becomes available current recommendations and guidelines are continually being updated and revised. The available information for the design of constructed wetlands for the treatment of wastewater is discussed in the following sections.

2.3 Design of constructed wetland wastewater treatment systems

2.3.1 Types of systems

Constructed wetland treatment systems can be divided into three types; surface flow systems, horizontal sub-surface flow systems and vertical sub-surface flow systems (Figures 2.1, 2.2 and 2.3). These may be used individually, in combination with each other or as an addition to conventional wastewater treatment systems (CIRIA, 1997).

Surface flow, or free-water systems, are wetlands in which water primarily flows above the ground surface and through the litter layer (Merritt, 1994, Figure 2.1). They simulate natural marshes, employing shallow channels and basins, planted with emergent, submergent and/or floating vegetation through which water flows at shallow depths and at low velocities.

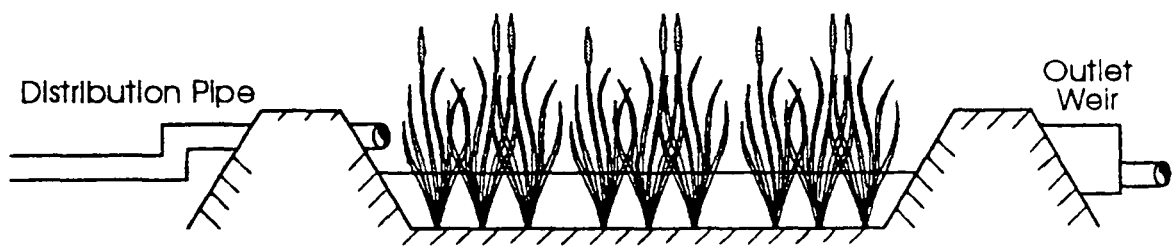

Figure 2. 1 Diagram of a surface flow wetland system (taken from Kadlec and Knight, 1996).

Surface flow systems tend to have four features in common; an inlet device, the wetland basin itself, wetland plants and an outlet device (Kadlec and Knight, 1996). However, there is a wide variety of configurations for each of these features, and therefore overall system design can vary greatly. Overland flow systems are easier to construct, are less demanding in terms of sediment requirements as the on-site substrate is often satisfactory, and have a simpler inflow structure than other system types. The principal design criteria are retention time, water depth, organic and hydraulic loading rates and aspect ratio (length:width) (Crites, 1994). They are potentially easier to maintain because hydraulic conductivity is not as critical as for other systems and continual surface flooding prevents colonisation by most unwanted plants. Also, surface flow systems

more closely resemble a natural reedbed, therefore offering a better quality wildlife habitat.

A constructed horizontal sub-surface flow system is a wetland in which wastewater flows horizontally through a lined basin or channel which is filled with a permeable substrate. This is planted with wetland plants, and flow remains below the media surface (George and Kemp, 1996, Figure 2.2). They are often constructed with a bed of gravel, making them more comparable to conventional biological filters than natural wetland systems.

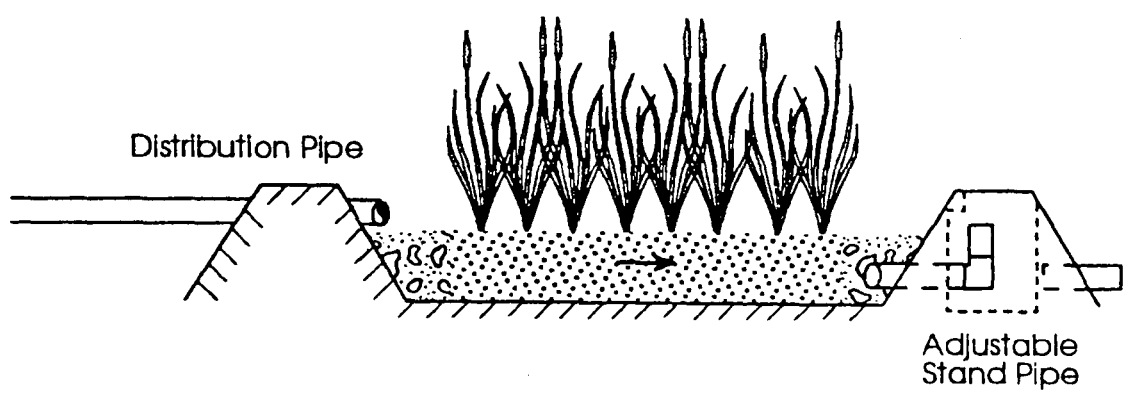

Figure 2. 2 Diagram of a subsurface flow wetland system (taken from Kadlec and Knight, 1996)

The water saturated substrates are generally anaerobic, except for the aerobic rhizospheres created by the leakage of oxygen from the root hairs and the transfer of oxygen from the atmosphere across the air-water interface. The rhizospheres support a diverse microbial community, which are essential for the removal and degradation of many pollutants. Treatment occurs during contact between the wastewater and the media surfaces and rhizospheres. As a greater percentage of this active zone is anaerobic in sub-surface flow systems, they have different performance characteristics to surface flow systems. For example, horizontal sub-surface flow systems remove more BOD and suspended solids per unit area than a surface flow system (Steiner and Freeman, 1989). Sub-surface flow systems theoretically require less land than surface flow systems because they incorporate a deeper active zone (CIRIA, 1997). However, they are more expensive to construct due to the cost of media, which usually has to be specially imported, and the complexity of inlet structures. Sub-surface flow systems are susceptible to blockage and a constant flow rate is necessary to prevent ponding and overland flow, hence they are more difficult to maintain. Theoretically, no effluent is exposed at the surface of the wetland and wildlife has less opportunity to come into contact with pollutants. The risk of insect vectors and odours is also eliminated (Reed

and Brown, 1995). The principal design criteria for sub-surface flow systems are retention time, solids and BOD loading rates, and substrate depth and grade (Crites, 1994). Overloading, surface flooding and media plugging (in particular by metal hydroxide precipitates and invasive weeds) have frequently been reported to reduce the effectiveness and loading rate treatment efficiency of these systems.

Vertical sub-surface flow systems have similar properties to horizontal sub-surface flow systems except that wastewater flows vertically up or down through the bed. Wastewater is generally introduced intermittently to the bed surface and then allowed to gradually drain vertically down through the substrate (Cooper *et al.*, 1996, Figure 2.3). In vertical upflow systems wastewater is fed in at the base of the system, and passes up through the bed (Farahbakhshazad and Morrison, 1998, Rowe, 1998). In vertical downflow systems dosing is timed so that the bed drains completely and refills with air before the next dosing occurs. This, in combination with the splashing caused by the rapid dosing of the bed, increases oxygen levels within both the wastewater effluent, and the system itself. As a result the ability of vertical sub-surface flow systems to remove pollutants through aerobic processes, such as nitrification, is greatly increased in comparison to both surface and sub-surface horizontal flow systems which tend to be oxygen limited. However, they are not as efficient in the removal of suspended solids and are therefore usually followed by a horizontal flow bed.

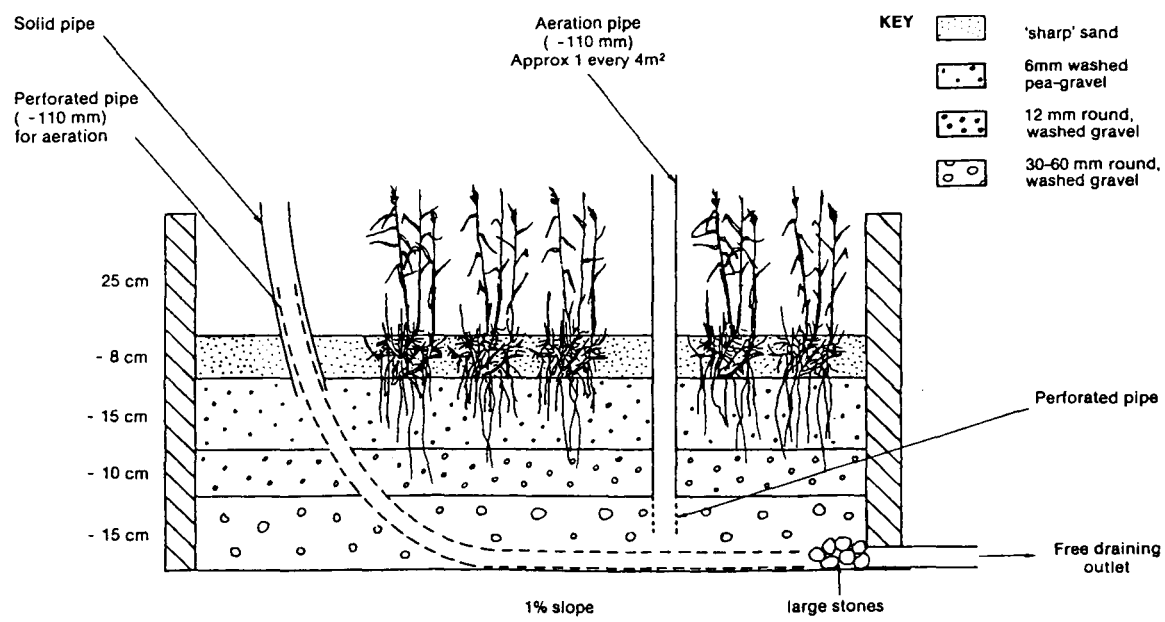

Figure 2. 3 Diagram of a vertical flow wetland system (taken from Cooper, 1990).

The selection of system type is usually based on a variety of factors such as pollutant removal requirements, land availability, substrate cost and climate. Although, as already

the most important factor in determining system choice is the size, shape and cost of the available land. Surface flow systems require more land than sub-surface flow systems due to hydraulic loading requirements. Hence, surface flow wetlands are usually large, long, narrow systems, whereas sub-surface systems require a smaller, more compact area (Reed and Brown, 1995; Steiner and Freeman, 1989). For both horizontal surface and sub-surface flow system designs it has been possible to demonstrate a correlation between performance (% removal of key pollutants) and surface area per population equivalent, and between performance and wastewater strength (CIRIA, 1997). Sub-surface flow systems are usually more expensive to construct due to the cost of excavation, and replacing on-site soil with a permeable bed substrate which may constitute over 50% of the total construction costs (Brown, 1994). However, surface flow systems are more susceptible to freezing in cold climates, reducing effective water depth, retention time and preventing surface reaeration (Kadlec, 1989). In a sub-surface flow system the litter layer insulates the bed, reducing freezing effects (Steiner and Freeman, 1989).

2.3.2 Sizing of beds

All constructed wetlands are attached-growth biological reactors (Watson and Hobson, 1989). Performance for biologically mediated reactions, such as organic degradation, nitrification and adsorption, can be described by 1st order plug flow kinetics if steady state conditions are assumed. Reactions are first order when the rate of change of the reactant (in this case the pollutant) is proportional to the amount of the reactant present. Reaction rates and constants are determined experimentally and appear site specific. Rates have been reported for BOD_5 only. The basic relationship is:

$$\frac{C_e}{C_o} = \exp[-K_T t]$$

where C_e = effluent concentration, mg/l

C_o = influent concentration, mg/l

K_T = temperature-dependent, first-order reaction rate constant, $days^{-1}$

t = hydraulic residence time, days

However, in wetland systems steady state conditions are not characteristic and modifications to this relationship are necessary. Various authors (e.g. Kadlec and Knight,

1996, Reed and Brown, 1995; Watson *et al.,* 1989) have produced general models by manipulating this equation to incorporate more parameters.

2.3.2.1 Surface Area

Brix *et al.* (1989) recommend the use of the following equation, derived from the above relationship, to establish the wetland surface area required for surface flow systems -

$$A_h = \frac{Q_d(\ln C_o - \ln C_t)}{K_{BOD}}$$

A_h = surface area of bed, m^2

Q_d = daily average flowrate of sewage, m^3/d

C_o = daily average BOD_5 of the feed, mg/l

C_t = required daily average BOD_5 of the effluent, mg/l

K_{BOD} = rate constant, m/d

K_{BOD} is the rate constant of the biodegradation process and should therefore vary only with temperature. However, various researchers have back-calculated this 'constant' from their own data, resulting in a range of values. Values calculated from UK and Austrian data for the treatment of domestic sewage generally range from 0.067-0.1, with lower values in winter for the treatment of domestic sewage (Cooper, 1990). Researchers in Denmark have calculated K_{BOD} values of 0.083 ± 0.017 for 49 systems (Schierup *et al.,* 1990). For the design of systems receiving normal strength domestic sewage with a BOD_5 in the range of 150-300mg/l, the value of K_{BOD} has generally been set at 0.1 in the UK (Cooper *et al.*, 1996), resulting in an area of approximately $5m^2$/pe for the secondary treatment of domestic sewage and $1m^2$/pe for tertiary treatment. CIRIA (1996) recommendations are similar, recommending design areas of 5-10 m^2/pe for secondary treatment of domestic wastewater, and 0.4-2.0 m^2/pe for tertiary treatment.

For both horizontal sub-surface flow and vertical flow systems, it has been recommended that the cross-sectional area is calculated using a form of Darcy's Law which describes the flow regime in a porous media (CIRIA, 1997). The equation is as follows

$$A_c = \frac{Q_s}{Kf \frac{dH}{ds}}$$

A_c = cross sectional area of the bed m^2

Q_s = average flow rate of sewage m^3/s

Kf = hydraulic conductivity of the fully-developed bed m/s

$\frac{dH}{ds}$ = slope of the bed m/m

When applying Darcy's Law, it is essential to accurately determine the hydraulic conductivity of the selected media to avoid undesirable surface flow, and several authors have therefore recommended that safety factors are incorporated into the initial design to allow for possible changes in media conductivity with time (Johansen and Brix, 1996, Reed and Brown, 1995). Using Darcy's Law, CIRIA (1998) recommend a design area of 3.7-5m^2/pe for secondary treatment of domestic wastewater and 1-2m^2/pe for tertiary treatment using horizontal sub-surface flow systems, and areas of 1m^2/pe for BOD removal only and 2m^2/pe for both BOD removal and nitrification in vertical flow systems. The latter figures are also recommended by Cooper *et al.* (1996). For vertical flow systems providing treatment for <100 people, Grant (1995) recommends that the system should involve two vertical flow beds which are sized using the following equation:

$$A_1 = 3.6P^{0.35} + 0.6P$$

A_1 = surface area of the first vertical flow bed (m^2)

P = population equivalent

The surface area of the second vertical flow bed (A_2) should be 50% of A_1 if the sewage is from a septic tank, and 60% if no septic tank is used.

For surface flow systems it is not necessary to consider the hydraulic conductivity of the media. However, Reed and Brown (1995) recommend using Manning's equation for hydraulic design with the inclusion of factors to account for resistance caused by dense stands of vegetation. Kadlec and Knight (1996) do not recommend design areas for either

horizontal or vertical flow systems, suggesting the use of general models to estimate design parameters using site specific characteristics. They also emphasise that, if using a wetland with high removal efficiencies as a design model for a similar system, it is essential to also take into account operational and ecosystem characteristics of the successful wetland as these aspects can also determine system success or failure.

As mentioned in Section 2.2.3, it is now believed that the use of the design approaches outlined above, which are calculated for an effluent of a typical quality and flow, may not be suitable for urban runoff treatment wetland design as both the hydrology and pollutant load of urban runoff are highly variable. Also, such design approaches are based on BOD as the key parameter to be removed and this is not generally the most important pollutant in urban runoff. Various recommendations have been put forward which try to take into account the variability of urban runoff. Wong and Somes (1995) have developed a method involving the stochastic generation of runoff sequences which are fed into a wetland behaviour simulation, resulting in a design chart which enables the selection of the appropriate storage volume and outlet characteristics on the basis of a long-term data set. Halcrow (1993) recommends systems be designed to retain the design storm event for a minimum of 30 minutes. The design storm event is the critical event involving a short intense storm following a dry period which mobilises the maximum pollutant load with highest inflows entering a system when the receiving watercourse flow is at a minimum. Persson *et al.* (1998) recently put forward a model to determine the hydraulic efficiency (a value which combines measures of flow uniformity and effective utilisation of storage volume) of stormwater wetland systems. This enables the effect of variations in wetland design, such as overall shape, location of inlet/outlet structures and basin morphology to be evaluated. Lawrence and Breen (1998) developed a process based model which can be adapted to local catchment conditions, with the wetland size and design being guided by the critical pollution reduction requirement recommended for the protection of downstream waters. Revitt *et al.* (1999) recommend that the system should be designed to retain the average storm event, which can be calculated from average rainfall, runoff coefficient and catchment area, for a minimum of 3-5 hours and preferably 10-15 hours to achieve good removal efficiencies. A maximum retention time of 24 hours has also been suggested (Shutes *et al.*, 1999). A final approach which has been used to size the surface area of wetland systems is based on a requirement of between 1-5% of the catchment area (Kadlec and Knight, 1996). It has been suggested that with adequate pre-treatment this

may be reduced to 0.2% of the catchment area (Halcrow, 1993). Although, Tilley *et al.* (1998) recommend using this as approach as a rough guide for wetland size in the initial design stages only as, for example, runoff from a predominantly industrial catchment area will contain higher loadings, and therefore require a larger wetland area, than runoff from a similar sized residential catchment area.

However, none of these approaches are universally agreed. There is no consensus as to optimal system design, water depth, substrate type or vegetation, and therefore the use of constructed wetlands to treat urban runoff currently involves a large degree of uncertainty.

2.3.2.2 Length and width

The length:width ratio (L:W) is critical with regard to minimising hydraulic short-circuiting and maximising wastewater contact with the entire area of surface flow systems and cross-sectional area of sub-surface flow systems. For surface flow systems, Steiner and Freeman (1989) recommend a L:W of at least 10:1 to ensure plug flow conditions, and an L:W of 1 or less for sub-surface flow systems. Watson and Hobson (1989) are in general agreement with these figures, although they suggest that for sub-surface flow gravel beds the ratio can be as high as 3:1. CIRIA (1997) recommends even higher L:W ratios for sub-surface flow systems, with values of between 4:1 to 10:1.

2.3.2.3 Depth of water and bed sediment/substrate

The recommended water depths for surface flow systems vary but the general consensus appears to be that water depth should be shallow at the inlet, to allow emergents to become established, and should not exceed 1m at its deepest point as few reed species can grow at depths greater than this (Merritt, 1994). Variation in water depth, from shallow marsh to open water, may also provide important new wildlife habitats (Shutes *et al.*, 1997). CIRIA (1996) recommend a bed depth of 20 cm for surface flow systems, although over time this will increase as the litter layer builds up.

The bed depth of sub-surface flow systems should be selected to match the depth of root penetration to ensure that all flow is within the root zone, maximising treatment in the aerobic rhizosphere (Watson *et al.,* 1989). This would mean that systems planted with *Phragmites* or *Scirpus* should not exceed 60 cm, and beds planted with *Typha* should not exceed 30 cm, as below these depths roots and rhizomes start to weaken. Almost all sub-surface systems in the UK are 0.6m deep and this is the average bed depth recommended by several authors (Cooper *et al.,* 1996, CIRIA, 1997, Johansen and Brix, 1996), although recommendations for inlet depth varies from 0.3-0.6m with the outlet depth being relatively deeper to accommodate the slope of the base of the bed. Maximum root and rhizome penetration can take up to 3 years to establish (Boon, 1985) and periodic draining is recommended in the autumn of the first two years to encourage downwards growth. A sufficient rooting depth is also required to prevent physical damage to the plants during high velocity inflows and freezing conditions (Shutes *et al.,* 1997). However, these recommendations assume that roots and rhizomes always penetrate to such depths. This may not always be the case as research carried out on sub-surface flow systems in the USA found that penetration was limited to the top 30cm irrespective of the plant species used (Reed and Brown, 1995).

Recommendations for the bed depth of vertical sub-surface flow systems range from as much 3-4m (CIRIA, 1997) down to 0.5-0.8m (Cooper *et al.,* 1996). The latter figure reflects the average bed depth of most vertical sub-surface flow systems in the UK.

2.3.2.4 Surface and bed slopes

Many of the early treatment systems were built with a sloping surface. However, this has resulted in a range of problems such as overland flow in sub-surface flow systems, erosion of the bed surface, uneven growth of reeds and difficulty in the use of flooding to control weeds. It is therefore now recommended that a level surface is used (Wood, 1991; Cooper, 1990).

For sub-surface flow systems, CIRIA (1996) recommend a base slope from inlet to outlet of between 1 and 5% to aid water movement through the media. Steiner and Freeman (1989) proposed a base slope of less than 2% for a sub-surface system, based on the initial hydraulic conductivity of the substrate according to Ergun's equation (a form of Darcy's

Law which adds a term to account for turbulent flow), and a slope of 0.5% in surface flow systems to enable the draining of cells for maintenance. Johansen and Brix (1996) also suggest using Darcy's law to calculate both bed width and slope of a sub-surface flow system. In the UK, a base slope of 0.5-1% is usual, although values as high as 8% have been used (Cooper *et al.,* 1996, Wood, 1991).

2.3.3 Pre-treatment

It was originally thought that pre-treatment was unnecessary and that screened or unscreened wastewater could be applied directly to wetland treatment systems (Cooper and Green, 1994). However, this caused problems such as the inlet blocking, blocking of the beds and odour. Now almost all wetland treatment systems are preceded by an appropriate preliminary treatment component. Several studies have shown that wetland systems receiving domestic sewage have a higher performance if preceded by a settlement tank or a septic tank, which can reduce influent wetland BOD loads by 20-30% (Cooper *et al.,* 1996).

Although urban runoff has different pollutant characteristics to domestic sewage, some form of pre-treatment is essential to prevent the beds from becoming blocked. Preliminary treatment can remove most heavy, large and floating debris by simple physical processes (Merritt, 1994). This is typically achieved using filters such as screens, trash racks, litter rakes and oil booms which trap large and floating objects and silt traps where the heaviest material, such as road grit, settles out. Shutes *et al.* (1997) recommend the use of pretreatment systems such as oil and grit interceptors, which can capture 70-90% of suspended particles >0.1mm and provide an outflow containing less than 0.1ppm oil. If space is available, the inclusion of settlement tanks or wetland forebays with additional booms are recommended to reduce the initial flush of sediments into the wetland, to prevent siltation in the inlet zone and to attenuate high velocity flows. Pre-treatment is therefore considered to be an essential component of an urban runoff constructed wetland treatment system, allowing for an even greater degree of treatment than is possible by the use of constructed wetlands alone.

2.3.4 Media employed in the construction of wetland treatment systems

2.3.4.1 Role of substrates

The choice of wetland substrates can be an important factor in determining whether a wetland treatment system is a success or failure. The physical and chemical natures of wetland soils and sediments vary greatly and determine many wetland 'conditions' such as nutrient retention, chemical transformations, porosity and permeability (Faulkner and Richardson, 1989). In a sub-surface flow system selected media must allow for a sufficiently high hydraulic conductivity (K_f) so that wastewater can flow fast enough to enable treatment without backing up and causing overland flow. Substrates can influence treatment capability through detention time, provision of contact surfaces between micro-organisms and wastewater, and oxygen availability. The media can provide reactive surfaces for the complexing of ions and other compounds, attachment sites for microbes which directly or indirectly utilise pollutants, support for emergent and submergent vegetation, and a source of nutrients for plant growth. They also provide a habitat in which benthic invertebrates can live, and a source of organic matter on which they can feed (Kadlec and Knight, 1996). The choice of substrate usually depends on cost, treatment requirements and system design. Organic soil has a high ion-exchange capacity, exhibiting a strong affinity for heavy metals (Yousef *et al.,* 1994), whereas inorganic soils have a greater capacity to retain phosphorus (Ewel, 1987). The hydraulic conductivity of the media will determine whether flow is mainly above the surface (surface flow system) or can permeate through the media (sub-surface flow system). Substrate types include organic soils, clay soils, sand, gravel and crushed rock (of various sizes and compositions) or mixtures of these various types.

2.3.4.2 Hydraulic conductivity

Soils are a combination of mineral and organic solids, water and open pore spaces. The spatial arrangement of these determines the soil structure and pore size which in turn significantly affect hydraulic conductivity (Faulkner and Richardson, 1989). Hydraulic conductivity is the rate at which water moves through the substrate. It is by far the most important factor influencing pollutant removal ability in sub-surface flow systems where removal processes are confined to the root zone. The pollutant removal ability of a substrate is a function of media-wastewater contact time; the lower the hydraulic conductivity the greater the contact time between wastewater and the media and the higher the pollutant removal efficiency of the media. However, substrates with a low hydraulic conductivity are poorly permeable and therefore their use in sub-surface flow systems tends to result in undesirable surface flow (Cooper, 1993).

Soil types can be broadly divided into organic soils (>20% organic matter) and mineral soils (<12% organic matter). Organic soils occur when the rate of organic matter deposition is greater than the rate of decomposition, and they have a lower pH, bulk density, and nutrient availability than mineral soils. Mineral soils are divided into clay soils and sandy soils, the classification depending on a range of factors such as particle size. Clays have the finest particle size resulting in an extremely low hydraulic conductivity and high adsorption capacity which increases the removal potential of pollutants such as phosphates and heavy metals (Kadlec and Knight, 1996). However, clay soils should be used with care as a lowering of pH can release adsorbed pollutants. Sandy soils have larger mineral particles which are more chemically inert decreasing the ability to bind nutrients but increasing the hydraulic conductivity. Most wetlands are initially dominated by mineral soils. However, as the wetland matures the organic content increases and soils will eventually become organic (Kadlec and Knight, 1996). It was initially believed that the hydraulic conductivity of a constructed wetland would increase over time, as decaying rhizomes would leave a series of open channels permeating the substrate (Boon, 1985). However, this was not found to be the case, and it is now recommended that the design hydraulic conductivity is equal to that of the selected media (Cooper *et al.*, 1996). It is essential that the hydraulic conductivity of the

on-site substrate is measured, and that, if the soil has a low conductivity, it is replaced with a more porous soil or with gravel.

Wetland plants grow optimally in deep rich soil, which allows maximum root penetration. However, the use of soil as a growth media has caused problems in many sub-surface flow systems, where the low K_f results in overland flow, channelling and scouring of the bed surface. This can result in some areas being water starved, affecting plant growth, and hence reducing treatment ability (Cooper, 1990). The use of gravel, either on its own or in combination with soil is now widely recommended (CIRIA, 1997, Cooper *et al.,* 1996). Gravel increases both hydraulic conductivity and acts as a silt trap during storm events (Mungur *et al.,* 1994). Howard *et al.* (1989) recommend gravel as the most suitable substrate for constructed wetlands stating that it provides adequate root growth, high conductivity and superior permeability. Soils have K_f values of 10^{-5} m/s or less whereas a uniform gravel bed in the range of 3 mm-6 mm or 5-10 mm will have an initial K_f value of 10^{-3}m/s or higher. Table 2.3, taken from Cooper (1990), lists the approximate hydraulic conductivities for a range of media types.

Table 2. 3 Typical Kf values for a range of soil types.

Soil texture	Typical Kf value (m/s)
Fine to coarse gravel	10^{-3}-1
Fine to coarse sand	10^{-7}-10^{-2}
Karst limestone	10^{-4}-10^{-2}
Sandstone	10^{-8}-10^{-4}
Silt, loess	10^{-9}-10^{-5}
Glacial till	10^{-12}-10^{-4}
Unweathered marine clay	10^{-12}-10^{-9}
Shale	10^{-13}-10^{-9}

CIRIA (1996) recommends the use of 10 mm graded gravel as a support media in sub-surface systems. A coarser gravel (4-10 cm stones) should be used at the inlet and outlet gabions to reduce clogging. The use of gravel has been successful, and initial concerns that gravel beds would be gradually blocked by solids do not appear to have been borne out. Gravel systems in the UK are still performing well after 8 years of operation

(Cooper *et al.,* 1996), and studies in the USA of gravel beds aged 2-5 years found that solids represented only 2% of the available void space (Reed and Brown, 1995). Analysis of the composition of these solids found them to be at least 80% inorganic matter, the primary source of which was believed to be fines associated with the media. Therefore, it is recommended that gravels are washed before use (Brown, 1994; Cooper *et al.,* 1996).

When using media such as gravel or pulverised fuel ash (PFA) which are lacking in nutrients, it is recommended that a small amount of standard fertiliser is added at planting time. Once established the plants should find enough nutrients in the sewage feed. Other wastewater types may require fertiliser initially, however, once the plants are established nutrients should be available through regeneration of the associated litter layer. Nutrient poor peat based organic soils are not recommended due to their acidic nature, and nutrient poor clays may be too compact for root penetration. It is recommended that prior to use all components of a substrate mixture should be analysed for hydraulic conductivity, buffering capacity, pH, plant nutrient levels and microbial activity (Cooper *et al.,* 1996).

2.3.5 Sealing and lining

If there is any risk that ground water may become contaminated through seepage from the treatment system, the bed should be sealed with an impermeable liner. The cheapest liner is the use of on-site clays. The performance of clay as a liner is related to how pure and fine the particles are. Omidi *et al.* (1996) state that if the saturated hydraulic conductivity of the on-site substrate is 10^{-7} cm s^{-1} it will form a suitable liner, whereas Cooper *et al.* (1996) recommend a minimum hydraulic conductivity of 10^{-8} cm s^{-1}. The clay should be at least 300 mm thick, and carefully compacted to eliminate cracks (Merritt, 1994). If the clay lining is too thin, deep rooted plants may penetrate it, or it may leak if allowed to dry out. Covering the clay with a layer of organic soil will help prevent drying out. However, Omidi *et al.* (1996) noted that only two desiccation cycles greatly increased the permeability of clay liners, and therefore its is recommended to avoid using clay in wetlands that have a highly fluctuating water level.

If the hydraulic conductivity of the on site media is greater than $1x10^{-8}$ cm s^{-1} then it is suggested that a plastic liner or membrane, such as high density polyethylene (HDPE) or low density polyethylene (LDPE), is used (Cooper *et al.,* 1996). Also widely used are liners made from bentonite (a very fine clay) sandwiched between a geotextile fabric. Sheet liners have the advantage of being easily transported and handled. They vary in their tear resistance and lifespan, though most types are likely to work satisfactorily for 20-25 years if fitted and treated properly. The main concern with liners is their vulnerability to puncture, and therefore it is recommended that liners are laid out over a layer of sand or matting 150 mm deep, and protected by at least the same depth of substrate above (Merritt, 1994). Concrete has also been widely used to create water tight tanks and ponds. An engineer should be consulted for all but very small ponds, and wetlands of an area $>3m^2$ should incorporate metal or PVC joints. Despite being a very robust material, concrete will crack if, for example, the ground subsides, and, as with other kinds of liners, leaks can be very difficult to trace and repair. Fresh concrete is toxic, and therefore newly constructed sites should either be washed or allowed to weather before being planted (Merritt, 1994).

2.3.6 Water level control

2.3.6.1 Inlet structures

It is essential to try to achieve an even distribution at the inlet in order to establish a good distribution of flow across the width of the bed and to avoid short-circuiting of wastewater (Cooper, 1990). Inlet design is more critical in sub-surface systems than surface flow systems to prevent scour and overland flow. Inflow can be distributed by gravity or pressurised flow; gravity-fed flow is preferable because it does not require energy input, reducing operation and maintenance costs. In wetlands treating urban runoff, a storm event can result in large volumes entering a system in a short period of time and ideally a distribution system is required to slow such high velocities. Ellis (1994) recommends that flow velocities should not exceed 0.3-0.5 ms^{-1} at the inlet to achieve effective sedimentation, and that flows exceeding 0.7 ms^{-1} will damage plants.

A number of different designs have been used in the UK, and choice of structure usually depends on wastewater characteristics and cost (Kadlec and Knight, 1996). For horizontal sub-surface flow systems, CIRIA (1996) recommends a pipe with tees or orifices which can be adjusted to produce an even distribution. The size of the pipes and tees will be dictated by the flow rate but the pipe is usually 70-150 mm diameter for small systems, with orifices at least 5 cm diameter to prevent plugging by solids, and feed points no more than 5m apart. Wire mesh gabions 0.5 m wide, containing evenly graded stones in the range 50-200 mm, reduce velocity of the incoming flow and help to reduce hydraulic short-circuiting. The inlet zone stones also serve as a secondary distribution zone, allowing the level of water across the bed to be equalised. If gravel is used as the bed media, it is possible that the use of the inlet zone stones may be avoided because of the porous nature of the gravel bed.

For surface flow systems a simple pipe or weir, with baffle zone, will reduce hydraulic short-circuiting and enable an even distribution across the bed (CIRIA, 1997). Castellated weirs are not recommended as they are expensive to construct and maldistribution of flow is often caused by material collecting in the castellations. Tipping troughs are another alternative which have been used successfully on small beds. It is essential that the inlet distributor be easily cleaned, for example by flushing.

2.3.6.2 Outlet Structures

Wetland outlet design is important for maintaining flow in the system and for monitoring flow rate. However, it is the control of water levels within the wetland which is the most important function of the outlet structure. The optimal water level is based on requirements for plant growth, hydraulic residence time and maintenance. The simplest and cheapest outlet design which provides water level control is a swivel pipe. This design consists of a length of plastic pipe set in a concrete dam with a moveable 90^0 bend attached to the upstream end. By moving the bend away from the vertical the level can be lowered. A calibrated water level marker enables the water height to be set more precisely. This design is suitable for most low flow wetlands. Another method is a vertical outlet pipe made up of a series of socketted sections which can be added or removed to raise or drop the water level. Pipes can easily become blocked by debris so it is recommended to

use a pipe with a large bore. Another widely used design uses boards that drop into a grooved spillway (stoplog sluice) or a chamber (monk sluice). Stop logs are not ideal because they tend to leak and make accurate control of water level difficult. The water may also drop a large distance at a fast rate resulting in scouring of the bed surface. However, this design has the advantage of being able to cope with relatively high rates of flow. It is recommended that the outlet arrangement allows the water level to be manipulated up to 200 mm above the bed surface and down to the base of the bed (liner level) for maintenance and management (Cooper, 1990). A recommended method of outlet control from sub-surface flow wetlands is a slotted or perforated pipe buried in a coarse gravel (CIRIA, 1997, Kadlec and Knight, 1996). The 1990 European guidelines recommend that, when using a soil medium, a 0.5m wide stone collector is used with a slotted pipe running along the base to prevent substrate being washed out into the receiving waters. If a gravel substrate is used this structure may not be necessary.

In designing a wetland for the treatment of urban runoff, it is important to take into account the potential impact of storm flooding. Where flooding of adjacent land is not acceptable and the wetland is not sufficiently large to cope with predicted storm events then an additional spillway must be incorporated into the design. In determining the size of the spillway it is necessary to take into account factors such as the size and nature of the catchment area, the storage capacity of the wetland, predicted frequencies and durations of storm events, and the discharge capacity of any sluices. Water flows and turbulence tend to be exaggerated around sluices and spillways, greatly increasing the potential for erosion, and therefore susceptible beds and banks should be protected.

2.3.6.3 Retention time

The retention time, defined as the average time that water occupies a given volume, is one of the most important considerations in the design of constructed wetland wastewater treatment systems (Kadlec and Knight, 1996). The length of time that water remains in the wetland can determine both the level of treatment of the wastewater and the types of pollutant removed. Generally, the longer the wastewater remains in the wetland, the more time there is for the various wetland pollutant removal processes to occur and the better the quality of the effluent leaving the system. Considerations affecting the residence time

include the length and width of a system, the vegetation, substrate porosity, hydraulic conductivity, depth of water, flow velocity and the slope of the bed. Water depth and substrate depth are the most important factors determining retention time in surface and sub-surface flow systems respectively. Several authors (Kadlec, 1989; Reed *et al.*, 1988; Kadlec and Knight, 1996) have produced general models to calculate theoretical residence times. The following model is based on the first order kinetic relationship discussed in Section 2.3.2.1 (Watson and Hobson, 1989):

$$t = \frac{LWdn}{Q}$$

where t = residence time, days

L = length of system (parallel to flow path), m

W = width of system, m

d = design depth of system, m

n = porosity of the system

Q = hydraulic loading rate of the system, m^3/day

Design estimates of residence time should take into account water losses due to evapotranspiration and seepage to ground water, as well as gains due to rainfall.

Estimates of required retention time for effective treatment vary greatly. CIRIA (1997) suggests a retention time of 16-24 hours for high performance treatment in surface flow systems, 2-24 hours for sub-surface horizontal flow systems and 1-12 hours for sub-surface vertical flow systems. Suggested retention times for the treatment of municipal wastewater range from 5 days to 14 days (Watson and Hobson, 1989). Guidelines for wetlands treating stormwater runoff in Florida require a minimum retention time of 120 hours, with no more than half the treatment volume discharged in 60 hours, whereas in Maryland a detention time of 24 hours for a stormwater treatment wetland is recommended (Livingston, 1989). As discussed in Section 2.3.2.1, Revitt *et al.* (1999) recommend a minimum retention time of 3-5 hours and preferably 10-15 hours for efficient treatment of urban runoff, and Shutes *et al.* (1999) suggest a maximum retention time of 24 hours.

2.3.6.4 Evapotranspiration

Evapotranspiration is the loss of water as a result of plant transpiration, coupled with evaporation from soil and water bodies (Merritt, 1994). Evapotranspiration is strongly diurnal and seasonal. Evapotranspiration causes concentration of contained pollutants and slows the flow which increases the retention time. Anoxic conditions and effluent deterioration may also result. These are the opposite to the effects caused by rainfall which produces dilution and increased flow. Evapotranspiration may be a limiting factor in some arid areas where the rate of evapotranspiration exceeds total water inflow (Kadlec and Knight, 1996).

2.4 Plants

2.4.1 Role of vegetation

Hydrophytes are plants adapted to wetlands. The most commonly used hydrophytes in wastewater treatment wetlands are *Typha* (reedmace; cattails), *Scirpus* (bulrush), *Phragmites* (common reed), *Carex* (sedges), *Lemna* (duckweed), *Phalaris* (reed canary grass) and *Juncus* (rush). However, less than 1% of the possible aquatic plants have been utilised in constructed wetlands (Kadlec and Knight, 1996).

Wetland vegetation can be split into two main groups; floating plants and rooted plants (Figure 2.4). Floating plants can be divided into those that float on the surface and those that float in the water column. Rooted plants are divided into emergents (those that have leaves that float or are above the surface), and submergents. Plant species combinations should be individual to each wetland and it is important to select species in relation to the local hydrological, nutrient and substrate conditions.

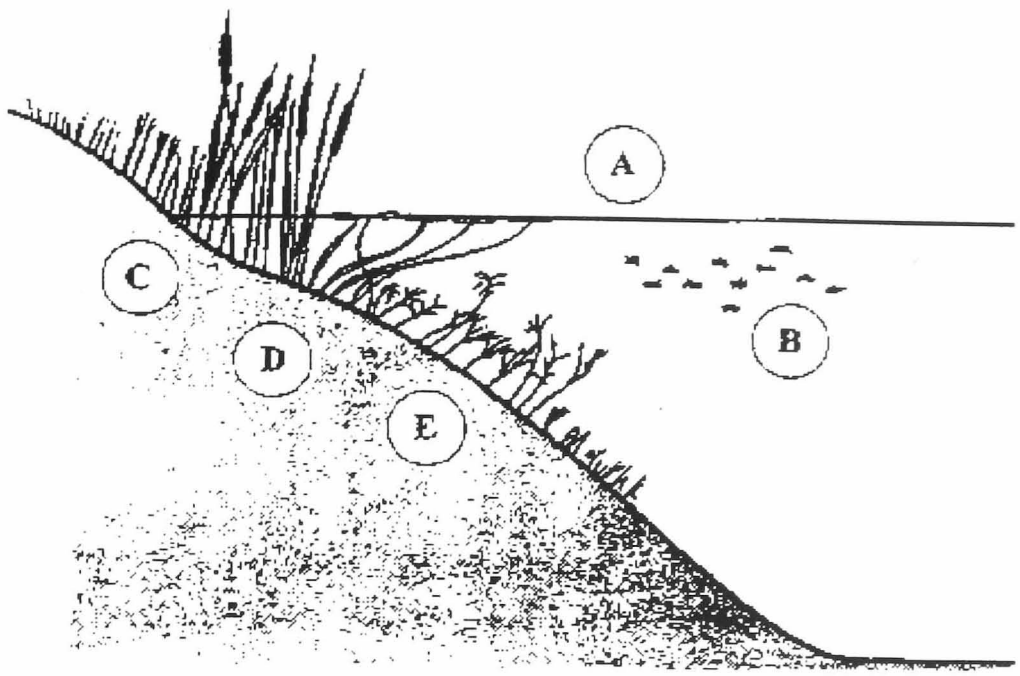

Figure 2. 4 Major life forms of aquatic macrophytes. A = free-floating at surface; B = free-floating beneath surface; C = emergent (e.g. *Typha latifolia*); D = with floating leaves; E = submerged (taken from Mungur, 1997).

Wetland vegetation has several functions, both direct and indirect. Firstly, and perhaps the most important for wastewater treatment, wetland plants have the ability to transport atmospheric oxygen through their leaves and down into their roots (hence their ability to grow in anaerobic conditions) (Brix and Schierup, 1989). Oxygen leaks out from the roots and results in thin aerobic zones around each root hair, which is known as the rhizosphere (Figure 2.5). The rhizosphere is the site of oxidation reactions in the otherwise mainly anaerobic sediment and supports large microbial populations that modify nutrients, metals and other compounds (Brix, 1996). The stems and leaves within the water column also provide a large surface area for the attachment of microbial populations. Many macrophytes have an extensive network of roots, penetrating down to a depth of approximately 0.6m, which help stabilise the sediment and reduce erosion. As discussed in Section 2.1.2.1, physical filtering of wastewater through stands of vegetation is not a significant removal process. However, the density of vegetation does enhance removal efficiencies through the buffering of incoming flow, reducing flow velocity thereby enhancing conditions for sedimentation.

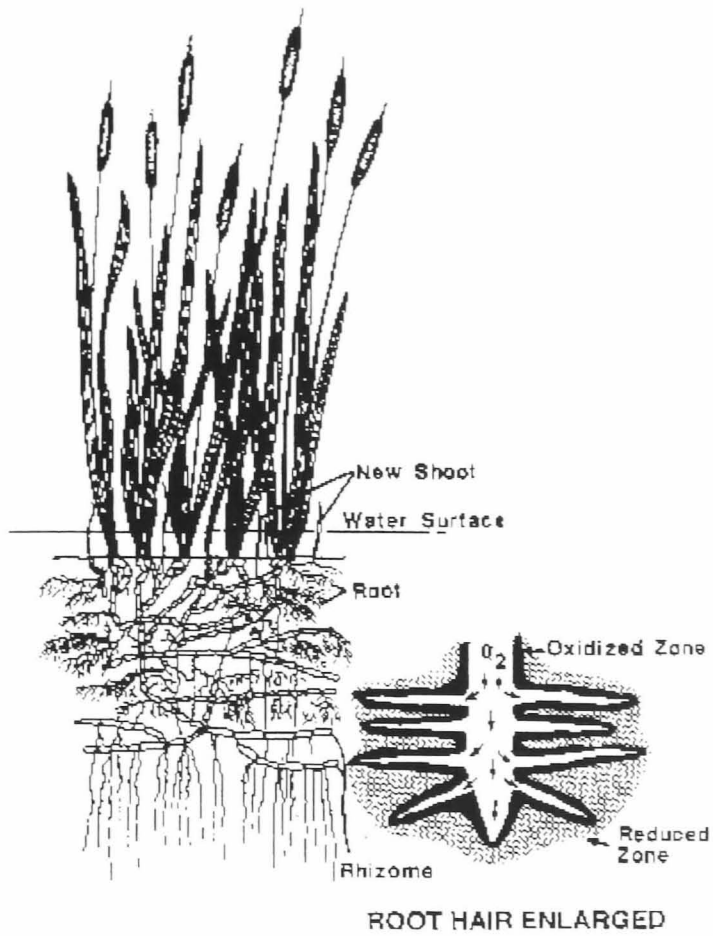

Figure 2. 5 Wetland plants have the ability to transport oxygen to support their roots growing in anaerobic substrates (taken from Hammer and Bastian, 1989).

There is also direct uptake of pollutants by the macrophytes, for example nutrients, which are used for growth, and heavy metals which are stored primarily in the below ground parts of the plant where they can be accumulated to high levels without causing damage. The bioaccumulation of metals and nutrients does result in removal of pollutants from the water column. However, these tend to be minor removal mechanisms in comparison to adsorption of metals and phosphates by the sediment, and nitrification and denitrifcation of ammonia and nitrates by micro-organisms in the sediment. Several authors have reported that metal accumulation is preferential in the order roots>rhizomes>leaves (Zhang, 1990; Mungur *et al.*, 1995 and 1998; Kadlec and Knight, 1996). However, although the roots tend to have the highest concentration of metals, they only account for a small proportion of the total plant biomass and therefore it is the rhizomes that are actually the largest store.

2.4.2 Planting and establishment

Reeds will potentially grow on all but the hardest and softest of substrates, especially where there are high levels of nutrients (Merritt, 1994). It is important when introducing vegetation to a new or existing wetland that the most appropriate species are selected. Only species that are typical of the local area and adapted to the site should be introduced. It is also important to identify plants that may be good for wastewater treatment and are not weed or alien species that produce undesirable environmental change by out-competing native plants and destroying habitat (Chambers and McComb, 1994). Other factors influencing species selection are expected water quality, normal and extreme water depths and maintenance requirements (Kadlec and Knight, 1996). At present, evidence suggesting that treatment performance varies between wetland macrophytes, in either surface flow or sub-surface flow systems, is inconclusive so selection can be made on the basis of growth potential, survivability and cost of planting and maintenance.

Constructed wetlands have been planted using various types of propagules; seedlings grown in a greenhouse, rhizome sections, clumps of reeds and sowing seed. All these methods have been successful but some are preferred. Propagation has been shown to be very successful using seedlings, which can easily be planted by hand (Cooper *et al.*, 1996). The Institute of Terrestrial Ecology recommend the use of seedlings because they provide more rapid cover than the other methods (Cooper, 1990). At the end of the first growing season a wetland planted with seedlings has substantially more cover, more uniformity of cover and a greater density of shoots (Cooper, 1990) with a usual survival rate of greater than 80% (Kadlec and Knight, 1996). Clumps of reeds have also proved to be successful. Several beds have used 20 cm^2 clumps of reeds from natural wetlands at distributions of 1 clump /m^2. In all cases the reeds survived and became successfully established but they tended not to spread outwards as rapidly as seedlings. This is also the case with the use of rhizome sections. Ye *et al.* (1997) recommend the use of seed as it has the advantage that a large number of genotypes can be used to create the wetland population, which could be very valuable in terms of long term establishment and survival. Kadlec and Knight (1996) recommended that seeds are derived from local, native stock, and that approximately $1.2x10^6$ seeds are required per hectare. Parr (1987) suggests that seeds should be collected from fertile stands in November/December, germinated in February/March with the aim of

planting as seedlings in May/June. A density of 4 seedlings/m^2 should be enough to obtain a good coverage by autumn, though the CIRA (1996) report recommends a planting density of 4-8 plants/m^2 for surface systems and a density of 4-6 plants /m^2 for sub-surface systems. Kadlec and Knight (1996) quote typical planting densities of 2-6 plants/m^2 for *Phragmites* and 1-2 plants/m^2 for *Typha* and *Scirpus*. It is possible to plant from March to the end of October but the most successful establishment has taken place with those planted in May/June. The time of year that planting is carried out may also determine the type of propagule selected.

Most reeds spread through their rhizomes (underground stems) extending across and through the substrate, with the aerial buds that form the plants growing at the rhizome tips. For the first month these buds are fed entirely with nutrients from the rhizome (Haslam, 1994). New roots then begin to grow and nutrients are absorbed for further growth. Under suitable conditions a clump of reeds can spread at a rate of 1.5m per year. Dense cover is essential for efficient treatment to occur through direct pollutant uptake, for attachment of microbial populations and to promote the settlement of suspended solids. When the reeds die back in winter a dense coverage will form a thick litter layer providing further treatment and odour control (CIRIA, 1997). Establishment of the litter layer takes 1-5 years, however it is possible to speed up the process by 'importing' a litter layer of straw approximately 10 cm thick in mid winter (Kadlec and Knight, 1996). Because reeds often grow in single species stands, they are susceptible to years of poor growth resulting from pest infestation or fungal infections. It is essential that the rhizomes always have access to water or the plants will die (Cooper, 1990). It is especially important to monitor water levels throughout the first growing season as young plants can be killed by even shallow flooding, whilst for older reeds at least the top third must be protruding above the water. However, if the bed is not flooded terrestrial plants are likely to become established and compete with the reeds (Section 2.5.2).

2.5 Routine management and maintenance

Constructed wetland treatment systems have a great advantage over conventional wastewater treatment systems in that they do not require the same skilled, intensive management and maintenance. However, some maintenance is required to keep the system performing optimally. The establishment of a workable management and maintenance plan should form an essential part of both the initial design and costing process, enabling both a regular maintenance programme and a prompt response to any unforeseen problems to be implemented.

2.5.1 Sediment removal

Initial settlement of suspended solids and associated micropollutants is an important wetland process. Settlement tanks or trenches need to be emptied regularly to sustain this wetland pollutant removal mechanism and to prevent resuspension of already settled sediments under increased flow conditions. Settlement tanks should therefore be designed to make dredging as easy as possible by, for example, providing a concrete approach. Silt should then be transported to landfill sites or an area where the possible high metal load will not cause further pollution problems.

2.5.2 Weed control

The use of a level bed surface is recommended to allow flooding for weed control (Wood, 1991). Weeds cause problems in constructed wetlands due to their rapid proliferation and the fact that in spring weeds start to grow earlier than some reed species, such as *Phragmites*, resulting in the shading of the reed shoots, retarding their growth (Cooper, 1990). Terrestrial plants are generally intolerant of saturated soils during the growing season and can be killed if shallowly flooded for two or three days during May or June (Merritt, 1994). Larger more robust plants such as docks or brambles may require prolonged or repeated flooding. However, prolonged flooding at other seasons may also make reeds more susceptible to fungal attacks and disease, thus checking their growth. For reed beds planted in spring/summer, weed control should be carried out by flooding to a

depth of 5 cm for the first 3 months of establishment. This will prevent most species from growing and allow the reeds to establish. For beds planted the previous autumn flooding should be delayed until the new reed spikes are through and flooding should not result in their submergence. A similar period of flooding in the second growing season may be beneficial. It should not be necessary to flood for weed control after this.

2.5.3 Plant harvesting

There is not complete agreement on the question of plant harvesting. On senescence, reeds may release nutrients and heavy metals stored in their leaves and shoots and it was originally believed that, if left unharvested, the build up of dead plant material may result in increased pollutant levels within the wetland (Merritt, 1994). However, harvesting is no longer believed to be as beneficial for several reasons. The harvesting of reeds would disturb sediments, causing resuspension and remobilisation of more pollutants than harvesting itself removes (Merritt, 1994). Studies have suggested that prior to senescence nutrients and metals stored in the leaves are translocated to the rhizomes which do not die-off in winter, thereby minimising pollutant release. Herskowitz (1986) reported that an average of only 2.5% of the total phosphorus removal in a surface flow wetland was achieved by harvesting. Ye *et al.* (1997) reported that little of the accumulated metal in *Phragmites australis* was translocated from the below ground part of the plants to the shoots and that therefore harvesting as a way of removing metals from the wetland system would be ineffective. Furthermore, the dead plants decompose to form the litter layer, which can bind metals (Sobolewski, 1996), acts as a sieve trapping suspended solids and associated pollutants, and insulates the bed preventing it from freezing in winter (Brix, 1996).

2.5.4 Monitoring

Wetland treatment systems need to be sampled sufficiently frequently for the operating authority to be confident that the required effluent standard is being achieved. For example, the minimum levels of data required to make an assessment of the performance of a constructed wetland treating domestic sewage are the levels of BOD, suspended

solids, ammonical-N in the influent and effluent, flow rate treated or population equivalent served, and surface area (CIRIA, 1997). The system should be inspected on at least a weekly basis to begin with, but after 2 or 3 growing seasons this may be reduced (Cooper, 1990). For sub-surface systems in particular, it is necessary to regularly inspect inlet and outlet flow control mechanisms to remove debris and set the correct water level. Routine monitoring and analysis of data is also important to establish and quantify the performance of components, processes, rates and designs, so that future systems can be designed and managed optimally. Hence further monitoring, such as the evaluation of the roles of soils, plants, micro-organisms and hydrological regimes, is recommended.

2.6 Wetlands and micro-organisms

Wetlands are known to provide ideal environments for a wide range of micro-organisms, providing a ready supply of carbon and other nutrients for growth. In addition, wetland plants and sediments provide ideal substrates for the attachment of micro-organisms. Studies of the microbiology of both constructed and natural wetlands have found microbial diversity and numbers were comparable (Duncan and Groffman, 1994), with bacteria being the most abundant group ($4.6x10^6$ cfu/g), followed by actinomycetes ($1.3x10^5$ cfu/g) and then fungi ($4.0x10^4$ cfu/g) (Hatano *et al.,* 1993 and 1994). These values are lower than typical soil counts, by approximately 2-3 orders of magnitude. Constructed wetland treatment systems are often viewed as a 'natural' microbial degradation systems, where the aim is to maximise contact time between micro-organisms and wastewater. Micro-organisms are involved in many important pollutant removal processes such as the breaking down of organic matter, nitrification and denitrification, binding of heavy metals, and indirectly causing precipitation of metals as insoluble salts which can then be further bound up into the sediment by physico-chemical reactions (Sections 2.1.2.1 and 2.1.2.2). Both aerobic and anaerobic micro-organisms are involved in these processes of pollutant removal, degradation and immobilisation.

Unlike organic pollutants, metals cannot be degraded to harmless products but persist in the environment. Micro-organisms have developed a range of resistance mechanisms to deal with metal toxicity such as extracellular precipitation, intracellular accumulation,

oxidation and reduction reactions, and binding to the cell surface and extracellular polysaccharides (Brierley, 1990). Analysis of heavy metal impacted soil microbial communities found that although heavy metals adversely affected biomass, metabolic activity and diversity, Pb and Cd resistant strains were found (Roane and Kellogg, 1996). It is thought that the presence of metals generates a strain selection or strain acclimation. However, it is not uncommon to find strains resistant to heavy metals in uncontaminated sites indicating that metal tolerance may be a widespread phenomenon. The ability of micro-organisms to tolerate high sediment concentrations of heavy metals has also been demonstrated to be associated with the 'shelter' effect of sediment and its organic matter content, whereby mechanisms such as adsorption, chelation and precipitation result in the binding of metals consequently lowering their bioavailability (Montuelle *et al.,* 1994). Babich and Stotzky (1986) reported that the limited toxic effect of high metal concentrations could be explained by the organic content of the sediment, demonstrating that organic composition of a specific growth medium influences the toxicity of the heavy metal tested. A study by Montuelle *et al.* (1994) also found that only 16% of isolates from a contaminated site were able to grow on agar containing a similar concentration of metals. However, it should be noted that the chemical forms of the metal in the sediment were probably different from those in the medium, where the soluble toxic fraction was more available.

2.7 Metal sorption by biomass materials

Conventional processes for the treatment and disposal of heavy metal contaminated wastes include chemical precipitation, electrode deposition and ion exchange, chemical oxidation and reduction, filtration, electrochemical treatment, membrane technologies and evaporation recovery (Zhang *et al.*, 1998; Pradhan and Levine, 1995; Williams *et al.*, 1998). Such techniques have been shown to be either ineffective or are too expensive for large volumes of dilute wastewater. In addition, when successful these techniques tend to generate large quantities of highly caustic sludges. Hence, over the past 15 years, interest in the phenomenon of metal sorption by biomass as a cost-effective process for removing heavy metals from wastewater has greatly increased as its potential has become apparent (Zhang *et al.*, 1998; Volesky and Holan, 1995). Sorption is usually a combination of biosorption, which is the physico-chemical binding

of metals to biological constituents, and bioaccumulation, which is defined as the metabolism-dependent intracellular uptake. Hence, biosorption can occur on both living and dead cells, whereas bioaccumulation occurs in living cells only (Gadd, 1990). The majority of studies have used either the microbial waste by-products of industrial processes or marine algae as the biomass, due to both economics and their ready availability. Seaweeds such as *Ascophyllum* and *Sargassum* have been reported to accumulate 30% of their dry weight in Pb and Cd, whereas the mycelia of fungi such as *Rhizopus* and *Absidia* have been reported to bind up to 25% of their dry weight for a range of heavy metals including Pb, Cd, Cu, Zn, and U (Volesky and Holan, 1995). Certain micro-organisms can perform as well as, or even better than, activated charcoal and ion exchange resins. Azab and Peterson (1989) reported that *Aspergillus niger* and *Mucor ramanniunus* exhibited higher sorption capacities for Cd than activated charcoal and an ion exchange resin, and *Penicillium* is reported to be approximately 14 times more efficient at accumulating Ra than activated carbon (Kapoor and Viraraghavan, 1995). Karavaiko *et al.* (1996) also reported that the Au biosorption efficiencies of a range of micro-organisms including *Aspergillus terreus, Rhizopus arrhizus*, and waste biomass producers of neomycin, gentamicin and ristomycin (94-98.8%) were comparable to that of activated carbon (98.8%). A further benefit was suggested in a study by Sing and Yu (1998) which found that unlike ion-exchange resins, which are negatively affected by the presence of various organic solvents, the presence of some organic solvents such as ethanol had a positive effect on the Cu adsorption capacity of *Phanerochaete chrysosporium.* Some micro-organisms are reported to be able to selectively scavenge certain metals from a mixed element solution, whereas others are able to bind a range of different metals. Another advantage of many biomass types is that sorbed metals can be readily desorbed by chelating agents such as EDTA, mineral acids (HCl and HNO_3) and K, Na and Ca salts, enabling both the recovery of metals and reuse of the biomass.

2.7.1 Biosorption

Biosorption by a microbial biomass is a function of the chemical composition of the cells it consists of (Holan and Volesky, 1995). It involves several mechanisms which differ, both quantitatively and qualitatively, according to the biomass used. Some of the

main mechanisms are ion exchange, physical adsorption, chelation, ion entrapment and diffusion through cell walls and membranes as a result of concentration gradients. There are several chemical groups that can attract and sequester metals in biomass, such as the acetomido groups of chitin (structural polysaccharides of fungi), amino and phosphate groups in nucleic acids, amino, amido, sulphydryl and carboxyl groups in proteins, and hydroxyls in polysaccharides (Volesky and Holan, 1995). However, the presence of these groups does not necessarily mean accessibility for sorption, due to steric or other barriers. Metal uptake can be a result of physico-chemical deposition via adsorption or precipitation. There are two types of adsorption - physical adsorption which occurs more readily but can easily be desorbed, and chemisorption, involving the formation of chemical bonds and less easily desorbed. Physical adsorption occurs as a monolayer, in comparison to chemisorption which has multilayer characteristics (Macaskie and Dean, 1990). Metals can be deposited on the cell surface or within the cell wall structure. This may result in the rupturing of the cell wall, increasing cell permeability and therefore resulting in intracellular accumulation due to exposure of internal bonding sites (Gadd, 1990). Studies have shown that more metal is associated with the cells than can be accounted for by the number of available binding sites, indicating that the initial metal-ligand binding sites act as a nucleation site around which further metal is then deposited. It has also been shown that some fungi can adsorb insoluble metal compounds e.g. sulphides (Gadd, 1990). *Mucor flavus, Aspergillus niger, Penicillum notatum* and *Fusarium solani* could all remove $Fe(OH)_3$ from acid mine drainage, and when *M. flavus* was grown with PbS for 2-5 day it converted the compound to a fine even suspension which was then completely adsorbed after 7 days (Wainwright *et al.*, 1986).

It is thought that biosorption occurs in two phases. The first phase is an initial fast reaction mechanism based predominantly on chemisorption, which may then be followed by residual and much slower additional metal deposition, indicating a different secondary metal binding mechanism. This may also be followed by a third slower phase of intracellular accumulation, depending on both the type of micro-organism and metal. Estimated times for each process to occur vary greatly, ranging from <4 seconds to 20 minutes for the initial biosorption to occur (Kuyucak and Volesky, 1988; Huang *et al.*, 1990; Zhang *et al.*, 1998), with processes of additional metal deposition and intracellular uptake reaching equilibrium in 1-4 hours (Strandberg *et al.*, 1981; Pradhan and Levine, 1995). However, this depends on a wide range of factors, as discussed in

Section 2.7.3. Several studies have found that the amount of metal accumulated varies with cell density. A higher cell density removes a greater amount of metal from solution, however, the amount of metal accumulated per gram of cells decreases with increasing cell density (Junghans and Straube, 1991; Horikoshi *et al.,* 1981). This lower uptake can be explained by adhesion between the cells at increasing cell densities reducing the relative amount of surface area available for binding (Kapoor and Virarahgavan, 1995; Kurek *et al.,* 1982).

Both living and dead cells can accumulate metals. The majority of biosorption studies have been carried out using dead biomass for several reasons (Kapoor and Viraraghavan, 1998). Firstly, dead biomass is more readily available, as a by-product from certain industrial processes such as the production of antibiotics (*Penicillium*) and brewing (*Saccharomyces cerivisciae*). Non-viable cells are not sensitive to adverse conditions such as high pH, temperature fluctuations, metal toxicity and low nutrient availability. It is also easier to desorb metals from dead cells enabling the biomass to be reused, as opposed to living cells were metals may be locked up intracellularly in living tissues. The biosorptive capacity of dead biomass may be greater than, equal to or less than the amount accumulated by living cells depending on both the type of micro-organism and the metal. However, living cells can offer several advantages in that they may also accumulate metals intracellularly, often to a higher concentration than dead cells (Gadd, 1990). Living cells can also precipitate metals in and around the cells walls, and in the external medium by various products of metabolism.

Kurek *et al.* (1982) examined the biosorption of Cd by soil micro-organisms in comparison with the involvement of other soils constituents in the same process. The study found that when the same mass of bacterial cells, clay and sand were incubated separately in media containing 10µg/ml Cd, bacterial cells removed the largest quantity of Cd, with dead cells removing more than living cells. However, although the bacterial biomass used in the experiment was similar to that found in the top layer of soil, the amount of sand and clay was much lower than in a typical soil. When the three constituents were used in approximately the same amount as they occur in soil and incubated separately, then clay removed most Cd (80%), and the cells removed 40%. This study also looked at Cd removal by a range of other soil micro-organisms (seven bacteria and four fungi) and found that all 11 strains were capable of removing

substantial amounts of Cd from the medium (40.5-90.0% of total Cd added). It was concluded that microbial sorption, particularly that of dead cells, could be an important factor for immobilising Cd in the soil environment and determining Cd availability to plants.

2.7.2 Bioaccumulation

Bioaccumulation is the metabolic-dependent uptake of metals, and can therefore only occur in living cells. It is usually a slower process than biosorption, and is generally a less significant process in terms of the overall amount of metal accumulated. Many metals are essential for growth and metabolism (e.g. Cu, Fe, Zn, Co, Mn) and micro-organisms can therefore accumulate them intracellularly from low external concentrations, although the mechanisms by which this occurs are complex and not fully understood (Gadd, 1990). Increases in metal concentrations beyond these essential requirements can result in cell lysis and loss of viability (Pradhan and Levine, 1995). However, many micro-organisms can tolerate levels greatly exceeding these threshold values, which they do in two main ways. Firstly, prevention of intracellular accumulation by precipitating metals on the cell wall, thereby protecting sensitive intracellular structures. Secondly, by binding metals and storing them intracellularly in a non-toxic form. Metals may be compartmentalised and/or converted to safer forms by precipitation or binding. Metals such as Ag, Co, Zn, Mg, Mn and K are often located in vacuoles, bound to low-weight polyphosphates. Some yeasts are reported to 'oxidatively detoxify' Tl to TlO_2 within mitochondria which can then be excreted from cells (Gadd, 1990).

A common response to metal exposure in many micro-organisms is the induction of intracellular, low-molecular weight, cysteine-rich, metal-binding proteins called metallothioneins, which have functions in the detoxification, storage and regulation of intracellular metal ions (Hughes and Poole, 1989). Metallothioneins may be induced by one metal and not another. They are known to be induced by Cu, Zn and Cd, and are reported to be able to bind Cu, Cd, Au, Ag, Hg and Zn. Fungal pigments have also been implicated in metal resistance and accumulation. Many extracellular fungal products can also complex or precipitate metals. Citric acid is an effective chelator (Hughes and

Poole, 1989) and oxalic acid can interact with metals to form insoluble oxalate crystals both around cell walls and in the medium (Murphy and Levy, 1983). The production of H_2S is common in many micro-organisms, and can confer metal resistance by the precipitation of insoluble metal sulphides in and around cell walls (Gadd and Griffith, 1978). However, recent studies carried out by Tsezos *et al.* (1997) on sorption sites of Pd, Y, Ag and Ni by 3 microbial strains (*Arthobacter* spp., *Pseudomonas mendocina* and *Alkaligenes eutrophus*) suggest that localisation of sorbed metals could be metal dependent rather than strain dependent.

2.7.3 Factors affecting uptake

The amount of metal accumulated varies greatly depending on a wide range of factors such as pH, competing ions, aerobic/anaerobic conditions, temperature, metal, species, age of culture and even on cell form within the same organism. pH affects the solution chemistry of the metals, the activity of functional groups in the biomass as well as the competition of metallic ions for the binding sites. At pH 4-5 almost all metallic species are ionised as cations, and the carboxylate groups of the proteins in the cell wall will be largely disassociated generating negatively charged cell surfaces. As the pH increases, some of the aminophosphate groups and lipoproteins of the cell wall are hydrolysed thus making some of the sites unavailable for metal binding (Pradhan and Levine, 1995). At low pH (<3), Zhang *et al.* (1998) reported that H^+ ions compete with metal ions for the adsorption sites. However, the optimal pH range varies greatly between different micro-organisms and metals. For example, U uptake by *Rhizopus, Asperigillus* and *Mucor* was optimal at pH 4-5 and substantially inhibited at pH 2.5, but uptake of U by *Penicillium* remained the same over a pH range of 2.5-9.5, indicating differences in fungal cell wall behaviour (Kapoor and Viraraghavan, 1995). The same researchers suggest that preferential sorption of metals by various fungal species may be attributed solely to variations in the fungal cell wall characteristics, whereby different cell wall polymers have different functional groups and different charge distributions and therefore different metal-binding affinities (Morely and Gadd, 1995).

Competing ions, such as other metals and ligands, can affect uptake by direct competition for adsorption sites, or through precipitation, e.g. of phosphates and

hydroxides. If the stability constants of the metal-ligand complexes are greater than the stability constants of the metal-biosorption site on the cell wall surface then biosorption will be considerably reduced. Several workers have reported that the biosorption of metals is relatively unaffected within a temperature range of 4-30°C (Gadd, 1990; Horikoshi *et al.,* 1981), unlike bioaccumulation which is inhibited at low temperatures as metabolic processes slow down. Tobin *et al.* (1984) found that higher uptake capacity tended to be observed for larger ions, and sorption has been reported to be preferential according to both ionic radius and charge with the order of metal binding stability decreasing in the order of Pb>Cu>Ni>Co>Zn>Cd>Fe>Mn>Mg. Mechanisms of sorption also greatly depend on whether cells are dead or alive, and if alive, whether they are growing (Gadd, 1990), with the genetic, biochemical and cellular characteristics of the biomass affecting binding mechanisms of the targeted metals. Townsley and Ross (1986) reported that Cu sorption by *Aspergillus niger* was 2.5 times greater in growing cultures than non-growing cultures. Zajic and Chiu (1972) found U uptake by *Penicillium* was culture age dependent, with 5 day old cultures being twice as effective as 15 day old cultures. Possible explanations for this are changes in cell-surface chemistry and morphology with age. Uptake by cells was also reported to be dependent on contact time and the initial metal ion concentrations (Kapoor and Virarahgavan, 1995). Hence, comparisons between studies are not always straight forward as many of the above factors vary greatly between different experiments. An additional problem in sorption studies is that some metals form microprecipitates, and the interpretation of results becomes complicated due to the fact that a collection of metal species is not due to sequestration only. Pb in particular, has a complex solution chemistry, and at pH>5 the formation of $Pb(NO_3)_2Pb(OH)_2$ and $Pb(NO_3)_25Pb(OH)_2$ complexes can destort sorption results (Volesky and Holan, 1995, Zhang *et al.,* 1998).

2.8 Fungi and yeast

Fungi are non-photosynthetic hyphal eukaryotes which absorb all their required nutrients from the substrate or host (Carlile and Watkinson, 1994). The hyphae, which grow apically, exert a mechanical force which can penetrate a leaf cuticle (Subramanian, 1983) and enable the efficient colonisation of substrates. Yeasts do not usually produce

hyphae but are also considered to be fungi on the grounds that they are plants that live by absorbing organic materials rather than by photosynthesis. They are generally unicellular and reproduce by budding though they may, on occasion, produce hyphae. The main factors affecting growth and activity are temperature, light, pH, moisture content and aeration. Growth is also affected by the availability of nutrients and trace metals, such as sulphur, phosphorus, potassium, zinc, boron, and the availability of carbon sources. However, many fungi and yeasts appear to be fairly robust, growing well in the pH range 3-11, and are capable of using a variety of carbon sources. They have the ability to grow in environments heavily contaminated with pollutants such as heavy metals and hydrocarbons. In a study of yeasts in sediments and soil, the highest total yeast counts were recorded at the most polluted sites (Vadkertiova and Slavikova, 1994).

The distribution of fungi and yeasts in soil appears to be both quantitatively and qualitatively variable, and may relate to differences in soil type and porosity, organic content, rainfall, pH, oxygen level, temperature and level of contamination (Montuelle *et al.,* 1994; Sage *et al.,* 1997). Both fungi and yeast are of great practical importance. Fungi are important in the breakdown and recycling of organic plant matter (decomposition of animals/micro-organisms is mainly carried out by bacteria), and their metabolic diversity has been exploited by the pharmaceutical industry for the production of antibiotics, enzymes and hormones (Carlile and Watkinson, 1994). Yeasts are present in soils in much lower numbers than bacteria, fungi and actinomycetes, and therefore are not thought to be as important in breaking down organic matter. However, they have been used for thousands of years in brewing and baking, and remain an important aspect of research in these industries.

2.8.1 Biosorption by fungi and yeast

As stated in Section 2.7, for reasons of both economics and ready availability many biosorption studies have focused on the metal-binding capacities of waste biomass by-products of the fermentation and pharmaceutical industries. These include a range of fungi such as *Rhizopus* and *Absidia* (steroid transforming fungi), *Penicillium* (antibiotic production), and yeasts, for example, *Saccharomyces cerevisiae* (brewing industry).

Table 2.4 identifies the metal binding ability of several metals by a range of fungi and yeast. The values vary greatly both between metals and biomass types. This is partly due to the fact that there is no uniform methodology for establishing metal-binding capacity, and therefore as discussed in Section 2.7.3, comparisons between studies are not always meaningful. However, it can be concluded that many types of both fungi and yeast have great potentials as cheap and efficient sorbents for a range of metals.

Table 2. 4 Some reported metal uptake values for a range of fungi and yeasts

Biomass type	Metal	Metal uptake	Reference
Absidia orchidis (f)	Pb	351 mg/g	Holan and Volesky, 1995
Penicillium chrysogenum (f)	Pb	122 mg/g	Niu *et al.*, 1993
Rhizopus nigricans (f)	Pb	166 mg/g	Holan and Volesky, 1995
Rhizopus arrhizus (f)	Pb	104 mg/g	Tobin *et al.*, 1984
Saccharomyces cerevisiae (y)	Zn	14-40 mg/g	Volesky and May-Phillips, 1995
Candida tropicalis (y)	Cu	2-61 mg/g	Junghans and Straube, 1991
Rhizopus arrhizus (f)	Zn	20 mg/g	Tobin *et al.*, 1984
Penicillium chrysogenum (f)	Zn	6.5 mg/g	Niu *et al.*, 1993
Aspergillus niger (f)	Pb	10.1 mg/g	Kapoor and Viraraghavan, 1998
Saccharomyces cerevisiae (y)	Cu	3-43 mg/g	Junghans and Straube, 1991
Saccharomyces cerevisiae (y)	U	55-140 mg/g	Volesky and May-Phillips, 1995
Rhodotorula spp (y).	Cu	3-49.1	Junghans and Straube, 1991
Penicillium chrysogenum (f)	Cd	56 mg/g	Holan and Volesky, 1995
Rhizopus arrhizus (f)	Cr	31 mg/g	Tobin *et al.*, 1984
Saccharomyces cerevisiae (y)	Cu	17-70 mg/g	Volesky and May-Phillips, 1995
Rhizopus arrhizus (f)	Ni	18 mg/g	Fourest and Roux, 1992
Rhodotorula mucilaginosa (y)	Cu	14.3 mg/g	Norris and Kelly, 1979
Aspergillus niger (f)	Ag	22.3 mg/g	Mullen *et al.*, 1992
Aspergillus niger (f)	Cd	3.2 mg/g	Kapoor and Viraraghavan, 1998
Rhodotorula mucilaginosa (y)	Cd	4.7 mg/g	Norris and Kelly, 1979
Rhodotorula glutinis (y)	U	21-37 mg/g	Horikoshi *et al.*, 1981
Rhodotorula rubra (y)	Ag	9.6 mg/g	Kierans *et al.*, 1991

Key: (f) = fungus

(y) = yeast

Polysaccharides represent about 90% of the fungal cell wall, and are present both as crystalline polysaccharides which form the cell wall 'skeleton', for example chitin and beta-glucans, and amorphous polysaccharides which form the 'cement' and carbohydrate component of extracellular enzymes (Farkas, 1979). The polysaccharides are complexed with proteins, lipids and pigments to form a multilaminate microfibular structure. Phosphodiester and carboxyl groups confer the electrical potential to the cell wall. Fungal cell walls contain chitin and chitosan, both of which have been shown to sequester metal ions. Unlike bacteria, fungal cell walls contain little protein and therefore chitin is likely to be the main Lewis base which is active in the cell wall (Zhang *et al.,* 1998). Chitin is a linear polymer of the acetylated amino sugar N-acetyl-glucosamine, and chitosan is the deacetylated derivative of chitin (Carlile and Watkinson, 1994). Chitin contains amine groups which bond with metals. The fungal cell wall can be thought of as a porous structure, and it has been suggested that both chemically bound and physically entrapped metals may act as nucleation sites for the further deposition of metals in the cell wall (Farkas, 1979). Tsezos and Volesky (1982) hypothesised that U biosorption by inactive fungal biomass involved firstly the formation of a complex between dissolved metal ions and chitin chains inside the cell wall, followed by the adsorption of additional U by the chitin network, close to that complexed by the chitin nitrogen. Finally hydrolysis of the U-chitin complex and precipitation of the hydrolysis product in the cell wall was thought to occur.

This is supported by work done by Zhang *et al.* (1998) on the accumulation of Pb by inactive *Rhizopus nigricans*, the cell wall of which contains 58% chitin. They found that blocking the chitin complexation sites reduced Pb uptake, whereas conversely increasing the amount of chitin complexation sites by deacetylating *R. nigricans* to form chitosan structures resulted in increased uptake. This study also demonstrated that the Pb uptake capacity of pure chitin was 13mg/g. However, uptake experiments demonstrated that *R. nigricans* was capable of accumulating as much as 74 mg/g of Pb. This value is far greater than that accumulated by pure chitin alone, confirming additional processes must be occurring. The amount of both chitin and chitosan in the cell wall can change during growth, and may account for the observed changes in metal accumulation with age. However, Volesky and Holan (1995), in a review of the biosorption of heavy metals, state that chitin and chitosan are not the major metal-

binding compounds and suggest that phosphate, hydroxyl and sulphydryl groups are equally as important.

2.8.2 Isolates utilised in laboratory experiments

A range of micro-organisms were isolated from the rhizosphere of plants collected at the Dagenham and Brentwood wetland systems. Two of these isolates were then studied in detail to determine their ability to tolerate and accumulate heavy metals. These were identified as the fungus *Beauveria bassiana* and the yeast *Rhodotorula mucilaginosa*, and are described in the following sections:

2.8.2.1 Beauveria bassiana

Beauveria bassiana is classified as an ascomycete fungus (personal communication: Dr J. Stephen, Center for Environmental Biotechnology, University of Tennessee, USA, 1998). Ascomycete fungi are higher fungi, classified as such through the presence of cross-walls in their hyphae (Carlile and Watkinson, 1994).

Beauveria bassiana is an entomopathogenic fungi (insect fungal parasite) of the migratory grasshopper (*Melanoplus sanguinipes*) (Bidochka and Khachatourians, 1993), and has been studied as a biopesticide against a range of insects such as grasshoppers (Inglis *et al.,* 1997), rice hispa (Hazarika and Puzari, 1997) and Colorado beetles (Subramanian, 1983). *B. bassiana* produces elastolytic proteases which electrostatically adsorb to free carboxyl groups on the insect cuticle proteins and breakdown the cuticle, thus enabling penetration by fungal hyphae (Bidochka and Khachatourians, 1994). *B. bassiana* is common in soil (Shimazu and Sato, 1996; Chandler *et al.,* 1997). It is a very robust fungus, being able to tolerate low sugar content, certain fungicides (Cyclohexamide (see Section 5.3) and Kasumin Bordeaux), elevated levels of $CuCl_2$, and can grow well over a pH range of 4-11 (Shimazu and Sato, 1996). Fargues *et al.* (1997) also reported that isolates from a variety of locations grew well over a wide temperature range of 8-30°C. Its abilities to biotransform hydrocarbons (Pietz *et al.,* 1997) and warfarin (Cannell *et al.,* 1997) have also received attention. *B. bassiana* is

grown on an industrial scale for the production of the antibiotic Beauvericin, a cyclohexadepsipeptide mycotoxin which has insecticidal properties and can induce apoptosis in mammalian cells (Logrieco *et al.,* 1998), and also for the production of numerous insecticides (Volesky, 1990).

2.8.2.2 Rhodotorula mucilaginosa

Rhodotorula mucilaginosa is a basidomycete yeast (personal communication: Dr J. Stephen, Center for Environmental Biotechnology, University of Tennessee, USA, 1998). *Rhodotorula* species have been isolated from a wide range of environments, including polluted soil (Middelhoven and Spaaij, 1997), streams (Pecanha *et al.,* 1996), lakes (Slavikova *et al.,* 1992), leaf surfaces (Middlehoven, 1997), acid mine drainage waters (Ehrlich and Fox, 1967), gold-mine effluents (where it was studied for its ability to assimilate ammonia (Andrade *et al.,* 1995)), and silver resistant strains from photographic sludge (Belly and Kydd, 1982). Vadkertiova and Slavikova (1994) found that *Rhodotorula* spp. formed a higher proportion of total yeast populations of both lake sediments and soil in summer in comparison to autumn counts. However, one species of *Rhodotorula*, *R. rubra,* was only isolated in soils during autumn. The proportion of *Rhodotorula* remain fairly consistent between sediment and soil samples, with four *Rhodotorula* species and two *Rhodotorula* species composing 15% and 16% of the total yeast population during summer in sediment and soil samples, respectively. Sage *et al.* (1997) reported that *R. rubra* was one of two yeasts more often isolated from heavily polluted sites than lightly polluted ones.

Rhodotorula spp. are known to degrade hydrocarbons (Subramanian, 1983), cresol (Middelhoven and Spaaij, 1997), and have been used in catalysing the hydrolysis of certain acids used in the synthesis of antibiotics (Subramanian, 1983). They also form a major component of slime in paper pulp mills. *Rhodotorula* spp. are coloured pink by carotenoid pigments, and studies have highlighted the potential of *R. rubra* to be used as a source of pigment and protein feed for aquacultured animals (Hari *et al.,* 1992). The ability of certain species to accumulate Ag, Cd, U and Cu has also been investigated (see Table 2.4).

2.8.3 Summary of biosorption by fungi and yeast

The ability of many species of fungi and yeast to accumulate and precipitate metals via a wide range of mechanisms has been clearly demonstrated. Recent studies have focused on the potential and practical application of microbial sorption as an efficient and cost effective method of removing metals from industrial effluents. However, the potential of fungi and yeast to remove and immobilise heavy metals as a treatment process in constructed wetland treatment systems receiving urban runoff has not been investigated.

CHAPTER 3 SAMPLING LOCATIONS, MATERIALS AND METHODS

3.1 Site descriptions

3.1.1 The Brentwood wetland

Both constructed wetlands were constructed between January and April 1995. This site is at Brentwood, a small town situated to the north east of London (Grid reference: 5585, 1928). The constructed wetland site includes a horizontal sub-surface flow system under normal flow conditions and a surface flow system in storm conditions. There is also an adjacent area of natural wetland (surface flow system) (Figs. 3.1, 3.2 and 3.3). The wetland system is built on the site of a flood basin receiving flow from a SWO (surface water outlet), which drains a catchment area of 150 ha, before joining the River Ingrebourne close to its source. At this point urban runoff constitutes the majority of flow within the River Ingrebourne. The wetland system has a total surface area of 360m^2 during low flow conditions. The constructed wetland has a surface area of 144m^2 and the natural wetland (which consists of a channel dug into the flood basin (see Fig. 3.1)) has a surface area of 60m^2 . However, during storm events the natural wetland can flood into the surrounding flood basin which has an area of approximately 2800m^2. Background water analyses carried out by the National Rivers Authority (now the Environment Agency for England and Wales) of runoff discharging from the SWO identified elevated BOD levels (up to 75 mg/l) and heavy metal concentrations, especially lead and zinc (maximum concentrations of 195 µg/l and 132 µg/l respectively). Runoff initially enters a settlement zone which reduces the water velocity and encourages settlement of suspended solids. Under normal flow conditions water passes through both the constructed wetland planted with *Phragmites australis* and through the natural wetland, colonised by *Typha latifolia*. The water level is controlled throughout the wetland by stoplogs. Both the constructed wetland and the natural wetland discharge into a combined outlet chamber before rejoining the River Ingrebourne.

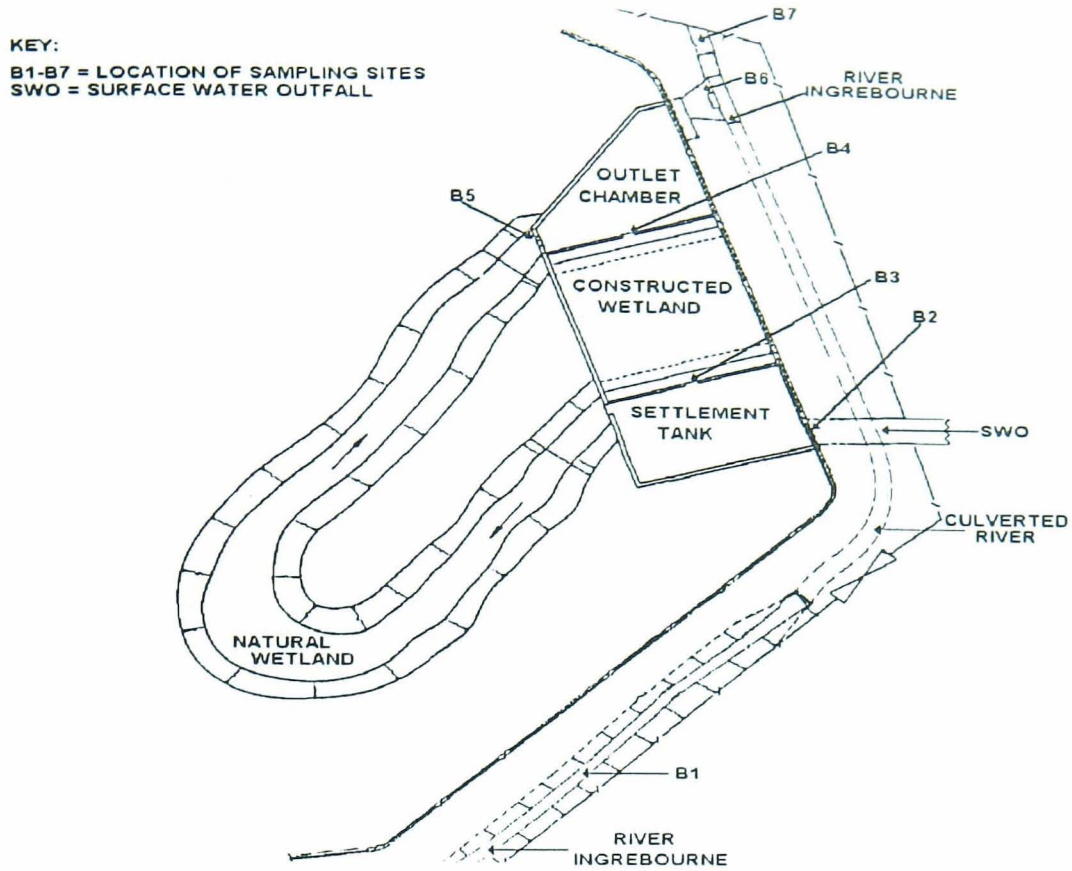

Figure 3. 1 Diagrammatic representation of the Brentwood wetland (scale: 2cm=12m)

Figure 3. 2 View from the final settlement tank looking back through the constructed wetland component (planted with *Phragmites australis*) towards the initial settlement tank, Brentwood wetland.

Figure 3. 3 View across final settlement tank looking into the natural wetland component (colonised by *Typha latifolia*), Brentwood wetland.

3.1.2 The Dagenham wetland

This site is at Dagenham, East London, and is located on the Wantz stream (Grid reference: 5502, 1842). The Wantz is a small watercourse which receives substantial discharges from the surrounding 440 ha urban catchment area. Water quality data collected by the National Rivers Authority prior to the construction of the wetland indicated elevated levels of BOD (up to 69.4 mg/l) and of heavy metals (total Pb 285 µg/l and total Zn 550 µg/l). The watercourse exhibits flashy characteristics during storm events owing to the highly impermeable nature of the catchment area, resulting in a visible deterioration in water quality. The wetland is 250m long and is built in a specifically widened area of the stream. A series of weirs control the flow into three separate beds to prevent hydraulic short-circuiting and to increase aesthetic appeal (Figs. 3.4, 3.5 and 3.6). In front of the first weir is a settlement zone for the initial removal of suspended solids. The first bed is planted with *Typha latifolia* followed by two beds planted with *Phragmites australis*, all at a planting density of $4/m^2$.

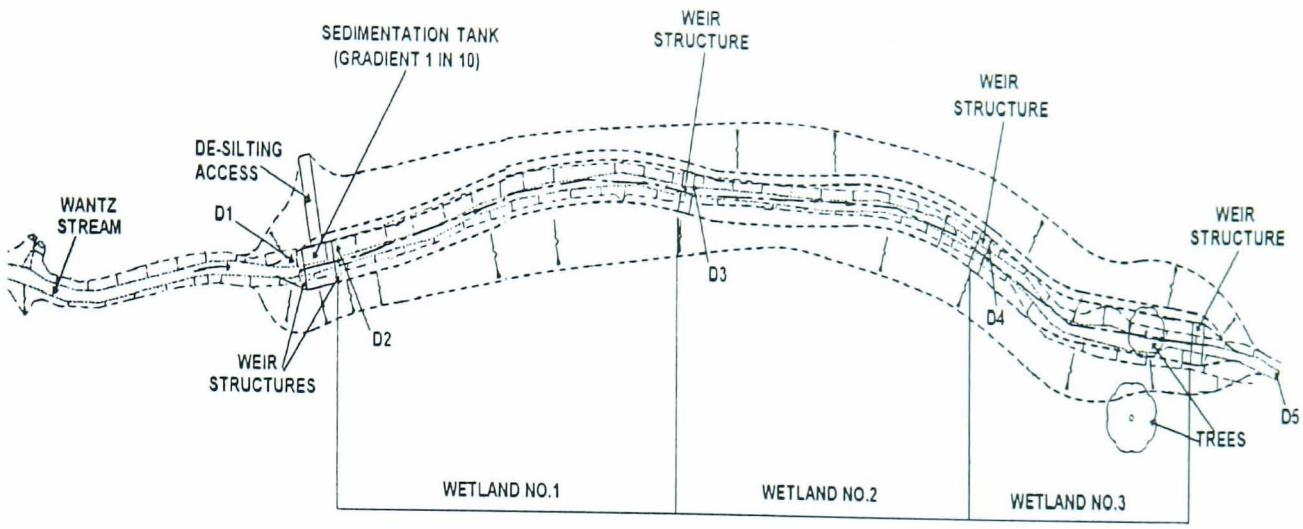

Figure 3. 4 Diagrammatic representation of the Dagenham wetland (scale: 2cm = 40m)

Figure 3. 5 View looking downstream across the settlement tank and the start of bed 1 (planted with *Typha latifolia*)

Figure 3. 6 View along wetland system looking up through bed 2 (planted with *Phragmites australis*) up to end of bed 1 (planted with *Typha latifolia*).

3.2 Sampling programme

Twelve sampling sites were identified, seven at Brentwood (labelled B1 - B7; Fig. 3.1) and five at Dagenham (labelled D1-D5; Fig. 3.4). These sampling sites were selected to cover all sections of each wetland treatment system in order to assess the performance of the individual wetland components, as well as that of the overall system. Sampling was carried out at bi-monthly intervals between October 1995 and October 1997. In addition, samples were also collected during seven storm events, of which three storm events were recorded at the Dagenham wetland (1 May 1996, 25 August 1997 and 28 November 1997) and four storm events were recorded at the Brentwood wetland (25 August 1997, 8 October 1997, 28 November 1997 and 26 May 1998).

3.3 Sample collection and field measurements

3.3.1 Water and sediment samples

Duplicate samples of water and sediment were collected from approximately the same position at each of the sample sites shown in Figures 3.1 and 3.4. Water samples were collected in acid washed (10% nitric acid) plastic bottles, and then stored at 4°C until analysis could be carried out. In addition, extra water samples were collected at each of the sample sites at Dagenham for analyses of trihalomethanes (carried out by the Environment Agency, Thames Region). Surface sediment samples were collected using either a plastic scoop or a sediment grab, transferred to plastic bags and stored in a freezer until analyses could be carried out.

3.3.2 Plant samples

Plants were collected four times (seasonally) between 1997-98 for tissue metal analysis. This involved the collection of *Typha latifolia* from the inlet of the first bed at Dagenham and *Phragmites australis* from the inlet of the constructed wetland at Brentwood. These were carefully removed using a fork to ensure sufficient sections of roots and rhizomes were collected, transferred to large plastic bags and stored in a freezer until analyses could be carried out.

3.3.3 Other parameters

Dissolved oxygen content (pHOX 62 meter), temperature (pHOX 62 meter) and pH (Checkes pH meter) were recorded at each sample site and on each occasion. Flow velocity (Ott propeller meter) was also measured at each of the sample sites at the Dagenham wetland and at B2, B5 and B6 at the Brentwood wetland, together with the water cross-sectional parameters to enable the calculation of flow rate (l/s).

3.3.4 Microbiological sampling

The samples for the microbial work were collected from the plant rhizospheres on 27 November 1996. This involved the collection of plants from five locations. The sites and species are as follows:

Brentwood - the constructed wetland (*Phragmites australis*)
- the natural wetland (*Typha latifolia*)

Dagenham - bed 1 (*Typha latifolia*)
- bed 2 (*Phragmites australis*)
- bed 3 (*Phragmites australis*)

Plants were carefully removed with a sterile trowel, placed in sterile bags for transport to the laboratory and analysed within four hours of collection.

3.4 Determination of retention time

The retention time was determined by measuring the length of time a fluorescent dye added to the flow at the inlet of the system took to appear at the outlet. In this case the dye used was Rhodamine WT (recommended by the Environment Agency). The amount of dye to be added was determined by the flow recorded at the inlet to each wetland system, with the maximum permissible concentration of Rhodamine WT being 15mg/l. Retention times were measured on two occasions at each wetland during dry weather flow conditions.

3.5 Extraction procedures for heavy metals

The measurement of total heavy metals in water (µg/l), sediment (µg/g) and plants (µg/g) involved the digestion of the sample with concentrated nitric acid followed by analysis using either Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) or Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Acid digestion was necessary to solubilize heavy metals by oxidizing organic matter and releasing

bound metals. The procedures for acid digestion of each sample type are described in the following sections.

3.5.1 Water samples

Duplicate water samples (100ml) were placed in acid washed (10% nitric acid) teflon beakers, to which 1ml of nitric acid was added, and then evaporated to dryness on a sand bath at 100^0C. The residues were dissolved in 1% nitric acid, filtered through a Whatman No. 42 filter paper and made up to a final volume of 100ml. The extracted samples were analyzed for Pb, Cu, Cd, Cr and Ni by Graphite Furnace Atomic Absorption Spectroscopy, and for Zn by Inductively Coupled Plasma Atomic Emission Spectroscopy.

3.5.2 Sediment samples

Sediment samples were dried in an oven at 100^0C for 24 hours, ground in a mortar and pestle, and sieved to a fraction of less than 250 μm. Duplicate 5g samples of sediment were weighed out, placed in acid washed (10% nitric acid) teflon beakers, and digested by adding 25ml of concentrated nitric acid and evaporating to dryness on a sandbath at 100^0C. The residues were dissolved in 1% nitric acid, filtered through a Whatman No. 42 filter paper and made up to a final volume of 100ml. The extracted samples were analyzed for Pb, Zn, Cu, Cd, Cr and Ni by Inductively Coupled Plasma Atomic Emission Spectroscopy.

3.5.3 Plants

Plant samples were washed thoroughly with tap water to remove all attached sediment and separated into 3 parts comprising the roots, the rhizomes and the leaves. The separate plant components were oven dried at 100^0C for 24 hours and ground in a mortar and pestle. Duplicate samples were weighed out, placed in acid washed (10% nitric acid) teflon beakers and digested with 10ml of concentrated nitric acid. The

residues were made up to a final volume of 100ml using the same procedure as for the water and sediment samples. The extracted samples were analyzed for Pb, Zn, Cu, Cd, Cr and Ni by Inductively Coupled Plasma Atomic Emission Spectroscopy.

3.6 Metal Determination

3.6.1 Inductively Coupled Plasma - Atomic Emission Spectroscopy

Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) (Perkin Elmer Model Plasma 40 Spectrometer) was used to determine the total metal concentrations of Pb, Zn, Cu, Cd, Cr and Ni in sediment and plant samples, and of Zn in the water samples. The published detection limits of Pb, Zn, Cu, Cd, Cr and Ni were 40, 3, 1, 2, 3 and 7 µg/l, respectively (Fifield and Kealey, 1989). The procedural blank was used as the calibration blank, and each sample was analysed three times with instrumental parameters at the default setting.

3.6.2 Graphite Furnace Atomic Absorption Spectroscopy

Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) (Perkin Elmer Zeeman Atomic Absorption Spectrometer, Model 4110 ZL) was used to determine the total metal concentrations of Pb, Cu, Cd, Cr and Ni in water samples. The detection limits given in the literature were 0.05, 0.02, 0.003, 0.01 and 0.1 µg/l for Pb, Cu, Cd, Cr and Ni, respectively (Fifield and Kealey, 1989). However, those determined in the laboratory (calculated as 3 x standard deviation of a procedural blank) were 0.18, 0.45, 0.27, 0.15, and 0.21 µg/l for Cd, Pb, Cr, Ni and Cu, respectively. The fact that these values were higher than the published limits of detection was thought to be associated with the fact that the GFAAS was located in a open laboratory rather than in a clean room. Each sample was analysed three times with the instrumental parameters at default setting, including the use of a mixed matrix modifier ($Mg(NO_3)_2.6H_2O$ and $NH_4H_2PO_4$) in the determination of total Pb concentrations. For each metal a procedural blank was

run as a sample, and the concentration of metal detected in the blank subtracted from the sample concentration to allow for possible contamination during sample preparation.

3.6.3 Analysis of standard reference material

Certified water and sediment reference materials were analysed to determine the efficiency of the extraction procedures outlined in Sections 3.5.1 and 3.5.2. The reference materials used were Surface Water (SPSW1) and Chinese Stream Sediment (GBW 07311), both of which were supplied by Promochem Ltd, Welwyn Garden City, Herts, England. These reference materials were selected as the quoted certified metal concentrations were within the range of values recorded in water and sediment samples collected at both sites during the monitoring programme. Table 3.1 gives the metal concentrations determined for each of the reference materials in comparison with the certified values.

Table 3. 1 Mean metal concentrations determined in standard reference materials using routine extraction procedure in comparison to certified values (±SD)

	Zn	Cd	Cr	Ni	Cu	Pb
Surface Water Sample (µg/l)						
determined values	19±2.8	0.5±0.0	1.8±0.28	9.9±1.9	21.4±2.3	2.9±0.17
certified values	20±1	0.5±0.01	2.0±0.01	10±0.1	20±1.0	5±0.1
Chinese Stream Sediment sample (µg/g)						
determined values	250±10.7	4.6±0.3	9±0.9	40.2±1.9	44±3	436±34
certified values	373±21	2.3±0.2	40±4	14.3±1.5	79±4	636±34

The values determined for the surface water reference material using the routine method agreed well with the certified values except for Pb where a comparison of the values indicated an extraction efficiency of only 58%. As the values for the other metals compared well, it was thought that this lower extraction efficiency for Pb may be a due to a problem with the reference material. To test this hypothesis, the extraction procedure was repeated on a Pb standard solution (5µg/l) which resulted in an Pb extraction efficiency of 83%. Although this value is less than the extraction efficiencies reported for the other metals it is considerably greater than the extraction efficiency reported for the reference material, supporting the suggestion that the lower extraction value is associated with the reference material itself rather than with the extraction procedure.

The values determined for the sediment reference material using the method vary markedly from the certified values (Table 3.1). The determined values given in the Table are the mean of four separate samples; two of the samples involved the digestion of 5g of reference material, and two samples involved the digestion of 10g of reference material. The SDs associated with the mean values indicate that the concentrations determined were consistent for all four samples. The reference material originates from China, and although the UK supplier states in their brochure that total dissolution techniques were used in the certification procedure, the company was not able to provide any further information on the extraction process. It is thought that possible differences in the extraction procedures employed are an explanation for the differences between the certified and determined values.

3.7 Other chemical and physical analytical techniques

3.7.1 Chlorides, nitrates, phosphates and sulphates

The concentrations of chlorides (mg/l), nitrates (mg/l), phosphates (mg/l) and sulphates (mg/l) in water samples were determined using Ion Chromatography (Dionex series 2000i chromatograph). 50µl of sample was injected from the sample loop into an AS4 column, and analysed at a flow rate of 2ml/minute at a pressure of 1800psi. The eluant used was a solution of sodium carbonate and sodium hydrogen carbonate.

3.7.2 Biochemical Oxygen Demand (BOD_5)

BOD_5 is a measurement of the amount of oxygen required by micro-organisms to biodegrade the organic matter in a sample, and gives an indication of the level of organic pollution. To determine BOD_5, each water sample was first diluted by a factor of 4 with dilution water (a nutrient solution, containing calcium chloride, iron chloride, magnesium sulphate and phosphate buffer solution, saturated with oxygen) to ensure excess dissolved oxygen was present during the whole experiment. The dissolved oxygen content was immediately measured using a Checkmate DO meter (mg/l).

Samples were then incubated in the dark at 20^0C and after 5 days the oxygen content was again measured using the same instrument. The difference between the initial and the final dissolved oxygen contents represented the BOD_5 value of the sample following incorporation of the dilution factor.

3.7.3 Total Ammonia

The concentrations of total ammonia (mg/l) were determined using Nessler's method which is based on converting the ammonia present in the sample to a coloured complex ($Hg_2NH_2I_3$) by the addition of Nessler's reagent. The intensity of the colour, which is proportional to the concentration of ammonia, was measured by comparison with Lovibond colour standards.

3.7.4 Unionised Ammonia

When ammonia is dissolved in water the following equilibrium is set up:

$$NH_3 + H_20 \Leftrightarrow NH_4^+ + OH^-$$

As the pH increases the concentration of free ammonia (NH_3), which is more toxic to aquatic life, increases. The concentration of Unionised Ammonia (µg/l) present in a sample was determined using the following calculation (Surface Waters (River Ecosystem) (Classification) Regulations, 1994):

$$\text{Unionised Ammonia} = \text{Total Ammonia}/(1.0 + 10.0^{(10.055 - (0.0324 * t) - pH)})$$

where t = temperature (^{0}C)

3.7.5 Suspended solids

To determine the concentrations of suspended solids, 500ml water samples were vacuum filtered through a pre-weighed filter paper (Whatman No.42) which had been washed in distilled water and dried at 100^0C for 24 hours. After filtering of the sample, the filter paper was again dried at 100^0C for 24 hours, and then reweighed. The

difference between the initial and the final mass of the filter paper gave the mass of suspended solids in the sample.

3.7.6 Trihalomethanes

To determine the concentration of total trihalomethanes in water samples collected at each of the sampling locations at the Dagenham wetland, water samples were sent to the Environment Agency Fobney Laboratory for analysis using Gas Chromatography with Electron-Capture Detector.

3.8 Microbial methodology

3.8.1 Initial isolation procedure

1g of soil was scraped from the roots of each plant using a sterile spatula, and suspended in sterile saline (9mls). These solutions were serially diluted down to 10^{-7} using sterile saline. Replicate plates of the 10^{-4}-10^{-7} dilutions were plated out on 3 kinds of media: glycerol yeast extract agar (glycerol 35g, yeast extract 4g, $NaNO_3$ 2g, KH_2PO_4 1g, $MgSO_4.7H_2O$ 0.5g, $FeSO_4.7H_2O$ 0.01g, agar 15g in 1 litre distilled water), actinomycete isolation agar and Rao and Subrahmanyan's agar (Dietz and Thayer, 1980) and incubated at 30^0C for 7 days. 1.6ml/l of the antifungal agent cycloheximide (1000mg/l) was added to each of the media prior to autoclaving. Three different media were used to enable the isolation of a range of colony types, and to examine whether the type of medium affected the total numbers of micro-organisms isolated from each plant rhizosphere. A number of strains representing a range of morphologies were selected to be screened for metal tolerance, and were stored on glycerol yeast extract agar slants at 4°C. The isolates were selected on the basis that they represented a range of colony types.

3.8.2 Selection of metal tolerant colonies

Actinomycete isolation agar (Dietz and Thayer 1980), selected for ease of use, was spiked with either Zn or Pb at the concentrations (μg/g) shown in Table 3.2

Table 3. 2 Concentrations of Zn and Pb (μg/g) used to identify metal tolerant colonies.

	Zn	Pb
Minimum concentration	30	40
Mid-point concentration	400	200
Maximum concentration	800	400

Zn and Pb were selected for the metal tolerance experiments as these were found to be the metals present in the highest concentrations in the sediments at both wetlands. The concentrations chosen correspond to the minimum, mid-point and maximum values which had been recorded at the two sites prior to the commencement of the microbiological experiments.

Zn and Pb, as nitrate salts, were dissolved in 10 ml of distilled water and sterile filtered before being added to the autoclaved media. The addition of metal caused some precipitation at higher concentrations but at this stage, where the aim was to identify isolates that could tolerate metals, this was not considered a problem. The isolated strains were streaked on replicate spiked plates (four colonies/plate), and incubated at 30^{0}C for 7 days. Growth of each colony on the various metal concentrations was recorded as good, medium, present or absent. Based on these results, seven strains were selected for further work. However, the growth of five of the seven isolates was inconsistent on the spiked medium. These were therefore discarded, and the experiments described below concentrated on the establishing the ability of the two remaining isolates to tolerate and accumulate metals.

3.8.3 Selection of media for metal experimental work

To assess the performance of the selected strains it was first necessary to identify an appropriate medium for the experimental work. The selected media needed to fulfil two criteria; firstly that the metals must dissolve completely at a pH of 6.5-8 (pH range measured in the wetland systems), and secondly, that the medium used was a defined medium so that results were directly attributable to the isolates being studied and were not the effects of an 'ingredient' of unknown composition. Following a review of the literature, in which no suitable media were identified, a large number of different media were tested. As a result, a modified glycerol asparagine (GA) medium based on the work by Dietz and Thayer (1980) was selected. The modified GA medium contained Asparagine 1.14g, glycerol 20ml, MOPS (3 [N-Morpholino] propanesulfonic acid) 1.37g and agar 20g in 1 litre distilled water, pH 7.0-7.4.

3.8.4 Experimental procedures

3.8.4.1 Identification of selected isolates

Both isolates were submitted to the Public Health Laboratory Service, Mycology Reference Unit, Bristol, where samples were identified using in-house methods based on isolate morphology. Samples were also sent to Dr. John Stephen, Center for Environmental Biotechnology, University of Tennessee, USA, to confirm the results of the initial identification by 5.8s and 18s rDNA sequencing.

3.8.4.2 Assessment of the level of metal tolerance

The level of Pb and Zn tolerance of the two selected strains was determined using the minimum inhibition concentration (MIC) technique. Plates of GA medium were spiked with progressively higher metal concentrations of Pb or Zn to identify the concentration which completely inhibited growth of the isolate.

3.8.4.3 Determination of metal-uptake ability of selected isolates

The aim of the this experiment was to determine the amount of Pb or Zn accumulated by each strain, and how this varied with time. Over a period of three weeks, the metal associated with the cells, the cell mass and the viable count were determined at the start of the experiment (viable count only) and at intervals of 1 day, 7 days, 14 days and 21 days. The cells counts were determined by viable count on GA medium.

The experimental procedure involved 100ml acid washed flasks containing 50ml of GA broth. For each strain, the experiment consisted of the following set of flasks for each of the designated time periods:

2 x isolate control (isolate but no metals)
2 x Pb spiked (Pb + isolate)
2 x Zn spiked (Zn + isolate)
1 x Pb control (Pb but no isolate)
1 x Zn control (Zn but no isolate)

The flasks were incubated in a shaker incubator at 30°C. After 1 day, the first set of flasks were removed from the incubator and the viable count determined. The broth was then vacuum filtered through autoclaved, preweighed Q-MA filters (particle retention 0.6μm). The filters and collected cells were dried at 105°C to a constant weight to determine cell mass. The cells and associated filter were then digested in 10ml concentrated nitric acid (see Section 3.5.1) and analysed for Pb or Zn by Inductively Coupled Plasma Atomic Emission Spectroscopy. Additional filters were also digested as blanks. The above procedure was repeated after 7 days, 14 days and 21 days. The concentrations of metals used to spike the broth are shown in Table 3.3.

Table 3. 3 Concentrations of Zn and Pb (μg/g) used in metal-uptake ability experiment.

	Zn	Pb
Isolate A	200	100
Isolate B	100	40

3.8.4.4 Effect of metals on colony growth at different temperatures

The aim of this experiment was to examine how metals affect colony growth at different temperatures [4 °C and 30°C]. Plates of each isolate were grown-up on GYEA media for 7 days to obtain good growth. A series of small squares (approximately 1cm^2) were cut out of each plate, and placed colony side down on replicate plates of GA which were spiked with either Pb (40µg/g, 200µg/g or 400 µg/g) or Zn (30µg/g, 400µg/g or 800µg/g), or were the control. The areas of colonies were measured at regular intervals to assess colony growth at the different temperatures.

3.8.4.5 Effect of metals on cell morphology

The aim of this experiment was to examine the effects of Pb and Zn on cell morphology using Scanning Electron Microscopy (SEM). Both isolates where grown up on plates of GA spiked with Pb or Zn at the concentrations shown in Table 3.4.

Table 3. 4 Concentrations of Zn and Pb (µg/g) used to spike plates for examination of isolates using Scanning Electron Microscopy.

	Zn	Pb
Isolate A	800	400
	1800	800
Isolate B	100	40

Isolates were also grown up on control plates. Samples were prepared for SEM analysis by Dr. Tony Brain, Electron Microscopy Unit, Kings College, University of London. This involved freeze-drying in liquid nitrogen (-195.8°C), and coating with gold to prevent charging of the sample during analysis. Samples were examined using scanning electron microscopy (Cambridge Instruments, Stereoscan 240), with an accelerating voltage of 25 kV. Measurements of hyphal width and cell width of isolates A and B, respectively, were recorded on Pb spiked plates, Zn spiked plates and control plates to determine if cell morphology was different in the presence of metals. Between 55 and 72 measurements were made per plate.

3.8.4.6 Transmission electron microscopy and x-ray analysis

To determine the distribution of heavy metals in isolate A, cross-sections of the hyphae were examined using transmission electron microscopy. Isolate A was grown on GA media spiked with either Pb (600µg/g and 800µg/g) or Zn (1800µg/g), or the control plates. The samples were also prepared by Dr. Tony Brain, Electron Microscope Unit, Kings College, University of London, by fixing the specimens with osmium tetroxide, staining with uranyl acetate and lead citrate and embedding in Spurr's resin. Samples were cut using a microtome to a thickness of 60-70nm. The samples were examined with a Philips EM301G Transmission Electron Microscope operated at an accelerating voltage of 80kV. X-ray analysis was used to determine if any electron dense areas were the result of metal accumulation. Samples were again prepared by Dr. Tony Brain, and x-ray analysis was carried out by Dr. Alice Warley, The Rayne Institute, St. Thomas' Hospital, London, using a transmission electron microscope (Zeiss EM10C) operating at an accelerating voltage of 80kV.

CHAPTER 4 AN ASSESSMENT OF THE TREATMENT PERFORMANCE OF TWO CONSTRUCTED WETLANDS RECEIVING URBAN RUNOFF.

Following the results of surveys carried out on several rivers in London which identified urban runoff as a major factor suppressing river water quality, and as part of the implementation of local Agenda 21, the Environment Agency for England and Wales, Thames Region, (EA) decided to build full-scale experimental wetland treatment systems at two selected sites receiving urban runoff (Scholes *et al.,* 1995). These systems have been monitored for a wide range of parameters including heavy metals, suspended solids and nutrients over a two year period, which has included a routine bimonthly monitoring programme and the sampling of 7 storm events.

Unfortunately, due to vandalism, it was not possible to leave automatic sampling equipment on site and therefore the sampling programme involved the collection of grab samples only. Water and sediment samples were collected at bimonthly intervals and plants were collected seasonally (for methods see Chapter 3). Sampling of storm events involved the collection of water samples at the inlet and outlet only, as it was often not possible to enter the system during storm events due to flooding.

The results are discussed with respect to system design, and comparisons are made between the performance of the systems during dry weather and during storm events. Recommendations are made to improve the treatment efficiency of the two studied systems, and also for the design and management of future urban runoff treatment wetlands which will be used by the EA at a national level.

4.1 Total aqueous metal concentrations recorded at the Dagenham and Brentwood wetlands

In this section total aqueous metal concentrations recorded at the Dagenham and Brentwood wetlands during dry weather (Section 4.1.1) and during wet weather (Section 4.1.2) are presented and discussed.

4.1.1 During dry weather conditions

Tables 4.1 and 4.2 show the mean concentrations (±SD) recorded at each sampling point at both the Dagenham (D1-D5) and Brentwood (B1-B7) wetlands during dry weather. The SDs show that concentrations of all the metals are highly variable which is characteristic of urban runoff (Wong and Soames, 1995).

Table 4. 1 Mean total aqueous concentrations (µg/l) of metals (±SD) at each sampling point during dry weather over the two year monitoring programme at the Dagenham wetland.

	Zn	Cd	Pb	Cr	Ni	Cu
D1	48.6±45.4	1.3±2.0	3.4±3.8	3.4±2.6	50.2±46.7	9.5±7.1
D2	84.1±93.6	2.0±2.7	10.0±13.0	3.5±2.9	40.5±28.0	16.2±10.3
D3	47.1±34.6	1.2±1.4	6.3±4.9	2.1±1.6	25.3±14.1	13.1±8.3
D4	34.0±26.2	1.7±2.7	4.7±4.3	2.6±3.8	24.3±15.0	12.4±8.2
D5	31.4±18.3	0.7±0.5	6.3±9.0	1.7±1.5	19.8±13.9	18.5±14.7

Table 4. 2 Mean total aqueous concentrations (µg/l) of metals (±SD) at each sampling point during dry weather over the two year monitoring programme at the Brentwood wetland.

	Zn	Cd	Pb	Cr	Ni	Cu
B1	22.4±16.4	1.2±1.8	5.2±4.7	0.9±0.8	10.7±6.7	8.8±6.8
B2	52.9±65.6	1.2±1.8	5.2±4.9	1.0±1.1	8.1±5.3	13.0±12.3
B3	23.6±27.1	0.6±0.5	2.7±3.5	0.8±1.1	7.9±6.8	10.5±10.8
B4	31.0±37.5	1.4±1.9	3.5±3.6	0.8±1.0	8.2±5.8	9.6±8.7
B5	18.0±14.7	0.7±0.6	2.9±4.7	0.8±1.1	6.8±4.6	8.5±9.6
B6	56.1±90.8	2.2±2.1	2.7±2.7	0.8±0.7	6.4±4.4	8.5±7.1
B7	19.9±16.1	0.8±0.8	3.6±3.4	1.2±1.3	6.2±4.3	10.1±10.5

Although metal concentrations during dry weather were generally low, on some sampling dates elevated values were reported at both sites. For example, samples collected on 6 March 1996 at site D1 (Dagenham wetland) exhibited a Ni concentration of 173µg/l (see Appendix I). The second highest Ni concentration recorded at this site was 65µg/l. During low flow conditions metals may enter the drainage system either as

a result of a spillage, for example from a garage or from other activities such as car washing. Under these conditions, metals accumulated on impervious surfaces could be mobilised and transported to the wetlands producing unexpected elevated concentrations. The existence of these peak metal concentrations are also a factor in the large standard deviations associated with the mean concentrations. The fact that such elevated concentrations were recorded during dry weather also emphasises the need for a robust year-round treatment system capable of coping with a wide range of concentrations under different conditions.

Table 4.1 shows that although concentrations were variable, all the metals except Ni show an increase in concentration from D1 (upstream site, Dagenham wetland) to D2 (settlement tank). This could be due to problems both with the management and maintenance plan, and with the design of the system. The former was not established until the second year of the monitoring programme, and the settlement tank was not emptied until June 1997. By this time, partly due to the erosion of stream banks, stripped of vegetation during the construction of the system, the settlement tank had completely filled with sediment. As will be discussed in Section 4.8, the settlement tank contains the maximum mean sediment concentrations for all the metals and this is thought to be an important factor influencing the increases in aqueous metal concentrations. The fact that the highest metal concentrations tended to be recorded in the settlement tank throughout the two year monitoring programme indicates that this component of the wetland system does enhance the transfer of metals from the water column to the sediment. However, the increases in aqueous metal concentrations indicate it is also a source, suggesting that the settlement tank is under-sized to prevent the resuspension of already settled sediments and associated pollutants, or that it is too infrequently maintained.

Another problem at the Dagenham wetland is that it is not a secure area. It is located on a flood plain with open access to the public. As a result of either direct dumping in the system or as a result of debris being washed downstream in storm events, a range of objects such as supermarket trolleys, tyres and a bike were recorded in the settlement tank. All of these items could contribute to increases in metal concentrations.

From D1 (inlet) to D5 (outlet) there is an overall decrease in Zn, Cd, Cr and Ni concentrations but Cu and Pb show an overall increase. In surface waters, Pb is adsorbed to suspended particles (Reinelt and Horner, 1995), and Cu is associated with organic matter (Sobolewski, 1996). Hence, the variable removal of suspended solids and BOD could be a factor in the overall increases of the these two metals (see Section 4.6). Comparison of sites D2 and D3 indicates changes in concentrations from the settlement tank to the outlet of the first bed planted with *Typha latifolia*. All of the metal concentrations show an overall decrease from D2 to D3, in contrast to the changes in concentrations associated with the second and third beds (D3 to D4 and D4 to D5 respectively) where mean metal concentrations show both increases and decreases. Both of these beds are planted with *Phragmites australis*, and a possible factor in this variable pattern is the incomplete plant cover in the *Phragmites* beds. These reeds were never as dense as in the *Typha* bed due to problems with initial establishment. This situation was further compounded by the grazing activity of horses. The construction of a fence has been recommended, and agreed by the Environment Agency, and will prevent horses from grazing the *Phragmites* which should fully recover as the system matures.

Table 4.2 shows the mean aqueous metal concentrations at each sampling point during dry weather at the Brentwood wetland over the two year monitoring programme. Again the SDs indicate that the concentrations are variable which is thought to be associated with the same factors described above for the Dagenham wetland. It can be seen for all the metals, and particularly for Ni, that concentrations tend to be lower than those recorded at the Dagenham wetland. This may be associated with Dagenham being a larger, industrial and residential catchment area, whereas Brentwood has a smaller catchment area which is only residential. Mean inlet concentrations (B2) are greater than those at the upstream site for Zn, Cr and Cu only which may indicate that the upstream site also receives some urban runoff. This was not previously thought to be the case.

All of the metals show a decrease in mean concentration from B2 (inlet) to B3 (inlet to the constructed wetland) suggesting that the settlement tank does result in a decrease in metal concentrations. This is considered to be due to its ability to reduce inflow

velocity and thereby enhance the conditions for the settlement of suspended solids and associated pollutants.

The changes in metal concentrations associated with the constructed wetland (B3 to B4) tend to show an overall increase, whereas changes in concentration from B2 (inlet) to B5 (outlet of the natural wetland) (the inlet to the natural wetland was not monitored) show an overall decrease in mean concentrations of all the metals. However, the B2 to B5 concentrations represent the changes in concentrations associated with both the natural wetland and the settlement tank and the latter has already been shown to demonstrate a positive metal removal efficiency. The retention time of the natural wetland is greater than that of the constructed wetland (see Section 4.7) which may explain its greater efficiency in comparison to the constructed wetland where the location of the inlet directly opposite the outlet is thought to encourage hydraulic short-circuiting. Overall (between B2 and B6), the wetland system shows a reduction in mean concentrations for all of the metals except for Zn and Cd. Zn and Cd have a greater affinity for the dissolved phase than the particulate phase (Morrison *et al.*, 1984) and this may explain their overall increase in concentration in comparison to the overall decrease in the mean concentrations of Pb, Cr, Ni and Cu.

4.1.2 During wet weather conditions

Table 4.3 gives the mean aqueous metal concentrations recorded during storm events at the inlet and outlet of the Dagenham and Brentwood wetlands (three storm events at Dagenham and four storm events at Brentwood). There is an increase in the magnitude of the mean concentrations of all the metals during wet weather in comparison to the mean concentrations during dry weather, except for Ni at the Dagenham wetland.

Table 4. 3 Mean total aqueous concentrations (µg/l) of metals (±SD) at each sampling point during storm events at the Dagenham and Brentwood wetlands.

	Zn	Cd	Pb	Cr	Ni	Cu
Dagenham wetland						
D1	279.2±305.8	5.4±4.7	25.9±8.2	6.9±6.3	33.0±24.6	29.4±16.9
D5	55.2±45.1	1.9±3.0	12.4±6.7	2.8±3.8	29.7±26.2	17.6±19.8
Brentwood wetland						
B2	136.4±87.5	2.3±1.8	55.8±40.0	7.9±5.5	10.7±6.5	28.5±11.5
B6	105.4±17.8	2.4±2.2	37.4±23.5	4.8±2.2	8.7±9.7	19.4±12.7

As in dry weather, mean storm event concentrations tend to be higher at the Dagenham site compared to Brentwood, except for mean Pb and Cr concentrations which are higher at Brentwood. The elevation in the concentrations of all metals during wet weather (except for Ni at Dagenham) clearly indicates the mobilisation of metals from within the catchment area. The large standard deviations associated with these mean values illustrate the large variations in metal concentrations between storm events. This variation is associated with a wide range of factors such as the length of the antecedent dry period, the catchment characteristics (Merritt, 1994) and the relative time of sampling within the storm event (see Section 4.3.2). Despite this variability in the concentrations, for each of the metals at both of the wetlands there is an overall decrease in mean concentration from the inlet to the outlet during storm events. The only exception is for Cd at the Brentwood wetland which shows a slight increase in mean concentrations which may be associated with the mobility of this metal.

4.1.3 Comparison with previous data

Table 4.4 shows the mean inlet concentrations for both wetlands during the dry weather monitoring period and during storm events in comparison with values reported for yard and street runoff in a study carried out in central Paris (GromaireMertz *et al.*, 1999), characteristic values for urban runoff in Denmark (Hvitved-Jacobsen *et al.*, 1994) values for rainwater and for runoff from undeveloped land (Bascombe, 1991; Kadlec and Knight, 1996) and the Dangerous Substances and Freshwater Fisheries Directive (Council of European Communities, 1976 and 1978 respectively).

Dry weather mean concentrations for Zn, Cd, Pb and Cu are comparable to the values reported at the lower end of the range for rainwater/undeveloped sites. During storm events, concentrations generally increase for all metals and are comparable to values given for yard, street and urban runoff. Table 4.4 also shows that during storm events at Dagenham, mean Cd concentrations can exceed the values set out in the Dangerous Substances Directive (Council of European Communities, 1976) and this metal is therefore of particular concern. The mean storm event concentrations of the other metals do not exceed these water quality criteria at either site. However, water metal concentrations of this magnitude are known to have a chronic effect, resulting in

elevated concentrations in sediments, plants and other biota (Wood and Shelly, 1999; Mulliss, 1994; Section 4.7 and 4.8).

Table 4. 4 Mean inlet total aqueous metal concentrations (µg/l) at both wetlands in comparison to values reported for other studies and the dangerous substances water quality directive (µg/l).

	Dagenham		Brentwood		Yard runoff	Street runoff	Urban runoff	Rainwater/ undeveloped	Dangerous substances directive
	d.w.	w.w	d.w.	w.w					
Zn	48.6	279.2	52.9	136.4	57-1359	246-3839	300-500	20-415	2000
Cd	1.3	5.4	1.2	2.3	0.2-1.3	0.3-1.8	0.5-3.0	<0.5-1.8	5
Pb	3.4	25.9	5.2	55.8	49-225	71-523	50-150	<5-36	250
Cu	9.5	29.4	13.0	28.5	13-50	27-191	5-40	<5-16	112
Cr	3.4	6.9	1.0	7.9	-	-			250
Ni	50.2	33.0	8.1	10.7	-	-			200

Key: d.w. = dry weather mean concentrations at the inlet

w.w. = wet weather mean concentrations at the inlet

4.2 Metal loadings during dry weather and wet weather conditions at the Dagenham and Brentwood wetlands

Although the concentrations discussed in the preceding section reveal some interesting trends, the data will also be analysed in terms of metals loadings which represent the actual changes in the mass of the pollutant through the wetland system and therefore give a clearer indication of the treatment performance. Loadings are calculated using the following equation:-

$$L = C \times Q$$

Where : L = Pollutant loading (µg/s)

C = Pollutant concentration (µg/l)

Q = Flow rate (l/s)

As discussed in Section 4.1, metal concentrations vary widely between sampling dates. This variability is also evident in the loading data where it appears to be magnified due to variations in flow, both between the wetland components and between sampling dates (see Appendix II). Table 4.5 shows the mean inlet loadings recorded during dry weather and storm events at the inlet of the Dagenham and Brentwood wetlands.

Table 4. 5 Mean metal inlet loadings (±SD) during dry weather and storm events at the Dagenham and Brentwood wetlands.

	Dagenham wetland		Brentwood wetland	
	mean inlet load (mg/s) dry weather	mean inlet load (mg/s) storm events	mean inlet load (mg/s) dry weather	mean inlet load (mg/s) storm events
Zn	1.58±1.52	45.18±10.26	0.84±1.19	24.13±26.00
Cd	0.04±0.07	1.73±1.64	0.01±0.12	0.30±0.18
Pb	0.11±0.11	7.58±8.01	0.08±0.12	8.53±6.74
Cu	0.38±0.33	10.72±12.19	0.16±0.14	4.46±3.00
Ni	1.73±1.77	14.15±17.36	0.10±0.08	1.55±0.99
Cr	0.12±0.10	3.28±4.18	0.01±0.01	1.18±0.82

The data clearly indicate that there is a substantial increase in metal loadings during storm events compared to dry weather, which is associated with both increases in concentration (Table 4.3) and flow rate (see Appendix II). This elevation in metal loads is consistent for both catchment areas, with loadings increasing by a factor of between 8 and 118. As with the concentration data, inlet loadings tend to be higher at the Dagenham wetland which is thought to be related to the land use within the respective catchment areas as discussed in Section 4.1. An exception to this is the mean storm event Pb inlet loading which is marginally higher at Brentwood than at Dagenham. This higher Pb loading is reflected in the sediment metal data, with the maximum Pb sediment concentrations being recorded at Brentwood (see Section 4.8).

To ascertain whether the differences between metal loadings at the inlet during dry weather and storm events are significantly different, the data were examined using two different statistical approaches. These are described in the following subsections.

4.2.1 Explanation of the adopted statistical approaches

For the statistical analysis of the data, it was decided to use parametric tests wherever possible as these are more powerful than nonparametric tests. To use parametric tests certain assumptions must be met (Samuels, 1990); firstly that the samples are random samples taken from their respective populations, and that the samples are independent of each other. Secondly, that population distributions are approximately normal. The collection of samples was carried out over a two year period, such that the collection of samples at one sampling point did not affect the collection of samples at another sampling point. Therefore all samples are considered to be randomly obtained and independent. To test for a normal distribution, the distribution of the data at each of the sampling points for each of the parameters was tested using the Ryan-Joiner test for normality (Minitab 10.5, Microsoft Windows v3.11). In this test, the null hypothesis states that there is no difference between a normal distribution and the distribution of the data set. The null hypothesis is rejected at $p<0.05$. If the data were found to be not normally distributed, they were transformed using an appropriate transformation. Where it was not possible to transform the data to a normal distribution, or to use the same transformation for all data sets, a nonparametric test was used. Although this is less powerful on a small data set, it is still valid. Table 4.6 summarises the determined distributions for the metal loadings, and identifies the transformations required to achieve a normal distribution.

Table 4. 6 Distribution of the raw data set (metal loadings) and transformations carried out.

	Dagenham wetland		Brentwood wetland	
	distribution of raw data	transformation	distribution of raw data	transformation
Zn	not normally distributed	log10	not normally distributed	log10
Cd	not normally distributed	log10	not normally distributed	log10
Pb	not normally distributed	log10	not normally distributed	log10
Cr	not normally distributed	log10	not normally distributed	log10
Ni	not normally distributed	log10	normally distributed	-
Cu	not normally distributed	log10	not normally distributed	log10

The normally distributed data were then subjected to the parametric statistical approaches outlined below:-

- t -tests - these involve formulating as a hypothesis a statement that the means of the two populations being compared do not significantly differ, and then assessing whether the data are consistent or inconsistent with this hypothesis. The null hypothesis is that there is no difference between metal loadings at the inlet during dry weather and storm events, and is rejected at $p < 0.05$.

- comparing mean dry weather data to each storm event individually. Dry weather values are considered to be significantly different from the storm event value if the value of the dry weather mean added to twice the SD is less than the inlet value recorded during the storm event. This enables each storm event to be examined individually, highlighting differences both between each storm event and between the different parameters within storm events.

4.2.2 Results of the statistical analyses carried out on dry weather and wet weather total metal loadings

Table 4.7 gives the results of t-tests carried out to examine whether there was a significant difference between mean inlet loadings during dry weather and storm events, rather than comparing dry weather loadings to each storm event individually. The t-tests were calculated using the unpooled method to calculate the standard error as outlined by Samuels (1989).

The results of the t-tests (Table 4.7) indicate that there is a significant difference between the inlet loadings during dry weather and storm events for Zn, Cd, Pb and Cu at the Dagenham wetland and for all six metals at the Brentwood wetland. This difference is due to an increase in loadings and is expected during wet weather conditions as a result of the mobilisation of pollutants deposited within the catchment area (Mulliss *et al.*, 1996). However, for Cr and Ni at the Dagenham wetland, there is not enough evidence to reject the null hypothesis that there is no difference in inlet loadings. Although three storm events were monitored at Dagenham, it was only possible to collect flow data for two of the events and therefore dry weather loads are being

compared to the metal loads of only two storm events at this site (four storm events were monitored at the Brentwood wetland). The loadings of both metals did not show a substantial increase at the inlet during the first storm event, although the inlet loadings of Cr and Ni did show a large increase during the second storm event (see Appendix II). As a result of the difference between the inlet loadings of the two storm events, there is not enough evidence to support the alternative hypothesis.

Table 4. 7 Results of t-tests and 2SD+mean to examine for significant differences between inlet loadings during dry weather and storm events.

	Dagenham wetland		Brentwood wetland	
	t-test	2SD + mean	t-test	2SD + mean
Zn	$p<0.05$	yes	$p<0.05$	yes
Cd	$p<0.05$	yes	$p<0.05$	yes
Pb	$p<0.05$	yes	$p<0.05$	yes
Cr	$p>0.05$	1 of 2 (1)	$p<0.05$	yes
Ni	$p>0.05$	1 of 2 (1)	$p<0.05$	yes
Cu	$p<0.05$	yes	$p<0.05$	yes

Key: Values in brackets denote which of the storm events were not significantly different from the dry weather values.

The above explanation is supported by the second statistical approach which compares the dry weather values to each storm event individually. There is again strong evidence that Zn, Cd, Pb and Cu inlet loadings significantly differ during dry weather and storm events at the Dagenham wetland, and for all metals at the Brentwood wetland (Table 4.7). The inlet loadings of Ni and Cr only differ significantly from the second and not from the first storm event at the Dagenham site. This could indicate that the approach of comparing dry weather data to the individual storm events is a more useful approach for a data set of this size. The reason for the difference in Ni and Cr loadings between the two storm events at the Dagenham wetland is unclear but it is possible that elevated loadings entering the system were missed.

4.3 Metal removal efficiencies during dry weather and wet weather conditions at the Dagenham and Brentwood wetlands

To enable comparisons to be made both between the removal of different metals, and the removal of metals by each wetland system, a series of removal efficiencies were calculated for various components of both wetlands, and for each system as a whole.

These were calculated as follows:-

$$RE = 100 - \left(\frac{B}{A} x \frac{100}{1}\right)$$

where: RE = removal efficiency (%)

B = total metal outlet loading (µg/s)

A = total metal inlet loading (µg/s)

4.3.1 Metal removal efficiencies during dry weather

Removal efficiencies were calculated for each of the components of the Dagenham wetland, and for three of the components of the Brentwood wetland (flow was not monitored at each sampling point at this site). The components for which the removal efficiencies were calculated are as follows:-

Dagenham wetland

D1-D2 = removal by settlement tank

D2-D3 = removal by bed 1 (planted with *Typha latifolia*)

D3-D4 = removal by bed 2 (planted with *Phragmites australis*)

D4-D5 = removal by bed 3 (planted with *Phragmites australis*)

D1-D5 = total removal for overall wetland system (inlet to outlet)

Brentwood wetland

B2-B5 = removal by initial settlement tank + natural wetland

B5-B6 = removal by final settlement tank

B2-B6 = total removal for overall wetland system (inlet to outlet)

Tables 4.8 and 4.9 summarise the dry weather removal efficiencies for each wetland component, as well as the total removal efficiencies for each system. The performances of all the components are highly variable, with some apparently substantial increases in metal loadings. However, it is important to emphasise that these values are calculated from very low loadings and the actual changes in concentrations associated with negative removal efficiencies are generally very small.

Table 4. 8 Mean percentage metal removal efficiencies with SD for each component, as well as the whole system, during dry weather at each wetland.

	Zn	Cd	Pb	Cr	Ni	Cu
Dagenham wetland						
settlement tank	-132±255	-234±543	-747±1705	-36±155	8±49	-79±123
bed 1	16±88	-24±137	-110±346	35±42	24±33	-10±87
bed 2	24±45	-27±77	-6±85	-20±84	9±28	10±37
bed 3	-55±121	-28±116	-56±102	0±54	12±22	-95±110
inlet to outlet	-31±170	-48±107	-278±631	34±51	28±66	-125±227
Brentwood wetland						
natural wetland	63±27	12±87	64±26	43±32	46±34	57±32
final settlement tank	-885±2060	-644±1115	-371±512	-195±366	-68±68	-116±180
inlet-outlet	-93±323	-322±612	-12±130	-18±111	21±66	24±43

Table 4. 9 Range of percentage metal removal efficiencies for each component, as well as that for the whole system, during dry weather at each wetland.

	Zn	Cd	Pb	Cr	Ni	Cu
Dagenham						
s.t.	-694 to 48	-1463 to 6	-4603 to -13	-378 to 55	-69 to 73	-345 to 14
bed 1	-182 to 85	-349 to 93	-946 to 95	-47 to 84	-27 to 59	-144 to 68
bed 2	-69 to 68	-193 to 55	-191 to 89	-157 to 75	-21 to 50	-53 to 56
bed 3	-282 to 40	-273 to 97	-209 to 80	-111 to 59	-15 to 49	-303 to 49
inlet to outlet	-437 to 78	-266 to 97	-1784 to 72	-44 to 86	-116 to 85	-681 to 19
Brentwood						
natural wetland	15 to 97	-207 to 93	31 to 97	-30 to 82	-17 to 97	-15 to 92
final s.t.	-6348 to 2	-3495 to 8	-1429 to 54	-1150 to 32	-180 to 31	-479 to 81
inlet-outlet	-975 to 90	-1810 to 62	-311 to 94	-317 to 67	-143 to 94	-26 to 99

Key: s.t. = settlement tank

Despite the large negative removal efficiencies recorded on occasion, none of the increases in loadings and concentrations associated with these negative removal efficiencies resulted in values that exceed the water quality directives given in Table 4.4. A factor in the negative removal efficiencies and associated large standard deviations is the way in which some of the removal efficiencies themselves were determined. In the calculation of loadings, metals that were occasionally not detected were assigned the working limit of detection (calculated as 3xSD of a procedural blank (see Section 3.6.2)) as a concentration to enable the use of as much data as possible. Where such a value was assigned at the outlet of a system or component a positive removal efficiency could be demonstrated for that metal. However, when such a value occurred at the inlet to a component or system the resulting removal efficiency indicated a substantial increase in mass of the particular metal. This is because the limits of detection were such low concentrations that even a small increase resulted in a substantial negative removal efficiency because the removal efficiency was calculated relative to a low concentration base. The range of removal efficiencies (Table 4.9) also demonstrates that each component has resulted in metal removal from the water column at some stage in the 2 year monitoring programme, except for Pb removal by the settlement tank at Dagenham which at its best resulted in a 13% increase. This highly variable performance is associated with the typically low concentrations monitored over the 2 year period. However, the maximum dry weather concentrations for Zn, Cd and Cr at Dagenham and Zn and Cr at Brentwood corresponded to their highest removal efficiencies recorded during dry weather. The maximum inlet concentrations for the other metals corresponded to net positive removal efficiencies ranging from 19-80%, except for the maximum Pb and Cu concentrations at Dagenham which corresponded to an overall increase.

These data were further analysed to examine whether differences between the removal efficiencies of each component of each system were significantly different. The distribution of the removal efficiencies for each component were first examined using the Ryan-Joiner test for normality. Although the Ni removal efficiencies at the Dagenham wetland and the Cu removal efficiencies at the Brentwood wetland were normally distributed for each component this was not the case for the other metals. It was therefore decided that a nonparametric test should be used to examine all of the data enabling comparisons between both the individual components for each metal, and

between the metals themselves (values in Table 4.10 in brackets give the p values calculated using ANOVA for metals that were normally distributed). The data were examined using the Kruskal-Wallis test, which is a nonparametric alternative to the ANOVA, and involves ranking data and then determining the degree of overlap between populations. The null hypothesis states that there is no significant difference between the removal efficiencies of each component, and it is rejected at $p<0.05$. Table 4.10 shows the p-values for each metal calculated using the Kruskal-Wallis test as outlined by Kitchens (1987).

Table 4. 10 Results of the Kruskal-Wallis test to examine for significant differences between the removal efficiencies of each component.

	Dagenham wetland	Brentwood wetland
Zn	0.031	0.000
Cd	0.454	0.002
Pb	0.157	0.001
Cr	0.440	0.000
Ni	0.807 (0.786)	0.001
Cu	0.059	0.035 (0.005)

Where the Kruskal-Wallis test indicated that there was a significant difference between the removal efficiencies of each component, Tukey's Honestly Significantly Different (HSD) test was carried out to see which components differed from which. The results of the Kruskal-Wallis test strongly indicate that the removal efficiencies of each component at the Dagenham wetland do not significantly differ from each other, with p-values ranging from 0.059 for Cu to 0.807 for Ni. This is probably associated with the highly variable performance of each component due to the typically low metal concentrations found during dry weather. The exception to this is Zn with a p-value of 0.031. However, the results of the Tukey's HSD test did not find the removal efficiencies of any of the components to be significantly different at the 0.05 significance level. These different outcomes calculated from the Zn data are thought to be due to the fact that, although the removal efficiencies of each component covered a wide range, the Kruskal-Wallis test identified that the removal efficiencies of the first and second beds were similar to each other and were higher than those of the settlement

tank and the final bed. However, these differences were not great enough to support rejection of the null hypothesis using Tukey's HSD.

At the Brentwood wetland there is a significant difference between the components for all the parameters. The results of Tukey's HSD indicated significant differences between the removal efficiencies of the final settlement tank and those of the natural wetland and the total removal efficiencies of the system for Zn, Pb, Cr, and Ni. For Cd, the removal efficiencies of the natural wetland component were significantly different from those of the final settlement tank and the total removal efficiencies, and for Cu there was a significant difference between the removal efficiencies of the natural wetland component and the final settlement tank only. To summarise, the results of Tukey's HSD indicated that:

- the performances of the natural wetland component and the whole system were significantly better than that of the final settlement tank for Ni, Cr, Pb and Zn.
- the performance of the natural wetland component alone was significantly better than that of both the final settlement tank and the total system for Cd, and significantly better than the final settlement tank only for Cu.

Table 4.2 shows that the natural wetland component (B2-B5) results in a decrease in mean concentrations for all the metals. As discussed in Section 4.1, this is thought to be associated with a number of factors;

- the value for the natural wetland component also includes the removal of pollutants by the initial settlement tank
- the size of the natural wetland in comparison to the constructed wetland and differences in retention time (see Section 4.7).

However, when the data are converted into loadings this reduction is enhanced due to the fact that the flow monitored at the outlet of the natural wetland (B5) tended to be less than that measured at either the inlet (B2) or the outlet (B6). The mean flow values were for B2, B5 and B6 14±6 l/s, 9±5 l/s, and 15±11 l/s respectively. The difference in flow volumes between B2 and B5 is thought to be primarily due to the fact that although there was flow leaving the constructed wetland component it was at a velocity which

was too low to measure and also due to the fact that the natural wetland could retain/detain incoming flow. The fact that flow leaving the constructed wetland was too low to measure is also a factor in the apparent poorer performance of the final settlement tank. Since the contribution of the constructed wetland to the pollutant loadings entering the final settlement tank could not be calculated, removal efficiencies for the final settlement tank were based on the loadings entering this component for the natural wetland only. However, the outlet value used, B6, is actually the combined outlet of the constructed wetland and the natural wetland following passage through the final settlement tank. Although flow from the constructed wetland was typically very low, the fact that it cannot be included as part of the inlet loadings into the final settlement tank is of importance, particularly when removal efficiencies are calculated from such low concentrations.

4.3.2 Metal removal efficiencies during storm events

As explained previously, it was only possible to collect samples at the inlet and outlet locations of each of the wetlands during storm events (Section 4). Table 4.11 shows the mean removal efficiencies for the two fully monitored storm events at the Dagenham wetland and for the four monitored storm events at the Brentwood site.

Table 4. 11 Mean percentage metal removal efficiencies (±SD) during storm events at the Dagenham and Brentwood wetlands.

Site	Zn	Cd	Pb	Cu	Ni	Cr
Dagenham wetland	71±39	72±38	69±19	66±38	34±4	81±21
Brentwood wetland	-20±94	4±51	16±51	8±71	22±62	23±36

At Dagenham, the removal efficiencies are generally higher and more consistent in comparison with those at the Brentwood wetland (Table 4.11). The higher treatment ability of the Dagenham wetland could be due to several factors. The Dagenham wetland has a greater surface area than that of the Brentwood system, with a surface area of 1750m^2 in comparison to a minimum area of 360m^2. These treatment areas represent 0.04% and 0.001% of the respective catchment areas although, as the Dagenham wetland is located on a flood plain and the Brentwood wetland in a flood basin, both

systems experience flooding increasing the potential surface area at both sites. Inlet metal concentrations and loadings tend to be higher at the Dagenham site during storm events (Tables 4.1, 4.2 and 4.5), supporting the results of a study by Mungur *et al.* (1997), which reported increased removal efficiencies with increasing inlet load.

The variability between the removal efficiencies of the two storm events at Dagenham could be associated with the fact that the two events monitored at this site were of very different magnitudes. Although the flow monitored at the inlet during the May storm event (1/5/96) was almost twice the mean dry weather value (60 l/s in comparison to 34 l/s), the inlet flow monitored during the August storm event (25/8/97) was 452 l/s (over 13 times the mean dry weather flow). The two events were also characterised by different metal concentration patterns;

- Zn and Cd concentrations were higher during the May storm event
- Cr, Ni and Cu concentrations were greater during the August storm event
- Pb concentrations showed little variation between the two events

This suggests that each storm event should be examined separately, and that to gain a fuller understanding of processes of metal removal it would be necessary to monitor further storm events of different magnitudes.

Changes in flow volumes during storm events also affect removal efficiencies in another way particularly at the Dagenham site. As shown in Table 4.3, all metals show a decrease in mean concentration between the inlet and the outlet during storm events at both sites (except for Cd at Brentwood). However, for both fully monitored storm events at Dagenham, flow at the inlet exceeded that at the outlet by 23% and 35% respectively. This was due to the fact that the Wantz stream is a narrow watercourse with high banks until it reaches the wetland system where the stream has been widened and the banks graded to a gentle slope. As a result, during increased flow this area floods resulting in the differences in flow between the inlet and outlet, which leads to increased metal removal efficiencies when calculated from loads in comparison to concentrations. Such results also indicate that it may be advisable to include a by-pass channel as part of the design of urban runoff treatment wetland systems to cope with increases in flow during storm events.

As with the dry weather monitoring, another important factor influencing the treatment ability of the Dagenham wetland system during storm events has been the delay in the establishment and operation of the management and maintenance plan. The most significant effects on treatment performance are thought to have arisen from the delay in the emptying of the settlement tank and the damage caused to the *Phragmites* beds by horses grazing the plants (see Section 4.1). The management and maintenance plan is now in operation, and metal removal efficiencies are expected to improve as these issues are dealt with. However, it is strongly recommended that the design of future systems includes a management and maintenance system as part of the initial wetland budget to ensure that a prompt response can be made to any problems that may arise.

The mean removal efficiencies given in Table 4.11 for the Brentwood wetland summarise the treatment performance of this system for four storm events (on 25 August 1997, 8 October 1997, 28 November 1997 and 26 May 1998). Important factors here are the variations between the storm events themselves and also the differences in the way in which the storms were monitored. For the first event monitored in August 1997, it was already raining when sampling commenced. Samples were collected with no delay between the inlet and outlet samples, and calculated removal efficiencies indicate that only Pb and Cr were removed, with removal efficiencies of 19% and 9% respectively. For the second storm event (October, 1997), sampling began just as rainfall commenced, and the changes in both the metal loadings and the flows at the inlet and outlet to the system during this event are shown in Figs. 4.1 and 4.2.

The maximum loadings for all the metals were recorded at the inlet 22 minutes into the storm event, and the corresponding maximum loadings at the outlet, for most metals, occurred after 73 minutes. This suggests that the retention time for this system under these conditions was approximately 50 minutes, and, based on this retention time, the removal efficiencies are as follows: Zn 55%, Pb 62%, Cu 85%, Ni 77%, Cr 63%. In contrast, an overall increase in Cd of 3% was observed which indicates that Cd may be mobilised at a different rate. The data in Figures 4. 1 and 4.2 also clearly show the significant effect that the relative time of sampling between inlet and outlet may have on metal removal efficiencies, demonstrating the value of monitoring the profile of the storm event.

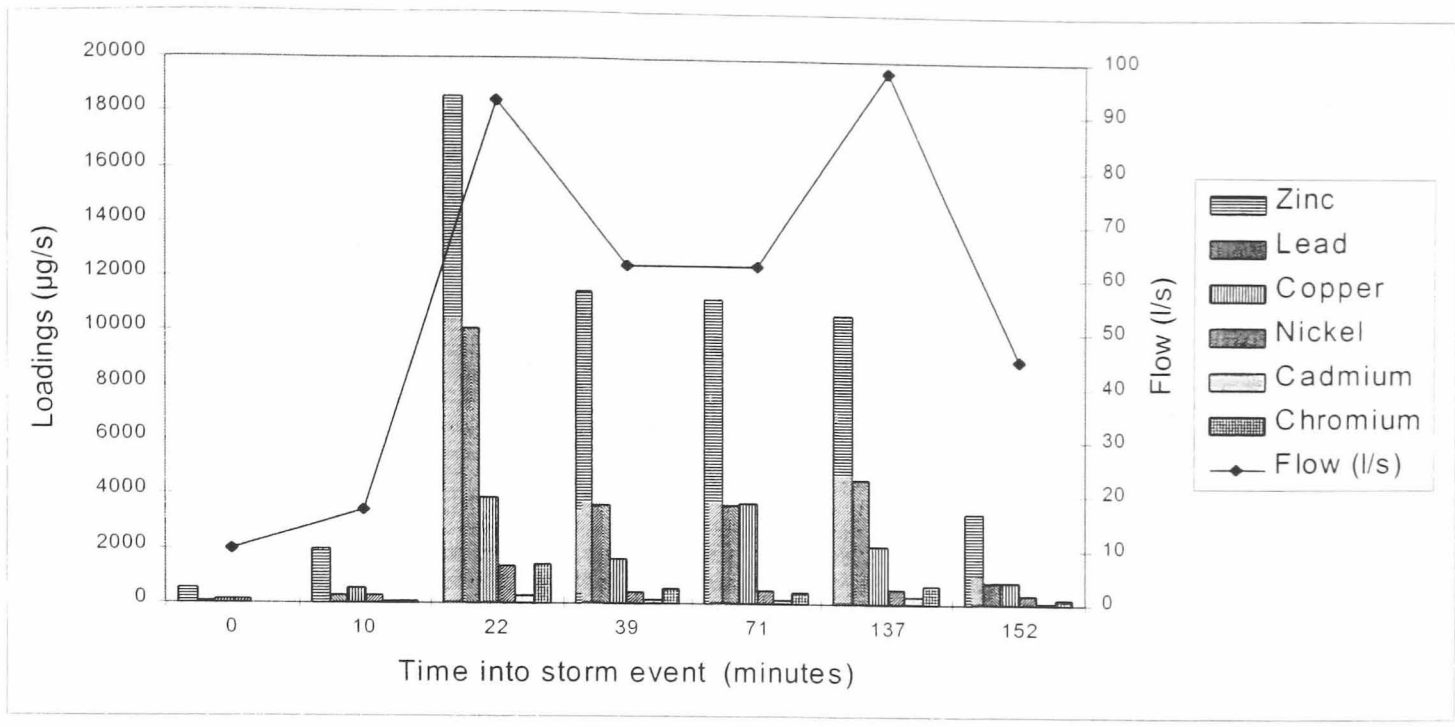

Figure 4. 1 Changes in total metal loadings and flow at the inlet during the October storm event at the Brentwood wetland

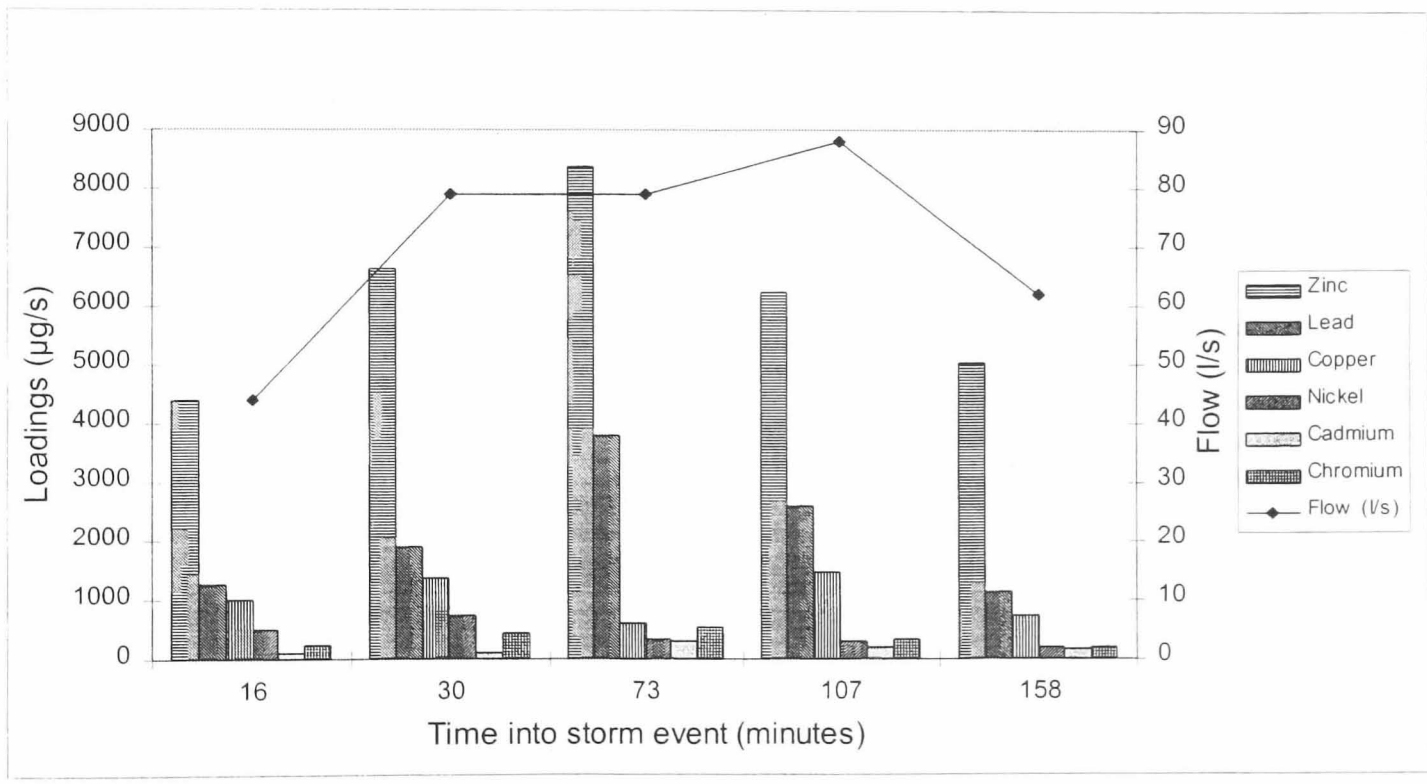

Figure 4. 2 Changes in total metal loadings and flow at the outlet during the October storm event at the Brentwood wetland

The third monitored storm event at Brentwood was in November 1997, and rainfall had commenced when sampling began. Based on the data obtained from the October storm, a period of 50 minutes was allowed between the collection of inlet and outlet samples. The removal within the wetland system of all metals was apparent (Pb 40%, Cd 74%, Cu 42%, Ni 72%, Cr 41%), except for Zn which showed an overall increase of 8%. The

final storm event at Brentwood was sampled in May 1998. Although it was raining heavily when the sampling of this storm event commenced, it could be seen that water levels within the wetland were falling. A total of six samples were collected (three at the inlet and three at the outlet) over a period of 65 minutes. Unlike the profile of the October storm event, this series of samples did not indicate any clear pattern in the timing of the maximum pollutant loadings recorded at the inlet and outlet. The maximum loadings of Zn, Pb and Cr were recorded at the inlet in the first sample collected (0 minutes into the storm event), whereas maximum loadings of Cd, Ni and Cu at the inlet were recorded 50 minutes into the event. The maximum loadings of Zn, Pb, Cr, Ni and Cu at the outlet were recorded 20 minutes into the storm event whereas the maximum Cd loading was recorded at the outlet 65 minutes into the storm event. As the data do not enable the estimation of a possible retention time for this storm, removal efficiencies were calculated using the approximate 50 minute retention time used for the previous two storm events to enable comparisons to be made between events. However, removal efficiencies calculated using this retention time show that only Zn was removed (29%), with Cd, Pb, Cr, Ni and Cu all showing an overall increase.

The magnitude of the storm events is considered to influence the removal efficiencies. During the first three storm events monitored at Brentwood, inlet flows increased from a dry weather mean value of 14 l/s to 117 l/s, 92 l/s and 135 l/s respectively. Although these are all substantial increases in comparison to the dry weather mean value, they are all of an approximately similar magnitude. However, the inlet flows recorded during the final May storm event ranged from 211 - 286 l/s, which are approximately twenty times the dry weather mean value and more than twice the previously recorded storm event maximum value. The negative removal efficiencies for most of the metals under these conditions suggest that this system is perhaps undersized to cope with an event of this magnitude. During the final May storm event, the flows measured at the inlet during the collection of the first two samples were markedly less than those recorded at the outlet (see Appendix II). This is thought to reflect the fact that the storm hydrograph was in a recessional phase during the collection of samples. The greater flow volume at the outlet may also indicate that the natural wetland is receiving runoff from surrounding agricultural land. Flow measured at the outlet was comparable to that

at the inlet during the collection of the third set of samples, and this may be a result of the flow volume equalising through the system as the storm event recedes.

As was observed with the metal concentrations recorded during the storm events at the Dagenham wetland, there was no clear pattern in elevation of concentration between the metals, with maximum and minimum concentrations being recorded during different events. For example, the maximum Pb, Cr and Cu concentrations were recorded during the October storm event, the maximum Cd and Ni concentrations were recorded during the August storm event, and the maximum Zn concentration was recorded during the May storm event.

The monitoring of the Brentwood site during both dry weather and storm events has highlighted problems with the design which are also affecting treatment performance and therefore need to be addressed. Firstly, the runoff entering the system directly discharges into the initial settlement tank which results in a visible deterioration in water quality during storm conditions due to the resuspension of previously settled particles. The installation of a stilling structure such as a gabion at the inlet to dissipate high flow velocities has been recommended. Secondly, the inlet to the constructed wetland component is directly opposite the outlet encouraging hydraulic short-circuiting and therefore reducing the treatment efficiency of this component. The effects of this can be most clearly seen following a storm event when the *Phragmites* were bent over in the direct path between the inlet and outlet. It is recommended that future systems have several inlet and outlet feed points, or that they are at least off-set to avoid this problem. It has been recommended that baffles should be placed within the constructed wetland to disperse flow across the entire bed. The absence of these design criteria could be an explanation of why this treatment wetland system including a subsurface flow component did not, as would be expected, perform better than the Dagenham wetland system which consisted of surface flow only. The variations in concentration and flow between the four storm events, in combination with the highlighted design problems are thought to be the primary factors influencing the variable performance of the Brentwood system.

4.4 Dissolved oxygen concentrations, temperature and pH recorded at the Dagenham and Brentwood wetlands

Table 4.12 identifies the mean values (±SD) of dissolved oxygen (DO), temperature and pH recorded over the two year monitoring programme at each sampling point at each of the two wetlands.

Table 4. 12 Mean values (±SD) of dissolved oxygen, temperature and pH at each sampling point during dry weather over the two year monitoring programme at the Dagenham and Brentwood wetlands.

	Dissolved oxygen (mg/l)	Temperature (°C)	pH
Dagenham			
D1	6.01±2.02	13.7±4.8	7.4±0.5
D2	6.20±1.81	14.0±4.7	7.4±0.6
D3	5.00±1.98	13.9±4.5	7.4±0.4
D4	6.48±2.07	13.4±4.6	7.4±0.4
D5	8.23±2.84	14.1±4.9	7.3±0.3
Brentwood			
B1	8.12±2.51	13.2±5.9	7.4±0.5
B2	7.99±2.76	13.4±5.4	7.4±0.6
B3	8.51±2.90	13.1±5.4	7.5±0.5
B4	6.34±3.74	12.3±4.3	7.4±0.3
B5	7.23±2.33	12.9±5.4	7.5±0.3
B6	7.85±2.49	12.9±5.4	7.7±0.2
B7	8.12±2.47	13.3±5.3	7.6±0.5

DO concentrations show little variation over the period of the monitoring programme, with mean DO concentrations showing an overall increase (D1-D5) at the Dagenham wetland but an overall decrease at the Brentwood wetland. Mean inlet DO concentrations are higher at the Brentwood wetland than at the Dagenham wetland, which may be associated with the fact that pollutant concentrations monitored at this site are generally lower than those at the Dagenham site (Tables 4.1, 4.2 and 4.3). Mean temperatures recorded are similar at both sites reflecting that the sampling of both wetlands always took place on the same day. The SDs associated with the mean temperatures indicate the seasonal temperature variations. pH values also show little variation, either between sampling trips, sampling points or the wetlands themselves, with all values well within the 6-9 pH range set down in the Directive for the Protection

of Coarse Freshwater Fish (Council of European Communities, 1978). These parameters were measured during dry weather conditions only.

4.5 Concentrations of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates, trihalomethanes and total and unionised ammonia

4.5.1 During dry weather conditions

The mean concentrations (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates and sulphates at each sampling point during dry weather over the two year monitoring programme are shown in Tables 4.13 (for Dagenham) and Table 4.14 (for Brentwood).

At Dagenham, mean concentrations of BOD showed little variation between the sampling points, although the mean concentrations at D3 were slightly higher. This could be associated with the fact that the bed immediately before the D3 sampling site had the densest plant growth, with decomposing plant matter contributing to the higher BOD values at this site. Mean BOD concentrations at Brentwood also showed little variation between the sampling points except at B3 where concentrations were slightly higher, possibly due to flow directly discharging into the first settlement tank and causing resuspension of organic matter. However, the differences between these concentrations are not large, and the SDs show that within the variability of these concentrations such a conclusion can only be tentative.

Table 4. 13 Mean concentrations (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates and sulphates at each sampling point during dry weather over the two year monitoring programme at the Dagenham wetland.

	BOD_5 (mg/l)	suspended solids (mg/l)	chlorides (mg/l)	nitrates (mg/l)	phosphates (mg/l)	sulphates (mg/l)
D1	7.1±4.8	86.6±117.5	95.7±24.1	41.7±28.6	2.0±0.9	90.1±23.7
D2	7.4±5.1	57.7±54.0	91.9±22.1	43.2±29.5	1.7±0.8	101.8±49.5
D3	9.3±8.8	34.5±24.6	92.5±27.2	29.4±9.5	1.7±0.7	89.4±31.3
D4	7.5±5.3	40.5±31.2	91.9±37.3	31.0±12.1	1.7±0.8	83.3±29.1
D5	7.8±5.3	28.1±20.0	94.7±33.3	29±10.6	1.8±0.6	87.5±31.7

Table 4. 14 Mean concentrations (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates and sulphates at each sampling point during dry weather over the two year monitoring programme at the Brentwood wetland.

	BOD_5 (mg/l)	suspended solids (mg/l)	chlorides (mg/l)	nitrates (mg/l)	phosphates (mg/l)	sulphates (mg/l)
B1	8.0±5.5	27.8±25.2	150±80.1	23.2±7.1	0.2*	167.8±88.4
B2	8.5±6.1	54.7±40.2	92.7±25.9	21.3±4.3	0.6*	144.4±53.3
B3	10.0±7.2	41.3±22.8	91.9±23.0	21.1±5.2	0.8±0.6	153.5±57.9
B4	7.5±5.2	26.0±19.6	80.3±22.1	18.7±5.5	0.1*	151.9±66.5
B5	8.4±6.3	43.2±25.7	95.9±37.4	30.4±33.6	0.3*	154.7±79.3
B6	7.8±4.8	37.9±25.7	91.8±30.9	19.6±5.4	0.5±0.4	148.8±69.1
B7	7.9±5.9	36.0±28.2	110.6±57.9	21.2±7.2	0.2*	149.3±73.8

* = only recorded once

The mean concentrations of suspended solids showed an overall decrease at both the Dagenham (D1-D5) and Brentwood wetlands (B2-B6). Mean concentrations at Dagenham were reduced to a value less than the 35mg/l maximum discharge level permitted by the Urban Wastewater Treatment Directive (Council of European Communities, 1991). However, mean concentrations at the outlet of the Brentwood wetland just exceeded this value, despite the fact that the mean inlet concentration at the Brentwood wetland (54.7mg/l) was lower than that at the Dagenham wetland (86.6mg/l). In addition, the Brentwood wetland has two settlement tanks which should enhance suspended solid removal. The difference in total surface area of the two systems may be significant (Section 4.3.2) with the Dagenham wetland having almost five times the surface area of the Brentwood system. The large SD associated with the mean suspended solids concentration at D1 is mainly due to a single elevated concentration of 350mg/l recorded on 4 April 1997. This pattern of generally low concentrations with occasionally elevated values during dry weather was also noted in the metal concentration data (Section 4.1.1). However, it is important to note that many of the mean concentrations of suspended solids exceed the 35mg/l maximum discharge (Council of European Communities, 1991) at both wetland systems in dry weather.

Mean concentrations of chlorides at both sites were similar (Tables 4.13 and 4.14) with neither site showing any marked increase or decrease between the inlet and the outlet. It was noted that the chloride concentrations did not show any marked increase during the winter months as would have been expected due to the application of rock salt to roads as an anti-icing agent (Halcrow, 1996). Elevated chloride concentrations during winter

have been recorded at a wetland receiving highway runoff (Protection of the Water Environment Using Balancing Facilities - Second Interim Report on Monitoring Results, 1999). The absence of any increase in chloride concentrations at these sites suggests that rock salt is not heavily applied within these catchment areas.

Mean nitrate concentrations showed an overall decrease at both sites (D1-D5 and B2-B6), with a greater decrease being recorded at Dagenham compared to Brentwood. This, again, may be associated with the respective sizes of the wetland systems. At the Brentwood site, both the mean nitrate concentration and SD at B5 were noticeably higher than the other recorded values. This was due to a single elevated concentration of 130.05mg/l recorded on the 11 September 1997 at this sampling point. The next highest concentration recorded at this sampling point was 33.30mg/l. It is interesting to note that elevated concentrations of Zn (309.5µg/l) and Cd (5.5µg/l) were recorded at the next sampling point (B6), and elevated concentrations of total ammonia at B2 (1.167mgN/l), B3 (1.868mgN/l), B5 (1.868mgN/l) and B6 (3.0mg/l) were also recorded on this same sampling visit suggesting that a pollution event was occurring. The inlet flow rate on this occasion (6 l/s) was the lowest value recorded during the whole two year monitoring programme, indicating that there had been no rainfall recently within the catchment area and supporting the hypothesis that, even during dry weather, activity within the catchment area can mobilise pollutants resulting in elevated concentrations.

At the Dagenham wetland, mean phosphate concentrations at the inlet (D1) equalled the maximum discharge level permitted according to the Urban Wastewater Treatment Directive (91/271/EEC 1998) of 2mg/l. However, the mean concentrations then showed a slight decrease through the system, to a mean concentration of 1.8mg/l at the outlet (D5). Phosphates are typically associated with the particulate phase (Revitt *et al.*, 1999), and this small decrease may therefore be associated with the decrease in the mean concentrations of suspended solids previously reported. The main sources of phosphates are thought to be washing powders (Tebbut, 1992) from washing machines that are wrongly plumbed into the surface water system instead of the sewer system. At the Brentwood site, mean phosphate concentrations were lower than those recorded at the Dagenham wetland. In fact, at five of the seven sites including the inlet (B2), phosphates were only recorded on a single occasion, suggesting that phosphates were not of particular concern at this site.

Mean sulphate concentrations showed little variation between sampling points at either site, although there was a slight increase below the settlement tank at the Dagenham site (D2) suggesting that it may be acting as a source rather than a sink for this determinand. Comparison of sulphate concentrations between the two wetlands indicates that concentrations at Brentwood tend to be much greater than at Dagenham throughout the whole system. Sulphates are naturally occurring, and the differences in concentrations between the two sites are thought to reflect differences in local geology between the two catchment areas.

Additional water samples were collected at each of the sampling points at the Dagenham wetland commencing in September 1996 (a total of seven sampling visits) and were analysed at the Environment Agency's Fobney Laboratory for trihalomethanes (THMs) using Gas Chromatography with Electron Capture Detector. This additional analysis was performed as background water quality analysis carried out by the EA prior to construction of the wetland system reported elevated concentrations of THMs at a site upstream of the wetland system. The main source of THMs is the chlorination of water containing organic matter (Tebbut, 1992). Chlorinated water is used in garages within the catchment area for the cleaning of car parts. Values in excess of the total THMs standard of 150µg/l (EU Drinking Water Directive, 98/83/EC) were recorded upstream of the wetland prior to the construction of the site; 151µg/l on the 9 August 1995, 158µg/l on the 16 January 1995 and 161µg/l on the 20 July 1994. However, none of the THM concentrations in any of the samples collected during this study exceeded this 150µg/l value (Table 4.15). Although the concentrations did vary at each sampling point, mean concentrations decreased from inlet to outlet (D1-D5), indicating that wetland systems are capable of decreasing the concentration of THMs. THMs were not monitored at the Brentwood site.

Table 4. 15 Mean concentration (±SD) of Trihalomethanes, Total ammonia, and Unionised ammonia at each sampling point during dry weather over the two year monitoring programme at the Dagenham and Brentwood wetland.

	Trihalomethanes (µg/l)	Total Ammonia (mgN/l)	Unionised ammonia (µgN/l)
Dagenham			
D1	47.6±16.9	0.35±0.27	2.86±2.67
D2	41.5±20.8	0.44±0.45	4.42±6.67
D3	35.3±18.1	0.34±0.31	2.95±4.94
D4	36.2±18.7	0.47±0.66	3.52±4.68
D5	29.9±25.4	0.29±0.23	1.77±1.88
Brentwood			
B1	-	0.21±0.28	1.94±2.28
B2	-	0.28±0.33	3.15±4.74
B3	-	0.34±0.52	4.46±7.39
B4	-	0.28±0.28	2.08±2.62
B5	-	0.31±0.52	2.80±4.60
B6	-	0.46±0.86	6.66±13.64
B7	-	0.24±0.19	4.60±9.28

Mean dry weather concentrations of total ammonia and unionised ammonia showed an overall decrease from the inlet to the outlet at the Dagenham wetland and an overall increase from the inlet to the outlet at the Brentwood sites. At both sites, mean concentrations demonstrated considerable variability throughout system. As for the dry weather metal concentrations, both total and unionised ammonia showed an increase in mean concentration from D1 to D2 in the Dagenham wetland indicating that the settlement tank was contributing to the total and unionised ammonia concentrations. Both parameters showed a decrease in concentration between the inlet and the outlet of the *Typha* bed, an increase between the inlet and the outlet of the first *Phragmites* bed, and a decrease between the inlet and the outlet of the second *Phragmites* bed. The performance of the different beds is thought to be associated with the different density of plants in each bed. The first *Typha* bed having the best coverage, followed by the second and third beds planted with *Phragmites*. However, the second bed appeared to

result in an overall increase in mean concentrations in comparison to the third bed which showed an overall decrease suggesting that there were other factors involved.

In relation to water quality standards, neither the mean concentrations of total ammonia or unionised ammonia exceeded the values set down in the Freshwater Fisheries Directive (Table 4.17). However, total ammonia concentrations exceeding these criteria were recorded on three occasions at different sites (see Appendix I) again demonstrating that elevated concentrations can occur during dry weather, which is a matter of concern.

At the Brentwood site, mean concentrations of total and unionised ammonia were similar to the values reported at Dagenham. However, mean concentrations of both parameters showed an overall increase between the inlet and outlet (B2-B6). Both the initial and final settlement tanks (B2-B3 and B5-B6) resulted in a mean increase in both total ammonia and unionised ammonia. This may also indicate that the settlement tanks are oxygen limited, and the ammonia is not being oxidised. This does not appear to agree with the dissolved oxygen data which show little difference between the dissolved oxygen concentrations before and after the settlement tanks at both sites. However, the dissolved oxygen measurements were always taken just below the water surface and they may decrease with depth within the tank. The poorer performance of the Brentwood system in dry weather in comparison to that of the Dagenham system may again be related to the differences in surface area between the two sites.

4.5.2 During wet weather conditions

Table 4.16 gives the mean concentrations (±SDs) of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia at the inlet and outlet of each wetland during three storm events at Dagenham and four storm events at Brentwood. As for the metal data, the standard deviations indicate the differences between the individual storm events.

At Dagenham, all the parameters showed a decrease in mean concentration on passing through the wetland system except for suspended solids which showed an overall increase. This is thought to be partly associated with the delays involved in the

emptying of the settlement tank. This became completely filled with sediment before it was emptied, and was an obvious source of resuspension. Other factors were the problems which occurred with the initial establishment of the *Phragmites* in the second and third beds, and the damage to the plants and the disturbance of sediments caused by grazing horses. All these issues are now being resolved through the implementation of a management and maintenance plan, and it is hoped that further monitoring of the system will indicate the benefits of these remedial measures. At the Brentwood site, mean concentrations of BOD_5, suspended solids and chlorides showed a decrease, whereas the mean concentrations of nitrates, sulphates and total ammonia all showed an increase. However, at both sites the differences between most of the inlet and outlet concentrations were small, and there do not appear to be any clear distinctive trends.

Table 4. 16 Mean concentrations (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates and sulphates at the inlet and outlet during storm events at the Dagenham and Brentwood wetlands.

	BOD_5 (mg/l)	suspended solids (mg/l)	chloride (mg/l)	nitrate (mg/l)	phosphate (mg/l)	sulphate (mg/l)	Total ammonia (mgN/l)
Dagenham							
D1	9.7±6.8	35.3±47.0	63.9±39.0	23.2±8.9	1.0±0.7	55.7±25.8	0.58±0.23
D5	8.8±7.1	41.8±38.7	56.7±28.6	17.1±1.2	0.9±0.6	45.0±15.3	0.29±0.21
Brentwood							
B2	9.3±10.3	100.6±64.8	69.5±77.7	12.3±7.5	0.2*	47.6±22.1	0.17±0.07
B6	8.3±9.2	96.5±52.4	47.0±41.0	12.9±8.4	0.2±0.2	57.2±32.4	0.23±0.12

* = only recorded once

Table 4.17 gives the mean concentrations (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia at the inlet during dry weather and storm events at both wetlands, in comparison to values reported by studies in the literature and for appropriate water quality standards. It can be seen that, at both sites, there was little difference between mean BOD concentrations during dry weather and during storm events, and that the concentrations of chlorides, nitrates, phosphates and sulphates showed a clear decrease between the different conditions with lower values in wet weather conditions.

Table 4. 17 Mean concentrations (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia at the inlet during dry weather and storm events at the Dagenham and Brentwood wetlands, in comparison to values reported for other studies and water quality criteria.

	Dagenham wetland (mg/l)		Brentwood wetland (mg/l)		Other studies (mg/l)	Water quality standards (mg/l)
	d.w.	w.w.	d.w.	w.w.		
BOD	7.1	9.7	8.5	9.3	9-143 c	25 a
Suspended solids	86.6	35.3	54.7	100.6	22-490 c	35 a
Chlorides	95.7	63.9	92.7	69.5	159-2174 d	200 e
Nitrates	41.7	23.2	21.3	12.3		50 b
Phosphates	2.0	1.0	0.6	0.2*	0.02-4.30 f	2 a
Sulphates	90.1	55.7	144.4	47.6		
Total ammonia (as N)	0.35	0.58	0.28	0.17	0.2-4.6 f	0.78 b

d.w. = dry weather

w.w. = wet weather

a = Urban Wastewater Treatment Directive

b = Freshwater Fisheries Directive

c= GromaireMertz *et al.*, 1999

d = Ellis and Revitt, 1991

e = Surface Water Abstraction Directive

f = Ellis 1993

It was expected that BOD concentrations would increase during wet weather due to the mobilisation of particles with associated organic matter. However, mean BOD concentrations do not show a marked elevation at either site during storm events, supporting the view that using design guidelines for sewage treatment wetlands based on the removal of BOD may not be appropriate for urban runoff treatment wetlands. The mean concentrations of chlorides, nitrates, phosphates and sulphates were lower during storm events than during dry weather. This is thought to be associated with the fact that these pollutants are not deposited widely within the catchment area, and are typically not principal components of urban runoff (except possibly chlorides during winter months). Therefore, during rainfall events the concentrations of these parameters decrease due to the dilution effects of increased flow, and do not show the same pattern

of increase in concentration due to mobilisation of accumulated pollutants that has been seen for metals.

The mean concentrations of suspended solids showed a marked reduction during storm events at Dagenham but an approximate doubling during wet weather at the Brentwood wetland. As described in Section 4.2, rainfall had begun prior to the commencement of sampling for all the storm events at the Dagenham site, and it is possible that early elevated concentrations of suspended solids were missed. At the Brentwood site, the mean inlet concentration of suspended solids increased during storm events as expected due to the mobilisation of particles accumulated on impervious surfaces during the antecedent dry period.

In comparison to values for urban runoff reported in other studies, the concentrations of BOD_5, chlorides and total ammonia recorded at both sites tend to be lower or at the lower end of the reported range suggesting that these pollutants are not of primary importance as components of the pollutant load within either catchment area. In contrast, the mean concentrations of phosphates and suspended solids are both well within the range of values recorded by other authors. The mean concentrations of suspended solids exceeded water quality standards during both dry weather and storm events which is of great concern. The concentrations of THMs and unionised ammonia were not determined during storm events at either site.

4.6 Loadings of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates, trihalomethanes and total and unionised ammonia

Although the changes in concentrations within each wetland system, and between dry and wet weather conditions highlight some interesting trends, the data also need to be examined in terms of pollutant loadings as these refer to changes in the actual masses of the pollutants. Loadings were calculated as described in Section 4.1, and mean inlet loadings (±SD) during dry and wet weather of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates, total ammonia, unionised ammonia and trihalomethanes for both wetlands are given in Table 4.18.

Table 4. 18 Mean loadings (±SD) of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates, total ammonia, unionised ammonia and trihalomethanes at the inlet during dry weather and storm events at the Dagenham and Brentwood wetlands.

	Dagenham wetland		Brentwood wetland	
	d.w. (g/s)	w.w. (g/s)	d.w. (g/s)	w.w. (g/s)
BOD	0.23±0.15	1.70±0.92	0.11±0.09	2.04±3.17
Suspended solids	3.62±4.9	2.97±3.86	0.95±1.19	18.63±19.19
Chlorides	3.50±0.85	7.25±2.33	1.22±0.12	9.66±10.33
Nitrates	1.64±1.10	4.56±3.64	0.28±0.12	1.77±0.96
Phosphates	0.08±0.02	0.08±0.00	-	-
Sulphates	3.30±0.88	7.95±5.27	1.99±1.11	6.91±3.33
Trihalomethanes	1.58±0.71	-	-	-
Total ammonia (as N)	0.01±0.01	0.09±0.08	3.60±3.98*	26.00±13.00*
Unionised ammonia	0.12±0.10*	-	-	-

* = mg/s

d.w. = dry weather

w.w. = storm event

As for the metals data, all of the parameters, except for suspended solids at the Dagenham wetland, showed a noticeable increase in loadings during storm events in comparison to the dry weather values. However, the increase in loadings of these parameters is primarily due to the increased flow associated with storm events (see Appendix II) as mean concentrations tended to remain approximately the same or show a decrease between dry weather conditions and storm events (Table 4.17). This is in contrast to the metal data where increased loadings tended to be associated with both increases in concentration and flow. The exceptional case of the suspended solids loadings at Dagenham, which showed a decrease in mean loadings during storm events despite the increased flow, supports the proposition that the initial elevated suspended solids loadings were missed as sampling commenced after the start of the rainfall events. Loadings increase by factors of between 2 and 20 although mean loadings of phosphates at Dagenham remained approximately equal. The elevation of storm event loadings was greater for metals with enhancement factors of between 8 and 118. This is thought to demonstrate the fact that the input of parameters such as nitrates and

phosphates into the wetland systems was of a more continuous nature, whereas the input of metals was more intermittent as their mobilisation and transportation into watercourses depended primarily on rainfall within the catchment areas.

Dry weather loadings for all the parameters were higher at Dagenham than at Brentwood, whereas storm event loadings for BOD, suspended solids and chlorides were greater at Brentwood than Dagenham. The higher loadings of BOD at Dagenham may be due to organic matter associated with the higher loadings of suspended solids. Loadings of nitrates, sulphates and total ammonia were higher at Dagenham than Brentwood. This was particularly true for nitrates and total ammonia reflecting the higher concentrations of these parameters at Dagenham, with the higher ammonia loadings suggesting that there could be a higher frequency of 'wrong connections' in this catchment area than within the Brentwood catchment.

4.6.1 Statistical analysis of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia loadings during dry weather and wet weather conditions

Following the approach used for the metals data, it was decided to analyse this data further to examine whether differences between the loadings of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia during dry weather and storm events were significantly different. Two different statistical procedures, involving the use of t-tests and comparison of mean dry weather data to each individual storm event were applied. Both these approaches have been explained in Section 4.2.1.

Table 4.19 summarises the distributions of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia during dry weather, and identifies the transformations carried out where necessary.

Table 4.20 describes the results of t-tests carried out to examine whether there was a significant difference between inlet loadings during dry weather and storm events, in contrast to comparing dry weather loadings for each storm event individually. The values in brackets in Table 4.20 denote which of the storm events were not significantly different from the dry weather values, where 1 = the first storm monitored at the site, 2 = the second storm event monitored etc. It is interesting to note that this varies for

different parameters, which is thought to be associated with factors such as the length of the antecedent dry period, activity within the catchment area and, as demonstrated by Figs 4.1 and 4.2, the relative times of sampling at the inlet and outlet within each storm event.

Table 4. 19 Distribution of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia, and transformations carried out.

	Dagenham wetland		Brentwood wetland	
	raw data	transformation	raw data	transformation
BOD	normal	-	not normal	log10
Suspended solids	not normal	log10	not normal	log10
Chloride	normal	-	not normal	log10
Nitrates	normal	-	normal	-
Phosphates	normal	-	not enough samples	
Sulphates	normal	-	normal	-
Total ammonia (N)	normal	-	not normal	square root*

* = values <1 resulted in -ve log values, and therefore a square root transformation was used.

Table 4. 20 Results of t-tests and 2SD + mean to examine for significant differences between inlet loadings during dry weather and storm events.

	Dagenham wetland		Brentwood wetland	
	t-test	2SD + mean	t-test	2SD + mean
BOD	p>0.05	yes	p<0.05	2 of 4 (1 and 3)
Suspended solids	p>0.05	no	p<0.05	3 of 4 (1)
Chloride	p>0.05	yes	p<0.05	3 of 4 (2)
Nitrates	p>0.05	1 of 2(1)	p<0.05	3 of 4 (2)
Phosphates	p>0.05	no	not enough samples	
Sulphates	p>0.05	1 of 2(1)	p<0.05	2 of 4 (1 and 2)
Total ammonia	p>0.05	yes	p<0.05	3 of 4 (2)

At the Dagenham wetland, the results of the t-test indicate that there are no significant differences between the inlet loadings during dry weather and storm events for all of the parameters (Table 4.20). However, comparison of the mean dry weather loads to those of each storm event individually suggests that there is a significant difference for BOD, chlorides and total ammonia, and between dry weather loads and the second storm event for nitrates and sulphates. This could be due to the fact that the t-test is based on

comparing mean values and due to a combination of the high variability within both the dry weather and the storm event data sets and the small sample sizes.

Results of the t-tests at the Brentwood site suggest that there is a significant difference between the dry weather and storm event loadings for all of the parameters, except for phosphates. Phosphates were only detected twice at the inlet during the whole monitoring programme; during dry weather (1 July 1996) and during the 25 August 1997 storm event. The mean concentrations tended to remain approximately similar or show decreases during storm events at both sites, and therefore the significant differences at this site are thought to be primarily associated with increased flow during storm events. At Brentwood, the inlet flow increased from the mean dry weather value of 14±7 l/s by factors of 8, 7, 10 and 21 for each of the four storm events respectively. At Dagenham, although the inlet flow increased compared to the mean dry weather flow (34±5 l/s) by a factor of 13 during the second storm event, the inlet flow during the first storm event was only twice the mean dry weather flow. This is an explanation of the higher mean inlet loads at this site for some of the parameters (Table 4.18). This explanation is also supported by the other statistical approach which suggests that four of the six parameters are not significantly different for the second storm event which had the lowest inlet flow of the four storm events (92l/s), whereas all of the parameters are significantly different for the 4th storm event which had the highest inlet flow of the four events (282l/s). This is in contrast to the metals data which indicated that there was a significant difference between loadings during dry weather and storm events for all of the metals irrespective of the flow, except for Cr and Ni at the Dagenham wetland.

4.6.2 Removal efficiencies of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates, trihalomethanes and total and unionised ammonia during dry weather

Dry weather removal efficiencies based on loadings were calculated for each of the components of the Dagenham wetland, and for 3 of the components of the Brentwood wetland (flow was not monitored at each sampling point at this site). The components for which the removal efficiencies were calculated are as follows:-

Dagenham wetland

D1-D2 = removal by settlement tank

D2-D3 = removal by bed 1 (planted with *Typha latifolia*)

D3-D4 = removal by bed 2 (planted with *Phragmites australis*)

D4-D5 = removal by bed 3 (planted with *Phragmites australis*)

D1-D5 = total removal for overall wetland system (inlet to outlet)

Brentwood wetland

B2-B5 = removal by initial settlement tank + natural wetland

B5-B6 = removal by final settlement tank

B2-B6 = total removal for overall wetland system (inlet to outlet)

Tables 4.21 and 4.22 summarise the dry weather removal efficiencies for each of these components, as well as the total removal efficiencies for each system. As for the dry weather metal removal efficiencies, the removal efficiencies of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia at both sites are highly variable, which is again considered to be related to the generally low recorded concentrations (Tables 4.13 and 4.14). Although many of the mean values for each component are negative at the Dagenham wetland, the mean removal efficiencies for the whole system for all of the parameters are positive, except for chloride which shows a small increase. At the Brentwood wetland it appears that the natural wetland shows the best performance, reporting the highest removal efficiencies for all of the parameters.

The value for the 'natural wetland component' also includes the performance of the initial settlement tank, and removal efficiencies calculated from loadings are enhanced by the fact that the flow leaving the natural wetland is generally lower than that at the inlet. This generally low flow value at the outlet of the natural wetland also affects the removal efficiencies of the final settlement tank, where differences in low concentrations are magnified by any variation in flow volume between the inlet and the outlet of the final settlement tank. In addition, as the flow from the constructed wetland was too low to be measured, loadings from this component could not be included in the inlet value used for the calculation of removal efficiencies of the final settlement tank. However, the outlet value used in the calculation of removal efficiencies for the final settlement tank does include loadings from the constructed wetland component as B6 is

the combined outlet for both the natural wetland and constructed wetland components. Although flow was always very low during dry weather, the fact that the contribution from the constructed wetland to pollutant loadings entering the final settlement tank is not included in the inlet value for the calculation of removal efficiencies but is included as part the outlet value may further explain the apparent poor performance of the final settlement tank.

Table 4. 21 Mean percentage removal efficiencies and SD for BOD_5, suspended solids, total and unionised ammonia and trihalomethanes for each component, as well as the whole system, during dry weather.

	BOD	Suspended solids	Total ammonia	Unionised ammonia	Trihalomethanes
Dagenham wetland					
settlement tank	8±42	-36±62	-17±53	-36±151	22±17
bed 1	-40±108	3±62	-52±123	-277±687	-4±20
bed 2	-35±166	-44±113	-129±477	-109±374	-8±37
bed 3	-12±79	-88±256	8±41	-17±131	17±39
inlet to outlet	23±48	35±45.3	20±45	6±74	32±37
Brentwood wetland					
natural wetland	33±33	40±39	39±29	-48±213	-
final settlement tank	-87±81	-30±79	-211±381	-399±454	-
inlet to outlet	-17±85	18±35	-50±167	-449±1063	-

Table 4. 22 Mean percentage removal efficiencies and SD for chlorides, nitrates, phosphates and sulphates for each component, as well as that for the whole system, during dry weather.

	Chloride	Nitrate	Phosphate	Sulphate
Dagenham wetland				
settlement tank	4±10	-26±56	19±31	-17±65
bed 1	-17±23	2±48	-23±45	-12±52
bed 2	9±20	-3±42	12±19	11±23
bed 3	-10±23	-3±34	-8±28	-10±19
inlet to outlet	-4±13	11±46	12±26	1±23
Brentwood wetland				
natural wetland	37±22	27±30	-	36±25
final settlement tank	-73±44	-51±52	-	-75±42
inlet to outlet	-2±41	3±45	-	-4±43

In comparison to the mean total removal efficiencies at the Dagenham site, only two of the seven parameters for which removal efficiencies could be calculated show a positive value at the Brentwood site. This is thought to be associated with both the generally lower concentrations and loadings recorded at the Brentwood wetland, which increases the variability within the data sets, and also with previously mentioned factors such as differences in the design and total surface area of the respective systems (Section 4.3.2).

Tables 4.23 and 4.24 show the ranges of removal efficiencies reported for each component, as well as the range of removal efficiencies for the whole system, over the two year monitoring programme. It can be seen that all of the components have resulted in the removal of each parameter except for the final settlement tank which at its best did not alter BOD and sulphate loadings (0% removal efficiency) and resulted in a slight increase in unionised ammonia (-2%).

Table 4. 23 Range of percentage removal efficiencies for BOD_5, suspended solids, total and unionised ammonia and trihalomethanes for each component, as well as the whole system, during dry weather.

	BOD	Suspended solids	Total ammonia	Unionised ammonia	Trihalomethanes
Dagenham wetland					
settlement tank	-60 to 55	-102 to 63	-133 to 15	-375 to 57	2 to 39
bed 1	-271 to 80	-101 to 83	-322 to 84	-2077 to 87	-32 to 22
bed 2	-472 to 83	-247 to 94	-1483 to 72	-1161 to 68	-72 to 22
bed 3	-173 to 87	-652 to 84	-55 to 73	-353 to 95	-38 to 75
inlet to outlet	-44 to 82	-16 to 96	-62 to 69	-130 to 93	-19 to 73
Brentwood wetland					
natural wetland	-28 to 79	-30 to 80	-19 to 83	-582 to 91	
final settlement tank	-240 to 0	-168 to 67	-1265 to 17	-1296 to -2	
inlet-outlet	-217 to 60	-24 to 83	-529 to 54	-3595 to 49	

Table 4. 24 Range of percentage removal efficiencies for chlorides, nitrates, phosphates and sulphates for each component, as well as that for the whole system, during dry weather.

	Chloride	Nitrate	Phosphate	Sulphate
Dagenham wetland				
settlement tank	-11 to 20	-125 to 22	-29 to 51	-157 to 50
bed 1	-59 to 28	-69 to 70	-93 to 36	-127 to 62
bed 2	-34 to 35	-79 to 42	-20 to 41	-36 to 52
bed 3	-46 to 25	-52 to 47	-79 to 19	-43 to 24
inlet to outlet	-21 to 19	-44 to 75	-16 to 48	-38 to 45
Brentwood wetland				
natural wetland	-14 to 66	-35 to 61	-	-26 to 65
final settlement tank	-154 to 2	-132 to 66	-	-155 to 0
inlet-outlet	-64 to 56	-73 to 63	-	-89 to 54

The data in Tables 4.21 and 4.22 were analysed further to determine whether differences between the removal efficiencies of each component were significant. The distribution of the removal efficiencies of each component was initially examined using the Ryan-Joiner test for normality. The removal efficiencies of chlorides, nitrates and THMs at the Dagenham wetland, and the removal efficiencies of suspended solids, chlorides and nitrates at the Brentwood wetland were normally distributed. However, as the distribution of the other parameters was not normally distributed it was decided to use a nonparametric test (Kruskal-Wallis test) so that all the data could be analysed in the same way, and thus enable comparisons between all of the parameters. ANOVA was also carried out on the normally distributed parameters (values in Table 4. 25 in brackets) and it can be seen that although these values do differ from the results of the nonparametric test, the significance of the result is not altered. As can be seen from the p-values reported in Table 4.25, there is only sufficient evidence to reject the null hypothesis of no significant difference between the removal efficiencies of each component for chlorides at the Dagenham wetland, whereas there is strong evidence of a significant difference for all of the parameters at the Brentwood wetland, except for suspended solids.

Table 4. 25 Results of Kruskal-Wallis test for significant differences between the removal efficiencies of each component.

	Dagenham wetland	Brentwood wetland
BOD	0.713	0.000
Suspended solids	0.700	0.079 (0.087)
Chloride	0.017 (0.034)	0.000 (0.000)
Nitrates	0.738 (0.616)	0.004 (0.000)
Phosphates	0.096	-
Sulphates	0.181	0.000
Total ammonia	0.418	0.000
Unionised ammonia	0.649	0.008
Trihalomethanes	0.334 (0.303)	-

Where the Kruskal-Wallis test indicated that there was a significant difference between the removal efficiencies of each component, Tukey's HSD test was carried out to see which component differed from which. The results indicated that at the Brentwood wetland the removal efficiencies of the natural wetland component and of the total system for BOD, total ammonia and nitrates were significantly different from those of the final settlement tank. Secondly, that the removal efficiencies of the natural wetland significantly differed from the final settlement tank for unionised ammonia, and that the removal efficiencies of all the components were significantly different from each other for chlorides and sulphates. To summarise, the results of the Tukey's HSD indicated that at Brentwood:

- the natural wetland component showed the highest removal efficiencies
- the final settlement tank showed the lowest removal efficiencies

Again, as mean concentrations showed little variation between the components, differences in flow are thought to be an important factor in these results.

At the Dagenham wetland, Tukey's HSD indicated that there was a significant difference in the removal of chloride between the first bed (planted with *Typha*) and the

second bed (planted with *Phragmites*). It is surprising that removal efficiencies appear to significantly differ between components for only one of the parameters, but this apparent difference may be due to the denser plant cover in the first bed in comparison to that in the second and third beds.

4.6.3 Removal efficiencies of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia during storm events

During storm events, samples were collected from the inlet and outlet only. Table 4.26 shows the mean removal efficiencies for the two fully monitored storm events at the Dagenham wetland and for the four storm events at the Brentwood site.

Table 4. 26 Mean percentage removal efficiencies of BOD_5, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia during storm events at the Dagenham and Brentwood wetlands.

	BOD	Suspended solids	Chlorides	Nitrates	Phosphates	Sulphates	Total ammonia
Dagenham	24±4	-99±98	32±21	44±19	-9±	30±6	38±58
Brentwood	15±33	-4±38	11±37	-17±33	48*	-22±16	-59±96

* = one storm event only

At the Dagenham wetland, the mean removal efficiencies of BOD were similar during both dry weather and storm events which could be associated with the fact that, despite the differences in flow between dry weather and storm events, mean BOD concentrations were similar (Table 4.13). Although suspended solids appeared to show a substantial increase at this site, the outlet concentrations recorded during both fully monitored storm events did not exceed the water quality standard of 35mg/l. However, loadings did show an increase and this is thought to be associated with delays in the implementation of the maintenance and management plan (Section 4.1). The mean removal efficiencies of total ammonia, chlorides and nitrates have increased in comparison to the dry weather values which is thought to be associated with the higher loadings during storm events, whereas the mean removal efficiencies of phosphates decreased during storm events which may be related to the net negative removal of suspended solids.

The mean removal efficiencies at the Brentwood site were lower than those at the Dagenham site, with only the removal of BOD and chloride showing a positive removal. Again, as with the metal removal efficiencies the standard deviations indicate the variability of the removal efficiencies between the individual storm events which are associated with the factors previously discussed in Section 4.3.2.

At the Brentwood wetland, the highest removal efficiencies for BOD_5 (48%), suspended solids (26%), chlorides (37%) and nitrates (28%) were recorded during the first storm event (25 August 1998), when samples at the inlet and outlet were collected with no time delay. The calculated removal efficiency for phosphate during this storm event was 48%, but this was the only storm event where phosphate removal efficiency could be calculated. This is in contrast to the removal efficiencies calculated for the metals data during this storm event which indicated that only Pb and Cr showed a positive removal. For the next three storm events removal efficiencies were calculated based on a possible retention time of 50 minutes which was based on the results of the metals analysis from the 8 October 1997 storm event. However, analysis of the data for BOD, suspended solids, chlorides, nitrates, phosphates, sulphates and total ammonia did not reveal a similar trend of maximum loadings at the inlet followed by maximum loadings at the outlet approximately 50 minutes later. This can be seen from Table 4.27, which gives the time in minutes into the storm events that the maximum loadings of BOD, suspended solids, chlorides, nitrates, sulphates and total ammonia occurred at the inlet and outlet to the wetland system. However, although these parameters do not appear to behave in a similar way to the metals, it is interesting to note that during both storm events the maximum loadings at the inlet and outlet of the wetland occurred at similar times to each other for BOD and suspended solids, and also at similar times to each other for chlorides, nitrates and sulphates suggesting that these pollutants may be behaving in similar ways with respect to each other (Table 4.27). Suspended solids rich in organic matter could explain the correlation between maximum BOD and suspended solids loadings, whereas chlorides, nitrates and sulphates are known to be highly mobile which may explain their similar behaviour patterns. The differences in the patterns of loadings between the various parameters could be attributed to factors such as differences in their mobilisation rates, and differences in the behaviour of pollutants in the wetland system, resulting in different retention times for individual pollutants or

pollutant groups. The fact that the maximum loadings of chlorides, nitrates and sulphates at the outlet during the 26 May 1998 storm event occur before the maximum loading at the inlet is thought to be associated with the fact that the storm event was receding when sampling commenced.

Table 4. 27 Time into storm event of maximum loadings at the inlet and outlet.

	8/10/97		26/5/98	
	maximum load at inlet	maximum load at outlet	maximum load at inlet	maximum load at outlet
BOD	71 minutes	107 minutes	0 minutes	20 minutes
Suspended solids	39 minutes	107 minutes	0 minutes	20 minutes
Chlorides	22 minutes	28 minutes	50 minutes	45 minutes
Nitrates	22 minutes	28 minutes	50 minutes	45 minutes
Sulphates	22 minutes	28 minutes	50 minutes	45 minutes
Total ammonia	97 minutes	73 minutes	0 minutes	45 minutes

These results clearly demonstrate the value of monitoring the profile of storm events in an attempt to understand the complex behaviour of pollutants within different storms. The use of automatic sampling equipment is strongly recommended to enable the performance of both these treatment wetlands, and future systems, to be fully evaluated during storm events of different magnitudes.

4.7 Retention times of the Dagenham and Brentwood wetland systems in different seasons

To determine the retention times of both systems, each wetland was dosed with Rhodamine WT dye (for methods see Section 3.4). Dosing was carried out twice at each site during dry weather conditions and the results are given in Table 4.28.

The results show that during dry weather conditions the retention time of both wetland systems during summer and winter was shorter than expected, with values noticeably lower than the 3-5 hour retention time recommended by Revitt *et al.*, (1999) (see Section 2.3.2.1). At the Dagenham wetland, the low retention time was thought to be

associated with both the damage caused by grazing horses and the establishment problems of the *Phragmites*. The low retention time at the Brentwood site is thought to be mainly due to the smaller size of the system.

Table 4. 28 Retention times of the Dagenham and Brentwood wetlands during dry weather conditions in summer and winter (minutes).

	Summer	Winter
Dagenham wetland	120	76
Brentwood wetland		
constructed wetland component	ND	62
natural wetland component	125	120

ND = not defined as no flow through system

At the Dagenham wetland the retention time is considerably greater during summer (120 minutes) than during winter (76 minutes) which is thought to be associated with the greater plant density during summer months. At the Brentwood site there is a little difference between the summer and winter retention times of the natural wetland component. This could be due to the fact that, during low flow conditions, flow through the natural wetland tended to be restricted to a narrow channel which was densely colonised by *Typha latifolia*. In winter, although the *Typha* died back, the channel remained full of senesced plants which also acted to retard flow hence the similar summer and winter retention times for this component. The retention time of the constructed wetland component was approximately half that of the natural wetland component during winter, which is thought to be primarily due to hydraulic short-circuiting resulting from the location of the outlet being directly opposite the inlet to the bed.

4.8 Sediment metal analysis

Sediment samples were collected at bimonthly intervals at the following sampling points within and adjacent to the Dagenham and Brentwood wetlands:

Dagenham wetland

D1 = upstream site

D2 = settlement tank

D3 = end of the first bed (planted with *Typha*)

D4 = end of the second bed (planted with *Phragmites*)

D5 = end of the third bed (planted with *Phragmites*)

Brentwood wetland

B1 = upstream site

B3 = first settlement tank

B4 = final settlement tank

B7 = downstream site

The results of the sediment metal analyses indicate that concentrations vary both between sites and between sampling points within each wetland system. Tables 4.29 and 4.30 give the mean concentrations of metals (±SD) recorded for each sampling point at each wetland. It can be seen that concentrations tend to be higher at Dagenham than Brentwood, reflecting the generally higher pollutant loadings at this site. At the Dagenham wetland, mean concentrations tend to decrease in magnitude in the order Zn>Pb>Ni>Cu>Cr>Cd, whereas aqueous metal concentrations during both dry weather and storm events showed a decrease in magnitude in the order Zn>Ni>Cu>Pb>Cr>Cd (Tables 4.1 and 4.3). The fact that Pb is accumulated in higher concentrations in the sediment when it tends to be present in lower aqueous concentrations than Ni and Cu is attributed to its strong association with the particulate phase (Morrison *et al.,* 1984). Similar trends were also noted at the Brentwood wetland where at B1 and B7 mean concentrations in the sediment decreased in the same order of magnitude (Table 30). At B3 and B4 the order of decrease in sediment metal concentration was Zn>Pb>Cu>Ni>Cr>Cd which is thought to reflect the fact that the mean Cu concentration is greater than the mean Ni concentration in flow entering the system (Table 4.2). Again, the mean sediment concentrations of Pb at all the sampling points are greater than the mean Cu and Ni sediment concentrations. However, another factor at this site is that during storm events mean aqueous metal concentrations decreased in the order of Zn>Pb>Cu>Ni>Cr>Cd (Table 4.3). This elevation in aqueous Pb

concentrations in relation to Ni and Cu is responsible for the higher Pb sediment concentrations, and also helps to explain the maximum Pb sediment concentrations recorded at this site when for the other parameters the highest aqueous and sediment concentrations are generally recorded at the Dagenham wetland.

Table 4. 29 Mean sediment metal concentrations (±SD) at each sampling point for the Dagenham wetland (µg/g).

	Cr	Zn	Cd	Pb	Ni	Cu
D1	31.1±11.3	239.3±146.0	5.7±1.7	132.5±46.5	60.7±28.2	58.5±18.7
D2	67.5±42.3	451.9±308.0	7.4±2.8	226.2±94.3	97.8±52.2	107.5±43.3
D3	29.4±14.6	141.1±95.4	5.4±1.2	78.8±38.5	52.6±27.8	45.8±20.1
D4	38.1±15.0	218.9±124.0	6.5±1.5	106.8±16.3	82.0±28.1	56.0±11.3
D5	26.4±15.9	271.9±311.9	4.3±2.3	106.5±80.4	70.9±41.8	51.4±48.3

Table 4. 30 Mean sediment metal concentrations (±SD) at each sampling point for the Brentwood wetland (µg/g).

	Cr	Zn	Cd	Pb	Ni	Cu
B1	9.1±2.7	131.0±84.9	4.4±0.8	62.2±12.4	58.7±36.1	38.2±9.1
B3	20.5±11.8	292.0±209.7	5.6±1.2	218.4±84.5	47.9±40.3	71.1±22.5
B4	21.0±8.3	320.1±213.4	6.4±1.6	215.9±48.9	51.5±35.0	65.0±24.3
B7	17.5±9.9	201.1±139.2	5.3±1.6	147.6±94.9	88.7±89.1	43.6±21.9

In comparison to values identified for low, moderately high and high levels of sediment metal contamination (Table 4.31), mean metal sediment concentrations are comparable to those associated with moderately high and highly contaminated sediment, reflecting the input of urban runoff into both systems.

Table 4. 31 Values given for low, moderate/high and high levels of sediment metal contamination (µg/g) (Swedish Environment Protection Agency, 1991).

	Low levels	Moderate/high levels	High levels
Cd	0.2-0.7	0.7-2.0	2-5
Pb	5-30	30-100	100-400
Cu	10-25	25-50	50-150
Cr	10-25	25-75	75-300
Ni	10-30	30-75	75-300
Zn	70-175	175-300	300-1000

Although the mean sediment concentrations of Zn and Cr recorded at the upstream site (B1) are within the range of values reported for low levels of sediment metal contamination, mean concentrations of Pb, Ni and Cu fall into the 'moderately/high' contaminated category, and mean concentrations of Cd are in the 'high' category. This suggests that the River Ingrebourne may also receive urban runoff inputs upstream of the wetland system.

Figure 4.3 shows the mean concentrations for each metal in the different components of the Dagenham wetland. The highest values for all metals can be seen to be associated with the settlement tank (D2). This indicates that, although there have been problems with resuspension, the settlement tank is still effectively removing metals from the water column.

At the Brentwood wetland, maximum mean concentrations of Pb and Cu were recorded in the initial settlement tank, with the maximum concentrations of Cr, Zn and Cd being recorded in the final settlement tank. This again suggests that both settlement tanks are capable of removing suspended solids and associated pollutants from the water column and retaining them in the sediment (Fig. 4.4). The mean maximum concentration of Ni was recorded at the downstream site (B7), suggesting that Ni is being transported through the system and accumulating in downstream sediment. However, the mean concentration of Ni in the sediment at the upstream site was also higher than the mean sediment concentrations within the system. This suggests that Ni may be entering the receiving stream from an unknown source, which is responsible for the elevated levels at B7, rather than preferential Ni transport through the system. This is also supported by the fact that the maximum mean concentration of Ni during dry weather was recorded at the upstream site (B1), rather than in flow entering the system via the surface water outlet (B2).

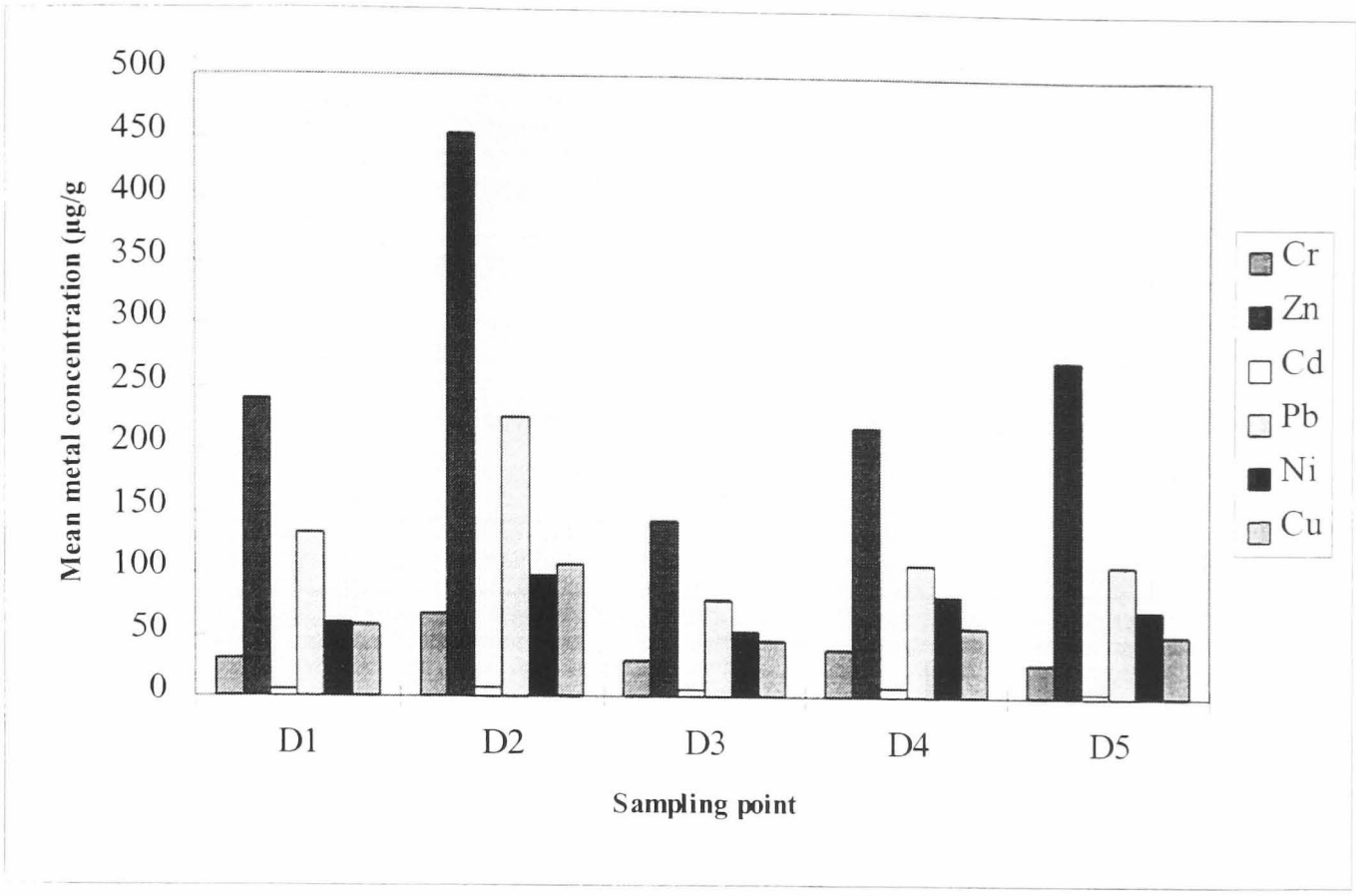

Figure 4. 3 Mean concentrations of metals in the sediment at each sampling point at the Dagenham wetland.

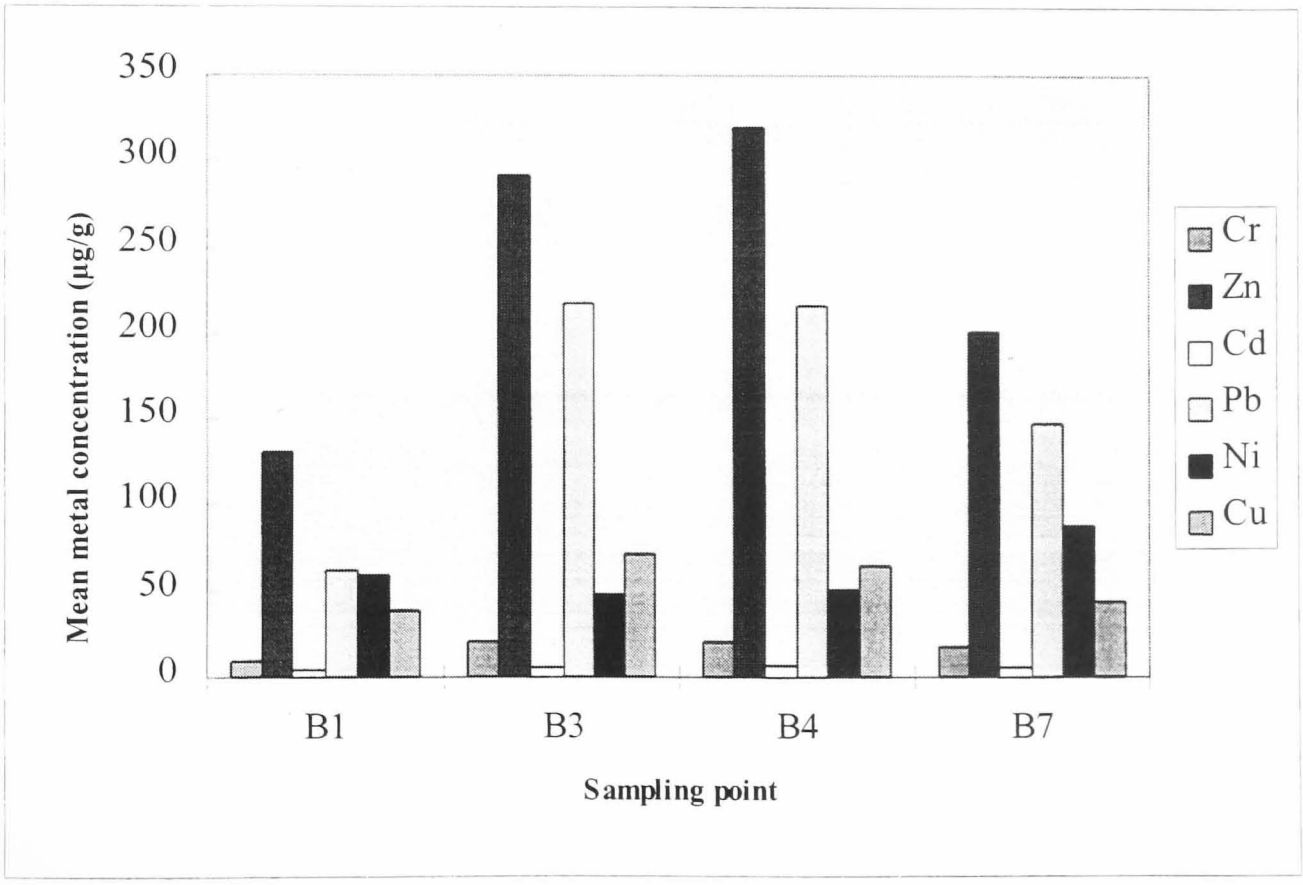

Figure 4. 4 Mean concentrations of metals in the sediment at each sampling point at the Brentwood wetland.

4.8.1 Statistical analysis of sediment metal concentrations at the Dagenham and Brentwood wetlands

To examine whether any of the differences between total metal concentrations at each sampling point were significant, it was decided to analyse the data further. As with the water pollution data, the first step was to examine the distribution of the data sets at each sample point using the Ryan-Joiner test for normality. Table 4.32 lists the distribution of the raw data sets and the transformations carried out.

Table 4. 32 Results of the Ryan-Joiner test on sediment metal concentrations at each site, and transformations carried out

	Dagenham wetland		Brentwood wetland	
	raw data	transformation	raw data	transformation
Cr	not normal	not transformable	not normal	log10
Zn	not normal	square root	normal	-
Cd	not normal	not transformable	normal	-
Pb	normal	-	not normal	square root
Ni	not normal	square root	not normal	log10
Cu	not normal	square root	not normal	log10

It was not possible to transform the Cr and Cd data sets at the Dagenham wetland to a normal distribution (possibly due to the small size of the data sets), and therefore for these two groups a nonparametric test was used. The normally distributed data were analysed using ANOVAs. The ANOVA is a parametric test based on means which determine whether a number of populations are significantly different given the variation within those populations. The results of the ANOVAs for each metal at each site are given in Table 4.33.

Table 4. 33 Results of ANOVAs to test for significant differences between sediment metal concentrations at each sampling site at both the Dagenham and Brentwood wetlands.

	Dagenham wetland	Brentwood wetland
Cr	0.008*	0.002
Zn	0.071	0.069
Cd	0.002*	0.025
Pb	0.000	0.000
Ni	0.100	0.293
Cu	0.000	0.021

* = Kruskal-Wallis test applied as data not normally distributed

Where the ANOVA indicated that there was a significant difference between the concentrations of metals in the sediment, Tukey's HSD was carried out determine where these differences lay. The results of the Tukey's HSD test indicated that at the Dagenham wetland, Cr concentrations at D2 significantly differed from those at D5, Cd concentrations at D2 and D4 significantly differed from those at D5, and Pb and Cu concentrations at D2 significantly differed from those at all the other sampling points. At the Brentwood wetland, Tukey's HSD indicated that the Cr and Pb concentrations at B1 were significantly different from those at all other sampling points, Cd concentrations at B1 were significantly different from those at B4, and that Cu concentrations at B3 were significantly different from those at B7. To summarise, the results of Tukey's HSD indicated that;

- At the Dagenham wetland, the settlement tank contains significantly higher metal concentrations for Cr (in comparison to D5), Cd (in comparison to D5), and Pb and Cu (in comparison to all four other sites).
- At the Brentwood wetland, B1 has significantly lower concentrations of Cr and Pb (in comparison to all other sites) and Cd (in comparison to B4), and that Cu concentrations at B7 were significantly lower than those at B3.

These results indicate that at the Dagenham wetland the settlement tank is accumulating the highest concentrations of metals and is therefore functioning as a treatment facility despite the associated problems of resuspension. The results also show that, at the

Brentwood site, B1 has significantly lower concentrations of Cr, Pb and Cd reflecting the fact that this site is thought to receive urban runoff only occasionally. There was not enough evidence to reject the null hypothesis for no significant difference between sediment metal concentrations for Zn and particularly Ni at either site. In the case of Zn this is thought to be associated with the fact that Zn is a very abundant and widespread metal in both catchment areas. It is the metal present in the highest aqueous concentrations during both dry weather and storm events at both sites, and in sediments at all sampling points. This suggests that it is readily accumulated and that therefore despite the noticeably higher concentrations in the settlement tanks at both sites, these observed differences are not significantly different. The ranges of Ni concentrations at each sampling point show a good deal of overlap within the respective wetland systems. This suggests that no individual component dominates the removal process, and that Ni may be behaving in a different manner to the other metals.

Another important point is that, although the maximum mean concentrations of all the metals were recorded in the settlement tank at the Dagenham wetland, the maximum concentrations of Zn (1035μg/g) and Cu (171μg/g) were actually recorded at D5 on 26 June 1997. On the same sampling visit, the sediment concentrations of Cr (62.8μg/g), Cd (10μg/g) and Pb (270μg/g) were also greatly elevated at D5 in comparison to their mean values at this site (Table 4.29). This is thought to be associated with the fact that the settlement tank was emptied at Dagenham a few days prior to 26 June 1997 sampling date. Following the emptying of the settlement tank, the concentrations of all the metals at this sampling point decreased. However, this decrease in the settlement tank also corresponds to an increase in all the metals at most of the sampling points downstream of the settlement tank, and in particular at D5. It is thought that the desludging of the settlement tank resulted in the resuspension of contaminated sediments. These sediments then settled out through the wetland, with the settlement of progressively finer particles occurring further down the system. The relationship between decreasing particle size and increasing pollutant concentration (Salomons and Forstner, 1984) could be an explanation for the increase in metal concentration at the sampling point at the end of the system. These results clearly demonstrate that although the emptying of the settlement tank resulted in a marked decrease in metal concentrations in this component, great care must be taken during the emptying process to prevent contaminated sediments being transported through the downstream system.

4.9 Plant metal analysis

Plants were collected seasonally over one year of the monitoring programme (spring 1997 - winter 1998) for tissue metal analysis. This involved the collection of *Typha latifolia* from the inlet of the first bed at the Dagenham wetland, and *Phragmites australis* from the inlet of the constructed wetland at Brentwood. Plants were divided into three sections comprising the roots, rhizomes and leaves. Total metal analysis of plant tissue showed that concentrations tended to vary both between the roots, rhizomes and leaves of each species, between the species themselves and seasonally.

Figures 4.5 and 4.6 show the mean metal concentrations in the roots, rhizomes and leaves of *Typha* and *Phragmites* for the four collected sample sets. The plant tissue data show that for all of the metals in *Typha*, and for Pb, Cu, Cr and Cd in *Phragmites*, metal uptake decreases in the order roots>rhizomes>leaves. This order of uptake has also been reported previously (Mungur *et al.*, 1995, Zhang *et al.*, 1990). For Zn and Ni in *Phragmites*, metal uptake decreased in the order roots>leaves>rhizomes. The higher concentration of Zn in the leaves of *Phragmites* may be associated with the fact that Zn is the most abundant metal in both the sediment and water samples indicating its common occurrence within the catchment area, and the higher leaf Zn concentrations may be due to dry deposition. The higher Ni leaf concentrations are more difficult to explain.

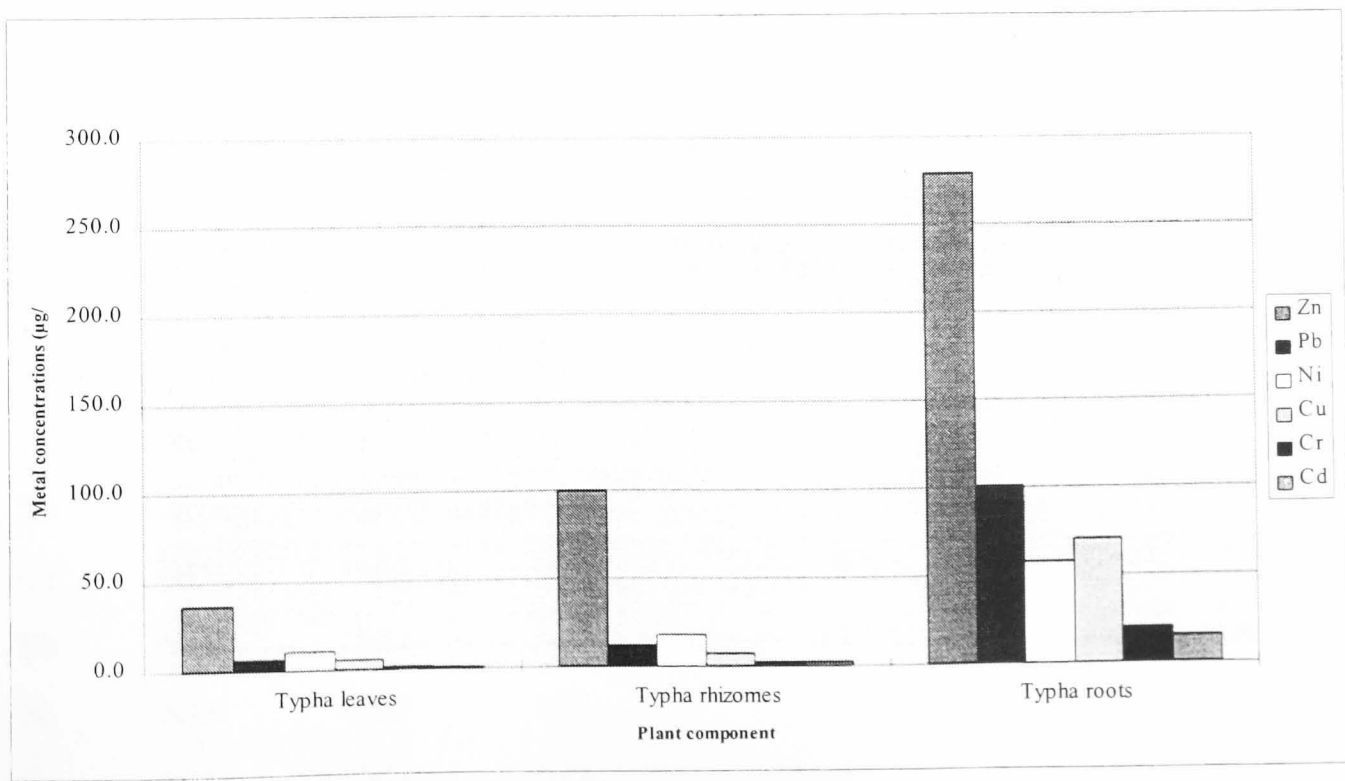

Figure 4. 5 Mean metal concentrations in the roots, rhizomes and leaves of *Typha latifolia* collected from the Dagenham wetland.

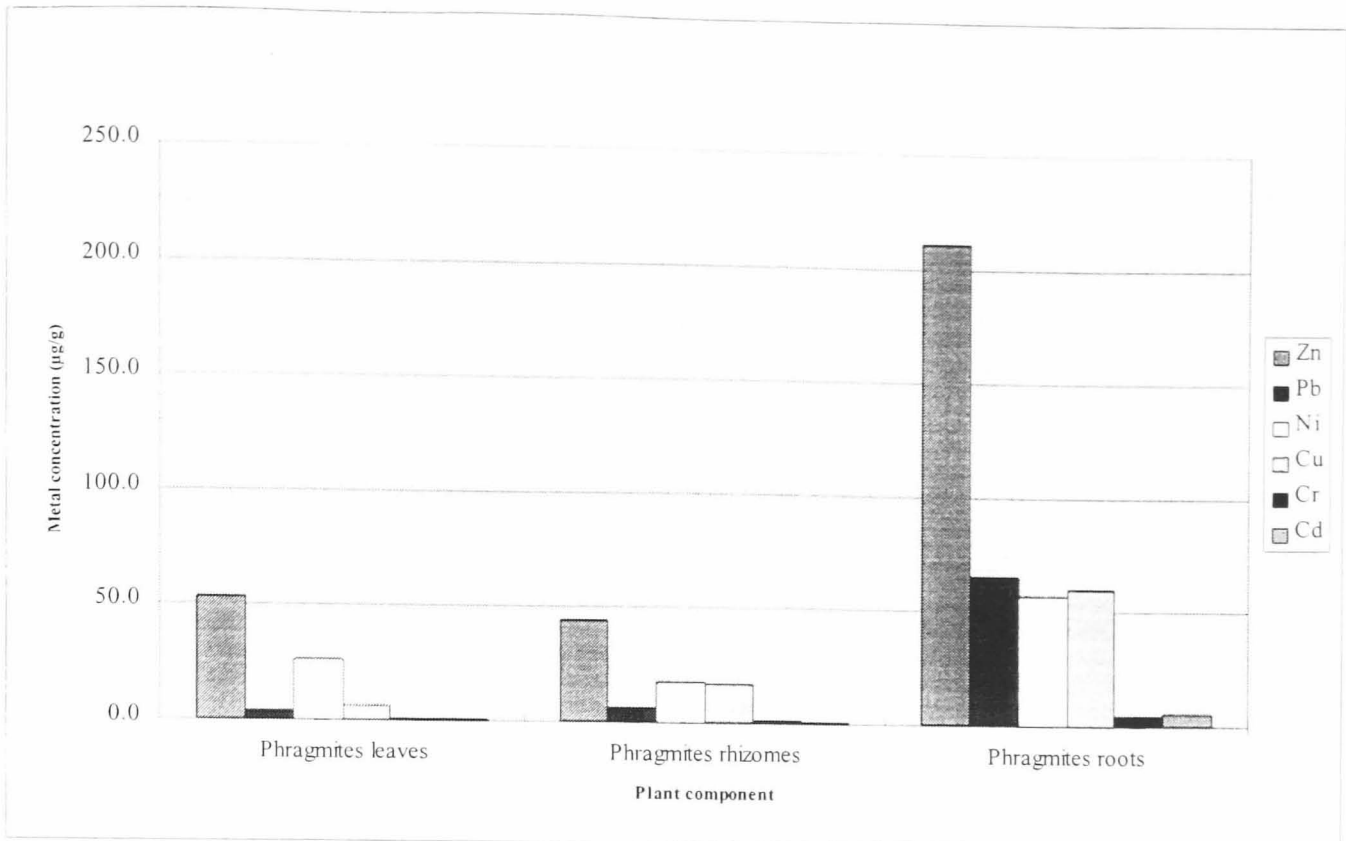

Figure 4. 6 Mean metal concentrations in the roots, rhizomes and leaves of *Phragmites australis* collected from the Brentwood wetland.

4.9.1 Statistical analysis of plant tissue metal concentrations

The data were analysed further to examine whether any of the differences in metal concentrations between the roots, rhizomes and leaves were statistically significant. Each data set was tested for normality using the Ryan-Joiner test, and where necessary the appropriate data transformations were carried out. Table 4.34 lists the distributions of the data sets, and transformations carried out where necessary.

Table 4. 34 Distribution and transformation carried out on plant components

	Typha latifolia			*Phragmites australis*		
	leaves	rhizomes	roots	leaves	rhizomes	roots
Cr	ND	ND	ND	ND	ND	ND
Zn	log10	log10	log10	ND	ND	ND
Cd	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	log10	log10	log10
Ni	ND	ND	ND	ND	ND	ND
Cu	ND	ND	ND	square root	square root	square root

ND = normally distributed

ANOVAs were carried out on the normally distributed data to test for significant differences between the components of each species. The results of the ANOVAs are listed in Table 4.35.

Table 4. 35 Results of ANOVAs to test for significant differences between plant components.

	Typha latifolia	*Phragmites australis*
Cr	0.103	0.067
Zn	0.059	0.018
Cd	0.004	0.002
Pb	0.030	0.000
Ni	0.001	0.079
Cu	0.009	0.015

Where the ANOVA indicated that there was a significant difference, Tukey's HSD was carried out to determine which component differed from which. The results indicated that for the *Phragmites* the concentrations of Zn, Cd and Pb in the roots were significantly different from those in the leaves and rhizomes, whereas for Cu, root concentrations were different from those in the leaves only. For *Typha*, Cd, Ni and Cu concentrations in the roots were significantly different from those in the leaves and rhizomes, whereas Pb concentrations in the roots significantly differed from those in the leaves only. There was insufficient evidence to support a significant difference between the concentrations in any of the components for Zn in *Typha*, Ni in *Phragmites* and for Cr in both species. Cr is typically present as Cr^{3+}, particularly in the presence of organic matter (Kadlec and Knight 1996). The ionic radius of Cr^{3+} (0.63A) is smaller than the ionic radii of Zn (0.74A), Cd (0.97A), Pb (1.20A), Ni (0.69A) and Cu (0.72A) (Weast and Astle, 1979), all of which are typically present in surface waters as divalent ions (Kadlec and Knight, 1996), and this size difference may be a factor in the more equal distribution of Cr concentration through the whole plant, in contrast to the other metals whose greater ionic size may prevent them from being transported as far. However, although the results of the statistical analysis seem to confirm that the metal concentrations in the roots are significantly higher than those in the rhizomes and leaves for most metals, it is important to remember that as the roots only form a small fraction

of the total plant biomass and leaves die-back in winter, it is actually the rhizomes which are the most important component for long-term storage in plants. The importance of the rhizome in metal bioaccumulation was demonstrated in a study by Zhang *et al.* (1990) in which metal concentrations in *Typha latifolia* were converted to metal loadings using biomass ratios. In this study plant biomass ratios were not determined, however, it is suggested that future plant bioaccumulation studies should include this as part of the analyses.

Table 4.36 gives the total seasonal plant tissue metal concentrations (sum of the root, rhizome and leaf components) of *Typha latifolia* and *Phragmites australis*. From Table 4.36 it can be seen that both species show marked seasonal variations in metal concentrations. Both *Typha* and *Phragmites* tend to contain highest concentrations in summer, followed by spring and winter, with lowest concentrations of all metals recorded in plants collected in autumn. The fact that the lowest metal concentrations were recorded in the autumn samples could be associated with die-back at the end of the growing season releasing metals at the time of the autumn sampling. Some of the released metals are then bound-up by the dead organic matter which may remain attached to the living plants (winter sampling).

Table 4. 36 Seasonal plant tissue metal concentrations for *Typha latifolia* (Dagenham) and *Phragmites australis* (Brentwood).

	Plant tissue metal concentrations (μg/g)							
	Spring		Summer		Autumn		Winter	
	T.l.	*P.a.*	*T.l.*	*P.a.*	*T.l.*	*P.a.*	*T.l.*	*P.a.*
Cr	23.5	7.2	52.1	11.5	6.8	2.3	11.3	4.2
Zn	571.1	346.8	775.4	404.7	150.7	165.6	167.6	318.8
Cd	31.2	6.2	21.4	10.4	9.0	4.4	12.8	8.7
Pb	153.7	52.5	224.6	107.5	32.7	35.9	65.7	106.2
Ni	76.8	ND	98.8	85.4	62.5	74.9	104.6	142.6
Cu	93.8	125.6	135.1	109.6	22.8	23.7	79.4	76.2

Key: *T.l. = Typha latifolia*,
P.a. = Phragmites australis
ND = not defined

When metal concentrations recorded in each species are considered relative to each other, *Typha* contains higher concentrations of metals than *Phragmites* in the spring and summer months, whereas in autumn and winter, concentrations of most metals are higher in *Phragmites* than in *Typha* (Table 4. 36). A possible explanation for this could be that the *Phragmites* has a longer growing season than the *Typha*, actively accumulating metals after the *Typha* has started to die-back in autumn, and then starting to grow earlier in the year. This may also suggest that it is advisable to include a mix of plant species in constructed wetland design to maximise the potential metal bioaccumulation.

4.10 Summary of results

This study demonstrates that two full-scale experimental wetland treatment systems, of differing design and surface area, were capable of reducing the pollutant loads associated with urban runoff. The results show that, during dry weather conditions, the concentrations of all the monitored pollutants were generally low and removal efficiencies were highly variable. During storm events, metal loadings increase, due to increases in both metal concentrations and flow rate, and removal efficiencies improve. The removal of BOD and nutrients also tended to be higher during storm events. However, the removal of suspended solids showed a mean net negative removal efficiency during storm events at both sites. The magnitude of storm events was found to be an important factor in removal efficiencies, with the Brentwood wetland system appearing to be undersized to treat the largest storm event recorded at this site.

Higher removal efficiencies were recorded at the Dagenham wetland, which is thought to be associated primarily with the fact that it has a greater surface area and that it received higher inlet loadings than the Brentwood wetland. The results also demonstrate that, despite problems of resuspension, the settlement tanks at both sites are efficient metal sinks, with these components tending to contain the highest mean sediment metal concentrations recorded at each wetland.

Plant tissue metal concentrations were found to vary both seasonally and between the species themselves suggesting that it may be advisable to include a mix of plant species

to maximise metal bioaccumulation in wetlands receiving metal contaminated wastewater. Both sites have suffered from several management and maintenance issues which could not be resolved immediately due to delays in the establishment of a management and maintenance plan. Such a plan is now in operation and removal efficiencies are expected to improve as recommendations made as a result of this study are implemented.

CHAPTER FIVE INVESTIGATION INTO THE ABILITY OF MICRO-ORGANISMS ISOLATED FROM URBAN WETLANDS TO ACCUMULATE HEAVY METALS

Wetlands offer an ideal environment for a range of micro-organisms, providing organic matter, nutrients, and a diversity of attachment sites in both aerobic and anaerobic environments. Micro-organisms may be involved in a number of pollutant removal processes occurring in wetland treatment systems, and their roles in processes such as the degradation of organic matter, nitrification and denitrification are now well understood (Cooper *et al.,* 1996).

The general ability of a wide range of bacteria, fungi and actinomycetes to bind heavy metals is well established (Holan and Volesky, 1995; Nakajima and Sakaguchi, 1986). More recently, researchers have been investigating the use of micro-organisms in the bioremediation of heavy metal contaminated wastewaters (Kapoor and Viraraghavan, 1998; Zhang *et al.,* 1998). However, the ability of micro-organisms isolated from full-scale constructed wetlands to accumulate heavy metals has received little attention. It is believed that this process may be an important year round pollutant removal process in the treatment of urban runoff by constructed wetland systems.

The results of laboratory experiments discussed in this Chapter describe the ability of selected fungal isolates to tolerate and accumulate Pb and Zn, and the effects of these metals on the growth of isolates at different temperatures. The location of Pb accumulated by one of the isolates is reported.

5.1 Isolation of micro-organisms from the Dagenham and Brentwood wetlands

Samples for microbial analyses were collected from the rhizosphere of wetland plants at both sites. The locations and species from which the micro-organisms were obtained were:-

Dagenham wetland — inlet to bed 1 (*Typha latifolia*)
inlet to bed 2 (*Phragmites australis*)
inlet to bed 3 (*Phragmites australis*)

Brentwood wetland — inlet to constructed wetland (*Phragmites australis*)
inlet to natural wetland (*Typha latifolia*)

The initial aim of the study had been to isolate actinomycetes, as a survey of the literature had indicated that actinomycetes possessed a higher metal binding capacity in comparison to bacteria and fungi (Nakajima and Sakaguchi, 1986). Hence, the media selected for the initial isolation were those recommended for aerobic mesophillic actinomycetes, to which the antifungal agent cycloheximide had been added. Table 5.1 gives the number of colony forming units per gram (cfu/g) of wet sediment at each location on each type of medium.

Table 5.1 Number of colony forming units per gram (wet weight) isolated from each location on glycerol yeast extract agar (GYEA), Rao and Subrahmanyan's agar (RSA) and Actinomycete Isolation Agar (AIA)

	GYEA	RSA	AIA
Dagenham			
Typha latifolia (bed 1)	$3.0x10^6$	$9.3x10^6$	$1.5x10^6$
Phragmites australis (bed 2)	$5.1x10^6$	$1.5x10^7$	$2.0x10^6$
Phragmites australis (bed 3)	$7.9x10^6$	$1.1x10^7$	$2.1x10^6$
Brentwood			
Phragmites australis (constructed wetland)	$1.3x10^6$	$3.9x10^6$	$8x10^5$
Typha latifolia (natural wetland)	$1.1x10^7$	$1.2x10^7$	$1.5x10^6$

For the Dagenham wetland, there was little variation in the number of micro-organisms isolated from both of the *Phragmites australis* beds (beds 2 and 3) within each medium. However, the total number of micro-organisms was slightly lower in the samples collected from the rhizosphere of *Typha latifolia* (bed 1). A possible explanation for this lower number of micro-organisms is that the *Typha latifolia* plants were collected from the inlet of the first bed next to the settlement tank, which at the time of sampling was

completely full and spilling sediment into the first bed. The settlement tank generally contained the highest sediment metal concentrations (see Section 4.8), and it is thought that these may be responsible for the lower total counts recorded at this site. The total number of colonies isolated was lowest using Actinomycete Isolation Agar (AIA), followed by Glycerol Yeast Extract Agar (GYEA), with the highest number of colonies being recorded on Rao and Subrahmanyan's Agar (RSA), suggesting that AIA is the most specific medium. The number of micro-organisms isolated with AIA compared well with the results of a study by Hatano *et al.* (1993) in an investigation of the microbial population of constructed wetland cells receiving domestic wastewater. The study reported actinomycete counts of $2.5x10^6$ cfu/g in the rhizosphere of *Typha angustifolia*, and $1.3x10^6$ cfu/g in the rhizosphere of *Phragmites australis* (both expressed as wet weight).

At the Brentwood site, there was a clear difference between the two locations, with the total number of micro-organisms isolated from the rhizosphere of *Typha latifolia* (natural wetland) being consistently higher than the total number of micro-organisms isolated from the rhizosphere of *Phragmites australis* (constructed wetland) on each of the different media. This difference in the number of micro-organisms is considered to be associated with several factors. The natural wetland was in existence before the constructed wetland component was built. Consequently, its microbial population has had the opportunity to develop and become more established in comparison to the constructed wetland which was less than two years old when the samples for the microbial analysis were collected. *Typha latifolia* in the natural wetland is growing in a soil substrate in comparison to the *Phragmites australis* in the constructed wetland which is planted in a gravel substrate that would initially be deficient in organic matter and nutrients. The differences between the macrophyte species may also contribute to the differences in microbial numbers. Wetland macrophytes can transport oxygen to their roots which diffuses into the substrate enabling the survival of aerobic micro-organisms in an otherwise anaerobic environment. Oxygen diffusion is therefore thought to be an important factor determining the size of the microbial population. McKee *et al.*, (1989) reported that *Typha glauca* had a greater potential for root aeration than *Phragmites australis*, which may affect the distribution of microbial populations between the two macrophyte species. A review by Brix (1996) has also suggested that a factor influencing root oxygen release rates was specific species differences.

5.2 Screening of isolates for metal tolerance

Following the initial isolation procedures approximately 27 strains from each location were screened for metal tolerance. A total of 136 colonies representing a range of morphologies were examined. These colonies were streaked out on replicate plates of Actinomycete Isolation Agar spiked with either Pb or Zn at concentrations which corresponded to the minimum (40μgPb/g or 30μgZn/g), mid-point (200μgPb/g or 400μgZn/g) and maximum (400μgPb/g or 800μgZn/g) sediment metal concentrations which had been recorded at the two wetland sites prior to the collection of samples for the microbial analyses (see Section 3.8.2).

Of the 136 isolates, the number of strains that could tolerate 400μgPb/g, 200μgPb/g and 40μgPb/g were 76, 91 and 100, respectively. For 800μgZn/g, 400μgZn/g and 30μgZn/g, the numbers were 9, 21 and 86, respectively. These results suggest that the ability of micro-organisms to tolerate Pb and Zn is widespread at low concentrations, with the numbers of isolates decreasing as the metal concentration increases. It appears that substantially more isolates can tolerate 400μg/g Pb (76 isolates) than 400μg/g Zn (21 isolates). This may indicate that Zn is more toxic to wetland microbial populations than Pb. However, it is important to remember that the conditions in the laboratory are different from those in the field. For example, the chemical form of the metal used in the laboratory medium could well be different from that in the wetland sediments, which may affect its bioavailability (Montuelle *et al.,* 1994), and therefore any conclusions made here are only tentative. Such a difference between the laboratory and field conditions could also be an explanation of why 50 isolates and 36 isolates did not grow on even the lowest concentrations of Zn and Pb, respectively.

5.3 Identification of selected isolates

Seven isolates were initially selected for further investigation due to their ability to tolerate Zn and Pb, and the fact that they were of differing morphologies to each other. However, five of the seven selected strains showed inconsistent metal tolerances (appearing to become less tolerant) and were discarded. The subsequent experiments concentrated on the two remaining isolates (labelled A and B). Isolate A forms a white,

filamentous, raised colony approximately 10mm in diameter with white aerial mycelium. It was isolated from the rhizosphere of *Typha latifolia* collected from the inlet of the first bed at the Dagenham wetland. Isolate B forms a circular, pink, flat colony approximately 5mm in diameter. It has a smooth, glistening surface with an entire edge, and was isolated from the rhizosphere of *Typha latifolia* collected from the inlet to the natural wetland at the Brentwood system.

It was initially thought that both isolates were actinomycetes due to the fact that they were isolated using selective media recommended for actinomycete isolation. In addition, the growth of each strain was not inhibited by the use of the antifungal agent cycloheximide (1.6ml/l of a 1000ppm solution). Samples were sent to the Public Health Laboratory, Mycology Reference Laboratory, Bristol, England, UK for identification and based on morphology studies, isolate A was identified as the ascomycete fungus *Beauveria bassiana*, and isolate B was identified as a basidomycete fungus of the *Rhodotorula* sp.

Both isolates were also sent to the Center for Environmental Biotechnology, University of Tennessee, USA, for identification using DNA sequencing. The DNA sequences amplified from the two isolates were then compared to reference sequences held on a national database. These comparisons revealed a 100% match between the 5.8S rDNA sequences of *Beauveria bassiana* (Shih *et al.,* 1996) and isolate A and between the 18s rDNA sequences of *Rhodotorula mucilaginosa* (Cai *et al.,* 1996) and isolate B. This confirms the results of the morphological identification of isolate A as *Beauveria bassiana* and that of isolate B as *Rhodotorula* sp., but further identifies isolate B to the species level as *Rhodotorula mucilaginosa*.

5.4 Assessment of the level of metal tolerance

The levels of Pb and Zn tolerances for *B. bassiana* and *R. mucilaginosa* were determined using the minimum inhibition concentration (MIC) technique (see Section 3.8.4.2). The inoculum size (cfu/ml) was determined at the start of the experiment for each strain. The experimental results are listed in Table 5.2 and indicate that despite the lower colony count, *B. bassiana* has a much greater tolerance of both Pb and Zn in

comparison to *R. mucilaginosa*. It is interesting to note that both strains appear to be able to tolerate approximately twice as much Zn as Pb, suggesting that, in contrast to the results of the initial isolation procedure, Pb appears to have a greater toxic effect than Zn on these selected isolates (Section 5.2).

Table 5.2 Inoculum size (cfu/ml) and the minimum inhibition concentrations (MIC) of *R. mucilaginosa* and *B. bassiana*.

	Number of cfu/ml	Minimum Inhibition Concentration	
		Pb (μg/g)	Zn (μg/g)
R. mucilaginosa	$4.5x10^6$	40-100	100-200
B. bassiana	$4.3x10^5$	800-1000	1800-2000

The MIC values for *B. bassiana* of 800-1000μgPb/g and 1800-2000μgZn/g are approximately double the maximum metal levels recorded in the sediment at both wetlands, and exceed the range of metal concentrations recorded for highly contaminated sediment (Table 4.30, Section 4.8) for both metals, indicating that this strain is extremely robust.

The levels tolerated by *R. mucilaginosa* are at the lower end of the ranges of values reported for Pb and Zn in wetland sediments at both sites. This apparently lower tolerance of elevated metal concentrations is thought to be associated with several factors. Studies in the literature have reported that the abilities of micro-organisms to tolerate elevated heavy metal sediment concentrations are due to the sheltering effect of sediment and its organic matter content. The sheltering effect can involve a range of processes such as adsorption to clay particles (Kurek *et al.*, 1982), chelation and precipitation (Montuelle *et al.*, 1994), all of which bind-up heavy metals effectively protecting micro-organisms from their toxic effects. Laboratory studies have demonstrated that the organic composition of the experimental medium influences heavy metal toxicity (Babich and Stotzky, 1986), and in a different study only 16% of the strains isolated from a river sediment containing high Cd concentrations where able to grow in the laboratory on a medium containing similar concentrations (Montuelle *et al.*, 1994). Such an effect could explain why the growth of *R. mucilaginosa* in the laboratory is inhibited at concentrations much lower than those typically recorded at

sites receiving urban runoff (Table 4.29, Section 4.8). However, another factor could be the removal of phosphates from the medium in order to avoid the precipitation of added metals (see Section 3.8.3). This may have compromised the growth of *R. mucilaginosa*, affecting its ability to tolerate metals under laboratory conditions.

5.5 Investigation into the effects of Zn and Pb on cell viability of *B. bassiana* and *R. mucilaginosa*, and the ability of both isolates to accumulate these metals

To examine the effects of Pb and Zn on the survival of the two selected isolates, and to determine their ability to accumulate these metals, a series of experiments were carried out as outlined in Section 3.8.4.3.

5.5.1 The effects of Zn and Pb on cell viability of B. bassiana

The results shown in Table 5.3 identify the viable counts determined at the start of the experiment, immediately after the flasks were inoculated (shown as the initial count), and then at each of the subsequent time intervals (shown as the final count). The percentage increase or decrease in cell viability between the initial count, and that determined after the relevant time period (one, seven, fourteen or twenty-one days) was calculated (%), and these values are also shown in Table 5.3.

Table 5. 3 Mean viable counts (cfu/ml) (±SD) for *B. bassiana* after different time intervals (duplicate samples).

	Control			Zn-spiked (200µg/g)			Pb -spiked (100µg/g)		
	initial count	final count	%	initial count	final count	%	initial count	final count	%
Day 1	$7.9\pm0.3\times10^4$	$2.7\pm0.8\times10^5$	241	$3.8\pm2.3\times10^4$	$2.1\pm0.7\times10^4$	-45	$4.3\pm1.3\times10^4$	$1.7\pm1.4\times10^4$	-60
Day 7	$4.6\pm0.4\times10^4$	$9.5\pm7.8\times10^3$	-79	$4.3\pm5.6\times10^4$	$1.4\pm1.2\times10^4$	-67	$5.4\pm1.8\times10^4$	$2.4\pm1.4\times10^3$	-96
Day 14	$6.9\pm1.1\times10^4$	$1.6\pm1.4\times10^4$	-77	$3.1\pm1.3\times10^4$	$1.7\pm0.8\times10^3$	-95	$5.8\pm0.8\times10^4$	18.0±9.9	-99.97
Day 21	$4.5\pm0.7\times10^4$	$5.1\pm4.0\times10^4$	13	$3.4\pm1.4\times10^4$	$6.8\pm7.4\times10^3$	-80	$5.4\pm0.4\times10^4$	0.0±0.0	-100

Key: Control = unspiked, inoculated flasks
Zn-spiked = spiked and inoculated flasks
Pb-spiked = spiked and inoculated flasks

It can be seen that the flasks were inoculated with a similar number of cells to within an order of magnitude. After one day, the viable count in the control flasks has shown a marked increase (241%) reflecting the growth of *B. bassiana* in unstressed conditions. However, the growth of *B. bassiana* in the flasks spiked with Zn or Pb showed that both metals were inhibiting further growth of the isolate, with decreases in cell viability of

45% and 60%, respectively. The decrease is greater in the Pb spiked flask, despite the lower Pb concentration (100μgPb/g in comparison to 200μgZn/g), supporting the results of the MIC experiment which found that Pb was more toxic to this isolate than Zn (see Section 5.4).

Following the increase in viability recorded after one day, the viable count of the control flasks showed mean decreases of 79% and 77% after seven and fourteen days, respectively, before showing an increase of 13% after twenty-one days. The decreases recorded in the control flasks is suprising. However, a possible explanation is that the medium used is very simple, and that the elevated growth rate recorded on day one may have exhausted the nutrient supply, resulting in the decreases in viability reported after seven and fourteen days. Following death, cells may begin to undergo autolysis, resulting in the release of nutrients, which may explain the increase in viability reported after twenty-one days. The viable counts continue to fall in both the Zn spiked and Pb spiked flasks, with the viable count in the Pb spiked flasks being reduced to 0cfu/ml after twenty-one days underlining the inhibitory effects of Pb on the growth of this isolate.

The large SD associated with some of the mean viable counts indicates that the growth rates in some of the replicate flasks were quite different. These differences are thought to be primarily due to the fact that flasks were inoculated from a plate rather than from a broth. This technique was used as *B. bassiana* tends to grow in pellets in a liquid medium rather than as an even suspension of cells which made it difficult to inoculate all of the flasks with a similar number of cells. However, using the inoculum from a plate means that a mixture of different growth stages may be selected, and hence result in differences between replicate flasks.

5.5.2 Metal accumulation by B. bassiana

Tables 5.4 to 5.7 give the results of the experiments carried out to determine metal accumulation by *B. bassiana* over one day, seven days, fourteen days and twenty-one days, respectively. Each Table gives the mean mass of cells collected from the replicate flasks over the exposure period, the mean concentration of metal associated with the extracted acidic solution, the mean concentration of metal associated with the cells and

the mean pH of the replicate flasks. Three sets of controls were established as described in each Table. In addition, blank filters were digested and analysed for Pb and Zn. The blank filter lost a mean mass of 1.04±0.39mg (average of seven filters) during the filtering process which was thought to be due to the loss of fibres caused by the filtering and rinsing procedure itself. To compensate for this, 1.04mg was added to all of the cell masses. The amount of Zn and Pb associated with the blank filters is also given in the Tables, and it can be seen that values were consistently <1µg/l for either metal. The amount of metal associated with the filter following the filtering of the uninoculated metal-spiked flasks (Controls B and C) varied between the different exposure periods used in the experiment, which is thought to be due to the differences between individual filters. The mean metal concentration of the extracted acidic solution was adjusted by subtracting the amount of metal associated with the relevant control filter to allow for this possible, though relatively minor, source of accumulation. This adjusted value is the value given in each Table (as concentration associated with extracted acidic solution), and is also the value used to calculate the amount of metal accumulated per gram of cells.

Table 5.4 indicates that after one day the mass of cells in both the Zn and Pb spiked flasks is markedly less than in the control flasks. This is thought to be due to the inhibition of cell growth by the metals, which was also indicated by the results of the viable count experiment (Table 5.3). The amount of Pb and Zn associated with the cells from the unspiked flasks (control A) was low in comparison to the values for cells collected from the spiked flasks for both metals, particularly for Pb. The amount of metal associated with cells collected from the Zn spiked flask was 21 times the amount associated with the cells collected from unspiked control A and 53 times the amount in the spiked but uninoculated control B. The amount of Pb associated with the cells collected from the Pb spiked solution was 619 times greater than the amount in the unspiked control flask and over 2000 times the amount associated with the spiked but uninoculated control C. These results strongly indicate that *B. bassiana* is capable of accumulating Zn, and to a much greater extent, Pb. The Zn concentrations are at the lower end of the range of values reported for the accumulation of this metal by a range of fungi and yeasts, whereas the Pb concentrations recorded are well within the range of values quoted in the literature (see Table 2.4, Section 2.8.1). Several other studies have also reported a greater binding capacity for Pb in comparison to other metals for a range

of biomass types, which may be related to its large atomic weight and ionic radius (Mattchuska and Straube, 1993, Pradhine and Levine, 1995, Gabriel *et al.*, 1994, Holan and Volesky, 1995).

Table 5.4 Accumulation of Zn and Pb (±SD) by *B. bassiana* over a one day exposure period (duplicate samples).

	cell mass (mg)	Metal concentration associated with extracted acidic solution		Metal concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	6.25±3.52	1.48±0.89	0.93±0.34	0.23±0.01	0.20±0.16	7.0±0.0
Zn 200µg/g	2.20±0.26	10.22±2.69	-	4.76±1.79	-	6.4±0.0
Pb 100µg/g	2.66±0.83	-	326.27±84.85	-	123.74±6.92	6.9±0.0
Control B	-	12.55	-	0.09*	-	6.4
Control C	-	-	9.13	-	0.06*	6.9
Filter blank	-	0.37	0.97	0.002*	0.006*	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined

The accumulation of metals by the isolate, in addition to substantial decreases in cell viability, could be interpreted in different ways. It could indicate that biosorption (metabolic-independent accumulation) was the primary mechanism of metal accumulation, whereby, for example, binding of Pb to cell walls caused them to rupture, resulting in both cell death and high levels of metal accumulation following the exposure of new, internal binding sites (Gadd, 1990). However, these results could also indicate that bioaccumulation (metabolic-dependent accumulation) was the major mechanism for metal accumulation, whereby cells bioaccumulated metals intracellularly to such a degree that a rapid reduction in cell viability resulted. However, the results of the experiment do not provide enough evidence to conclude whether biosorption or bioaccumulation is the more important mechanism in *B. bassiana*.

The pH of the medium prior to any inoculation or spiking was 7.0. After one day the pH of the control remained unchanged (Table 5.4). The pH of both the inoculated and uninoculated flasks spiked with Pb had decreased slightly (from pH 7.0 to 6.9), whereas the pH of both the inoculated and uninoculated flasks spiked with Zn had decreased from 7.0 to 6.4 (Table 5.3). A fall in pH on the addition of metal salts was also noted by Jones and Muehlchen (1994) and Mattchuska and Straube (1993) but was not explained.

As both the inoculated and uninoculated spiked flasks showed a similar fall in pH, it could be ascertained that this reduction was not due to the growth of the isolate. In addition, as the amount of metal removed from the uninoculated spiked flask was low in comparison to the amount removed by the inoculated spiked flask it could be determined that the removal of metal was not directly due to changes in pH, and was not occurring independently of the inoculum. Despite the fact that a greater reduction in pH was recorded in the Zn-spiked samples than the Pb spiked samples, Zn did not appear to have a greater toxic effect than Pb, which may have been expected due to the relationship between decreasing pH and increasing metal toxicity (Table 5.3).

After seven days, increases in cell masses were recorded in all of the flasks (Table 5.5). The mean mass was greatest for the control flasks, followed by the Zn spiked flasks which was markedly higher than the mean cell mass of the Pb spiked flasks. These results again indicate that Pb was more toxic to this isolate than Zn. The large standard deviation associated with the mass of cells from the flask spiked with Zn was probably due to the lower initial inoculum size of one of the replicate flasks (Table 5.3). The amount of Zn removed from the spiked medium had increased after seven days corresponding to the increase in cell mass whereas the amount of Pb removed from the solution remained almost exactly the same despite the slight increase in cell mass. The amount of Zn accumulated per gram of cells increased whereas the amount of Pb per gram of cells showed a decrease. The pH of the control and the Pb spiked flasks remained the same between day one and seven but the pH of the inoculated Zn spiked flask exhibited a small decrease to 6.2.

Table 5.5 Accumulation of Zn and Pb (±SD) by *B. bassiana* over a seven day exposure period (duplicate samples).

	cell mass (mg)	Concentration associated with the extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	13.91±5.68	0.7±0.25	1.74±0.33	0.06±0.04	0.14±0.08	7.0±0.1
Zn 200µg/g	10.07±10.55	43.97±28.51	-	6.39±3.86	-	6.2±0.1
Pb 100µg/g	3.35±1.53	-	326.15±135.06	-	98.39±4.54	6.9±0.0
Control B	-	10.93	-	0.07*	-	6.4
Control C	-	-	11.05	-	0.08*	6.9
Filter blank	-	nd	0.41	nd	0.003*	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined
nd = not detected

After fourteen days, the mass of cells in the control flasks continued to increase whereas the cell masses in the Zn and Pb spiked flasks remained approximately the same as those determined after seven days growth. The amount of Zn removed from the spiked medium showed a small increase from day seven to fourteen, whereas the amount of Pb removed per gram of cells (dry weight) increased substantially over the same time period. The fact that a greater amount of metal was accumulated by approximately the same cell mass could indicate that the initial binding of the metal to the cell components acts as a nucleation site around which further metals are then deposited (see Section 2.8.1).

Table 5.6 Accumulation of Zn and Pb (±SD) by *B. bassiana* over a fourteen day exposure period (duplicate samples).

	cell mass (mg)	Concentration associated with the extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	18.52±0.61	0.84±0.45	2.15±2.33	0.04±0.02	0.11±0.22	6.7±0.0
Zn 200µg/g	10.51±2.93	57.25±31.78	-	5.23±1.57	-	6.2±0.1
Pb 100µg/g	3.11±0.78	-	447.52±306.32	-	135.83±64.52	6.9±0.0
Control B	-	4.91	-	0.03*	-	6.4
Control C	-	-	24.28	-	0.16*	6.9
Filter blank	-	nd	0.61	nd	0.004*	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined
nd = not detected

After twenty-one days, the mean cell mass in the control had decreased considerably compared to day fourteen. The mean mass of cells from the Zn-spiked flasks showed a slight increase whereas the mean mass of cells from the Pb-spiked flasks also showed a decrease. The decreases in mass are thought to be associated with cell lysis, with the amount of Pb removed from solution dropping presumably as cells break-up and the precipitated metal is no longer associated with the cell mass. The cell mass of the Zn spiked flasks showed an increase in one of the replicate flasks (flask A) from fourteen to twenty-one days to 19.96mg, whereas the cell mass in the other replicate flask (flask B) decreased to 3.91mg over the same exposure period, resulting in the large SD seen in

Table 5.7. A noticeable difference in viable counts was also reported between the two flasks after twenty-one days (Table 5.3), which suggests that the cells in flask A were better able to tolerate elevated Zn concentrations than those in flask B. This could be due to the origin of the inoculum, which came from a plate rather than a broth, whereby a mixture of different growth stages may have been selected with the effects of heavy metals varying between these growth stages (Kapoor and Viraraghaven, 1995).

Table 5.7 Accumulation of Zn and Pb (±SD) by *B. bassiana* over a twenty-one day exposure period (duplicate flasks).

	cell mass (mg)	Concentration associated with extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	7.67±1.98	1.56±0.87	0.57±0.29	0.19±0.06	0.07±0.02	6.9±0.0
Zn 200µg/g	11.94±11.35	32.76±32.85	-	2.62±0.26	-	6.2±0.2
Pb 100µg/g	1.57±1.08	-	105.89±98.14	-	60.42±20.94	6.9±0.0
Control B	-	15.70	-	0.011*	-	6.4
Control C	-	-	37.62	-	0.26*	6.9
Filter blank	-	0.54	0.68	0.004*	0.005*	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined

Several studies in the literature have found that as cell mass increases, the amount of metal removed from the solution also increases, but the actual amount of metal accumulated per gram of cell decreases (Horikoshi *et al.,* 1981, Junghans and Straube 1991, Gadd 1993). It is thought that as cell density increases, the adhesion between cells reduces the relative amount of surface area available for binding. In this study, although higher cell mass corresponded to a higher amount of metal removal from solution it also corresponded to a higher amount of metal per gram of cells. The mass of cells used in the experiment may have influenced the result. For example, the cell mass used in the study by Junghans and Straube (1991) ranged from 100-600 mg for four species of yeast, whereas, the mean cell masses used in this study range from 2.20-11.94mg and 1.57-3.35mg for the Zn and Pb spiked samples respectively (Tables 5.4-5.7).

5.5.3 The effects of Zn and Pb on the cell viability of R. mucilaginosa

Table 5.8 gives the results of the viable counts carried out in the inoculated flasks at the start of the experiment (initial count) and at each subsequent time interval (final count) to determine the effects of metals on the viability of *R. mucilaginosa*.

Table 5.8 Mean viable count (cfu/ml) (±SD) for *R. mucilaginosa* after different time intervals (duplicate samples).

	Control			Zn-spiked (100µg/g)			Pb-spiked (40µg/g)		
	initial count	final count	%	initial count	final count	%	initial count	final count	%
Day 1	$2.0\pm0.3\times10^7$	$4.7\pm0.6\times10^7$	135	$1.5\pm0.1\times10^7$	$1.8\pm0.6\times10^6$	-88	$1.5\pm0.7\times10^7$	$5.6\pm4.0\times10^5$	-96
Day 7	$1.8\pm0.5\times10^7$	$5.8\pm2.9\times10^7$	222	$2.2\pm1.0\times10^7$	$3.9\pm1.0\times10^3$	~100	$1.5\pm0.1\times10^7$	$4.9\pm5.2\times10^3$	~100
Day 14	$3.1\pm0.9\times10^7$	$4.8\pm0.1\times10^7$	55	$1.9\pm0.1\times10^7$	3.2×10^2	~100	$1.9\pm0.6\times10^7$	$5.2\pm2.5\times10^2$	~100
Day 21	$1.9\pm0.0\times10^7$	$2.8\pm0.5\times10^7$	47	$3.2\pm1.1\times10^7$	56 ± 31	~100	$2.4\pm0.6\times10^7$	*	*

Key: Control = unspiked and inoculated flasks
Zn-spiked = spiked and inoculated flasks
Pb spiked = spiked and inoculated flasks

It can be seen from the Table that all the flasks were inoculated with a similar number of cells to within an order of magnitude. The initial inoculation of 10^7 cfu/ml was approximately 3 orders of magnitude greater than the initial viable counts in the *B. bassiana* experiment (Table 5.3). After one day the viable count of the control had increased by 135%, which is thought to reflect the growth of *R. mucilaginosa* under unstressed conditions. After seven days, the viability had continued to increase (222%). However, the rate of increase then started to fall, with increases of 55% and 47% after fourteen days and twenty-one days respectively, presumably as the nutrient supply began to be used up.

These increases are in clear contrast to the changes in cell viability reported for the Zn and Pb-spiked flasks after one day which show substantial decreases of 88% and 96%, respectively, clearly demonstrating the toxic effects of these metals (Table 5.8). As with *B. bassiana*, Pb appears to have a greater inhibitory effect on *R. mucilaginosa* than Zn. It is also interesting to note that the percentage decreases in viability were greater for *R. mucilaginosa* after one day (Table 5.8) in comparison to *B. bassiana* (Table 5.3), despite the lower concentrations of Pb and Zn used in this experiment. This underlines the lower tolerance of *R. mucilaginosa*, as previously indicated in Section 5.4. The viability continued to fall throughout the course of the experiment in both the Zn and Pb spiked

flasks, with a viable count of only 56cfu/ml being recorded in the Zn-spiked flasks after twenty-one days.

The greater ability of *B. bassiana* to tolerate Zn and Pb in comparison to *R. mucilaginosa* after one day, despite the higher metal concentrations used in the *B. bassiana* experiment, may be explained by factors such as:

- differences in morphology - *B. bassiana* is a filamentous fungus and *R. mucilaginosa* is a yeast, and therefore *B. bassiana* is thought to have a greater surface area for binding metals, thereby preventing the potential damage caused by intracellular accumulation, compared to *R. mucilaginosa*.
- cell wall composition - in most filamentous fungi, chitin and glucans are important components of cell walls, whereas the major components of the cell walls of yeasts are glucans and mannans (Carlile and Watkinson, 1994), and metal binding affinity may vary between these different cell wall polymers (Morley and Gadd, 1995).
- production of extracellular products - *B. bassiana* is known to produce citric and oxalic acids (Bidochka and Khachatourians, 1991) and *R. mucilaginosa* is reported to produce a fucose-containing extracellular polysaccharide (Golubev and Churkina, 1997). Differences in the metal-binding capacity of such products may explain differences in the ability of these isolates to tolerate and accumulate metals.

5.5.4 Metal accumulation by R. mucilaginosa

The results of the experiments carried out to determine the amount of Pb and Zn which could be accumulated by *R. mucilaginosa* are described in Tables 5.9 to 5.12. The data in the Tables are set-out as previously outlined for *B. bassiana* (Section 5.5.2). The lower metal concentrations (100μgZn/g and 40μg/gPb) were selected to represent concentrations which did not totally inhibit growth (Section 5.4), whilst still being readily detectable using ICP-AES. Table 5.9 indicates that after one day the mass of cells in the control flasks was over twice the cell mass in the Zn-spiked flasks and almost three times the mass in the Pb spiked flasks, reflecting the inhibitory effects of both metals on the growth of *R. mucilaginosa*, and that Pb was comparatively more toxic than Zn.

Table 5.9 Accumulation of Zn and Pb (±SD) by *R. mucilaginosa* over a one day exposure period (duplicate flasks).

	cell mass (mg)	Concentration associated with extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	23.39±4.57	1.34±0.36	0.84±0.26	0.06±0.03	0.04±0.02	7.1±0.1
Zn 100µg/g	9.12±1.34	103.71±23.98	-	11.3±0.96	-	6.7±0.0
Pb 40µg/g	8.23±1.03	-	132.47±5.66	-	16.28±2.72	6.9±0.0
Control B	-	7.64	-	0.05*	-	6.7
Control C	-	-	17.53	-	0.12*	6.9
Filter blank	-	0.16	1.61	0.001*	0.01*	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined

The amount of Zn associated with cells from unspiked flasks (control A) remained low throughout the entire experiment, with a maximum mean value of 1.34µg/l. However, the amount of Pb associated with these cells, although still relatively low, showed a gradual increase throughout the experiment from 0.84-7.61µg/l. The reason for this is not clear. Similar amounts of Zn and Pb were removed from the spiked media after one day (Table 5.9). When calculated as the amount of metal removed per gram of cells (dry weight), the amount of Zn and Pb associated with cells from the spiked flasks was 188 times and 407 times higher respectively than the amount associated with cells from the unspiked control flasks (control A), and 226 times and 135 times higher respectively than the amount of Zn and Pb associated with filters through which an uninoculated Zn or Pb spiked solution was filtered (controls B and C). These results indicate that *R. mucilaginosa* was also capable of accumulating both Zn and Pb. The amount of Zn accumulated per gram of cells is approximately twice the amount associated with *B. bassiana* after the same time period, despite the lower Zn concentration used in this experiment. This may be related to the higher cell mass of *R. mucilaginosa* in comparison to that used in the *B. bassiana* experiment. However, the amount of Pb associated with *R. mucilaginosa* is almost eight times less than that associated with a lower mass of *B. bassiana*, suggesting that the explanation is much more complex. The initial pH of the medium prior to spiking or inoculation was 7.0. After one day the pH of

the control and Pb spiked flasks had remained approximately the same, with the pH of the Zn spiked solution having decreased slightly further which is a similar pattern to that reported for the *B. bassiana* experiments.

After seven days (Table 5.10) the mean cell mass in the control flasks had remained approximately the same whereas the masses in the Zn and Pb spiked flasks had shown a decrease. The amount of Zn associated with the cells fell by over 50% whereas the amount of Pb increased by 285%. This suggests that Zn accumulation by *R. mucilaginosa* is metabolic-dependent, with Zn being accumulated primarily within living cells. Hence, as the viability of the isolate decreases (as indicated by Table 5.8), the amount of Zn removed from the spiked medium also decreases. The Mg^{2+} transport systems of both the yeast *Saccharomyces cerevisiae* and the bacteria *Escherichia coli* have been reported to take-up Zn^{2+}, amongst other ions (Portier and Palmer, 1989), and this may be a possible mechanism for Zn accumulation by *R. mucilaginosa*.

Table 5.10 Accumulation of Zn and Pb (±SD) by *R. mucilaginosa* over a seven day exposure period (duplicate flasks).

	cell mass (g)	Concentration associated with extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	22.04±2.94	0.78±0.13	1.68±0.21	0.04±0.01	0.08±0.00	7.7±0.2
Zn 100µg/g	7.72±1.39	49.00±0.52	-	6.44±1.09	-	6.8±0.1
Pb 40µg/g	7.13±0.04	-	331.29±19.09	-	46.49±2.45	7.0±0.0
Control B	-	9.71	-	0.07*	-	6.7
Control C	-	-	27.21	-	0.18*	7.1
Filter blank	-	nd	0.55	nd*	0.003*	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (only one flask)
Control C = flask containing broth + Pb only (only one flask)
* = amount of metal associated with filter (mg/g)
- = not determined
nd = not detected

In contrast to the Zn results, the amount of Pb accumulated by the cells showed a large increase from one to seven days. As discussed for *B. bassiana* in Section 5.5.2, it is possible that the initial accumulation of Pb acted as a nucleation site around which further metals were then deposited. The amount of Zn accumulated by both strains per gram of cells is approximately the same after seven days, however, the amount of Pb is

less than twice that reported for *B. bassiana*. The pH in the control has increased to 7.7, but has remained approximately the same in the Pb and Zn spiked flasks. The reason for the increase in pH reported for the control is unclear.

After fourteen days the mean cell mass of the control remained approximately the same, whereas those in the Zn and Pb spiked flasks both fell slightly. The amount of Zn removed from the spiked medium had also decreased which supports the theory that Zn accumulation may be primarily a metabolic-dependent process. The amount of Pb removed from the spiked medium also showed a decrease. However, this could be due to lysis of cells following death which resulted in the release of metals back into solution. An alternative explanation is that precipitated metals are no longer associated with cell matter and are therefore not collected by the filter. The pH of all the flasks fell slightly, and the pH in the inoculated spiked flasks remained similar to that of the uninoculated spiked flasks. Therefore, it is thought that any changes in metal accumulation are not directly due to changes in pH as the amount of metal accumulated by the filters themselves (controls B and C) remains very low in comparison to values reported for the cells.

Table 5.11 Accumulation of Zn and Pb (±SD) by *R. mucilaginosa* over a fourteen day exposure period (duplicate flasks).

	cell mass (mg)	Concentration associated with extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	21.11±0.21	0.36±0.08	3.2±2.84	0.02±0.00	0.15±0.14	7.4±0.1
Zn 100µg/g	6.72±0.15	33.32±11.29	-	4.98±1.79	-	6.5±0.0
Pb 40µg/g	6.79±0.64	-	216.78±25.10		31.92±0.67	6.8±0.0
Control B	-	nd	-	nd*	-	6.6
Control C	-	-	22.57	-	0.15*	6.9
Filter blank	-	-	-	-	-	-

Key: Control A = flask containing broth + isolate only
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined
nd = not detected

After twenty-one days the mass of cells in all of the flasks had shown an increase (Table 5.12). This is particularly surprising in the case of the Zn spiked flasks where the viable count decreased dramatically to only 56 cfu/ml. The increase in mass may be associated with the fact that, of all the Zn and Pb spiked flasks, the twenty-one day replicate flasks were inoculated at a slightly higher cfu/ml value than the other flasks (Table 5.8). It is also possible that the non-viable yeast cells had not yet lysed, resulting in the low viable count with a mass similar to that at the earlier stages of the experiment.

Table 5.12 Accumulation of Pb and Zn (±SD) by *R. mucilaginosa* over a twenty-one day exposure period.

	cell mass (mg)	Concentration associated with extracted acidic solution		Concentration associated with cells		pH
		Zn (µg/l)	Pb (µg/l)	Zn (mg/g)	Pb (mg/g)	
Control A	26.15	0.91	7.61	0.03	0.29	7.6
Zn 100µg/g	8.82±0.42	26.55±2.14	-	3.02±0.39	-	6.7±0.1
Pb 40µg/g	8.09±0.35	-	236.64±6.79	-	29.26±0.44	6.7±0.1
Control B	-	2.96	-	0.02*	-	7.0
Control C	-	-	15.16	-	0.10*	6.9
Filter blank	-	0.10	1.10	0.0007*	0.007*	-

Key: Control A = flask containing broth + isolate only,
Control B = flask containing broth + Zn only (one flask)
Control C = flask containing broth + Pb only (one flask)
* = amount of metal associated with filter (mg/g)
- = not determined

The amount of Zn removed from the spiked medium was the lowest for day twenty-one which correlates with the lowest viable count recorded. However, the fact that this value did not fall to approximately zero suggests that some Zn was also accumulated by biosorption, as well as metabolic-dependent mechanisms. The amount of Pb removed from the solution increased in comparison to the value after fourteen days probably due to the increase in cell mass whereas the amount of Pb removed per gram of cells remained approximately the same. The pH in the control flasks had increased slightly in comparison to that recorded after fourteen days. The pH in the Zn and Pb spiked flasks remained approximately the same. Over the course of the experiment the greatest variation in pH was recorded in the control flasks, ranging from pH 7.0-7.7, which may be associated with differences in growth of the isolate in unspiked and spiked

conditions. The addition of Zn tended to have a greater effect on pH than the addition of Pb. This pattern was also noted in the *B. bassiana* experiment.

Overall, the amounts of Zn accumulated per gram of cells by *R. mucilaginosa* (3.02-11.3mg/g) and *B. bassiana* (2.62-6.39 mg/g) were of similar ranges. However, there is a clear difference in the amount of Pb accumulated per gram of cells between the two strains, with the amount accumulated by *R. mucilaginosa* (16.28-46.49mg/g) being substantially lower than that accumulated by *B. bassiana* (60.42-135.83mg/g). Differences between the amounts of metals accumulated by each strain are thought to be associated with a wide range of factors such as differences in morphology, cell wall composition and structure, type, concentration, ionic radius and atomic weight of the selected metal, production of extracellular products and mechanisms of cell transport and compartmentation (Gadd, 1993; Section 5.5.3). Although the ability of both strains to accumulate Zn and Pb (and in particular the accumulation of Pb by *B. bassiana*) has been clearly demonstrated, it is difficult to speculate at this stage which of these factors are primarily responsible for the differences in the amount of Zn and Pb accumulated by each isolate.

5.6 The effects of Zn and Pb on colony growth of *B. bassiana* and *R. mucilaginosa* at different temperatures

The aim of these experiments were to examine the effects of Pb and Zn on colony growth, and to determine whether the responses of the isolates to these metals varied with temperature.

5.6.1 The effects of Zn and Pb on the colony growth of B. bassiana at different temperatures

Figure 5.1 shows the growth of squares of *B. bassiana* colony (see Section 3.8.4.4), expressed as colony area, at 30°C on replicate plates that were spiked with progressively higher concentrations of Zn in comparison to the growth on unspiked plates (control). The concentrations selected were those used in the initial isolation procedure, and

corresponded to the minimum (30μgZn/g), midpoint (400μgZn/g) and maximum (800μgZn/g) concentrations recorded at both wetland sites when samples for microbial analyses were collected. The initial sizes of the colonies were approximately the same for all the plates (~160mm^2). *B. bassiana* showed the best growth rate on the control plates, with colony growth decreasing on the Zn spiked plates in the order 400μg/g > 30μg/g > 800μg/g (Fig. 5.1). It is surprising that a higher colony growth was recorded for colonies exposed to a Zn concentration of 400μg/g compared to 30μg/g. However, the difference is not marked. In addition, the initial squares of colony may contain parts of the colony which were at different stages of growth. It is possible that the squares used on the 30μg/g Zn spiked plates were from an older part of the colony than those used on the 400μg/g Zn spiked plates which could explain the slower growth rate. Although growth on the Zn spiked plates is inhibited in comparison to the control, colony growth was clearly visible on all of the spiked plates. After thirty-five days, the colony surface area on the spiked plates was 66%, 76% and 58% of the growth recorded on the control, for` Zn concentrations of 30μg/g, 400μg/g and 800μg/g respectively. This indicates that *B. bassiana* is very robust with even the highest Zn concentration reducing the colony size by less than 50%.

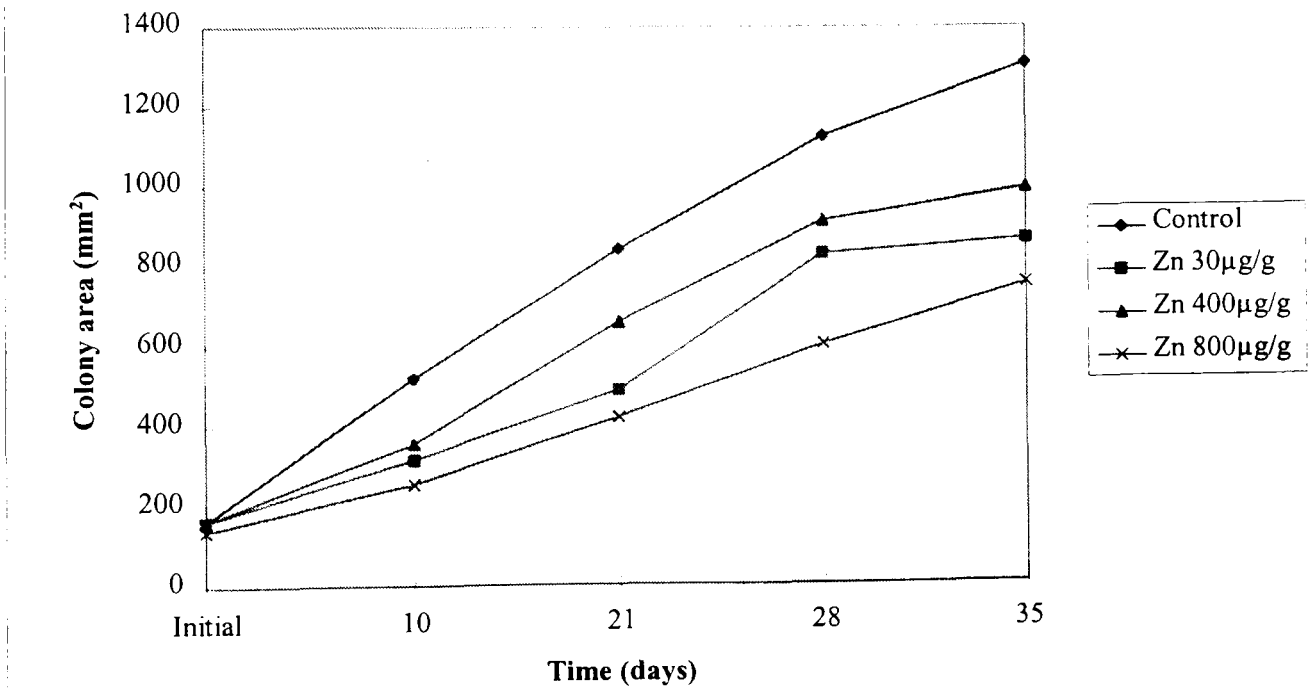

Figure 5. 1 Growth of *B. bassiana* on control and Zn-spiked plates at 30°C.

Figure 5.2 shows the results of *B. bassiana* on plates spiked with Pb (40µg/g, 200µg/g. 400µg/g) in comparison to the growth on unspiked control plates at 30°C. The initial colony sizes were similar on all the plates. Good growth was recorded on all of the plates with colony growths on the spiked plates after 10 and 21 days showing little difference from those of the control. After 21 days, although the growths recorded on plates spiked with Pb concentrations of 200µg/g and 400 µg/g remained almost the same as those on the control, the growth on plates spiked with a Pb concentration of 40µg/g was greater suggesting that low concentrations of Pb are actually stimulating the growth of *B. bassiana*. The stimulation of growth of various biota by heavy metals has also been reported in the literature; 2mM Zn resulted in an initial stimulation of growth of *Rhizobium* (Leung, 1992), whereas a Cd concentration of 10µg/l was reported to stimulate the growth of some algal species (Kadlec and Knight, 1995).

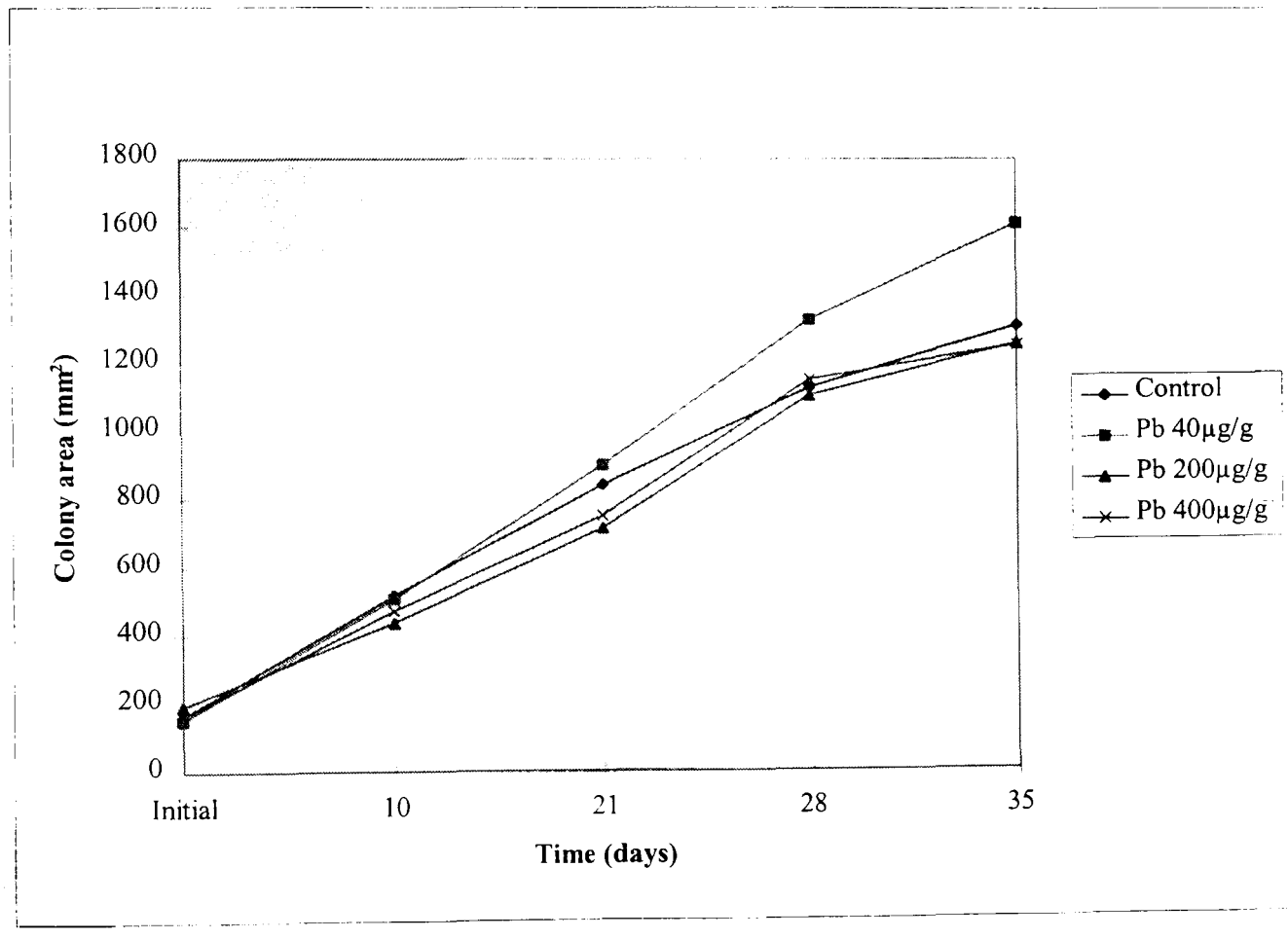

Figure 5. 2 Growth of *B. bassiana* on control and Pb-spiked plates at 30°C.

The stimulation of growth observed in this study may be associated with enhanced growth in an attempt to colonise a new, uncontaminated substrate. After thirty-five days, the colony size on plates spiked with a Pb concentration of 40µg/g was 23% greater than that of the control, whereas the colony sizes on both the 200µg/g and 400µg/g Pb spiked plates were 96% of the size of the control. All these results indicate that *B. bassiana* is

highly metal tolerant. Throughout the course of the experiment, the colony sizes on the Pb spiked plates appear to be greater than those reported for the Zn spiked plates (Figures 5.1. and 5.2). The colony size on plates spiked with Zn at a concentration of 400µg/g was 991mm^2 after a period of thirty-five days, which is markedly less than the colony size of 1248mm^2 recorded on plates spiked with the same concentration of Pb. This is surprising as the results of the minimum inhibition concentration experiment (Section 5.4) indicated that *B. bassiana* has a much greater tolerance to Zn compared to Pb. However, the actual minimum inhibition concentrations for both Zn and Pb were considerably greater than the concentrations used in this experiment, and differences in both the response of the isolate to higher metal concentrations and the effect of higher metal concentrations on the isolate could be an explanation of this different result at lower metal concentrations.

Figures 5.3 and 5.4 show the results of the growth of *B. bassiana* when exposed to the same concentrations of Zn and Pb but incubated at 4 °C as opposed to 30 °C. The growth of the isolates on all of the plates showed a clear lag phase lasting between ten and twenty-one days. The lag phase at 30°C was considerably shorter as after ten days, colonies on all the plates were showing a clear increase in size (Figures 5.1 and 5.2). The longer lag phase of the colonies incubated at 4°C is thought to be due to the lower temperature. Between ten and twenty-one days, colonies on all of the plates began to show a marked increase in size, and continued to grow strongly over the next fourteen days until the end of the experiment.

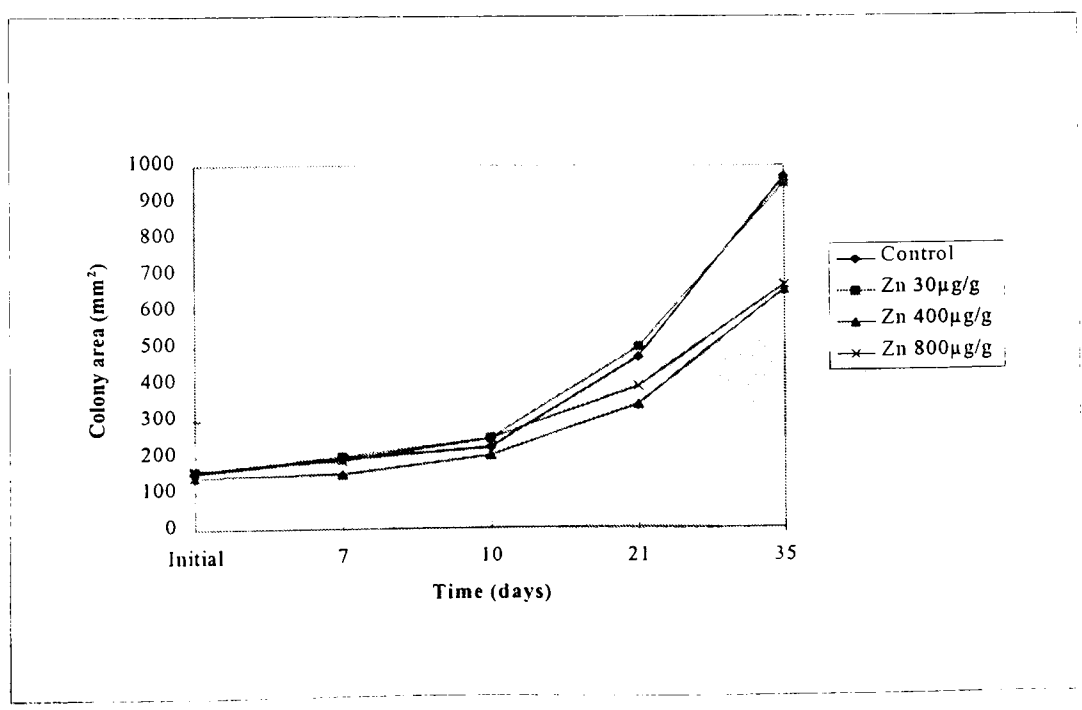

Figure 5.3 Growth of *B. bassiana* on control and Zn-spiked plates at 4°C.

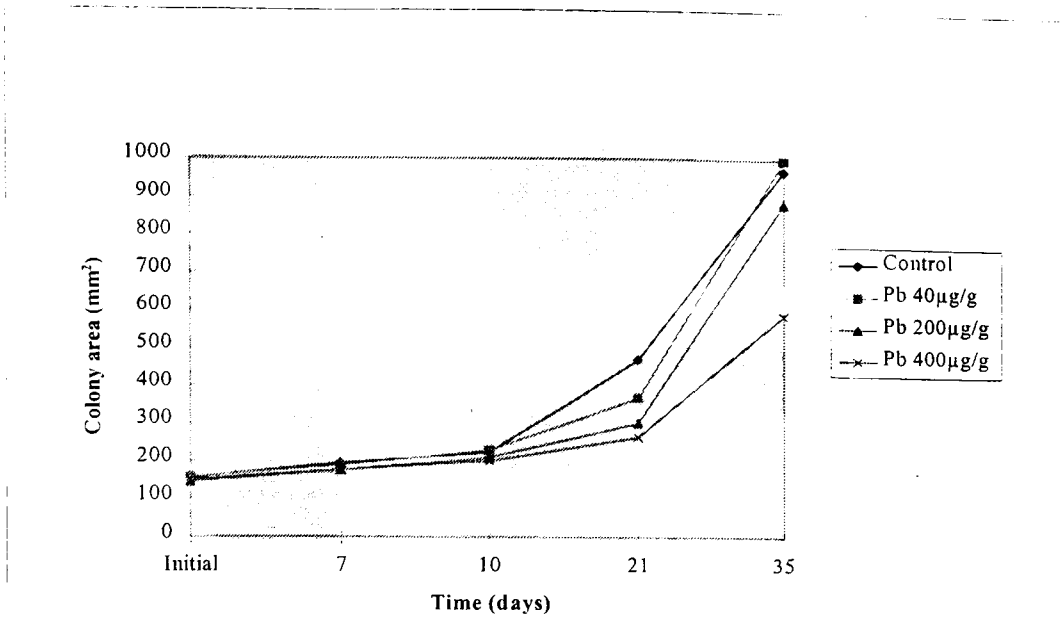

Figure 5.4 Growth of *B. bassiana* on control and Pb spiked plates at 4°C.

Growths of the colonies on the Zn spiked plates at 4°C (Figure 5.3) differed from those at 30°C in that at 4 °C the growths of the control and plates spiked with a Zn concentration of 30µg/g were very similar. Growth on plates spiked with a Zn concentration of 400µg/g was similar to that on 800µg/g plates, but both measurements were markedly lower than the growths on the control and 30µgZn/g plates. The sizes of the colonies recorded after thirty-five days at 4°C were 74% (control), 110% (Zn 30µg/g), 66% (Zn 400µg/g) and 89% (Zn 800µg/g) of the colony areas recorded for the same Zn concentrations when plates were incubated at 30°C.

Growths of *B. bassiana* on the Pb spiked plates (Figure 5.4) differed from those at 30°C in that the lowest Pb concentration did not appear to stimulate growth. After thirty-five days, the colony sizes on the control and on plates spiked with a Pb concentration of 40µg/g were approximately the same, followed by, in decreasing size, the growth on plates spiked with 200µg/g and 400µg/g of Pb. In comparison with the growths on plates incubated at 30°C, the growths at 4°C were again lower but still substantial after 35 days, comprising 74% (control), 62%(Pb 40µg/g), 70% (Pb 200µg/g) and 88% (Pb 400µg/g) of the growth recorded for the same Pb concentrations at 30°C.

These results indicate that, although the lag phase is longer, the growth of *B. bassiana* on plates spiked with Zn and Pb is reduced by less than 40% at 4°C in comparison to

growth at the same metal concentrations at 30°C. This is of great interest with respect to the year round treatment ability of constructed wetland treatment systems receiving urban runoff. The minimum water temperature recorded at the locations where samples were collected were 5.3°C (21st January 1997) and 5.6°C (21st January 1997) at the Dagenham and Brentwood wetland sites respectively. At the Dagenham site, where flow is generally low, the temperature at the sediment surface would be approximately the same as that in the overlying water, with sediment temperature increasing relative to the water temperature with increasing sediment depth. Therefore, it could be assumed that the sediment temperature (at least at the time when samples were collected) was at a minimum of 5.3°C. Studies in the literature have suggested that at temperatures of 4-5°C the activity of nitrifying and denitrifying bacteria is very slow, if occurring at all (Cooper *et al.,* 1996). However, the results of this study suggest that this may not be the case for all micro-organisms, with *B. bassiana* continuing to both grow and tolerate metals at 4°C (66%-110% and 62-88% of the growth recorded at 30°C for Zn and Pb, respectively).

5.6.2 The effects of Zn and Pb on the colony growth of R. mucilaginosa at different temperatures

Figure 5.5 shows the growths of *R. mucilaginosa* on plates spiked with a Zn concentration of 30μg/g, a Pb concentration of 40μg/g and on unspiked control plates incubated at 30°C. The growth of *R. mucilaginosa* was inhibited at the other metal concentrations (400μg/g and 800μg/g for Zn and 200μg/g and 400μg/g for Pb) used in the *B. bassiana* experiment (Section 5.4), and therefore these concentrations were not included in this experiment.

It can be seen that the growth rate of *R. mucilaginosa* was not high on any of the plates. It is interesting to note that, as with *B. bassiana* at 30°C, a Pb concentration of 40 μg/g appeared to stimulate growth. However, even this stimulated growth resulted in only a doubling of the colony size over a thirty-five day period, in comparison to the 11-fold increase in colony size exhibited by *B. bassiana* at the same Pb concentration. This comparison of colony growth of *B. bassiana* and *R. mucilaginosa*, under the same nutrient conditions, at the same temperature and in the presence of the same

concentrations of Zn (30μg/g) or Pb (40μg/g), clearly demonstrate the greater tolerance and robustness of *B. bassiana* to these concentrations of Zn and Pb.

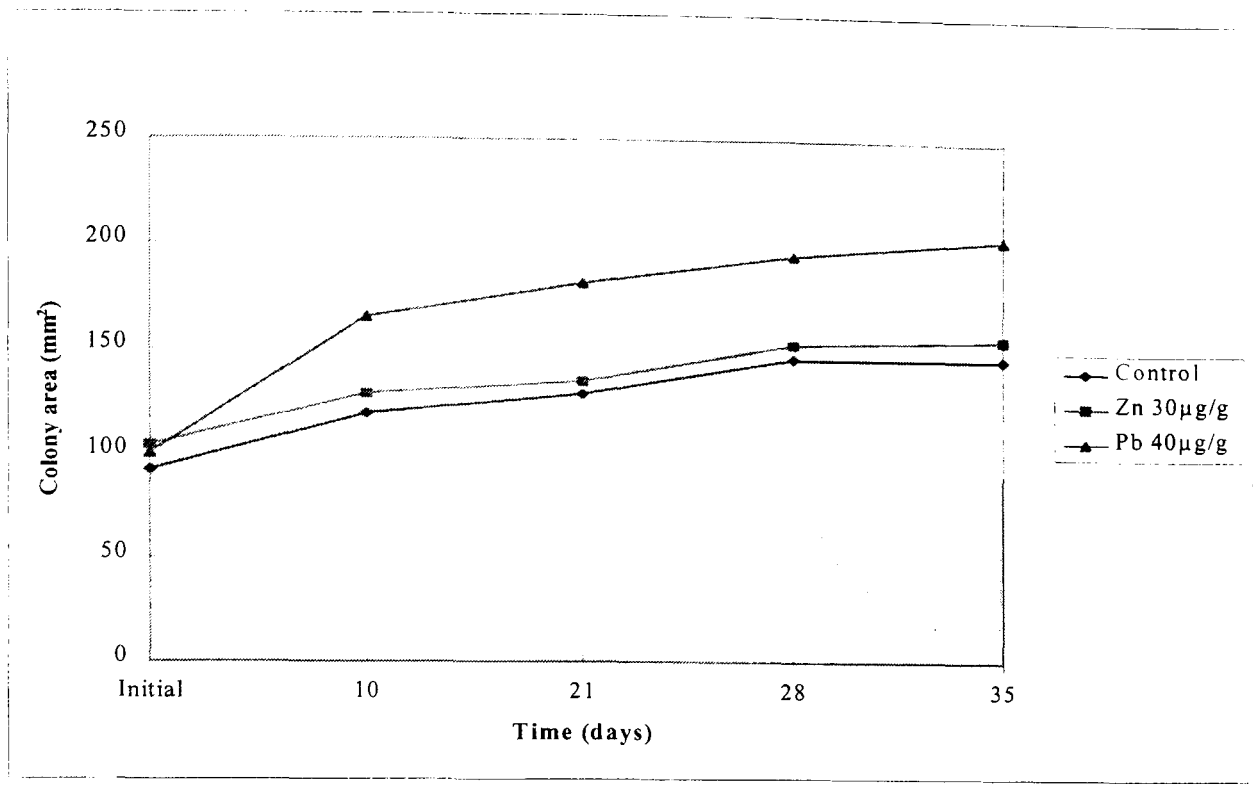

Figure 5.5 Growth of *R. mucilaginosa* on control, Zn and Pb-spiked plates at 30°C.

Figure 5.6 shows the growths of *R. mucilaginosa* on plates spiked with the two concentrations of either Zn or Pb in comparison to the growth on unspiked plates at 4°C. Very little growth occurred, even on the control, suggesting that the growth of this strain was negligible at 4°C.

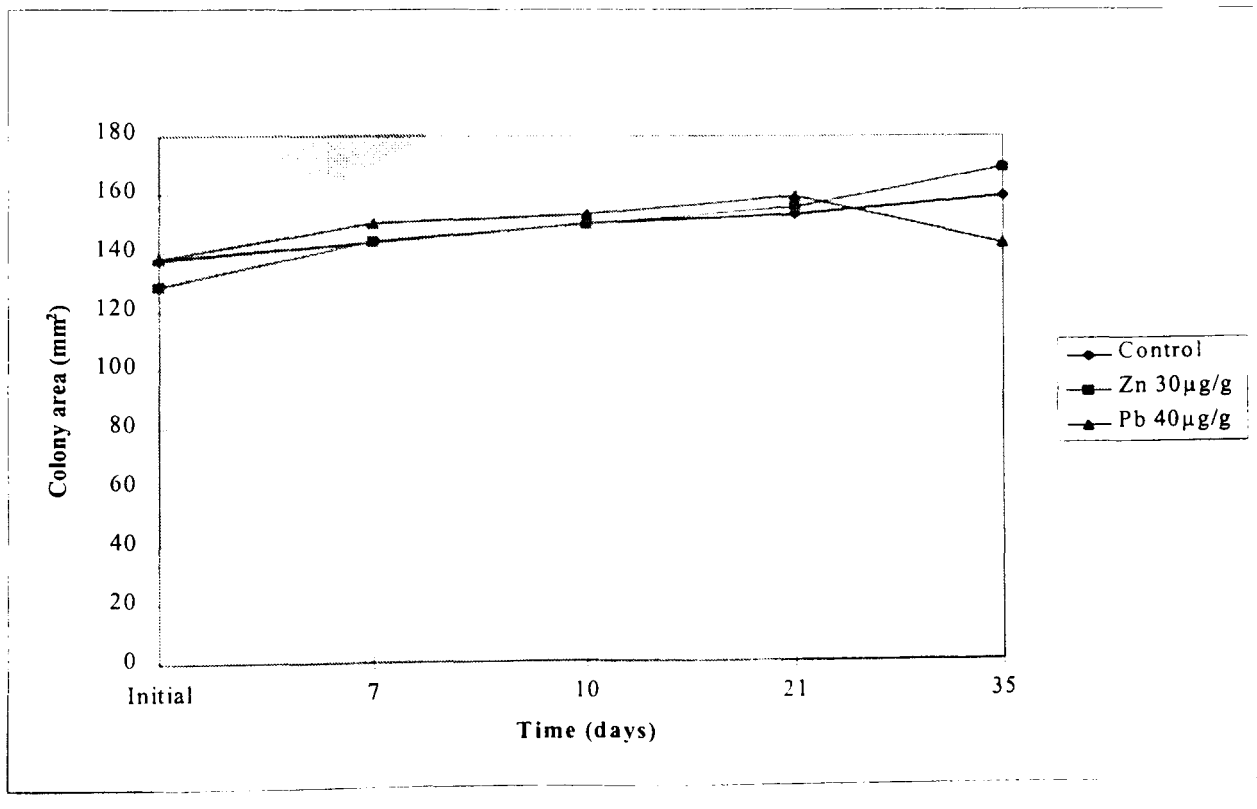

Figure 5.6 Growth of *R. mucilaginosa* on control, Zn and Pb-spiked plates at 4°C.

5.7 Examination of the effects of Zn and Pb on morphology using Scanning Electron Microscopy.

B. bassiana and *R. mucilaginosa* were examined using Scanning Electron Microscopy (SEM) to investigate the effects of Zn and Pb on morphology. Figures 5.7-5.9 represent scanning electron photomicrographs of *B. bassiana* growing on an unspiked plate, and on plates spiked with 800µgZn/g or 400µgPb/g, respectively. Figure 5.7 shows the branching hyphae *of B. bassiana* growing on unspiked medium. The hyphae varied in diameter along their length but tended to appear quite rounded. In contrast, the hyphae appeared to be narrower and had a dried-out appearance on plates spiked with Zn (Figure 5.8) and Pb (Figure 5.9), suggesting that the presence of both metals affects the morphology of *B. bassiana*. Similar colony morphology was observed on plates spiked with higher concentrations of Zn and Pb (data not shown).

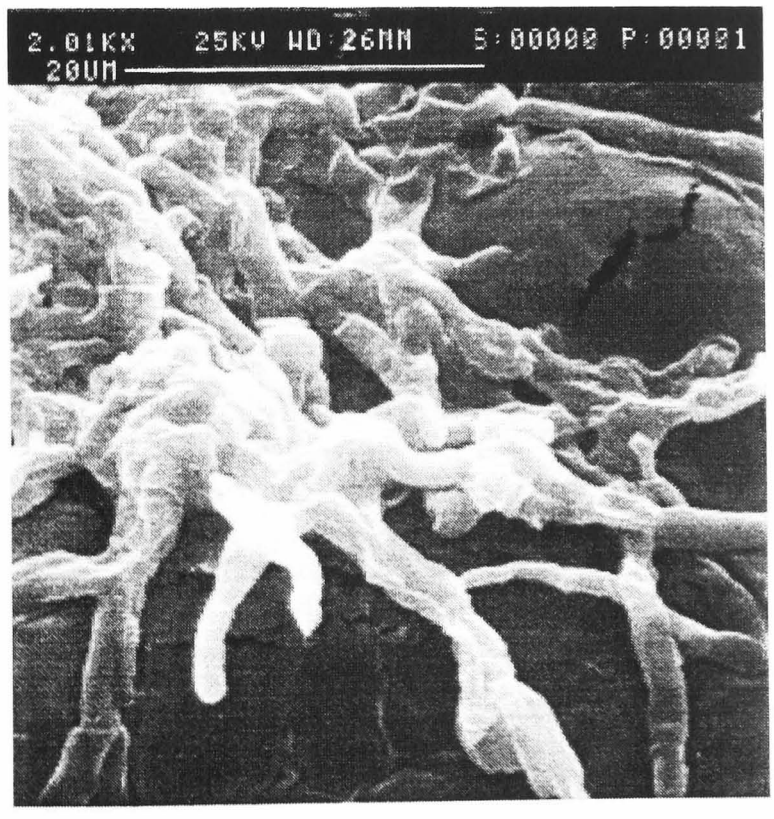

Figure 5.7 Scanning electron photomicrograph of *B. bassiana* grown on unspiked medium.

Figure 5.8 Scanning electron photomicrograph of *B. bassiana* grown on plates spiked with 800µgZn/g.

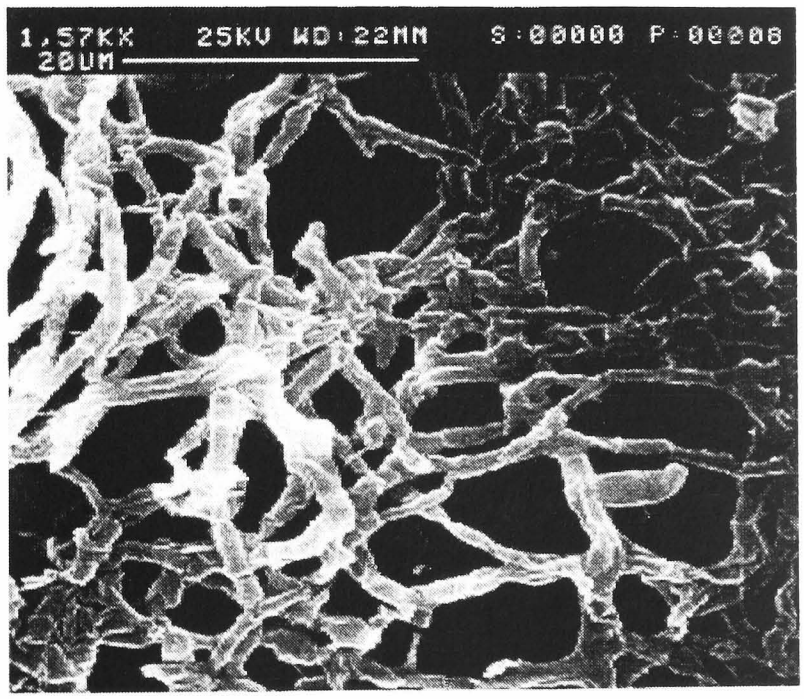

Figure 5.9 Scanning electron photomicrograph of *B. bassiana* grown on plates spiked with 400µgPb/g.

Figures 5.10 and 5.11 show the growths of *R. mucilaginosa* on an unspiked medium (control) and on a medium spiked with 40µgPb/g. Figure 5.10 shows both individual cells and colonies growing on a control plate. The individual cells appear to be smooth and rounded, whilst those in the colony appear to be surrounded by some kind of extracellular product. *R. mucilaginosa* is known to produce fucose-containing extracellular polysaccharides (Golubev and Churkina, 1997), and these are visible in the photomicrograph.

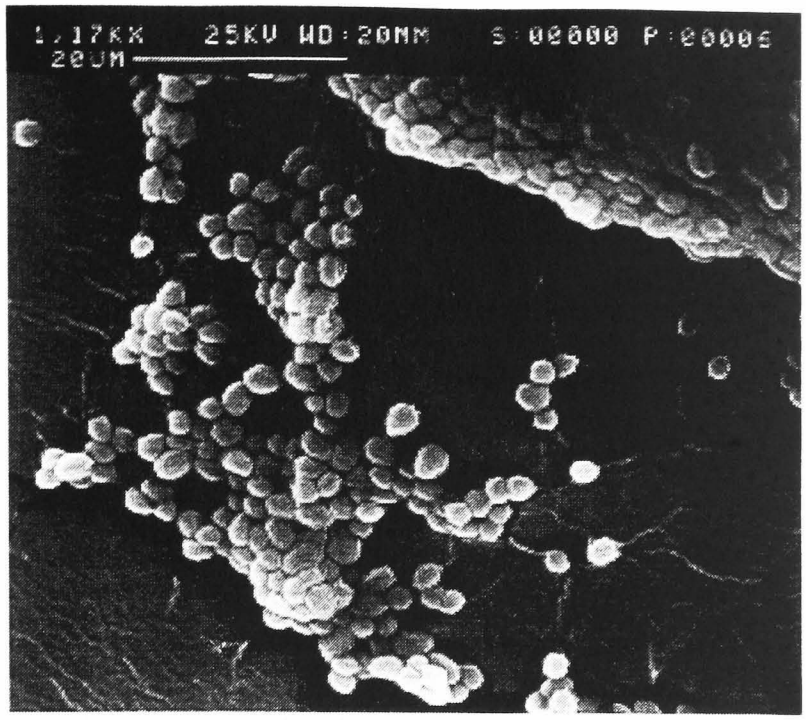

Figure 5.10 Scanning electron photomicrograph of *R. mucilaginosa* grown on unspiked medium.

The growth of *R. mucilaginosa* on plates spiked with 40µgPb/g is shown in Figure 5.11. Cells appear to be less densely packed in comparison to the control. The colonies did not appear to be as extensive as those on the control plate, and the presence of the extracellular polysaccharide is not as apparent. The surfaces of the individual cells also had more wrinkled/dimpled appearances than those in the control. The growth and appearance of *R. mucilaginosa* on a medium spiked with 100µgZn/g was similar to that on the Pb spiked plate (data not shown).

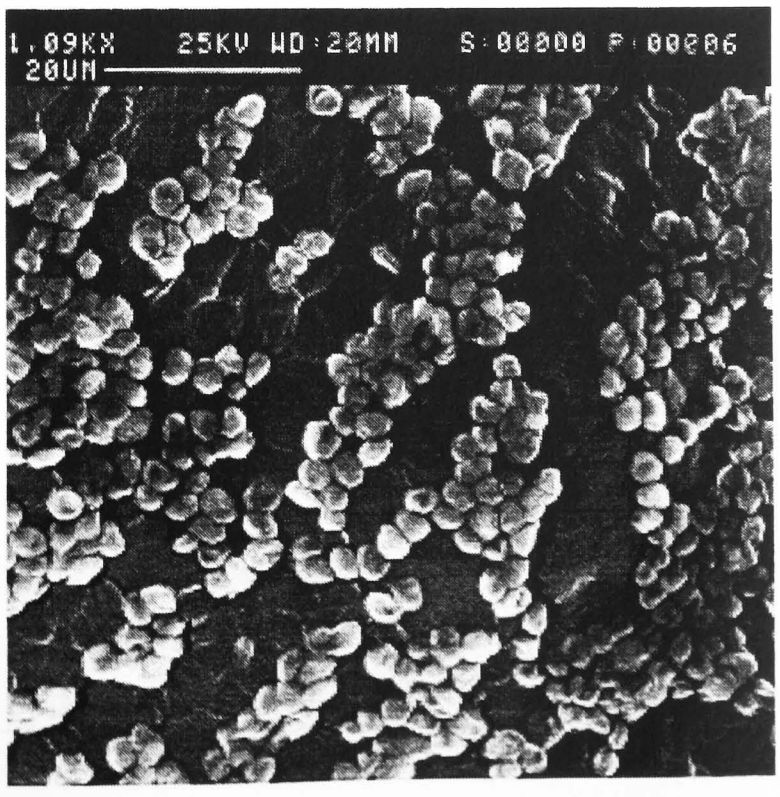

Figure 5.11 Scanning electron photomicrograph of *R. mucilaginosa* grown on plates spiked with 40µgPb/g.

In an attempt to quantify the differences in growth between isolates growing on an unspiked medium and a medium spiked with Zn or Pb, a series of measurements of either hyphal width or cell width were made for *B. bassiana* or *R. mucilaginosa*, respectively. Table 5.13 lists the mean widths of hyphae (±SD) of *B. bassiana* growing on an unspiked medium, and on media spiked with either Zn (at concentrations of 800µg/g and 1800µg/g) or Pb (at concentrations of 400µg/g and 800µg/g). Between 55 and 72 measurements of hyphae width were made for each plate.

Table 5.13 Mean width (µm) (±SD) of hyphae of *B. bassiana* grown on an unspiked medium, in comparison to growth on a medium spiked with either Zn or Pb.

	Control	Zn 800µg/g	Zn 1800µg/g	Pb 400µg/g	Pb 800µg/g
Hyphal width	2.84±0.62	1.58±0.37	2.27±0.40	1.74±0.40	1.44±0.27

The data in Table 5.13 shows that the hyphae growing on the control plates have the greatest mean width followed by, in order of decreasing size, hyphae growing on plates spiked with 1800µgZn/g>400µgPb/g>800µgZn/g>800µgPb/g. It is surprising to note that the mean width of hyphae growing on plates spiked with the highest concentration of Zn (1800µg/g) was greater than that growing on lower concentrations of both Zn and Pb. It is possible that the elevated metal concentrations may be resulting in strain acclimation or selection. Therefore, whilst overall growth is greatly reduced, some cells are better able to tolerate such elevated concentrations, thereby enabling growth similar to that of the control. The width of hyphae growing on plates spiked with either 800µg/g Zn or Pb was reduced by 51% and 56%, respectively, in comparison to growth of the control. This indicates that a reduction in hyphal width is associated with the presence of both metals and that Pb has a greater effect than Zn, which supports the results of the experiments reported in Sections 5.4, 5.5 and 5.6.

Table 5.14 gives the mean widths (±SD) of individual cells of *R. mucilaginosa* growing on an unspiked medium, and those growing on media spiked with 100µgZn/g and 40µgPb/g. The results suggest that the presence of both Zn and Pb result in an apparent increase in cell size, which is more marked for the medium spiked with Pb.

Table 5.14 Mean width (µm) (±SD) of cells of *R. mucilaginosa* grown on an unspiked medium, in comparison to growth on a medium spiked with either Zn or Pb.

	Control	Zn 100µg/g	Pb 40µg/g
Cell width	2.89±0.39	3.22±0.52	3.52±0.54

The increased size of cells on the Pb spiked medium supports the findings of the colony growth experiment which also found that the same concentration of Pb stimulated growth (Section 5.6). However, growth stimulation was not observed on Zn-spiked plates, and the explanation for the greater size of cells on both metal spiked plates is unclear. It is recommended that future research should include further investigation in this area to enable these results to be fully understood.

To determine whether any of the differences in hyphae and cell widths between the control, Zn and Pb spiked plates for *B. bassiana* and *R. mucilaginosa* were significantly different, the data were examined statistically. The distribution of each data set was first tested for normality using the Ryan-Joiner test (Minitab 10.5, Microsoft Windows v3.11), as described in Section 4.2.1. However, it was not possible to transform all the data sets for *B. bassiana* to a normal distribution using the same transformation and therefore it was decided to analyse the data using a Kruskal-Wallis test (nonparametric test). As the raw data sets for *R. mucilaginosa* were normally distributed, the data sets were analysed using ANOVA (parametric test).

The results of the Kruskal-Wallis test indicate that the differences between the hyphal width of *B. bassiana* on unspiked and spiked medium were statistically significant ($p = 0.000$). The data were analysed further using Tukey's Honestly Significantly Different test (Tukey's HSD). These results indicated that the width of the hyphae on the control was significantly different from the hyphae growing on all the other plates, as were the hyphae growing on plates spiked with a 1800µgZn/g and 400µgPb/g. Hyphae growing on plates spiked with 800µgZn/g and 800µgPb/g were not significantly different from each other, but were significantly different from all other groups. To summarise, the results of the statistical analysis strongly suggest that the width of hyphae show significant differences between the plates, decreasing in width in the order control > Zn

1800µg/g > Pb 400µg/g > Zn 800µg/g = Pb 800µg/g (where > means *significantly* greater than).

The results of the ANOVA on the cell widths of *R. mucilaginosa* strongly support the alternative hypothesis that the hyphae growing on unspiked and spiked media are significantly different ($p = 0.000$). The data were also analysed using Tukey's Honestly Significantly Different test (Tukey's HSD). The results indicated that each of the data sets were significantly different from each other, with the width of cells decreasing in size in the order Pb 40µg/g > Zn 100µg/g > control (where > means *significantly* greater than).

The results of the statistical analysis strongly indicated that there are significant differences between the growths of both *B. bassiana* and *R. mucilaginosa* on unspiked media, and in the presence of varying concentrations of either Zn or Pb. However, some of these differences vary markedly from what may have been expected. It is important therefore to use as much data as possible to fully interpret the results of each experiment. For example, the results of the statistical analysis on the hyphae of *B. bassiana* strongly indicate that hyphae growing on a medium spiked with 1800µgZn/g are significantly wider than hyphae growing on a medium spiked with 800µgZn/g. This suggests that the higher concentration of Zn stimulates growth of the isolate. However, the results of the minimum inhibition concentration experiment (Section 5.4) showed that at this concentration hardly any growth occurs. Therefore the greater hyphal width may be due to some form of strain selection, whereby certain cells are better able to tolerate elevated concentrations, rather than a stimulatory effect on growth for the entire population. These results emphasise the importance of using all the available data to enable a full interpretation.

5.8 Examination of *B. bassiana* using Transmission Electron Microscopy and Energy Dispersive X-ray Spectrometry

The results described in Sections 5.4 and 5.6 indicated that, of the two strains studied, *B. bassiana* had a greater tolerance of Pb and Zn, and a much greater capacity to accumulate Pb (Section 5.5). To examine *B. bassiana* further, the growths of the isolate on Zn spiked, Pb spiked and unspiked media were examined using Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray Spectrometry (EDX).

5.8.1 Results of Transmission Electron Microscopy

Figures 5.12 - 5.15 show the micrographs of *B. bassiana* on an unspiked medium (Figure 5.12), on a medium spiked with 800µgPb/g (Figures 5.13 and 5.14), and on a medium spiked with 800µgZn/g (Figure. 5.15). Figure 5.12 shows a cross-section through hyphae growing on an unspiked medium. The magnification was x10 000 and the hyphae diameter was estimated to be approximately 2.84µm. Fungal cell walls are clearly apparent, as are various internal structures such as nuclei, mitochondria, vacuoles and lipid droplets. The empty-looking cell in the centre of the picture is likely to be an older, dead cell. However, it is interesting to note that the cell wall is still intact.

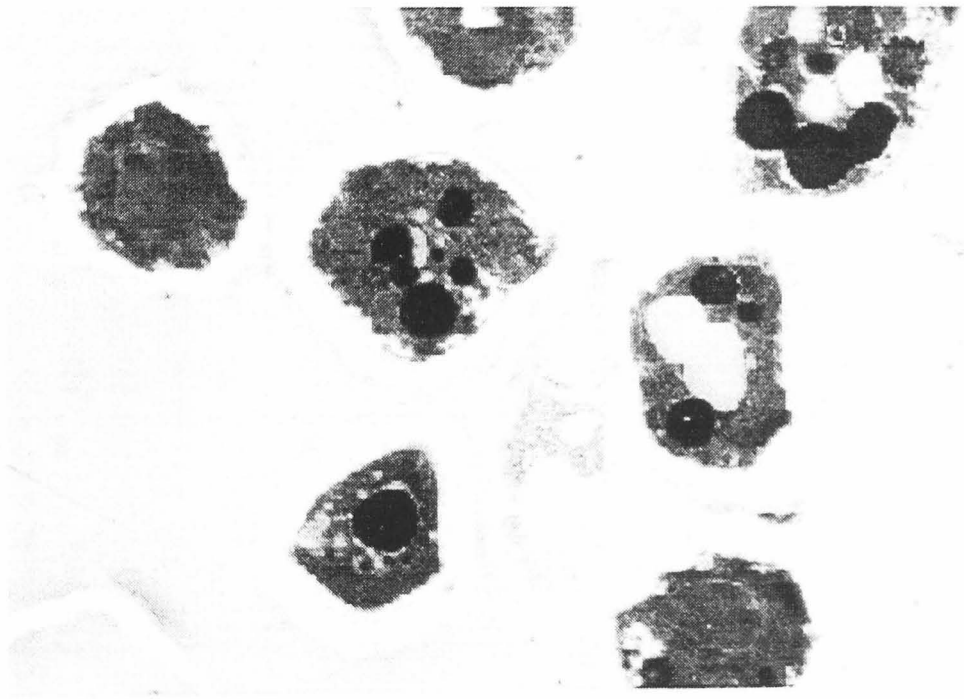

Figure 5. 12 Cross-section of hyphae of *B. bassiana* grown on a unspiked medium (x10 000)

Figures 5.13 and 5.14 show cross-sections of the hyphae grown on a medium spiked with 800µgPb/g and taken at magnifications of 10 000 and 19 000 times respectively. The hyphae are approximately 1.44µm in diameter. Again, the cell walls and some internal structures are apparent. However, the pictures in Figures 5.13 and 5.14 indicate the presence of a number of electron dense areas, associated with the cell wall, inside the cell itself, and in the medium surrounding the cells. These electron dense areas were not present on any of the control samples, and are thought to be a Pb salt precipitated both intracellularly and extracellularly with respect to *B. bassiana*. The cell contents

appeared to be much lighter in colour and some cells were completely empty suggesting that these cells are struggling to survive at this Pb concentration. This supports the results of the MIC experiment, where inhibition of growth was found to be between 800-1000µgPb/g (Section 5.4).

Figure 5. 13 Cross-section of hyphae of *B. bassiana* grown on a medium spiked with 800µgPb/g (x10 000)

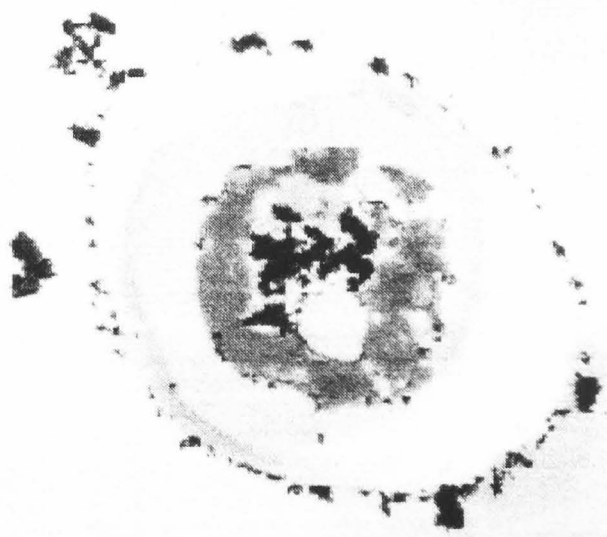

Figure 5. 14 Cross-section of a hypha of *B. bassiana* grown on a medium spiked with 800µgPb/g (x19 000)

Figure 5.15 shows cross-sections of hyphae grown on a medium spiked with 800µgZn/g. In comparison to Figures 5.13 and 5.14, cells in Figure 5.15 appear to be

much healthier, with cell contents appearing to be darker and internal structures clearly visible. Electron dense areas were not observed on any of the Zn spiked plates and were not expected as the results of the metal uptake experiments indicated that *B. bassiana* did not have a great capacity to bind Zn (Section 5.5.2). In addition, Zn has a lower density than Pb (7.130g/cm^3 compared to 11.360g/cm^3, respectively) and is therefore less easy to detect by transmission electron microscopy. The healthier appearance of cells at 800µgZn/g in comparison to the appearance of cells grown at the same concentration of Pb again supports the results of the MIC experiment, which found that Zn was less toxic to the isolate (MIC of 1800-2000µgZn/g).

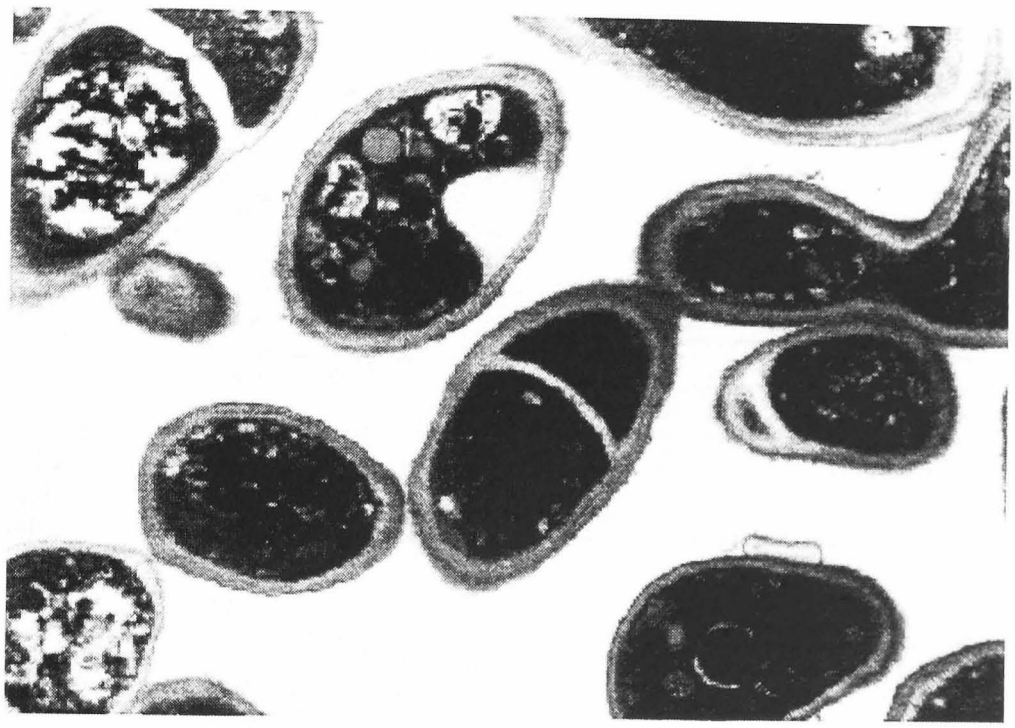

Figure 5. 15 Cross-section of hypha of *B. bassiana* grown on a medium spiked with 800µgZn/g (x10 000)

5.8.2 Results of Energy Dispersive X-ray Spectrometry

To determine if the electron dense areas observed in Figures 5.13 and 5.14 were due to the presence of Pb, samples were analysed using Energy Dispersive X-ray Spectrometry (EDX) which enables the identification of all of the elements present in the sample. The area analysed is shown in Figure 5.16, which shows a cross-section of hyphae grown on a medium spiked with 800µgPb/g. The image is slightly blurred due to the high magnification (x50 000). The hyphae are approximately 1.44µm in diameter. The spectrum of the initial analysis indicated that Pb was present in the sample, and the area

was then mapped to determine its location. Figure 5.17 is the elemental map of the same area shown in Figure 5.16.

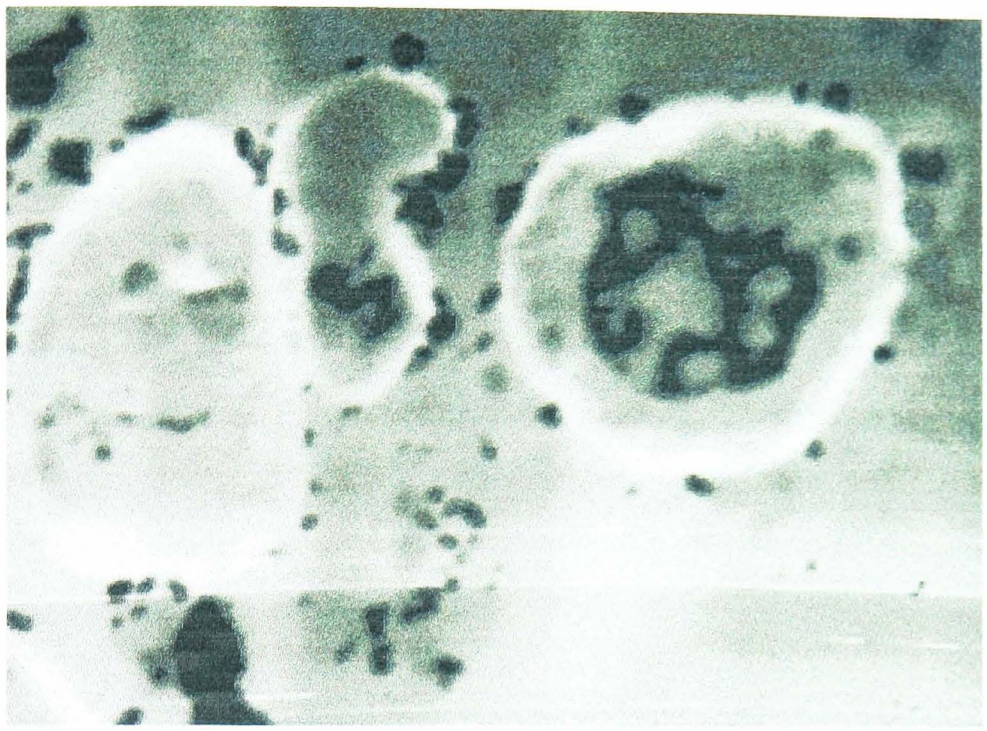

Figure 5. 1 Cross-section of hyphae of *B. bassiana* grown on a medium spiked with 800µgPb/g (x50 000)

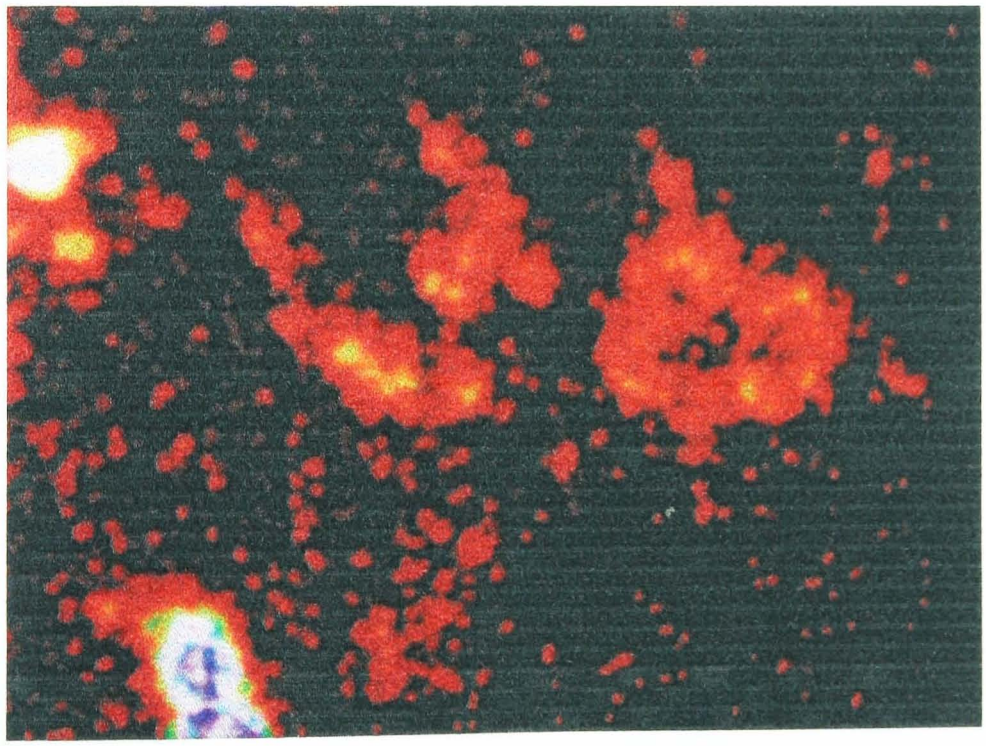

Figure 5. 2 Elemental map of a cross-section of hyphae of *B. bassiana* grown on a medium spiked with 800µgPb/g (x50 000)

The 'hot spots' of Pb are clearly visible (highly coloured areas), and their location closely corresponds to the main electron dense areas seen in Figure 5.16 confirming their identity as Pb. These results also confirm the ability of *B. bassiana* to accumulate

and precipitate Pb intracellularly (red/yellow areas in the middle and middle right of Figure 5.17). In addition, sorption of Pb to the cell wall and subsequent precipitation (red/yellow area upper middle of Figure. 5.17) is also demonstrated. The precipitation of Pb in the surrounding medium occurs which could possibly be due to the release of metabolites by the isolate (blue/white areas bottom left and top left of Figure 5.17). The colour brightness increases with the increasing number of counts recorded, which, in turn, increases with increasing Pb density. The two biggest areas of Pb precipitation in the medium (bottom left and top left) appear to be brighter than the areas within the hyphae and those associated with the cell walls, which may suggest that the precipitation of Pb extracellularly is a more important and significant process.

To examine the distribution of Pb in more detail, EDX spot analyses were carried out on several electron dense areas both inside and outside hyphae grown on a medium spiked with 800µgPb/g. Areas of the medium where electron dense areas were not visible were also scanned for comparison. Figure 5.18 shows the location of 9 spot analyses which were carried out, and the results are shown in the spectra labelled as Figures 5.19 - 5.27.

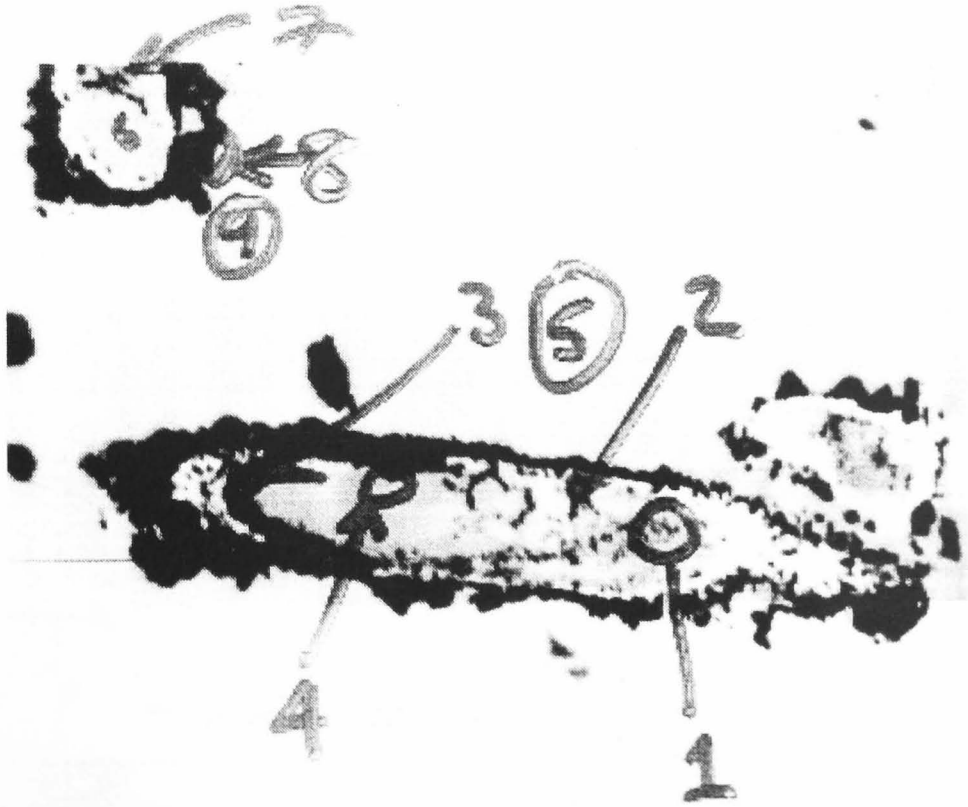

Figure 5. 18 Cross-section of hyphae of *B. bassiana* grown on a medium spiked with 800µgPb/g (x20 000). The numbers indicate the locations of spot analyses.

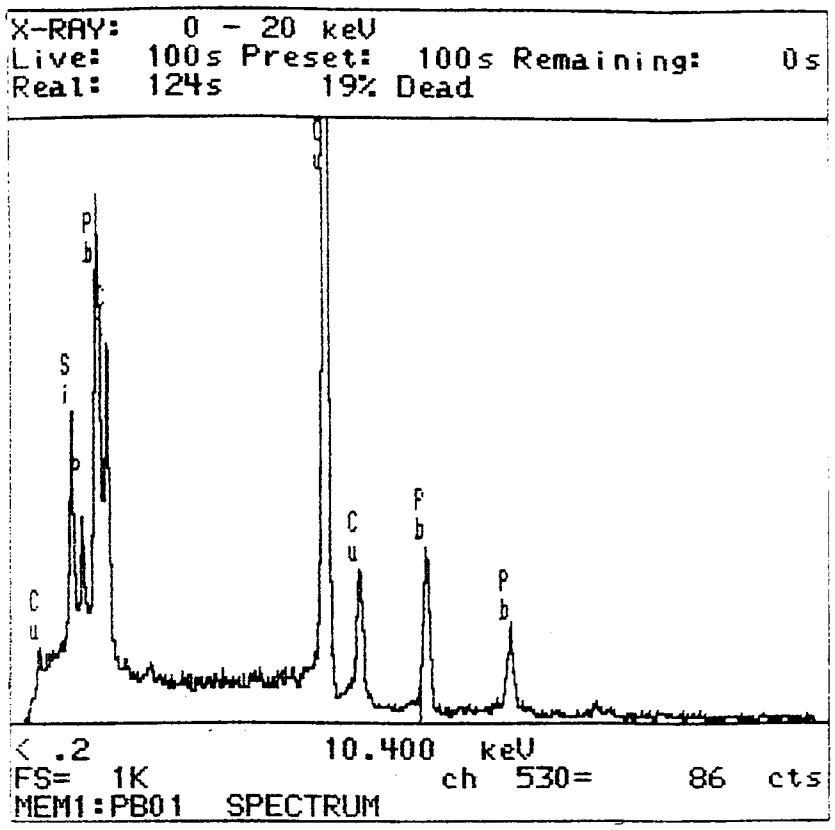

Figure 5. 19 EDX spectrum obtained at location 1 (see Figure 5.18)

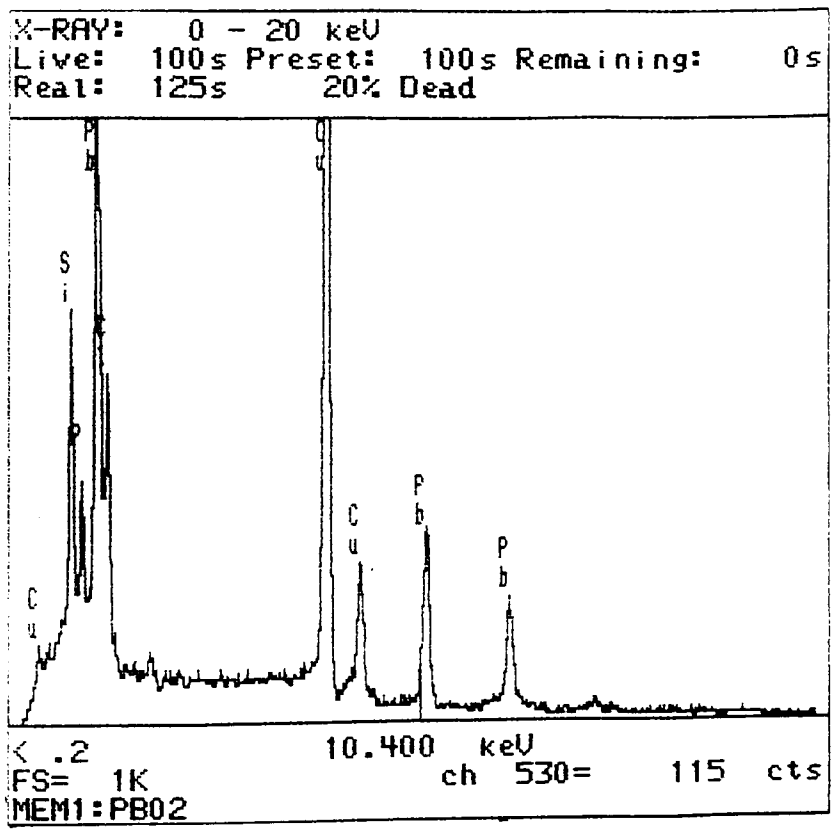

Figure 5. 20 EDX spectrum obtained at location 2 (see Figure 5.18)

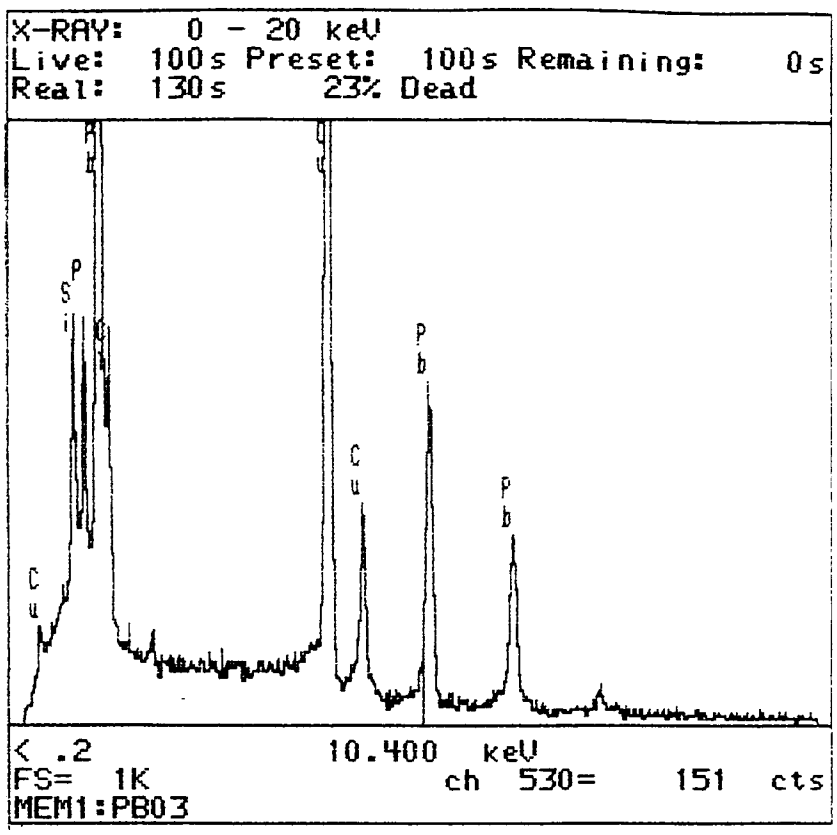

Figure 5. 21 EDX spectrum obtained at location 3 (see Figure 5.18)

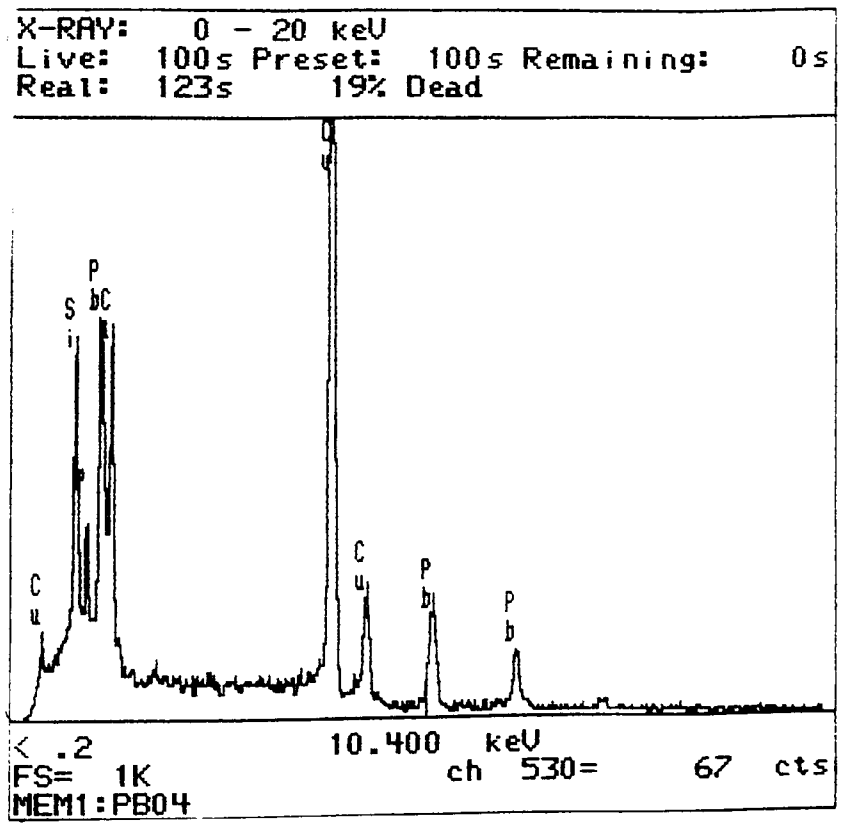

Figure 5. 22 EDX spectrum obtained at location 4 (see Figure 5.18)

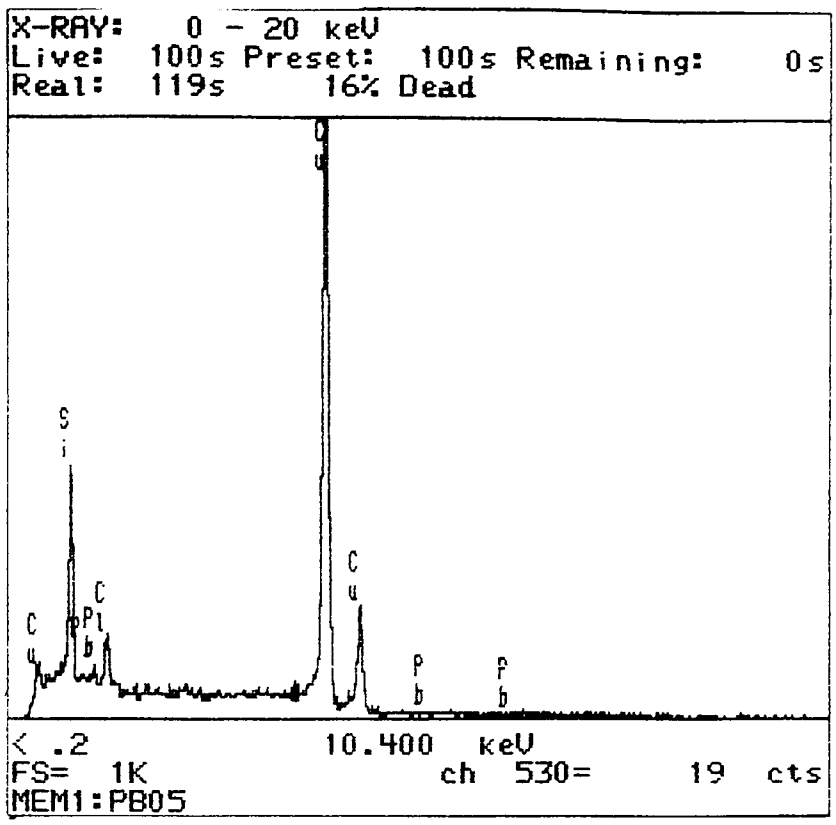

Figure 5. 23 EDX spectrum obtained at location 5 (see Figure 5.18)

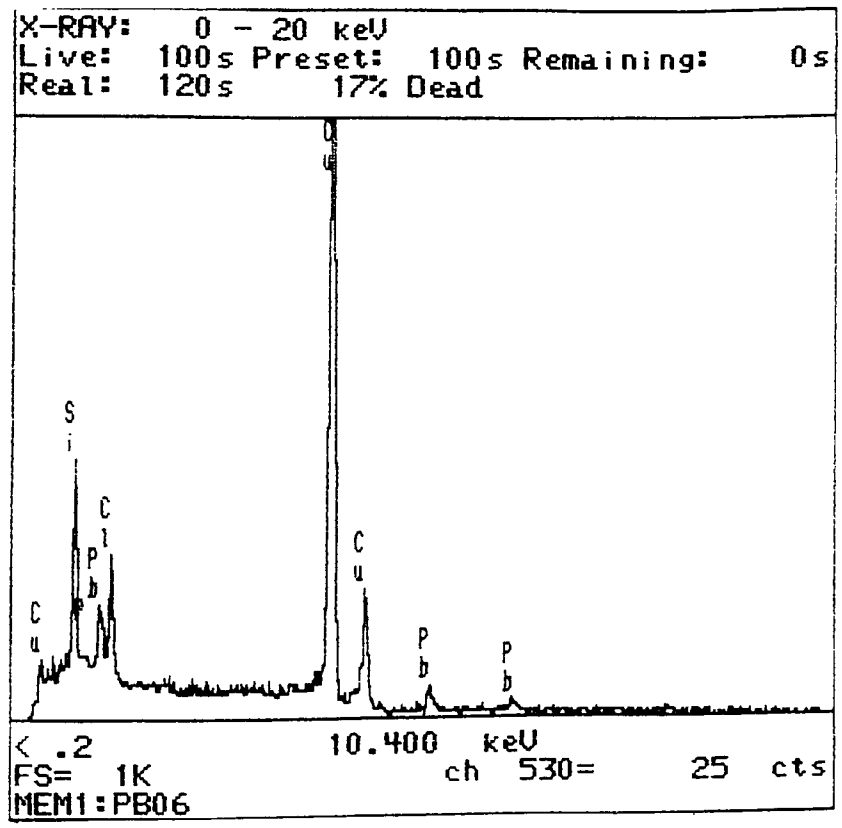

Figure 5. 24 EDX spectrum obtained at location 6 (see Figure 5.18)

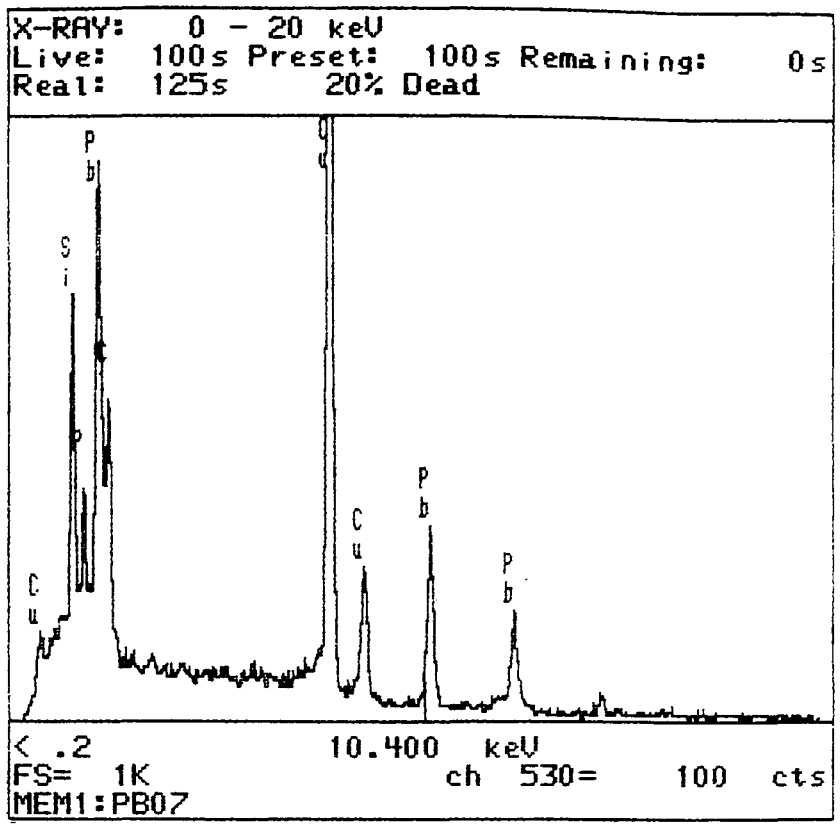

Figure 5. 25 EDX spectrum obtained at location 7 (see Figure 5.18)

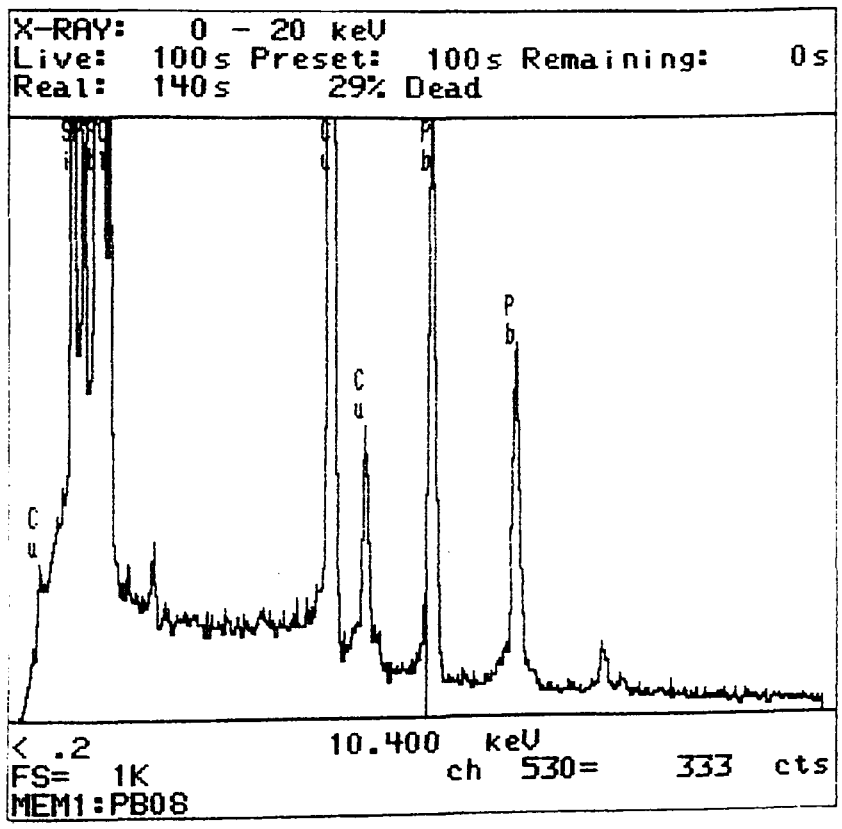

Figure 5. 26 EDX spectrum obtained at location 8 (see Figure 5.18)

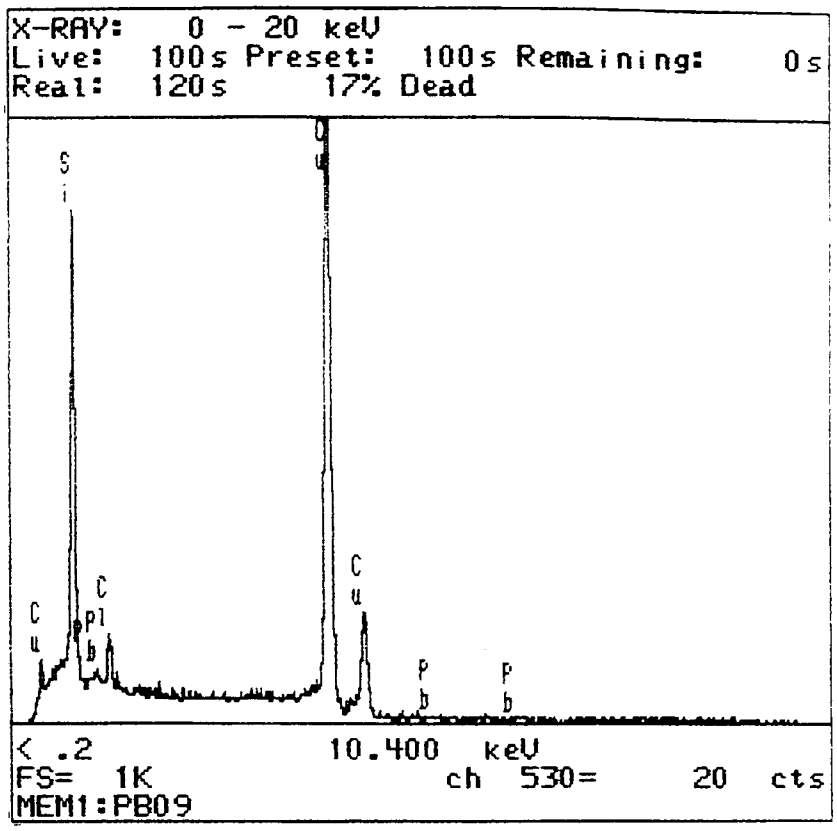

Figure 5. 27 EDX spectrum obtained at location 9 (see Figure 5.18)

Each spectrum gives the identity of all the elements present under the 'spot', which has a diameter of a few nanometers. Spot analyses carried out on locations 1-4 (Figs 5.19-5.22), 7 and 8 (Figs 5.25-5.26) were electron dense areas associated with the cell wall and inside the hyphae, whereas location 6 (Fig. 5.24) was inside a hyphae, but electron dense areas were not visible. In all of these spectra Pb peaks were apparent, with peak height appearing to decrease with decreasing crystal density. Spot analysis carried out on locations 5 (Fig. 5.23) and 9 (Fig. 5.27) were on areas of the media outside the hyphae where electron dense areas were not visible. On both these spectra, Pb peaks are not apparent, confirming the results of elemental mapping which identified electron dense areas as being due to the presence of Pb.

The peak heights of Pb in the spectra decrease in size in the order of location 8>3>7>2>1>4>6>9=5. This order closely reflects the decrease in crystal density. For example, the very dense precipitate associated with the cell wall at location 8 in comparison to the speckled appearance of the precipitate within the hyphae at location 1. At location 6, although electron dense areas are not visible on the photomicrograph, small Pb peaks were visible on the spectrum (Fig. 5.24). The amount of Pb accumulated

small Pb peaks were visible on the spectrum (Fig. 5.24). The amount of Pb accumulated and precipitated at location 6 may be too small to be visually detected at this magnification (x20 000) but present in sufficient quantity to be detected using EDX (detectability of approximately 0.1-0.5%). This accumulation may have been the result of either metabolic dependent accumulation, a process suggested by the localisation of precipitates within hyphae (e.g. at location 7), or as a result of passive accumulation following increasing cell permeability and/or cell death.

The other peaks present in the EDX spectra are due to Cu, Cl, P and Si. Although this technique is capable of identifying which elements are present, it is not capable of determining in what form they occur. For example, Cl may be present as chloride, chlorite or chlorate. The Cu peaks are present at approximately the same height in all the spectra, and are thought to be due to the fact that the grid used to support the samples was made of Cu. The Cl and Si peaks vary in height between spectra but they do not vary in a similar way to the Pb peaks. For example, although the greatest peak height for all of the elements was recorded at location 8 (Figure 5.26), the second highest Si peak was recorded at location 9 where no Pb was detected (Figure 5.27), and the second highest Cl peak was recorded at location 4 which was the 6th highest peak for Pb (Figure 5.22). As the Cl and Si peak heights do not vary in a similar way to the Pb peak heights, it is assumed that Cl and Si are not directly associated with the Pb precipitates.

Following discussions with the manufacturers of the agar, it is thought that their presence may be due to chloride and silicon ions within the seaweed used to make the agar. However, the peak heights of P appear to vary in size in approximately the same way as the Pb peaks, decreasing in size in the order of location 8>3>2>7>1>4>6=9=5, in comparison to the Pb peaks which decrease in the order 8>3>7>2>1>4>6>9=5. Although it is not possible to determine the exact form of P, the similar trends in peak height strongly suggest that P is involved in the precipitation of Pb. Pb could be binding to the phosphate groups present in many cell constituents. Gadd (1993) suggested that, although there are many potential sites capable of binding metals, such as amines, hydroxyls and sulphydryls, primary metal-cell wall interactions probably involve the binding of metals to both phosphate and carboxyl groups. Phosphates are present in large amounts as part of galactans which are polymers present in both cell walls and exocellular polysaccharides (Holan and Volesky, 1995) and in the abundant metabolite

glucose-6-phosphate which is converted to uridine diphosphate N-acetylglucosamine which is a precursor of chitin (Carlile and Watkinson, 1994). Phosphates are also present in nucleic acids where they form part of the sugar-phosphate backbone. However, due to the complex nature of the cell wall composition and structure it is unlikely that all the insoluble Pb is present as insoluble Pb phosphate.

Chitin and chitosan are important components of fungal cell walls and both have been shown to sequester metal ions (Tsezos and Volesky, 1982, Zhang *et al.*, 1998). Pb uptake by *Rhizopus nigricans* was reported to be primarily due to the binding of Pb to the amine-N of chitin, which then acted as a nucleation site for the further deposition of Pb (Zhang *et al.*, 1998). Almost all filamentous fungi contain chitin in their cell walls, and such a mechanism may therefore have also been involved in the accumulation of Pb by *B. bassiana*. The presence of precipitated Pb in the medium surrounding hyphae may indicate that the release of metabolites by the isolate is also resulting in the precipitation of Pb.

5.9 Potential application of results

B. bassiana is an insect pathogen and is known to produce citric and oxalic acids, which are thought to reduce protein bonding in the insect cuticle thereby enabling the penetration of hyphae (Bidochka and Khachatourians, 1991). In a study on *Aspergillus niger*, which also produces oxalic and citric acids, Sayer and Gadd (1997) demonstrated that the isolate was capable of solubilising insoluble Zn and Ca phosphate and Zn oxide which was subsequently precipitated as oxalate crystals in the medium under and around the colony. Therefore, the release of oxalic acid by *B. bassiana* may be responsible for the precipitation of Pb in the medium surrounding the hyphae. The precipitation of metals as oxalate crystals is highly desirable as insoluble metal oxalates are resistant to further solubilisation, with only a few bacteria and fungi being known that are able to readily degrade them (Sayer and Gadd, 1997). The precipitation of metals as insoluble metal-oxalate crystals by oxalic acid producing fungi such as *B. bassiana* would be an important mechanism in urban runoff treatment wetlands, for the removal of metals from urban runoff and as a long-term storage 'sink' within wetland substrates.

Although the importance of micro-organisms in many wetland treatment processes has been recognised, there have been few studies of the ability of wetland micro-organisms to accumulate metals. This study has demonstrated that micro-organisms isolated from urban runoff wetland systems are capable of tolerating Zn and Pb at concentrations typical of sites receiving urban runoff, and also showed that selected strains were capable of accumulating these metals. In treatment wetland systems receiving urban runoff, or other metal contaminated wastewater, the understanding and enhancement of such processes would clearly be of considerable value. Samples for microbial analyses were collected during winter (November 1997), indicating that such processes may occur throughout the year. In addition, many of the samples (including *B. bassiana*) were isolated from sites that were less than two years old at the time of sampling. The results indicate that metal tolerant micro-organisms either quickly become established at sites receiving metal contaminated wastewaters, or that they form a natural component of the plant and substrate microbial population. The microbial populations of urban runoff treatment wetlands have great potential in terms of year round metal accumulation, and it is strongly recommended that further research is carried out in this area. Such results may also suggest new approaches to urban runoff treatment wetland design, whereby wetland plants and substrates are selected on the basis of the microbial populations they can support.

5.10 Summary of results

The results of this study have demonstrated that a range of micro-organisms isolated from the rhizosphere of *Typha latifolia* and *Phragmites australis* (collected from the Dagenham and Brentwood wetlands) were able to tolerate Zn and Pb, with the numbers of tolerant isolates decreasing with increasing metal concentration. Subsequent experiments focused on the ability of two isolates, which were identified as *Beauveria bassiana* (filamentous ascomycete fungus) and *Rhodotorula mucilaginosa* (basidomycete yeast) to tolerate and accumulate Zn and Pb. Of the two strains, *B. bassiana* demonstrated a much greater tolerance to both metals, and could tolerate levels of almost twice the maximum Zn and Pb concentrations recorded at both wetland sites. Both species could accumulate Zn and Pb, with the accumulation of Pb by *B. bassiana* being particularly efficient. The colony growths of *B. bassiana* on Zn and Pb-spiked

media compared well with the growth of the isolates on unspiked media at 30°C and at 4°C. The colony growth of *R. mucilaginosa* was poor at 30°C and negligible at 4°C on both metal-spiked and unspiked media. Examination of the isolates using Scanning Electron Microscopy indicated that the presence of Zn and Pb resulted in a significant reduction in the hyphal width of *B. bassiana* but resulted in a significant increase in the cell size of *R. mucilaginosa*. *B. bassiana* was also examined using Transmission Electron Microscopy. Examination of the isolate grown on Pb-spiked medium indicated the presence of electron dense areas associated with cell walls, within the cells and in the medium surrounding the cells. These electron dense areas were not present on any of the control or Zn-spiked samples. Elemental mapping and Energy Dispersive X-ray analysis were carried out, and confirmed that these electron dense areas were due to the presence of Pb. The application of these results to urban runoff treatment wetlands is also discussed, with regards to the potential of microbial accumulation and precipitation of metals as treatment processes which may occur throughout the year.

CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Treatment performance and design of constructed wetlands receiving urban runoff

Two full-scale experimental treatment wetlands were monitored for a wide range of parameters, including heavy metals, BOD, suspended solids and nutrients, over a two year period to determine their ability to treat urban runoff. The results of the monitoring programme indicate that both wetlands were capable of reducing the pollutant loads, demonstrating that the use of this technology to treat urban runoff is appropriate. This study also shows that it is possible to locate and successfully establish treatment wetlands within urban areas, and the use of wetland systems at other urban sites to improve and protect the water quality of receiving waters is supported. The results will be used by the Environment Agency in the development of guidelines for the use of urban runoff treatment wetlands in England and Wales.

The two wetland systems monitored are of different designs and surface areas. Although removal efficiencies were variable, both systems demonstrated positive removal efficiencies during specific storm events for a range of parameters. Of the two systems, the mean removal efficiencies were higher at the Dagenham wetland during storm events, which is thought to be associated with several factors. The Dagenham wetland has a greater surface area than the Brentwood wetland, and inlet loadings at the Dagenham site were generally higher. The construction of the Dagenham wetland involved widening an existing watercourse which was then planted with *Typha latifolia* and *Phragmites australis*, and the results of this study support the use of this 'retro-fitted wetland' design approach for future systems.

As a result of this study, several recommendations have been made with regards to the design, management and maintenance of these wetland treatment systems. These include the installation of a stilling structure at the inlet to the settlement tank at the Brentwood wetland to reduce the velocity of the incoming flow, and the use of baffles in the constructed wetland to reduce hydraulic short-circuiting in this component. At the Dagenham wetland, the construction of a fence will enable the re-establishment and

recovery of damaged plants, increasing plant density and hence improving the retention time of the whole system.

Both wetland systems flooded during storm events, but as the Dagenham wetland was constructed on a flood plain and the Brentwood wetland in a flood basin this was not found to be a problem. However, this factor must be considered in the design of future systems, and where flooding is not acceptable then it is recommended that a by-pass channel is included as part of the design to cope with elevated flows. This would also help reduce sediment remobilisation during high flow conditions, which was reported at both wetland sites during storm events.

The treatment performance of both wetland systems has been adversely affected by several management and maintenance issues which could not be resolved immediately due to the delays in the establishment of a management and maintenance plan. The management and maintenance requirements of the systems following their construction were not considered during the initial wetland design process. Since there was no plan in operation and no funding available, there were considerable delays before recommendations, such as the emptying of the settlement tank, could be implemented. A management and maintenance plan should clearly identify who has responsibility for the various operational issues which may arise, and ensure that adequate funding will be available to enable prompt responses to such issues. It is considered essential that a management and maintenance plan is included in the initial design and costings of future constructed wetland treatment systems to enable treatment wetland systems to perform optimally.

An important and common factor in determining the location of the treatment wetlands is the availability of land. However, the results of the monitoring programme showed that, in addition to land availability, who access to the site and use of the surrounding land should also be considered. This aspect was not investigated at the Dagenham site, where the system was located on a flood plain which was also an area of common land used for grazing and the illegal dumping of solid waste. Such considerations at an initial stage would have enabled suitable precautionary measures, such as the construction of a fence to prevent horses grazing the plants, to have been incorporated into the wetland design.

To summarise, based on the results of this study, the following recommendations for the design of urban runoff treatment wetlands are made:

- **location** - when deciding where to locate an urban runoff treatment wetland, it is important to consider who has access to the land and surrounding area, with regards to preventing damage and/or conflict of use over the land area.

- **pre- and post-treatment** - the results of this study indicate that the settlement tanks at both sites resulted in the removal of metals from the water column, and they are therefore considered to be an essential component of urban runoff treatment wetlands. However, their design should ensure that inlet flows do not result in the resuspension of already settled sediment, that they are emptied regularly as required, and that great care is taken during this process to prevent contaminated sediments from being transported through the system.

- **wetland design** - of the two wetland systems studied, the retro-fitted wetland appeared to result in the greater treatment efficiency, and this design could readily be incorporated into streams in other urban areas. However, the further monitoring of both systems during storm events of different magnitudes, following the implementation of the recommendations made earlier in this section, should be carried out to enable a fuller evaluation of the performance of each system.

- **surface area** - the quality of urban runoff entering the monitored wetland systems, although very variable, differed between the catchment areas in that pollutant concentrations and loadings tended to be higher at Dagenham than Brentwood during both dry weather and storm events. This was thought to reflect the fact that the Dagenham catchment area (440ha) was larger and contained both industrial and residential areas, whereas the Brentwood catchment is smaller (150ha) and is residential only. This emphasises the importance of incorporating local catchment characteristics into the sizing of urban runoff wetland systems, and that the approach of sizing wetland systems based on a percentage of the catchment area may be modified to reflect local pollutant characteristics. The necessary information could be ascertained by background water quality analysis.

- **wetland vegetation** - plant metal tissue concentrations in *Typha latifolia* and *Phragmites australis* were found to vary both seasonally and between the species themselves. This may reflect differences in the onset and duration of the growing season of each plant species and therefore it is recommended that a mix of plant species is included to maximise potential metal bioaccumulation. A mix of plant species will also increase the aesthetic appeal and wildlife value of the wetland system.

- **management and maintenance plan** - as already emphasised earlier in this section, the inclusion of a management and maintenance plan as part of the initial wetland design and budgeting process is essential to enable systems to perform optimally.

6.2 Ability of micro-organisms, isolated from urban wetlands, to accumulate heavy metals

The results of this study demonstrate that a range of micro-organisms isolated from the rhizospheres of *Typha latifolia* and *Phragmites australis*, collected at the Dagenham and Brentwood wetland sites, are capable of tolerating Zn and Pb at concentrations typical of sites receiving urban runoff. In addition, results showed that two selected strains (identified as *Beauveria bassiana* and *Rhodotorula mucilaginosa*) were also capable of accumulating both metals. In particular, *B. bassiana,* demonstrated a high capacity to bind Pb. Results of this study also showed that the colony growths of *B. bassiana* on Zn and Pb-spiked media incubated at 4°C compared well with the growths recorded on Zn and Pb-spiked media incubated at 30°C. This may indicate that processes of biosorption and bioaccumulation can potentially occur year round, with the results of the uptake experiments suggesting that both living and dead cells may accumulate metals. Although the experimental conditions are undoubtedly much simpler than conditions in the field, it is felt that the results of the laboratory experiments clearly suggest the potential importance of microbial biosorption and bioaccumulation as processes of heavy metal removal in wetlands receiving urban runoff.

Within wetland treatment systems, sediments are considered to be the most important long-term 'sink' for heavy metals. The roots, rhizomes and leaves of many wetland plant species are also known to bioaccumulate metals, although, as the roots only form a

small fraction of the total plant biomass, and the leaves die-back in winter, only the rhizomes are considered to be of importance in terms of long-term metal storage. The potential of micro-organisms to both remove metals from the water column and act as metal 'sinks' within wetland treatment systems has yet to be quantified in the field. However, it is suggested that the microbial population should be considered as a biomass component capable of binding metals and hence should be included in models of metal removal and dynamics within wetland systems. The question of whether the removal of metals by micro-organisms acts as a long term or short term 'sink' would require further investigation.

A comparison of the amount of metal removed by each of the examined wetland components, in terms of the amount of metal accumulated per gram, shows that, for example, Pb concentrations decrease in the order of *B. bassiana*> *R. mucilaginosa*> sediment> plants. However, if the data were examined in terms of loadings then this trend would be reversed as sediment and plants represent a much greater proportion of the total mass of the system than the two isolates studied. To fully evaluate the ability of these, and other isolates, to remove metals would clearly require much further research. However, the results of this study strongly suggest that such work is warranted.

6.3 Recommendations for further research

The results of the monitoring programme show the ability of two constructed wetland systems to treat urban runoff during their first three years of development. It would be useful to continue the monitoring programme at both sites to examine whether removal performances change with the maturation of the wetland systems. This is particularly important with respect to determining the operational life span of urban runoff treatment wetlands. Several recommendations were also made to improve the treatment performance at both sites, and further monitoring would be necessary to determine the effectiveness of these measures. Further storm events of different magnitudes should also be monitored, to improve the understanding of pollutant behaviour and removal during storm events, and to determine the treatment efficiencies during storm events of various magnitudes and intensities at both systems. Although automatic sampling and flow monitoring equipment could not be left on-site at the Dagenham and Brentwood

wetlands due to vandalism, it is strongly recommended that automatic equipment is used at other urban runoff treatment wetland sites wherever possible, to enable sampling to begin as rainfall commences and to allow the collection of the maximum number of samples.

The development and assessment of further constructed wetland systems for the treatment of urban runoff is recommended. A Decision Support System (DSS) could then be produced which would incorporate the criteria for selecting, designing and monitoring these systems. This study has shown that information on catchment size alone is insufficient to determine the area of a constructed wetland system. Catchment and pollutant characteristics are required at the design stage and a model could be used to evaluate the area of the constructed wetland from catchment and water quality data. Best Management Practice (BMP) guidelines for urban runoff wetland treatment systems could also be developed for future use by Environment Agency staff .

The results of the microbiological experiments show that a range of micro-organisms isolated from urban runoff treatment wetlands are tolerant to high concentrations of Zn and Pb, and that selected isolates are capable of accumulating both metals. This work should be extended to test the ability of the identified isolates to tolerate and accumulate other heavy metals, both in single and multi-metal solutions, in an attempt to further evaluate and quantify the relevant processes, and to elucidate methods of microbial metal accumulation.

Although it would be interesting to screen other individual wetland microbial strains for metal tolerance and to determine their ability to accumulate a range of metals, it is suggested that it would be more valuable to determine the ability of a consortium of micro-organisms isolated from one of the monitored sites to tolerate and accumulate metals in continuous culture. This would have several advantages over the single strain, batch culture experiments used in the research so far. The use of a microbial consortium isolated from an urban wetland site would have the advantage that it is closer to the actual conditions in the field where a wide range of bacteria, fungi and actinomycetes would be competing with each other, and the responses of microbes within a consortium to the presence of heavy metals may be different from those of a single strain in isolation.

In batch culture experiments exponential growth only lasts for a few generations as nutrients are depleted and/or toxic waste products build-up. In continuous culture techniques, cultures can be maintained in the exponential phase through the continual provision of nutrients and the removal of wastes. This would enable experiments to be run over a much longer period of time, and would help to answer the question of whether microbial metal accumulation is potentially a long-term or short-term metal sink. Growth parameters, such as temperature, pH, oxygen concentration and nutrient levels, can be precisely controlled, and varied one parameter at a time. This would enable the optimal conditions for growth and metal accumulation to be identified, and to determine the effects of altering any or all of these parameters. Such optimal conditions could then be compared to the actual values of these parameters reported for wetland systems, and any differences in performance under these conditions evaluated.

These experiments could be taken a step further, using column experiments to provide a closer representation of a wetland environment. This would involve the collection of substrate from the Dagenham or Brentwood wetlands, which would then be sterilised using gamma radiation. The sterile substrate would then be placed in a series of columns and planted with surface-sterilised *Typha* and *Phragmites* seeds, also collected from the monitored sites. Selected columns could then be inoculated with the microbial consortium evaluated in the continuous culture experiment described above. The ability of these columns to remove metals from a metal-spiked solution could then be compared with the ability of columns containing the same composition of sterile substrate and wetland plants, but which had not been inoculated with the microbial consortium.

REFERENCES

Anderson, I. (1996). World's wetlands sucked dry. *New Scientist* **2023**, 9.

Andrade, M. C., Figueira, M. M. M., and Linardi, V. R. (1995). Utilization of ammonia, generated from abiotic cyanide degradation, by *Rhodotorula rubra*. *World Journal of Microbiology and Biotechnology* **11**, 343-344.

Azab, M. S., and Peterson, P. J. (1989). The removal of cadmium from water by the use of biological sorbents. *Water Science and Technology* **21**, 1705-1706.

Babich, H., and Stotzky, G. (1986) in **Montuelle, B., Latour, X., Volat, B., and Gounot, A. M.** (1994). Toxicity of Heavy Metals to Bacteria in Sediments. *Bulletin of Environmental Contamination and Toxicology* **53**, 753-758.

Barko, J. W., and Smart, R. M. (1980). Mobilisation of sediment phophorous by submersed freshwater macrophytes. *Freshwater Biology* **10**, 229-238.

Bascombe, A. D. (1991). Macroinvertebrate Biomonitoring of Urban Runoff Pollution. PhD Thesis. (Middlesex University: Urban Pollution Research Centre).

Belly, R. T., and Kydd, G. C. (1982). Silver resistance in microorganisms. *Developments in Industrial Microbiology* **23**, 567-577.

Berezowsky, M. (1995). Constructed wetlands for the remediation of urban waste waters. *Geoscience Canada* **22**, 129-141.

Bidochka, M. J., and Khachatourians, G. G. (1991). The Implication of Metabolic Acids Produced by *Beauveria bassiana* in pathogenesis of the Migratory Grasshopper, *Melanoplus sanguimipes*. *Journal of Invertebrate Pathology* **58**, 106-117.

Bidochka, M. J., and Khachatourians, G. G. (1993). Oxalic acid hyperproduction in *Beauveria bassiana* mutants is related to utilizable carbon source but not to virulence. *Journal of Invertebrate Pathology* **62**.

Bidochka, M. J., and Khachatourians, G. G. (1994). Basic proteases of entomopathogenic fungi differ in their adsorption properties to insect cuticle. *Journal of Invertebrate Pathology* **64**, 26-32.

Boon, A. G. (1985) in **Cooper, P. F.** (1990). European Design and Operations Guidelines for Reed Bed Treatment Systems. (Swindon: WRc).

Brierley, C. L. (1990). Bioremediation of metal-contaminated surface and groundwaters. *Geomicrobiology* **8**, 201-223.

Brix, H. (1993). Wastewater treatment in constructed wetland systems: system design, removal processes and treatment performance. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 9-22.

Brix, H. (1996). Role of macrophytes in constructed wetlands. *Proceedings of the 5th International Conference on Wetland Systems for Water Pollution Control* , 2-1-2-6.

Brix, H., Schierup, H. H., and Lorenzen, B. (1989) in **Cooper, P. F.** (1990). European Design and Operations Guidelines for Reed Bed Treatment Systems. (Swindon: WRc).

Brix, H., and Schierup, H. H. (1989). Sewage-treatment in constructed reed beds - Danish experiences. *Water Science and Technology* **21**, 1665-1668.

Brodie, G. A. (1989). Selection and Evaluation of Sites for Constructed Wastewater Treatment Wetlands . In *Constructed Wetlands For Wastewater Treatment - Municipal, Industrial and Agricultural.*, D. A. Hammer, eds (Michigan: Lewis), pp. 307-318.

Brown, D. S. (1994). Constructed Wetlands in the USA. *Water Quality Improvement* **4**, 24-28.

Cai, J., Roberts, I. N., and Collins, M. D. (1996). Phylogenetic relationships amongst members of the ascomycetous yeast genera *Brettanomyces*, *Debaryomyces*, *Dekkera* and *Kluyveromyces* deduced by small-subunit rRNA gene sequences. *International Journal of Systematic Bacteriology* **46**, 542-549.

Cannell, R. J. P., Rashid, T., Ismail, I. M., Sidebottom, P. J., Knaggs, A. R., and Marshall, P. S. (1997). Novel metabolites of warfarin produced by *Beauveria bassiana* and *Streptomyces rimosus*. *Xenobiotica* **27**, 147-157.

Carlile, M. J., and Watkinson, S. C. (1994). The Fungi. (London: Academic Press).

Chambers, J. M., and McComb, A. J. (1994). Establishing Wetland Plants in Artificial Systems. *Water Science and Technology* **29**, 79-84.

Chandler, D., Hay, D., and Reid, A.P. (1997). Sampling and occurrence of entomopathogenic fungi and nematodes. *Applied Soil Ecology* **5**, 133-141.

Chong, S., Garelick, H., Revitt, D. M., Shutes, R. B. E., Worral, P., and Brewer, D. (1999). The microbiology associated with glycol removal in constructed wetlands. *Water Science and Technology (in Press).*

CIRIA (1997). Design Guidance. *Design and Management of Constructed Wetlands for the Treatment of Wastewater*, 21-49.

CIRIA (1986). Sediment Movement in Combined Sewage and Stormwater Drainage Systems. (London: Research Project 366, CIRIA/HR).

Cole, R., Frederick, R., Healy, R., and Rolan, R. (1984). Preliminary findings of the priority pollutant monitoring project of the nationwide urban runoff program. *J. Water Pollut. Control Fed.* **56**, 898-908.

Cooper, P. F. (1990). European Design and Operations Guidelines for Reed Bed Treatment Systems. (Swindon: WRc).

Cooper, P. F. (1993). The use of reed bed systems to treat domestic sewage: the European design and operation guidelines for reedbed treatment systems. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 203-217.

Cooper, P. F., and Green, B. (1995). Reed Bed Treatment Systems For Sewage Treatment In The UK - The First 10 Years Experience. *Water Science and Technology* **32**, 317-327.

Cooper, P. F., Job, G. D., Green, M. B., and Shutes, R. B. E. (1996). Reed Beds and Constructed Wetlands for Wastewater Treatment. (Swindon: WRc).

Crites, R. W. (1994). Design Criteria and Practice for Constructed Wetlands. *Water Science and Technology* **29**, 1-6.

Dietz, A., and Thayer, D. W. (1980). Actinomycete Taxonomy (Procedures for Studying Aerobic Actinomycetes with Emphasis on the *Streptomyces*). (Special Publication Number 6: Society for Industrial Microbiology).

Duncan, C. P., and Groffman, P. M. (1994). Comparing Microbial Parameters in Natural and Constructed Wetlands. *Journal on Environmental Quality* **23**, 298-305.

EC Directive on Quality Requirements for Surface Waters Intended for the Abstraction of Drinking Water 1975 (75/440/EEC) - Surface Water Abstraction Directive.

EC Directive on Pollution Caused by Dangerous Substances Discharged in the Aquatic Environment on the Community (1976) (76/464/EEC) - Dangerous Substances Directive.

EC Directive on the Quality of Fresh Waters Needing Protection or Improvement to Support Fish Life 1978 (78/659/EEC) - Freshwater Fisheries Directive.

Eger, P. (1994). Trace Metal Removal From Acid Mine Drainage. *Water Science and Technology* **29**, 249-256.

Ehrlich, H. L., and Fox, S. I. (1967). Copper Sulphide Precipitation by Yeasts from Acid Mine-Waters. *Applied Microbiology* **15**, 135-139.

Ellis, J. B. (1993). Pollution of stormwater and combined wastewater: a discussion. *Dimensioning of Sewers and Stormwater Treatment Systems,* EWPCA/ATU Workshop, Munich.

Ellis, J. B. (1994). Highway Discharge Quality: Pollution Potential and Treatment. . In *Proceeding of Standing Conference on Stormwater Source Control Quantity and Quality.*, C. J. Pratt, eds (Coventry: School of the Built Environment).

Ellis, J. B., and Revitt, D. M. (1991). Drainage From Roads: Control and Treatment of Highway Runoff in Report *NRA 43804/MID.012*, Commissioned by Thames NRA, Technical Services Administration.

Ellis, J. B., Revitt, D. M., Shutes, R. B. E., and Langley, J. M. (1994). The Performance of Vegetated Biofilters for Highway Runoff Control. *Science of the Total Environment* **146**, 543-550.

Ewel, K. C. (1987). The Use Of Wetlands For Wastewater Treatment. *ERC-015a,* Ecosystems Research Centre, Cornell University, 21-25.

Farahbakhshazad, N., and Morrison, G. M. (1998). A constructed vertical macrophyte system for the return of nitrogen to agriculture. *Proceedings of the 6th International Conference on Wetlands for Water Pollution Control.*

Fargues, J., Goettel, M. S., Smits, N., Ouedraogo, A., and Rougier, M. (1997). Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different environments. *Mycologia* **89**, 383-392.

Farkas, V. (1979). Biosynthesis of cell walls of fungi. *Microbiological Reviews* **43**, 117-144.

Faulkner, S. P., and Richardson, C. J. (1989). Physical and Chemical Characteristics of Freshwater Wetland Soils. In *Constructed Wetlands for Wastewater Treatment - Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 41-72.

Fifield, F. W., and Kealey, D. (1989). Principles and Practice of Analytical Chemistry. (Glasgow: Blackie).

Fortin, D., Davis, B., Southam, G., and Beveridge, T. J. (1995). Biogeochemical phenomena induced by bacteria within sulphidic mine tailings. *Journal of Industrial Microbiology* **14**, 178-185.

Fourest, E., and Roux, J. C. (1992). Heavy metal biosorption by fungal mycelial by-products: mechanism and influence of pH. *Applied Microbiology and Biotechnology* **37** , 399-403.

Fuganti, C., Minut, J., Fantoni, G. P., and Servi, S. (1998). On the microbial transformation of alpha, beta-saturated aryl ketones by the fungus *Beauveria bassiana*. *Journal of Molecular Catalysis B: Enzymatic* **4**, 47-52.

Gabriel, J., Mokrejs, M., Bily, J., and Rychlovsky, P. *Folia Microbiology* **39**, 115-118.

Gadd, G. M. (1990). Fungi and Yeasts for Metal Accumulation. In *Microbial Mineral Recovery*, H. L. Ehrlich, and C. L. Brierley, eds (New York: McGraw-Hill Publishing), pp. 249-275.

Gadd, G. M.24 (1993). Transley Review No. 47. Interactions of fungi with toxic metals. *New Phytologist* **124**, 25-60.

Gadd, G. M., and Griffiths, A. J. (1978). Microorganisms and heavy metal toxicity. *Microbial Ecology* **4**, 303-317.

George, D. B., and Kemp, M. C. (1998). Design of subsurface flow wetlands treating municipal wastewater. *Proceedings of the 5th International Conference on Wetlands for Water Pollution Control* , 3,1-3,8.

Gersberg, R. M., Elkins, B. V., and Goldman, C. R. (1984). Wastewater Treatment by Artificial Wetlands. *Water Science and Technology* **17**, 443-450.

Golubev, V. I., and Churkina, L. G. (1997). Sensitivity to mycocins and morphological and physiological characteristics of authentic strains of *Rhodotorula mucilaginosa* synonyms. *Microbiology* **66**, 209-215.

Grant, N. (1995) in **Cooper, P. F., Job, G. D., Green, M. B., and Shutes, R. B. E.** (1996). Reed Beds and Constructed Wetlands for Wastewater Treatment. (Swindon: WRc plc).

GromaireMertz, M. C., Garnaud, S., Gonzalez, A., and Chebbo, G. (1999). Characterisation of urban runoff pollution in Paris. *Water Science and Technology* **39**, 1-8.

Halcrow Environmental Services (1993). *Good Roads Guide: The Water Environment.* (Swindon: Sir William Halcrow and Peter Brett Associates)

Halcrow Environmental Services (1996). *The Design and Performance of Vegetated Treatment Systems for Highway Runoff - Literature Review and Feasibility Study*. (Swindon: Sir William Halcrow and Partners Ltd.).

Hammer, D. A., and Bastian, R. K. (1989). Wetlands Ecosystems: Natural Water Purifiers?. In *Constructed Wetlands for Wastewater Treatment*, D. A. Hammer, eds (Michigan: Lewis), pp. 5-19.

Hari, R. P. K., Patel, T., and Martin, A. (1992). A new strain of *Rhodotorula rubra* isolated from yogurt. *Journal of Industrial Microbiology* **11**, 43-51.

Harrison, R. M., and De Mora, S. J. (1996). Introductory Chemistry for Environmental Sciences. (Cambridge: Cambridge University Press).

Haslam, S. M. (1994). Wetland Habitats. DOE Report N0. DoE/HMIP/RR/040.

Hatano, K., Trettin, C. C., House, C. H., and Wollum, A. G. (1993). Microbial Populations and Decomposition Activity in Three Subsurface Flow Constructed Wetlands. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 541-548.

Hatano, K., Frederick, D. J., and Moore, J. A. (1994). Microbial Ecology of Constructed Wetlands Used for Treating Pulp Mill Wastewater. *Water Science and Technology* **29**, 233-240.

Hazarika, L. K., and Puzari, K. C. (1997). Field efficiency of white muscardine fungus (*Beauveria bassiana*) on rice hispa. *Indian Journal of Agricultural Sciences* **67**, 463-465.

Hemond, H. F., and Benoit, J. (1988). Cumulative impacts on water quality functions of wetlands. *Environmental Management* **12**, 639-653.

Herskowitz, J. (1986) in **Kadlec, R. H., and Knight, R. L.** (1996). Treatment Wetlands. (Boca Raton: Lewis).

Higgins, M. J., Rock, C. A., Bouchard, R., and Wengrezynek, B. (1993). Controlling agricultural runoff by the use of constructed wetlands. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 359-367.

Hiley, P. D. (1994). The reality of sewage treatment using wetlands. *Proceedings of the 4th International Conference on Wetland Systems for Water Pollution Control*, 68-83.

Hoffman, E. J., and Quinn, J. G. (1987). Chronic Hydrocarbon Discharges into Aquatic Environments: 2. Urban Runoff and Combined Sewer Overflows. In *Oil in Freshwater, Chemistry, Biology and Countermeasure Technology.*, J. H. Vandermeulen, and S. E. Hrudey, eds (New York: Pergamon), pp. 114-137.

Holan, Z. R., and Volesky, B. (1995). Accumulation of cadmium, lead and nickel by fungal and wood biosorbents. *Applied Biochemistry and Biotechnology* **53**, 133-146.

Horikoshi, T., Nakajima, A., and Sakaguchi, T. (1981). Studies on the Accumulation of Heavy Metal Elements in Biological Systems. XIX. Accumulation of Uranium by Microorganisms. *European Journal of Applied Microbiology and Biotechnology* **12**, 90-96.

Howard, E., Wildeman, T. R., Laudon, L. S., and Machemer, S. D. (1989) in **Mungur, A. S.** (1997). The Performance and Perception of Wetland Systems for the Treatment of Highway Runoff. PhD Thesis. (Middlesex University: Urban Pollution Research Centre).

Huang, C. P., Huang, C. P., and Morehart, A. L. (1990). The removal of Cu (II) from dilute aqueous solutions by *Saccharomyces cerevisiae. Water Research* **24**, 433-439.ar

Hughes, M. N., and Poole, R. K. (1989). Metals and microorganisms. (London: Chapman and Hall).

Hvitved-Jacobsen, T., Johansen, N. B., and Yousef, Y. A. (1994). Treatment systems for urban and highway run-off in Denmark. *The Science of the Total Environment* **146**, 499-506.

Inglis, G. D., Johnson, D. L., and Goettel, M. S. (1997). Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. *Canadian Entomologist* **129**, 171-186.

Johansen, N. H., and Brix, H. (1996). Design criteria for a two-stage constructed wetland. *Proceedings of the 5th International Conference on Wetlands for water Pollution Control.*

Jones, D., and Muehlchen, A. (1994). Effects of potentially toxic metals aluminium, zinc and copper on ectomycorrhizal fungi. *J. Eviron. Sci Health* **A29**, 949-966.

Junghans, K., and Straube, G. (1991). Biosorption of copper by yeasts. *Biology of Metals* **4**, 233-237.

Kadlec, R. H. (1989). Hydrologic Factors in Wetland Water Treatment. In *Constructed wetlands for Wastewater Treatment - Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 21-40.

Kadlec, R. H., and Knight, R. L. (1996). Treatment Wetlands. (Boca Raton: Lewis).

Kapoor, A., and Viraraghavan, T. (1995). Fungal Biosorption - An Alternative Treatment Option for Heavy Metal Bearing Wastewaters: A Review. *Bioresource Technology* **53**, 195-206.

Kapoor, A., and Viraraghavan, T. (1998). Removal of heavy metals from aqueous solutions using immobilized fungal biomass in continuous mode. *Water Research* **32**, 1968-1977.

Karavaiko, G. I., Zakharova, V. I., Avakyan, Z. A., and Strizhko, L. S. (1996). Selective extraction of noble metals from solutions by micro-organisms. *Applied Biochemistry and Microbiology* **32**, 507-510.

Karpiscak, M. M., Freitas, R. J., Gerba, C. P., Sanchez, L. R., and Shamir, E. (1998). Management of dairy waste in the Sonoran desert using constructed wetland technology. *Proceedings of the 6th International Conference on Wetland Systems for water Pollution Control.*

Kierans, M., Staines, A. M., Bennet, H., and Gadd, G. M. (1991). Silver Tolerance and Accumulation in Yeasts. *Biology of Metals* **4**, 100-106.

Kitchens, L. J. (1987). Exploring Statistics: A Modern Introduction to Data Analysis and Inference. (St. Paul: West Publishing Company).

Knight, R. L., Ruble, R. W., Kadlec, R. H., and Reed, S. C. (1993). Wetlands for wastewater treatment: performance database. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 35-58.

Kurek, E., Czaban, J., and Bollag, J. M. (1982). Sorption of Cadmium by Micro-organisms in Competition with other Soil Constituents. *Applied and Environmental Microbiology* **43**, 1011-1015.

Kuyucak, N., and Volesky, B. (1988). Biosorbents for recovery of metals from industrial solutions. *Biotechnology Letters* **10**, 137-142.

Lan, C. Y., Chen, G. H., Li, L. C., and Wong, M. H. (1992). Use of cattails in treating wastewater from a Pb/Zn mine. *Environmental Management* **16**, 75-80.

Lawrence, I., and Breen, P. (1998). Design guidelines: Stormwater pollution control ponds and wetlands. (Canberra: The Cooperative Research Centre).

Leung, D. (1992). Field and Laboratory Studies of the Effects of Heavy Metals on *Rhizobium* and its Interaction with White Clover. (Kings College, London).

Livingston, E. H. (1989). Use Of Wetlands For Urban Storm Water Management. In *Constructed Wetlands For Wastewater Treatment - Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 253-264.

Logrieco, A., Moretti, A., Castella, G., Kostecki, M., Golinski, P., Ritieni, A., and Chelkowski, J. (1998). Beauvericin production by *Fusarium* species. *Applied and Environmental Microbiology* **64**, 3084-3088.

Macaskie, L. E., and Dean, A. C. R. (1990) in **Gadd, G. M.** (1993). Transley Review No. 47. Interactions of fungi with toxic metals. *New Phytologist* **124**, 25-60.

Mackney, B. J. (1990). The Design of Wetlands for Wastewater Treatment: An Australian Perspective. In *Constructed Wetlands in Water Pollution Control.*, P. F. Cooper, and B. C. Findlater, eds (Oxford: Pergamon Press), pp. 471-479.

Maltby, L., Forrow, D., Boxall, A., Calow, P., and Clifford, C. (1994). The Effects of Motorway Runoff on Freshwater Ecosystems: 1. Field Study. *Environmental Toxicology and Chemistry* **14**, 1079-1092.

Martin, C. D., Johnson, K. D., and Moshiri, G. A. (1998). Performance of a constructed wetland leachate treatment system at the Chunchula Landfill Mobile County, Alabama. *Proceedings of the 6th International Conference on Wetland Systems for water Pollution Control.*

Mattuschka, B., and Straube, G. (1993). Biosorption of Metals by a Waste Biomass. *Journal of Chemical Biotechnology* **58**, 57-63.

McKee, K. L., Mendelssohn, I. A., and Burdick, D. M. (1989). Effect of long-term flooding on root metabolic response in five freshwater marsh plant species. *Canadian Journal of Botany* **67**, 3446-3452.

Meiorin, E. C. (1989). Urban runoff treatment in a fresh/brackish water marsh in Fremont, California. In *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural.* D. A. Hammer, eds (Michigan: Lewis), pp. 677-685.

Merritt, A. (1994). Wetlands, Industry and Wildlife - A Manual of Principles and Practices. The Wildfowl and Wetlands Trust. (Newcastle upon Tyne: Hindson Print Limited).

Middelhoven, W. J. (1997). Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* **72**, 81-89.

Middelhoven, W. J., and Spaaij, F. (1997). *Rhodotorula cresolica* sp. nov, a cresol-assimilating yeast species isolated from soil. *International Journal of Systematic Bacteriology* **47**, 324-327.

Montuelle, B., Latour, X., Volat, B., and Gounot, A. M. (1994). Toxicity of Heavy Metals to Bacteria in Sediments. *Bulletin of Environmental Contamination and Toxicology* **53**, 753-758.

Morely, G. F., and Gadd, G. M. (1995). Sorption of toxic metals by fungi and clay minerals. *Mycology Research* **12**, 1429-1438.

Morrison, G. M., Revitt, D. M., Ellis, J. B., Svenson, G., and Balmer, P. (1984). The physico-chemical speciation of zinc, cadmium, lead and copper in urban stormwater. In *Planning and Control of Urban Storm Drainage*, P. Balmer, P. A. Malmquist, and A. Sjoberg, eds (Gothenburg: Chalmers University of Technology), pp. 989-1000.

Mullen, M. D., Wolf, D. C., Beveridge, T. J., and Bailey, G. W. (1992). Sorption of heavy metals by the soil fungi *Aspergillus niger* and *Mucor rouxii*. *Soil Biology and Biochemistry* **24**, 129-135.

Mulliss, R. M., Revitt, D. M., and Shutes, R. B. E. (1996). The impacts of urban discharges on the hydrology and water quality of an urban watercourse. *The Science of the Total Environment* **190**, 385-390.

Mulliss, R. M. (1994). The Ecotoxicological Impacts of Urban Discharges. PhD Thesis (Middlesex University: Urban Pollution Research Centre).

Mungur, A. S. (1997). The Performance and Perception of Wetland Systems for the Treatment of Highway Runoff. PhD Thesis. (Middlesex University: Urban Pollution Research Centre).

Mungur, A. S., Shutes, R. B. E., House, M. A., Revitt, D. M., and Robinson, M. T. (1994). A Constructed Wetland for the Treatment of Highway Runoff in the United Kingdom. *International Association on Water Quality, Newsletter* **No.11**, 7-12.

Mungur, A. S., Shutes, R. B. E., Revitt, D. M., and House, M. A. (1995). An Assessment of Highway Runoff Treatment by a Natural Wetland. *Water Science and Technology* **32**, 169-175.

Mungur, A. S., Shutes, R. B. E., Revitt, D. M., and House, M. A. (1997). An Assessment of Metal Removal by a Laboratory Scale Wetland. *Water Science and Technology* **35**, 125-133.

Mungur, A. S., Shutes, R. B. E., Revitt, D. M., House, M. A., and Fallon, C. (1998). A constructed wetland for the treatment of urban runoff. In *Proceedings of the International Conference on the Remediation and Management of Degraded Lands, Hong Kong*, M. H. Wong, eds.

Murphy, R. J., and Levy, J. F. (1983). Production of copper oxalate by some copper tolerant fungi. *Transactions of the British Mycological Society* **81**, 165-168.

Nakajima, A., and Sakaguchi, T. (1986). Selective accumulation of heavy metals by microorganisms. *Applied Microbiology and Biotechnology* **24**, 59-64.

Protection of the Water Environment Using Balancing Facilities (1999). Second Interim Report on Monitoring Results. (Middlesex University: Urban Pollution Research Centre).

Niu, H., Xu, X. S., Wang, J. H., and Volesky, B. (1993). Removal of Pb from aqueous solutions by *Penicillium* biomass. *Biotechnology and Bioengineering* **42**, 785-787.

Norris, P. R., and Kelly, D. P. (1979). Accumulation of metals by bacteria and yeasts. *Developments in Industrial Microbiology* **20**, 299-308.

Olson, R. K. (1992). Evaluating the role of created and natural wetlands in controlling non-point source pollution. *Ecological Engineering* **1**, xi-xv.

Omidi, G. H., Thomas, J. C., and Brown, K. W. (1996). Effect of Desiccation Cracking on the Hydraulic Conductivity of a Compacted Clay Liner. *Water, Air and Soil Pollution* **89**, 91-103.

Pecanha, M. P., Pagnocca, F. C., Rugani, C. A., and Neves, F. A. (1996). Yeasts and other parameters of pollution of the Ribeirao Claro stream in Rio Claro, Sao Paulo. *Revista de Microbiologica* **27**, 177-181.

Persson, J., Somes, N. L. G., and Wong, T. H. F. (1998). Hydraulic efficiency of constructed wetlands and ponds. *Proceedings of the 6th International Conference on Wetlands for Water Pollution Control.*

Pietz, S., Wolker, D., and Haufe, G. (1997). Selectivity of the biooxygenation of the N-phenylcarbamates by the fungus *Beauveria bassiana*. *Tetrahedron* **53**, 17067-17078.

Portier, R. J., and Palmer, S. J. (1989). Wetlands microbiology: form, function and processes. In *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 89-103.

Pradhan, A. A., and Levine, A. D. (1995). Microbial Biosorption of Copper and Lead from Aqueous systems. *The Science of the Total Environment* **170**, 209-220.

Reed, S. C., Middlebrooks, F. J., and Crites, R. W. (1988). Natural Systems for Waste Management and Treatment. (New York: McGraw-Hill).

Reed, S. C., and Brown, D. (1995). Subsurface flow wetlands - a performance evaluation. *Water Environment Research* **67**, 244-248.

720

Reinelt, L. E., and Horner, R. R. (1995). Pollutant removal from stormwater runoff by palustrine wetlands based on comprehensive budgets. *Ecological Engineering* **4**, 77-97.

Revitt, D. M., Shutes, R. B. E., and Scholes, L. N. L. (1999). The use of constructed wetlands for reducing the impacts of urban surface runoff on receiving water quality. In *Impacts of Urban Growth on Surface Water and Ground Water Quality* , J. B. Ellis, eds (Wallingford: IAHS Press), pp. 349-356.

Roane, T. M., and Kellogg, S. T. (1996). Characterization of bacterial communities in heavy metal contaminated soils. *Canadian Journal of Microbiology* **42**, 593-603.

Rowe, C. B. (1998). The upflow option for vertical flow rootzone wetlands, a New Zealand experience. *Proceedings of the 6th International Conference on Wetlands for Water Pollution Control.*

Sage, L., Bennasser, L., Steiman, R., and Seigle Murandi, F. (1997). Fungal microflora biodiversity as a function of pollution in Oued Sebou (Morocco). *Chemosphere* **35**, 751-759.

Salomons, W., and Forstner, U. (1984). Metals in the Hydrocycle. (Berlin: Springer-Verlag).

Samuels, M. L. (1989). Statistics for the Life Sciences. (San Francisco: Dellen).

Sayer, J. A., and Gadd, G. M. (1997). Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspegillus niger*. *Mycology Research* **101**, 653-661.

Schiffer, D. M. (1989). Water-Quality Variability in a Central Florida Wetland Receiving Highway Runoff. In *Water: Laws and Management*, F. E. Davis, eds (Bethesda: American Water Research Association).
i-720

Scholes, L. N. L., Shutes, R. B. E., Revitt, D. M., Forshaw, M., Andrews, K., and Purchase, D. (1995). Constructed wetlands and Sustainable Management in the UK. *International Association on Water Quality, Newsletter* **No. 13**, 11-13.

Shih, H. L., Lin, C. P., Liou, R. F., and Tzean, S. S. (1995). Complete nucleotide sequence of *Beauveria bassiana* 5.8s rRNA coding gene and flanking internal transcribed spacers. *DNA Sequencing* **5**, 381-383.

Shimazu, M., and Sato, H. (1996). Media for the selective isolation of an entomogenous fungus, *Beauveria bassiana* (Deuteromycotina: Hyphomycetes). *Applied Entomology and Zoology* **31**, 291-298.

Shutes, R. B. E., Revitt, D. M., Mungur, A. S., and Scholes, L. (1997). The design of wetland systems for the treatment of urban runoff. *Water Science and Technology* **35**, 19-25.

Shutes, R. B. E., Revitt, D. M., Lagerberg, I. M., and Barraud, V. C. E. (1999). The design of vegetative constructed wetlands for the treatment of highway runoff. *The Science of the Total Environment* **235**, 189-197.

Sing, C., and Yu, J. (1998). Copper adsorption and removal from water by living mycelium of white-rot fungus *Phanerochaete chrysosporium*. Water Research **32**, 2746-2752.

Slavikova, E., Vadkertiova, R., and Kockovakratochvilova, A. (1992). Yeasts isolated from artificial lake water. *Canadian Journal of Microbiology* **38**, 1206-1209.

Smith, A. J. (1989). Wastewaters: A Perspective. In *Constructed Wetlands for Wastewater Treatment.*, D. A. Hammer, eds (Michigan: Lewis), pp. 3-4.

Sobolewski, A. (1996). Metal species indicate the potential of constructed wetlands for long-term treatment of metal mine drainage. *Ecological Engineering* **6**, 259-271.

Steiner, G. R., and Freeman, R. J. (1989). Configuration and substrate design considerations for constructed wetlands for wastewater treatment. In *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 363-377.

Strandberg, G. W., Shumate, S. E., and Parrott, J. R. (1981). Microbial cells as biosorbents for heavy metals; accumulation of Uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* **41**, 237-245.

Subramanian, C. V. (1983). Hyphomycetes: Taxonomy and Biology. (London: Academic Press).

Surface Waters (River Ecosystem)(Classification) Regulations. (1994) SI 1994 N0. 1057.

Swedish Environmental Protection Agency (1991). Quality criteria for lakes and watercourses: a system for classification of water chemistry and sediment and organism metal concentrations. Swedish Environmental Protection Agency (ISBN: 91-620-1107-3).

Tebbut, T. H. Y. (1992). Principles of Water Quality Control. (Oxford: Butterworth-Heinman Ltd.).

Tettleton, R. P., Howell, F. G., and Reaves, R. P. (1993). Performance of a constructed marsh in the tertiary treatment of bleach kraft pulp mill effluent. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 437-440.

Tilley, D. R., and Brown, M. T. (1998). Wetland networks for stormwater management in subtropical urban watersheds. *Ecological Engineering* **10**, 131-158.

Tobin, J. M., Cooper, D. G., and Neufield, R. J. (1984). Uptake of metal ions by *Rhizopus arrhizus* biomass. *Applied Environmental Microbiology* **47**, 821-824.

Tomljanovich, D. A., and Perez, O. (1989). Constructing the Wastewater Treatment Wetland - Some Factors to Consider. In *Constructed Wetlands for Wastewater Treatment - Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 399-404.

Townsley, C. C., and Ross, I. S. (1986). Copper uptake in *Aspergillus niger* during batch growth and in non-growing mycelial suspensions. *Experimental Mycology* **10**, 281-288.

Tsezos, M., and Volesky, B.24 (1982). The mechanism of uranium biosorption by *Rhizopus arrhizus*. *Biotechnology and Bioengineering* **24**, 385-401.

Tsezos, M., Remoudaki, E., and Angelatou, V. (1997). Biosorption sites of selected metals using electron microscopy. *Comparative Biochemistry and Physiology* **118A**, 481-487.

UNCED (1992). Agenda 21. United Nations Conference on the Environment and Development, Conches, Switzerland.

Urban Wastewater Treatment Directive (1991). Council of European Communities - 91/271/EEC.

Vadkertiova, R., and Slavikova, E. (1994). Yeasts from sediments and soil along the Lake Jakubov. *Biologia, Bratislava* **49**, 841-847.

Volesky, B. (1990). Biosorption of heavy metals. (Boca Raton: CRC press).

Volesky, B. (1994). Advances in biosorption of metals: Selection of biomass types. *FEMS Microbiology Reviews* **14**, 291-302.

Volesky, B., and Holan, Z. R. (1995). Biosorption of heavy metals. *Biotech. Prog.* **11**, 235-250.

Volesky, B., and May-Phillips, H. A. (1995). Biosorption of heavy metals by *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology* **42**, 797-806.

Wainwright, M., Grayston, S. J., and De Jong, P. M. (1986). Adsorption of insoluble compounds by the mycelium of the fungus *Mucor flavus*. *Enzyme and Microbial Technology* **8**, 597-600.

Watson, J. T., and Hobson, J. A. (1989). Hydraulic Design Considerations and Control Structures for Constructed Wetlands for Wastewater Treatment. In *Constructed Wetlands for Wastewater Treatment - Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 379-392.

Watson, J. T., Reed, S. C., Kadlec, R. H., Knight, R. L., and Whitehouse, A. E. (1989). Performance Expectations and Loading Rates for Constructed Wetlands. In *Constructed Wetlands for Wastewater Treatment - Municipal, Industrial and Agricultural*, D. A. Hammer, eds (Michigan: Lewis), pp. 319-351.

Weast, R. C., and Astle, M. J. (1979). Handbook of Chemistry and Physics. (Boca Raton: CRC Press, Inc.).

Webb, J. S., McGinness, S., and Lappin-Scott, H. M. (1998). Metal removal by sulphate-reducing bacteria from natural and constructed wetlands. *Journal of Applied Microbiology* **84**, 240-248.

Wetzel, R. G. (1993). Constructed wetlands: Scientific foundations are critical. In *Constructed Wetlands for Water Quality Improvement*, G. A. Moshiri, eds (Boca Raton: Lewis), pp. 3-7.

White, C., Sayer, J. A., and Gadd, G. M. (1997). Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiology Reviews* **20**, 503-516.

Williams, C. J., Aderhold, D. and Edyvean, R. G. J. (1998). Comparison between biosorbents for the removal of metal ions from aqueous solutions. *Water Research* **32**, 216-224.

Wong, H. F., and Somes, N. L. G. (1995). A Stochastic Approach to Designing Wetlands for Stormwater Pollution Control. *2nd International Conference on Innovative Technologies in Urban Stormwater Drainage.* 303-310.

Wong, T. H. F., and Geiger, W. F. (1997). Adaptation of wastewater surface flow wetland formulae for application in constructed stormwater wetlands . *Ecological Engineering* **9**, 187-202.

Wood, A. (1991). Constructed Wetlands for Wastewater Treatment and Design Considerations. In *Constructed Wetlands in Water Pollution Control.*, D. A. Hammer, eds (Oxford: Pergamon Press.), pp. 481-494.

Wood, A. (1994). Constructed wetlands for water pollution Control: Fundamentals to their understanding. *Proceedings of the 4th International Conference on Wetland Systems for Water Pollution Control* , 25-34.

Ye, Z. H., Baker, A. J. M., Wong, M. H., and Willis, A. J. (1997). Zinc, lead and cadmium tolerance, uptake and accumulation by the common reed, *Phragmites australis* (Cav) Trin ex Steudel. *Annals of Botany* **80**, 363-370.

Yousef, Y. A., Lin, L., Lindeman, W., and Hvitved-Jacobsen, T. (1994). Transport of heavy metals through accumulated sediments in wet ponds. *The Science of the Total Environment* **146**, 485-491.

Zajic, J. E., and Chiu, Y. S. (1972). in **Kapoor, A., and Viraraghavan, T.** (1995). Fungal Biosorption - An Alternative Treatment Option for Heavy Metal Bearing Wastewaters: A Review. *Bioresource Technology* **53**, 195-206.

Zhang, L., Zhao, L., Yu, Y., and Chen, C. (1998). Removal of lead from aqueous solution by non-living *Rhizopus nigricans*. *Water Research* **32**, 1437-1444.

Zhang, T. T. (1990). The Use of Macrophytes for Heavy Metal Pollution Control in Urban Wetlands. MPhil Thesis. (Middlesex University: Urban Pollution Research Centre).

Zhang, T. T., Ellis, J. B., Revitt, D. M., and Shutes, R. B. E. (1990). Metal Uptake and Associated Pollution Control by Typha latifolia in Urban Wetlands. In *Constructed Wetlands in Water Pollution Control*, P. F. Cooper, and B. C. Findlater, eds (Oxford: Pergamon Press), pp. 451-459.

Appendix I

The following Tables give the aqueous, sediment and plant tissue concentrations of all of the monitored determinands at each of the identified sampling points at the Dagenham and Brentwood wetland systems. In the determination of metal concentrations, duplicate samples were analysed. In order to appreciate variability between replicate samples, the standard deviations associated with the mean values are given in the Table immediately following the Table of mean values. The following key applies for all of the Tables:

ND = not detected

NS = sample not collected

* = only one sample

Table I- 1 pH values recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	7.9	8.1	6.4	7.3	7.5	7.4	7.5	7.1	7.2	7.5
D2	7.0	7.9	8.3	6.3	7.1	7.8	7.4	7.4	6.8	7.2	7.8
D3	7.0	7.7	8.3	7.4	7.2	7.5	7.5	7.4	6.9	7.2	7.7
D4	6.9	7.6	8.1	7.7	7.5	7.4	7.4	7.5	6.8	7.0	7.9
D5	6.7	7.5	7.6	7.4	7.9	7.5	7.5	7.6	7.0	7.0	6.8
B1	6.9	7.6	6.1	7.7	7.6	7.9	8.0	7.7	7.7	7.4	7.2
B2	7.0	6.3	8.4	7.4	7.6	8.1	7.8	7.0	7.3	7.4	7.0
B3	7.0	7.6	8.5	7.6	7.6	8.0	7.8	6.9	7.3	7.4	7.1
B4	7.0	7.6	8.2	7.6	7.2	7.5	7.4	7.4	7.2	7.2	7.4
B5	6.9	7.3	8.2	7.6	7.5	7.7	7.8	7.6	7.1	7.2	7.5
B6	7.6	7.8	8.0	7.6	7.8	8.1	7.8	7.5	7.4	7.4	7.5
B7	7.3	7.7	6.1	7.6	7.6	8.2	7.9	7.5	8.0	7.5	7.7

Table I- 2 Dissolved oxygen concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	ND	7.80	3.66	3.60	7.20	9.10	7.40	5.80	3.74	5.80
D2	5.23	ND	7.90	4.06	4.40	6.65	8.98	7.10	6.45	3.55	7.64
D3	3.75	ND	7.20	2.24	2.93	3.80	7.22	6.39	4.83	3.90	7.74
D4	8.52	ND	7.90	6.45	6.40	2.12	7.07	8.30	8.26	4.00	5.80
D5	12.10	7.80	8.80	9.85	ND	4.44	8.30	11.16	10.44	3.81	5.55
B1	10.81	11.44	10.60	5.91	ND	5.19	10.19	5.56	5.55	8.79	7.20
B2	8.99	11.49	10.70	4.52	7.57	5.48	12.45	5.77	5.70	9.50	5.77
B3	8.67	11.50	10.60	4.55	ND	5.44	11.94	5.74	5.60	9.62	11.40
B4	10.67	11.45	10.40	2.95	ND	3.47	1.92	1.92	8.50	6.66	5.49
B5	9.84	10.34	10.40	3.85	ND	4.76	7.71	5.50	6.33	6.40	7.15
B6	9.10	10.85	10.60	6.50	5.02	9.30	11.25	4.87	7.80	6.31	4.80
B7	8.96	11.15	10.80	5.85	6.90	9.55	11.83	5.92	5.05	7.82	5.45

Table I- 3 Temperatures (°C) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	ND	8.50	18.10	17.20	11.90	5.30	13.40	18.20	19.00	11.60
D2	15.00	ND	8.30	19.30	17.70	12.40	5.30	13.00	18.20	19.00	12.00
D3	15.60	ND	8.40	16.60	18.50	12.60	5.10	13.60	18.20	18.20	12.10
D4	13.90	ND	7.10	16.30	18.80	12.60	4.70	12.70	17.40	18.20	12.30
D5	16.60	10.00	8.60	16.30	21.10	13.00	5.10	15.50	19.20	17.80	11.90
B1	12.10	8.10	6.70	13.00	15.20	24.90	6.90	8.40	16.80	21.00	12.00
B2	13.00	8.70	7.90	14.30	18.00	24.50	5.50	11.60	16.00	16.90	11.20
B3	13.00	7.80	7.70	14.50	15.30	25.10	5.60	12.10	14.90	17.30	10.70
B4	12.60	8.10	8.30	14.70	15.10	19.80	5.40	10.40	14.20	17.20	10.00
B5	14.20	7.90	7.90	14.70	14.90	24.90	5.60	11.20	14.20	17.00	9.30
B6	14.40	8.10	8.10	15.80	14.10	24.80	5.80	10.10	14.40	17.40	9.00
B7	14.60	8.20	8.50	15.80	14.10	24.90	6.40	12.10	14.50	18.40	9.20

Table I- 4 BOD_5 concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	14.8	6.0	10.4	4.0	2.4	4.0	5.2	ND	2.8	14.0
D2	17.6	14.8	7.6	8.8	2.4	2.4	6.8	4.0	ND	4.0	5.6
D3	16.4	19.2	8.4	27.2	1.3	2.8	1.2	7.6	ND	2.8	5.6
D4	15.2	18.4	6.4	6.0	1.2	3.2	7.6	6.4	ND	4.0	6.4
D5	17.6	17.6	8.4	13.2	1.6	0.4	4.0	4.4	ND	5.6	5.6
B1	16.4	19.2	8.0	5.6	2.0	6.0	5.2	11.2	3.6	3.2	7.6
B2	18.4	18.8	13.2	9.2	2.0	8.0	2.4	7.2	4.0	2.0	8.0
B3	28.0	16.0	12.4	11.2	1.6	7.6	8.8	9.2	3.6	5.6	6.4
B4	15.6	15.2	13.2	7.6	2.4	5.2	2.0	10.0	2.0	4.8	4.0
B5	21.6	14.8	12.8	6.4	6.0	6.4	2.4	12.4	1.2	4.0	4.0
B6	16.4	14.8	7.6	9.2	3.2	6.4	4.8	12.0	1.6	4.8	5.2
B7	18.8	16.8	2.4	8.4	3.6	8.0	3.6	13.2	2.0	3.2	6.4

Table I- 5 Suspended solids concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	32.0	ND	30.6	53.2	ND	ND	350.2	74.6	40.8	24.6
D2	66.8	53.0	4.8	23.0	89.2	ND	11.2	130.6	160.4	11.6	26.8
D3	75.0	53.2	3.2	38.6	ND	ND	ND	22.8	51.6	8.0	23.2
D4	73.8	68.4	5.4	nd	47.0	ND	ND	76.8	3.4	9.6	39.8
D5	75.0	36.0	10.8	10.6	45.0	ND	12.9	14.6	24.4	29.4	22.6
B1	76.6	35.6	11.2	23.8	11.0	ND	0.8	65.0	11.0	31.6	11.0
B2	67.6	52.0	ND	45.0	18.4	ND	ND	28.4	146.8	33.4	46.0
B3	65.0	50.0	0.4	58.1	ND	ND	ND	28.0	46.2	62.0	20.8
B4	61.8	22.4	6.8	36.8	ND	ND	12.4	30.2	45.4	1.2	16.6
B5	61.2	55.4	28.6	57.6	ND	ND	6.9	29.0	56.2	10.2	83.4
B6	75.8	45.0	5.6	65.4	ND	ND	2.7	26.4	71.4	4.2	44.4
B7	71.8	26.6	ND	3.0	ND	ND	5.1	44.2	80.2	30.8	26.2

Table I- 6 Mean total aqueous cadmium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	ND	ND	2.37	6.58	0.98	ND	0.60	0.75	0.65	0.25
D2	ND	2.99	1.41	9.26	1.81	ND	1.25	0.85	1.45	0.30
D3	ND	ND	1.18	4.79	0.65	ND	0.95	0.65	1.90	1.15
D4	0.29	ND	0.68	9.24	2.11	0.29	0.85	0.70	2.30	0.65
D5	0.33	0.64	1.15	ND	1.31	ND	0.90	1.35	0.44	ND
B1	ND	0.67	0.47	6.19	1.49	ND	0.55	0.49	1.25	ND
B2	0.22	ND	1.63	5.99	1.43	1.95	0.24	ND	0.24	0.29
B3	0.29	0.32	0.52	1.07	0.92	1.74	0.19	ND	0.45	0.24
B4	0.28	0.65	0.71	0.86	1.69	5.87	0.19	ND	3.45	0.30
B5	ND	ND	0.39	1.34	1.17	1.87	ND	0.24	1.15	0.40
B6	3.81	0.25	3.18	5.59	1.08	5.22	0.75	ND	2.05	0.25
B7	ND	ND	1.79	0.23	1.49	1.76	0.30	1.80	ND	ND

Table I- 7 Standard deviations associated with mean total aqueous cadmium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	ND	ND	0.47	0.76	0.11	ND	0.14	0.07	0.35	0.07
D2	ND	0.16	0.76	0.08	0.16	ND	0.07	0.49	0.07	0.00
D3	ND	ND	0.29	2.52	0.00	ND	0.35	0.07	0.42	0.64
D4	0.03	ND	0.01	0.05	0.91	0.15	0.07	0.42	0.42	0.49
D5	0.21	0.65	0.13	0.00	0.04	ND	0.14	1.20	0.37	ND
B1	ND	0.41	0.40	0.00	0.81	ND	0.07	0.44	0.92	ND
B2	0.06	0.01	1.34	0.20	0.98	1.35	0.08	ND	0.08	0.16
B3	0.15	0.20	0.48	0.28	0.11	0.20	0.01	ND	0.21	0.08
B4	0.14	0.66	0.74	0.46	0.08	0.90	0.01	ND	0.07	0.14
B5	ND	ND	0.23	0.07	0.42	0.08	ND	0.08	0.21	0.28
B6	0.24	0.10	0.04	0.13	0.01	0.10	0.21	ND	0.07	0.07
B7	ND	ND	0.32	0.07	0.52	0.75	0.14	0.28	ND	ND

Table I- 8 Mean total aqueous chromium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	2.27	1.26	5.59	3.92	3.88	1.92*	2.05	9.70	1.65	2.15
D2	2.27	0.61	2.05	2.83	6.23	1.17	9.80	5.35	1.30	2.95
D3	1.56	ND	ND	1.01	4.58	0.85	3.20	3.65	2.35	3.70
D4	2.77	ND	ND	2.22	13.03	1.61	1.40	1.45	1.80	1.00
D5	1.65	0.32	ND	2.10	5.03	0.83	3.45	1.35	1.05	0.70
B1	0.28	0.30	ND	ND	2.63	1.31	1.65	0.44	0.95	0.80
B2	ND	ND	0.50	ND	3.79	0.84	0.35	ND	1.65	1.30
B3	ND	ND	ND	ND	3.82	1.11	ND	0.60	1.05	ND
B4	0.56	ND	ND	ND	3.65	0.89	0.44	ND	1.05	ND
B5	ND	ND	ND	ND	3.68	1.69	ND	0.29	1.10	0.34
B6	ND	ND	0.34	1.35	2.50	0.88	0.35	ND	0.85	0.70
B7	ND	ND	ND	ND	4.13	1.80	0.95	2.50	0.40	0.65

Table I- 9 Standard deviations associated with mean total aqueous chromium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	0.89	0.11	0.16	0.87	0.33	*	0.64	0.28	1.06	0.64
D2	0.53	0.21	0.58	1.96	1.00	0.52	0.57	1.63	0.14	1.91
D3	1.82	ND	ND	0.23	0.86	0.19	0.42	0.07	0.78	0.28
D4	0.32	ND	ND	1.71	1.28	0.65	0.28	0.49	0.28	0.28
D5	0.55	0.07	ND	0.52	1.20	0.28	0.49	0.49	0.49	0.14
B1	0.01	0.04	ND	ND	0.41	0.42	0.07	0.23	0.35	0.42
B2	ND	ND	0.18	ND	0.90	0.20	0.07	ND	0.21	0.85
B3	ND	ND	ND	ND	0.64	0.13	ND	0.42	0.07	ND
B4	0.41	ND	ND	ND	0.84	0.12	0.23	ND	0.78	ND
B5	ND	ND	ND	ND	1.12	0.04	ND	0.02	0.00	0.09
B6	ND	ND	0.10	0.85	1.13	0.01	0.07	ND	0.35	0.00
B7	ND	ND	ND	ND	0.69	0.04	0.49	0.71	0.00	0.49

Table I- 10 Mean total aqueous copper concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	5.09	3.74	3.84	6.00	23.12	5.92	20.10	14.35	7.25	5.80
D2	5.08	5.23	8.79	27.61	31.76	12.41	29.70	13.15	19.95	7.85
D3	10.72	2.57	3.70	11.00	15.12	7.18	18.70	14.05	31.30	16.20
D4	5.46	2.01	3.35	15.83	16.3*	12.17	17.55	13.85	29.45	7.80
D5	6.51	5.58	6.28	46.84	32.88	9.77	27.75	29.20	15.55	4.90
B1	4.10	3.08	3.38*	ND	16.94	11.46	20.35	4.15	14.45	10.05
B2	4.96	2.65	4.89	12.44	45.16	14.00	10.45	5.30	18.40	11.60
B3	10.48	1.50	3.18*	31.24	23.78	7.80	0.25	4.70	19.75	2.10
B4	4.06	2.71	3.53	12.21	27.10	16.86	0.45	3.35	17.95	7.75
B5	1.81	2.52	3.26	2.82	32.43	7.46	3.80	8.80	17.35	5.15
B6	4.52	1.98	5.21	ND	21.94	10.62	13.20	4.55	17.60	5.05
B7	2.05	3.67	3.67	ND	34.50	10.65	18.15	6.60	16.65	5.25

Table I- 11 Standard deviations associated with mean total aqueous copper concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	1.77	1.92	0.38	1.70	2.53	2.61	4.10	3.04	4.31	3.54
D2	1.41	0.81	2.67	4.24	3.97	4.67	10.75	1.77	3.89	0.49
D3	2.19	0.60	0.22	1.81	1.68	0.86	2.40	0.07	4.38	2.83
D4	0.36	0.55	0.32	3.99	*	6.97	2.05	7.14	4.88	2.69
D5	3.77	0.91	5.40	0.24	9.71	1.22	3.89	9.33	2.19	3.39
B1	0.51	1.12	*	ND	3.10	0.66	7.57	0.49	0.07	6.01
B2	0.18	2.49	1.64	0.42	0.07	7.30	1.63	0.85	3.54	5.94
B3	7.22	0.01	*	0.91	6.90	0.32	0.07	0.99	0.92	0.14
B4	3.59	3.36	0.36	4.02	3.66	3.99	1.34	1.34	1.77	4.31
B5	0.36	2.28	0.47	0.67	2.14	1.09	3.96	0.85	2.90	2.05
B6	0.21	0.89	0.10	ND	1.61	0.30	3.96	2.90	4.10	0.64
B7	0.21	3.55	1.14	ND	4.08	1.36	5.30	1.41	4.88	3.89

Table I- 12 Mean total aqueous nickel concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	29.87	173.03	64.3*	53.10	23.61	16.32	27.45	65.40	29.45	19.85
D2	31.12	111.28	60.35	28.49	37.68	17.48	46.30	18.95	29.00	23.90
D3	13.34*	39.50	28.50	22.05	32.99	19.22	54.85	8.95	11.55	21.10
D4	16.43	52.99	20.40	22.89	43.32	25.36	33.70	8.75	7.90	11.50
D5	12.93	33.65	13.25	19.05	46.88	12.30	35.15	9.30	3.50	11.75
B1	14.59	9.79	12.75	5.22	20.69	9.13	21.80	2.43	4.35	5.85
B2	13.44	5.35	10.15	6.04	15.65	6.03	16.00	3.70	2.25	2.55
B3	8.41	5.64	9.35	5.74	24.73	6.07	12.55	3.40	1.70	1.35
B4	7.15	11.35	11.55	6.03*	17.64	6.12*	16.00	0.53	3.80	1.80
B5	6.21	9.61	8.65	5.67	16.20	6.48	9.45	0.18	2.85	2.40
B6	7.01	11.79	5.90	6.34	11.25	6.68	11.95	ND	1.50	1.30
B7	6.95	11.08	6.65	4.15	8.19	9.25	12.70	ND	1.00	2.20

Table I- 13 Standard deviations associated with mean total aqueous nickel concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	0.80	12.94	*	0.64	0.98	0.92	2.05	5.94	0.64	2.47
D2	0.59	7.92	2.05	0.85	5.37	0.72	0.14	1.34	1.98	0.57
D3	*	0.52	2.12	1.01	0.81	0.28	0.64	0.07	0.92	1.13
D4	0.12	0.01	2.40	3.18	6.95	1.20	1.56	1.48	0.99	0.57
D5	0.35	5.76	0.21	0.01	6.03	0.91	1.20	0.14	2.97	1.20
B1	3.04	2.02	0.92	1.05	7.97	3.14	1.27	3.22	1.91	3.75
B2	0.36	0.89	2.05	0.09	0.96	0.44	3.68	1.13	0.35	0.64
B3	0.64	0.69	0.07	1.19	2.55	0.68	2.19	0.85	1.56	0.35
B4	0.94	0.62	2.62	*	1.14	*	1.70	0.53	0.42	0.42
B5	0.08	0.03	1.48	0.36	1.36	1.70	0.07	0.04	0.35	0.71
B6	0.88	0.08	0.14	0.37	2.52	1.68	0.49	ND	0.28	0.14
B7	0.51	0.88	1.20	0.08	0.79	1.81	3.54	ND	0.00	0.71

Table I- 14 Mean total aqueous lead concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	ND	2.76	8.93	11.42	4.34	1.48	0.93	2.20	ND	1.55
D2	0.73	3.76	6.04	20.29	3.96	5.68*	43.50	3.75	8.85	1.58
D3	0.87	4.32	4.25	12.07	6.11	ND	2.40	7.05	11.85	14.05
D4	1.2*	4.48	3.74	14.77	5.21	0.73	3.30	0.88	8.85	1.83
D5	8.24	2.06	4.89	31.14	2.86	1.13	5.70	1.73	4.85	ND
B1	ND	2.42	5.13	14.52	6.11	1.60	5.45	ND	11.60	4.65
B2	0.64	2.38	1.46	7.54	9.14	1.03	7.10	ND	15.65	6.50
B3	4.62*	1.37	0.56	1.53	5.14	ND	ND	ND	10.75	0.58
B4	6.59	4.69	1.07	1.74	6.46	1.51	ND	ND	11.30	0.93
B5	ND	0.7*	1.05	1.56	7.34	ND	ND	ND	14.80	ND
B6	2.37	1.91	0.57	1.41	3.36	2.37	ND	ND	9.55	ND
B7	2.62	0.63	1.97	0.59	7.61	8.48	0.78	3.95	8.70	ND

Table I- 15 Standard deviations associated with mean total aqueous lead concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	ND	2.19	1.72	2.74	0.39	1.45	0.67	1.13	ND	1.48
D2	0.40	1.53	1.90	6.62	0.42	*	0.85	0.35	2.47	1.59
D3	0.59	1.75	1.22	2.79	0.85	ND	0.57	0.07	1.77	4.74
D4	*	1.71	0.67	2.93	0.35	0.39	0.99	0.60	0.64	1.94
D5	0.21	1.58	0.36	8.02	0.99	0.95	0.57	1.80	2.33	ND
B1	ND	0.16	1.11	8.32	0.71	0.02	1.77	ND	1.41	5.16
B2	0.26	1.07	0.78	0.67	0.74	0.82	0.14	ND	2.05	0.00
B3	*	0.66	0.16	0.42	0.53	ND	ND	ND	1.06	0.18
B4	2.47	2.47	0.87	0.74	2.62	0.42	ND	ND	4.95	0.67
B5	ND	*	0.57	0.25	0.39	ND	ND	ND	1.98	ND
B6	0.54	2.06	0.17	0.27	0.78	1.62	ND	ND	0.07	ND
B7	1.65	0.25	0.08	0.19	0.71	0.66	0.46	0.21	1.27	ND

Table I- 16 Mean total aqueous zinc concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	14.0	38.0	41.5	167.5	15.0	ND	66.0	32.0	47.0	52.5
D2	16.0	320.5	58.0	89.5	20.0	17.0	161.0	53.5	42.5	62.5
D3	39*	47.0	13.0	17.0	48.0	ND	121.0	27.5	83.5	63.0
D4	20.0	21.5	ND	30.0	17.0	15.5	52.0	30.5	99.5	41.5
D5	73.0	16.5	32.5	37.0	21.0*	12.5	49.5	20.0*	21.0	31.0
B1	ND	55.5	15.5	ND	ND	24.0	20.0	ND	49.0	15.0
B2	47.0	17.5	36.0	24.0	ND	ND	234.0	55.0	43.5	47.5
B3	97.0	ND	12.5	ND	ND	ND	15.5	ND	38.5	12.5
B4	ND	ND	ND	17.0	27.5	25.0	14.5	ND	133.5	44.5
B5	ND	ND	ND	ND	ND	ND	ND	ND	58.0	26.0
B6	55.0	ND	22.0	309.5	ND	32.5	27.5	ND	62.0	16.0
B7	ND	ND	ND	13.0	ND	ND	ND	62.0*	32.0	20.0

Table I- 17 Standard deviations associated with mean total aqueous zinc concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	2.8	5.7	4.9	3.5	4.2	ND	5.7	1.4	14.1	7.8
D2	1.4	55.9	1.4	13.4	7.1	4.2	12.7	3.5	0.7	9.2
D3	*	9.9	1.4	7.1	9.9	0.0	29.7	2.1	7.8	1.4
D4	0.0	7.8	ND	1.4	7.1	4.9	1.4	6.4	10.6	12.0
D5	9.9	6.4	3.5	8.5	*	0.7	9.2	*	8.5	2.8
B1	ND	7.8	4.9	ND	ND	1.4	5.7	4.2	1.4	4.2
B2	11.3	6.4	2.8	5.7	ND	ND	8.5	9.9	6.4	3.5
B3	18.4	ND	0.7	ND	ND	ND	4.9	ND	6.4	0.7
B4	ND	ND	ND	0.0	3.5	ND	3.5	ND	4.9	2.1
B5	ND	ND	ND	ND	ND	ND	ND	ND	4.2	4.2
B6	14.1	ND	4.2	29.0	ND	7.8	6.4	ND	1.4	5.7
B7	ND	ND	ND	1.4	ND	ND	ND	*	5.7	4.2

Table I- 18 Mean total cadmium concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	5.5*	5.3	4.0	9.0	5.2	6.4	5.9	5.2	2.9	7.2
D2	2.6	13.5	6.3	5.9	8.6	6.5	7.3	9.0	6.0	7.5	8.7
D3	6.5	4.7	6.6	5.1	4.6	5.0	4.6	7.3	6.6	3.4	5.2
D4	7.3	9.6	6.7	4.3	7.4	5.2	5.7	5.7	7.2	5.6	6.7
D5	3.0	2.3*	3.7	3.0	5.3	4.8	6.0	3.1	10.0	3.1	3.5
B1	4.8	4.2	5.1	3.3	6.1	NS	4.2	3.6	4.5	4.4	4.2
B3	NS	5.2	4.8	3.5	7.0	7.1	6.3	4.4	6.5	5.7*	5.8
B4	NS	6.9	5.2	4.1	7.8	7.7	3.7	8.2	7.0	5.6	7.3
B7	4.8	7.4	5.3	NS	3.9	6.3	8.7	4.4	4.4	4.0	4.1

Table I- 19 Standard deviations associated with mean total cadmium concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	*	0.1	0.3	0.2	0.7	0.4	1.2	0.8	0.2	0.1
D2	0.1	3.3	0.1	1.9	1.4	0.3	0.1	0.6	0.5	0.2	0.0
D3	0.3	0.0	0.4	0.2	0.5	0.3	0.1	0.1	0.5	0.2	0.7
D4	0.6	1.7	0.7	0.0	1.5	0.3	0.4	0.7	2.5	0.1	0.3
D5	0.0	*	0.6	0.1	0.4	0.1	0.1	0.2	3.3	0.0	0.2
B1	0.1	0.7	0.4	0.4	0.4	NS	0.2	0.5	0.4	0.7	0.5
B3	NS	1.0	0.5	0.4	0.0	0.6	0.8	0.1	1.2	*	0.2
B4	NS	0.6	0.1	0.3	2.1	0.5	0.1	0.2	1.6	0.1	0.4
B7	0.1	0.1	0.5	NS	0.2	0.1	0.4	0.3	0.1	1.0	0.0

Table I- 20 Mean total lead concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	87.5*	124.0	117.8	190.4	148.0	196.7	169.5	78.3	63.3	149.6
D2	44.5	309.2	233.2	208.2	281.2	331.6	291.8	330.0	125.5	133.2	199.7
D3	106.1	55.5	131.1	132.3	41.0	52.0	39.5	66.8	122.9	32.9	86.5
D4	108.7	119.6	83.9	108.9	93.6	89.2	137.8	114.2	123.2	101.4	93.9
D5	49.6	22.9*	38.0	87.2	140.8	149.6	224.2	56.7	269.7	58.4	73.9
B1	59.5	40.7	59.7	48.4	76.8	NS	70.6	58.5	58.8	82.0	66.7
B3	NS	60.9	234.5	201.1	235.7	281.1	209.5	163.7	225.5	391.8*	180.4
B4	NS	219.2	216.6	193.4	195.9	260.7	126.6	317.4	204.9	203.1	220.7
B7	350.5	124.4	151.9	NS	120.4	109.3	286.2	82.8	68.1	114.7	68.1

Table I- 21 Standard deviations associated with mean total lead concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	*	0.0	1.3	10.7	15.9	4.9	6.5	15.5	0.9	0.6
D2	1.3	87.4	14.5	13.0	35.8	4.5	1.2	13.9	3.0	15.0	13.7
D3	3.2	6.4	4.4	2.0	3.9	10.3	0.4	2.3	3.0	1.0	19.7
D4	6.7	21.4	8.8	0.6	10.7	0.5	6.6	8.5	30.9	2.3	2.3
D5	2.9	*	1.4	3.6	15.6	3.0	12.0	4.2	44.5	9.1	0.6
B1	1.3	6.7	2.4	6.1	0.5	NS	1.4	6.4	6.9	18.2	10.0
B3	NS	10.0	65.7	3.3	1.6	14.8	27.4	2.7	4.8	*	1.8
B4	NS	22.6	4.0	35.9	22.8	22.1	0.2	1.8	29.3	8.2	0.1
B7	23.2	9.4	11.1	NS	0.1	5.8	6.1	3.8	8.0	13.6	3.0

Table I- 22 Mean total chromium concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	27.9*	31.2	23.2	39.0	16.6	49.7	38.3	25.8	16.3	43.4
D2	21.2	84.2	35.0	65.1	167.2	106.5	65.2	72.8	32.2	27.1	65.6
D3	46.7	26.3	37.3	39.6	52.1	3.0	14.4	22.8	35.3	16.9	28.5
D4	70.3	50.7	39.2	33.3	45.1	9.2	40.2	33.8	36.5	30.6	29.8
D5	24.2	10.8*	16.7	16.7	42.4	42.9	17.6	18.3	62.8	18.9	19.0
B1	13.7	11.7	10.3	ND	10.5	NS	6.2	7.1	6.0	9.7	7.1
B3	NS	8.5	19.6	9.9	24.4	26.1	50.0	14.0	16.1	21.3*	15.5
B4	NS	18.4	19.2	ND	20.0	25.3	9.1	39.9	16.8	19.4	20.5
B7	31.7	16.7	16.8	ND	11.7	38.3	18.0	10.4	8.8	14.1	8.8

Table I- 23 Standard deviations associated with mean total chromium concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	*	1.8	1.7	7.2	6.4	0.8	3.1	6.4	0.9	0.0
D2	1.2	23.6	1.1	13.8	5.3	4.2	7.1	5.1	1.9	5.9	7.1
D3	0.0	0.3	1.4	6.4	2.5	1.3	0.5	1.0	0.8	1.2	4.5
D4	2.6	7.0	1.2	1.7	13.1	3.6	4.7	5.0	13.2	1.6	1.0
D5	3.5	*	1.2	0.4	6.6	3.7	0.5	1.5	20.2	1.0	0.2
B1	1.3	5.6	0.6	ND	0.2	NS	0.6	0.8	0.4	1.6	0.7
B3	NS	0.2	3.0	3.9	1.2	11.4	8.0	0.5	0.0	*	1.2
B4	NS	0.7	2.5	ND	1.6	3.4	0.0	0.7	3.9	0.8	0.1
B7	0.4	0.5	1.1	NS	0.0	17.8	0.2	0.7	0.4	2.3	0.0

Table I- 24 Mean total zinc concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	68.0*	65.9	58.0	461.3	264.1	361.9	301.9	298.9	141.2	372.0
D2	21.3	165.7	132.5	108.2	699.1	760.9	675.6	829.5	279.0	694.6	604.3
D3	54.5	50.7	92.4	82.0	132.0	164.5	89.9	133.3	372.6	127.6	252.3
D4	85.0	86.6	59.2	68.7	236.4	277.7	382.5	278.8	382.1	314.9	236.0
D5	32.8	41.8*	23.0	44.1	468.0	565.1	305.5	133.1	1034.9	141.1	201.9
B1	39.3	45.8	45.8	37.5	243.1	NS	172.7	139.0	161.6	260.6	164.6
B3	NS	34.2	76.6	58.4	348.5	463.3	655.8	223.7	336.5	515.5*	209.4
B4	NS	73.3	80.7	79.1	347.7	504.6	158.1	674.7	493.2	447.1	342.4
B7	114.6	52.7	62.7	NS	172.0	328.6	501.4	150.7	294.4	216.8	117.2

Table I- 25 Standard deviations associated with mean total zinc concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	*	1.6	2.8	1.3	22.8	7.3	11.7	49.8	7.0	9.9
D2	0.3	22.6	1.5	23.9	72.8	9.4	24.5	0.2	14.5	12.3	13.1
D3	1.3	3.1	0.4	6.1	13.2	1.2	2.0	4.0	41.9	2.3	37.2
D4	0.2	11.3	6.3	0.4	33.2	6.4	18.1	23.6	75.6	21.7	13.8
D5	0.6	*	0.0	1.4	52.3	31.0	14.7	10.8	248.9	18.2	4.2
B1	1.7	14.4	1.6	3.7	5.1	NS	5.6	18.5	16.4	4.8	22.0
B3	NS	6.0	1.9	2.8	16.7	18.8	78.5	0.6	24.7	*	3.8
B4	NS	0.9	5.4	9.0	40.5	36.3	0.6	21.6	13.6	10.7	1.1
B7	3.6	2.2	2.4	NS	0.6	17.4	20.3	7.7	16.0	9.0	0.2

Table I- 26 Mean total nickel concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	125.2*	38.5	53.6	65.1	75.9	65.0	51.5	38.4	22.2	7[illegible]
D2	5.4	118.3	73.8	63.8	74.4	127.1	165.7	187.2	48.9	102.5	108.6
D3	57.2	120.1	64.1	66.1	23.4	35.4	27.3	30.1	60.0	32.0	62.4
D4	93.8	140.0	49.6	104.8	46.5	94.3	101.1	77.8	71.6	67.7	55.3
D5	99.8	124.5*	48.8	89.0	58.2	141.1	23.8	34.1	100.8	27.1	32.9
B1	95.2	146.7	36.9	51.4	53.0	NS	40.4	30.4	33.4	58.9	40.4
B3	NS	118.4	21.3	62.3	28.9	33.1	122.0	17.4	24.3	34.4*	16.7
B4	NS	123.1	30.7	85.9	40.5	37.9	19.4	89.7	29.3	31.3	27.2
B7	87.0	140.1	104.4	NS	22.3	56.3	34.3	44.3	319.4	47.2	32.0

Table I- 27 Standard deviations associated with mean total nickel concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	*	2.0	2.9	3.1	8.9	2.7	0.3	3.7	0.6	0.4
D2	0.5	5.7	1.0	9.1	11.2	1.7	6.9	0.6	1.2	4.2	1.3
D3	1.3	0.8	12.7	11.0	2.3	0.1	0.7	0.9	6.7	0.4	9.3
D4	0.5	2.9	3.0	39.3	2.4	0.4	6.5	6.4	3.9	0.2	2.4
D5	5.8	*	5.9	33.3	7.0	12.1	0.3	3.8	2.6	0.8	0.6
B1	11.4	11.0	0.6	12.4	0.7	NS	1.0	3.1	2.6	2.4	5.8
B3	NS	19.0	0.8	4.0	1.1	0.2	12.2	0.1	1.0	*	0.7
B4	NS	1.9	1.2	2.8	3.9	2.1	0.3	8.4	0.1	0.4	0.2
B7	0.9	4.5	6.0	NS	0.0	2.5	0.3	1.6	23.6	1.9	0.3

Table I- 28 Mean total copper concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

Site	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	43.5*	50.1	45.9	75.3	54.0	85.1	79.3	48.5	28.5	34.6
D2	21.7	121.0	81.5	94.8	131.8	147.2	138.7	177.7	63.0	91.6	113.2
D3	49.7	37.1	63.8	64.8	21.5	29.0	31.0	68.5	74.7	16.9	46.5
D4	52.7	67.8	50.1	51.9	43.0	48.2	73.5	59.2	75.5	45.9	48.6
D5	19.2	9.8*	17.3	32.1	76.3	71.6	72.7	29.2	178.1	24.4	35.1
B1	36.7	24.9	38.5	28.3	52.2	NS	40.0	29.7	38.1	52.0	42.0
B3	NS	30.7	65.1	48.5	69.4	85.1	101.3	53.8	94.8	91.4*	70.5
B4	NS	59.7	62.9	43.8	51.6	74.5	30.7	121.7	75.9	60.6	68.6
B7	76.6	33.3	50.4	NS	32.1	40.0	87.4	25.4	37.5	32.2	21.0

Table I- 29 Standard deviations associated with mean total copper concentrations (µg/g) in sediment from identified sampling points at the Dagenham and Brentwood wetlands.

	18/10/1995	10/01/1996	06/03/1996	01/05/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	26/06/1997	28/08/1997	30/10/1997
D1	NS	*	2.3	1.0	3.3	1.2	2.6	1.4	13.9	1.1	1.6
D2	0.5	31.2	3.7	29.7	12.2	9.2	6.1	6.5	3.3	1.0	3.2
D3	0.5	0.4	1.2	3.0	3.3	3.7	0.7	3.5	2.6	1.6	7.3
D4	0.5	11.5	3.8	1.0	0.7	0.7	6.2	5.3	28.1	2.6	1.5
D5	0.9	*	1.3	1.4	9.0	4.0	4.1	1.9	57.7	4.9	0.9
B1	1.3	12.3	0.7	3.8	1.5	NS	1.4	3.8	5.4	8.7	6.9
B3	NS	11.7	1.8	1.1	3.3	6.5	14.6	1.2	17.4	*	2.5
B4	NS	3.9	2.6	11.9	6.5	5.7	1.0	4.2	12.3	0.7	2.1
B7	1.1	0.5	2.0	NS	1.6	4.0	2.8	1.8	3.3	3.4	0.8

Table I- 30 Mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in spring, 1997.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	1.6	32.3	1.1	7.7	7.0	5.4
Typha rhizomes	4.3	299.7	5.6	31.5	29.2	12.6
Typha roots	17.6	239.1	24.5	114.5	40.6	75.8
Phragmites leaves	0.7	47.4	1.0	3.2	NS	11.0
Phragmites rhizomes	2.1	46.6	0.8	2.1	NS	45.8
Phragmites roots	4.4	252.8	4.5	47.2	NS	68.8

Table I- 31 Standard deviations associated with mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in spring, 1997.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	0.0	7.0	0.1	0.3	2.4	1.3
Typha rhizomes	0.0	1.9	0.4	1.4	0.1	0.1
Typha roots	2.2	0.2	0.0	5.8	13.2	1.6
Phragmites leaves	0.1	4.8	0.1	0.6	0.3	1.5
Phragmites rhizomes	0.0	0.7	0.3	3.4	1.7	0.4
Phragmites roots	*	*	*	*	*	*

Table I- 32 Mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in summer, 1997.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	1.6	44.8	0.3	11.0	8.3	5.7
Typha rhizomes	0.7	60.2	2.4	9.4	11.1	5.6
Typha roots	50.2	670.4	18.7	204.2	79.4	123.8
Phragmites leaves	0.6	31.5	0.4	2.7	4.9	8.4
Phragmites rhizomes	1.3	48.6	0.8	11.5	11	10.6
Phragmites roots	9.6	324.6	9.2	93.3	69.5	90.6

Table I- 33 Standard deviations associated with mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in summer, 1997.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	0.0	7.0	0.1	0.3	2.4	1.3
Typha rhizomes	0.0	1.9	0.4	1.4	0.1	0.1
Typha roots	2.2	0.2	0.0	5.8	13.2	1.6
Phragmites leaves	0.1	4.8	0.1	0.6	0.3	1.5
Phragmites rhizomes	0.0	0.7	0.3	3.4	1.7	0.4
Phragmites roots	*	*	*	*	*	*

Table I- 34 Mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in autumn, 1997.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	2.3	34.3	0.8	4.3	13.5	3.8
Typha rhizomes	1.0	17.3	1.3	1.9	6.6	2.5
Typha roots	3.5	99.1	6.9	26.5	42.4	16.5
Phragmites leaves	0.2	99.4	0.8	6.1	17.4	5.6
Phragmites rhizomes	0.6	27.4	0.9	4.5	12.2	4.2
Phragmites roots	1.5	38.8	2.7	25.3	45.3	13.9

Table I- 35 Standard deviations associated with mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in autumn, 1997.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	0.7	1.4	0.1	1.9	2.7	0.0
Typha rhizomes	0.1	2.1	0.8	0.1	0.7	0.3
Typha roots	*	*	*	*	*	*
Phragmites leaves	0.0	1.4	0.1	1.4	3.4	0.4
Phragmites rhizomes	0.1	4.0	0.2	0.1	1.8	0.2
Phragmites roots	*	*	*	*	*	*

Table I- 36 Mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in winter, 1998.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	0.8	38.2	0.6	3.2	14.3	6.6
Typha rhizomes	2.1	23.4	1.4	4.9	23.1	7.2
Typha roots	8.4	106.0	10.8	57.6	67.2	65.6
Phragmites leaves	0.4	37.1	0.4	2.8	55.8	1.6
Phragmites rhizomes	1.6	55.8	0.9	7.3	30.3	8.3
Phragmites roots	2.2	226	7.35	96.1	56.53	66.33

Table I- 37 Standard deviations associated with mean total metal tissue concentrations (µg/g) in *Typha laitfolia* (Dagenham wetland) and *Phragmites australis* (Brentwood wetland) collected in winter, 1998.

	Chromium	Zinc	Cadmium	Lead	Nickel	Copper
Typha leaves	0.7	1.4	0.1	1.9	2.7	0.0
Typha rhizomes	0.1	2.1	0.8	0.1	0.7	0.3
Typha roots	*	*	*	*	*	*
Phragmites leaves	0.0	1.4	0.1	1.4	3.4	0.4
Phragmites rhizomes	0.1	4.0	0.2	0.1	1.8	0.2
Phragmites roots	*	*	*	*	*	*

Table I- 38 Chloride concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1998	28/08/1997	30/10/1997
D1	ND	63.80	106.00	67.69	89.77	94.36	132.45	118.41	112.95	64.51	107.13
D2	84.06	61.41	100.62	67.05	96.59	95.65	113.11	113.60	117.28	54.76	106.61
D3	85.39	84.31	107.61	68.21	87.56	84.64	107.87	124.80	126.86	31.84	108.62
D4	82.15	60.41	105.52	63.99	97.96	83.18	159.96	116.26	104.41	17.28	119.58
D5	78.57	61.97	124.11	75.40	101.89	85.26	147.50	111.77	116.78	27.12	111.00
B1	147.25	75.59	90.77	288.50	100.75	260.00	257.81	115.76	80.26	143.89	89.29
B2	114.34	72.34	95.99	60.62	103.50	85.35	110.39	83.70	85.48	59.62	148.12
B3	120.59	84.58	86.34	58.82	110.52	70.18	115.48	84.08	89.76	65.33	125.53
B4	110.49	78.27	94.28	55.09	100.17	73.79	31.70	93.90	89.85	73.49	82.71
B5	128.95	74.01	93.35	64.25	105.10	76.70	193.28	82.32	88.01	62.68	86.13
B6	129.67	72.38	88.92	60.69	106.87	75.44	164.30	83.31	85.71	65.84	76.46
B7	269.94	79.18	103.97	64.08	143.54	88.64	118.67	109.22	90.31	62.24	86.34

Table I- 39 Nitrate concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	37.45	36.18	22.53	28.76	55.35	27.01	57.53	113.13	18.60	20.08
D2	27.87	30.95	64.37	19.84	66.86	31.51	25.41	44.63	117.28	19.91	26.63
D3	32.64	45.15	41.29	24.83	32.64	21.39	22.66	36.21	30.31	12.94	23.25
D4	27.98	31.44	34.80	18.22	39.41	36.38	45.05	50.76	25.89	9.03	22.56
D5	31.35	34.14	50.45	20.47	37.27	20.48	33.46	31.43	27.04	10.44	22.53
B1	29.68	25.01	29.26	14.80	24.74	36.72	17.05	23.78	18.86	22.69	12.61
B2	21.88	18.28	25.33	19.84	32.05	20.54	19.23	22.34	19.18	16.94	18.17
B3	27.91	21.88	31.76	17.02	20.54	14.35	19.14	23.79	16.08	22.24	17.30
B4	22.62	20.27	26.63	12.07	14.93	15.16	26.29	15.81	17.46	23.87	10.99
B5	26.67	21.76	24.69	14.34	130.05	15.97	33.30	21.90	14.51	19.54	11.95
B6	25.71	21.56	23.01	14.69	17.87	16.45	30.09	21.70	15.96	17.73	10.94
B7	36.54	29.43	24.53	14.61	16.25	23.52	21.29	22.30	17.21	15.11	12.42

Table I- 40 Phosphate concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1998	28/08/1997	30/10/1997
D1	ND	1.10	1.52	1.60	3.52	2.20	2.07	2.24	ND	0.81	3.20
D2	1.93	0.57	1.78	1.72	2.42	2.35	2.85	1.47	ND	0.36	1.96
D3	1.73	0.83	1.69	2.21	1.95	2.32	1.80	2.92	1.11	0.66	1.95
D4	1.33	0.91	1.65	1.67	2.44	2.46	1.79	3.05	0.79	0.49	1.92
D5	1.25	0.92	1.55	1.64	1.83	2.34	1.75	3.00	1.35	ND	1.92
B1	ND	0.18	ND	ND	ND	ND	ND	ND	ND	ND	ND
B2	ND	ND	ND	0.60	ND	ND	ND	ND	ND	ND	ND
B3	ND	0.19	ND	0.67	ND	ND	ND	ND	1.40	ND	ND
B4	ND	0.15	ND	ND	ND	ND	ND	ND	ND	ND	ND
B5	ND	0.29	ND	ND	ND	ND	ND	ND	ND	ND	ND
B6	ND	ND	ND	ND	0.19	ND	ND	ND	ND	ND	0.72
B7	ND	ND	ND	ND	0.24	ND	ND	ND	ND	ND	ND

Table I- 41 Sulphate concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	140.94	105.30	63.98	94.34	81.63	87.32	100.90	97.79	64.90	64.33
D2	120.05	74.65	98.04	62.50	105.04	91.03	238.43	104.14	100.55	58.67	66.95
D3	128.12	146.77	103.53	59.56	86.64	84.97	79.39	102.39	89.99	33.05	69.00
D4	121.33	78.67	98.66	59.77	94.25	82.00	119.93	99.31	71.91	18.17	71.87
D5	137.03	75.01	111.53	60.66	98.99	78.99	117.32	109.98	82.76	23.36	67.34
B1	326.25	102.54	149.17	85.12	132.20	296.69	278.43	150.25	98.03	127.80	99.02
B2	286.40	109.79	140.90	88.45	138.84	131.56	132.65	179.10	133.74	97.02	149.68
B3	302.80	200.13	138.87	89.22	145.66	106.77	142.80	161.00	138.97	108.43	153.47
B4	274.77	109.15	144.52	79.04	133.33	122.93	285.94	151.53	138.86	118.90	112.31
B5	352.96	106.41	130.99	96.79	144.02	121.68	257.64	149.59	130.48	100.79	110.14
B6	323.88	110.89	136.66	90.05	146.85	121.66	227.43	147.40	131.68	98.32	101.51
B7	360.95	124.09	148.87	96.02	143.54	125.83	159.44	149.40	135.68	86.33	111.69

Table I- 42 Total trihalomethane concentrations (µg/l) recorded at identified sampling points at the Dagenham wetland during dry weather.

Site	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/07/1997	28/08/1997	30/10/1998
D1	20.60	47.10	71.80	46.70	52.80	60.50	33.60
D2	18.80	48.10	75.10	28.60	46.00	55.30	18.40
D3	22.70	30.70	69.70	38.80	42.00	31.00	12.30
D4	31.00	58.40	62.80	33.40	36.50	17.80	13.30
D5	21.00	13.60	82.90	32.40	36.20	13.80	9.60

Table I- 43 Total ammonia concentrations (mgN/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/96	11/09/1996	05/11/96	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	0.333	0.467	0.083	0.300	0.800	0.100	0.117	0.400	0.100	0.800
D2	0.600	0.333	0.534	0.100	0.367	0.534	0.100	0.100	0.367	0.083	1.668
D3	0.667	0.333	1.084	0.167	0.133	0.133	0.083	0.434	0.400	0.083	0.234
D4	0.600	0.267	0.800	0.100	0.117	2.334	0.117	0.117	0.333	0.083	0.333
D5	0.534	0.100	0.734	0.117	0.133	0.600	0.117	0.117	0.333	0.083	0.267
B1	0.133	1.084	0.133	0.117	0.083	0.133	0.083	0.083	0.167	0.117	0.167
B2	0.200	0.167	0.434	0.100	1.167	0.117	0.117	0.100	0.534	0.083	0.100
B3	0.400	0.200	0.300	0.117	1.868	0.333	0.100	0.083	0.133	0.083	0.167
B4	0.167	0.167	0.333	0.800	0.867	0.117	0.117	0.083	0.200	0.100	0.117
B5	0.133	0.200	0.267	0.117	1.868	0.200	0.083	0.100	0.133	0.100	0.167
B6	0.167	0.200	0.267	0.100	3.000	0.167	0.667	0.083	0.167	0.100	0.100
B7	0.133	0.434	0.300	0.117	0.534	0.600	0.100	0.117	0.167	0.083	0.100

Table I- 44 Unionised ammonia concentrations (µgN/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	4.84	7.62	0.07	1.89	5.38	0.33	0.88	1.72	0.58	5.26
D2	1.61	4.84	8.60	0.07	1.51	7.39	0.33	0.58	0.79	0.48	22.40
D3	1.87	3.07	17.58	1.27	0.73	0.94	0.34	2.64	1.09	0.45	2.52
D4	1.18	1.96	11.79	1.47	1.31	13.14	0.37	0.83	0.68	0.28	5.74
D5	0.81	0.58	4.86	0.87	4.35	4.38	0.47	1.29	1.22	0.28	0.36
B1	0.23	6.91	0.02	1.35	0.89	5.71	1.20	0.68	2.54	1.23	0.57
B2	0.46	0.06	6.79	0.64	15.46	6.08	0.97	0.21	3.08	0.65	0.20
B3	0.93	1.25	4.62	1.20	20.31	18.04	0.84	0.14	0.71	0.66	0.41
B4	0.38	1.06	5.36	8.31	3.72	1.41	0.39	0.40	0.80	0.50	0.54
B5	0.27	0.63	4.18	1.22	15.69	5.51	0.69	0.80	0.42	0.49	0.93
B6	1.70	2.01	4.23	1.12	47.01	8.87	5.67	0.49	1.08	0.25	0.87
B7	0.69	3.51	0.06	1.32	5.31	32.09	1.11	0.80	4.23	0.90	0.57

Table I- 45 BOD_5 concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	17.6	5.2		6.4	
D5	16.8	6.4		3.2	
B2		1.6	8.8	2.8	24.0
B6		0.8	9.6	2.0	20.8

Table I- 46 Suspended solids concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	4.0	12.6		89.4	
D5	14.0	25.4		86.0	
B2		25.8	67.3	149.2	160.2
B6		18.4	123.9	128.6	115.2

Table I- 47 Mean total aqueous zinc concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	632.0	116.0		89.5	
D5	ND	102.0		51.5	
B2		51.5	185.0*	71.0	221.0*
B6		126.0	71.0*	84.0	107.0*

Table I- 48 Standard deviations associated with mean total aqueous zinc concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	34.5	8.5		0.7	
D5	ND	9.9		3.5	
B2		3.5	*	0.0	*
B6		14.1	*	8.5	*

Table I- 49 Mean total aqueous cadmium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	9.59	6.45		0.30	
D5	ND	5.40		ND	
B2		4.60	3.0*	0.65	1.1*
B6		4.85	3.6*	ND	1.1*

Table I- 50 Standard deviations associated with mean total aqueous cadmium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	3.27	0.64		0.00	
D5	ND	0.57		ND	
B2		0.57	*	0.21	*
B6		0.64	*	ND	*

Table I- 51 Mean total aqueous lead concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	31.85	29.3		16.6	
D5	7.07	19.95		10.25	
B2		19.8	109.8*	32.2	65.5*
B6		15.45	48.0*	20.85	65.4*

Table I- 52 Standard deviations associated with mean total aqueous lead concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	3.38	4.53		0.85	
D5	1.24	3.18		2.19	
B2		0.85	*	2.69	*
B6		2.62	*	2.33	*

Table I- 53 Mean total aqueous chromium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	6.69	13.55		1.40	
D5	0.41	7.25		1.00	
B2		2.85	15.6*	5.55	7.8*
B6		2.50	6.8*	3.50	6.4*

Table I- 54 Standard deviations associated with mean total aqueous chromium concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	0.69	1.48		0.14	
D5	0.19	1.34		0.14	
B2		0.07	*	0.78	*
B6		0.85	*	0.42	*

Table I- 55 Mean total aqueous nickel concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	31.23	58.45		9.45	
D5	28.11	49.15		4.35	
B2		18.80	15.2*	2.00	9.4*
B6		22.70	4.1*	0.60	7.4*

Table I- 56 Standard deviations associated with mean total aqueous nickel concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	1.76	1.34		0.35	
D5	3.16	1.20		0.35	
B2		1.56	*	0.42	*
B6		3.54	*	0.28	*

Table I- 57 Mean total aqueous copper concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	35.02	39.60		10.50	
D5	3.06	40.10		9.50	
B2		14.45	42.3*	26.25	30.9*
B6		16.35	7.4*	16.40	37.4*

Table I- 58 Standard deviations associated with mean total aqueous copper concentrations (µg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1999	28/11/1997	26/05/1999
D1	12.6	7.8		0.1	
D5	0.2	4.5		1.1	
B2		0.2	*	1.8	*
B6		2.2	*	2.4	*

Table I- 59 Chloride concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	93.33	19.69		78.61	
D5	64.41	25.10		80.68	
B2		42.67	28.97	185.29	21.15
B6		25.77	35.07	107.73	19.35

Table I- 60 Nitrate concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	33.02	15.78		20.84	
D5	18.26	17.07		15.95	
B2		16.32	5.44	20.88	6.63
B6		11.30	8.60	25.24	6.59

Table I- 61 Phosphate concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	1.41	0.18		1.32	
D5	1.50	0.38		0.73	
B2		0.22	ND	ND	ND
B6		0.11	ND	ND	0.33

Table I- 62 Sulphate concentrations (mg/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	70.38	25.83		70.74	
D5	60.50	29.83		44.68	
B2		35.43	44.59	79.55	30.65
B6		36.96	59.15	102.14	30.48

Table I- 63 Total ammonia concentrations (mgN/l) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	0.600	0.333		0.800	
D5	0.167	0.534		0.167	
B2		0.167	0.117	0.267	0.133
B6		0.200	0.400	0.200	0.133

Appendix II

The following Tables give the loadings calculated for each of the parameters at each of the identified sampling points at the Dagenham and Brentwood wetlands. The following key applies for all of the Tables:

ND = not defined

NS = sample not collected

- = parameter not determined

Table II- 1 Flow volume (l/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

	18/10/1995*	10/01/1996*	06/03/1996*	01/07/1996	11/09/1996	05/11/1996	21/01/1997*	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	34	34	34	ND	30	45	34	35	34	ND	26
D2	32	32	32	35	29	ND	32	35	32	ND	29
D3	37	37	37	42	23	51	37	34	37	ND	34
D4	33	33	33	33	22	46	33	35	33	ND	31
D5	35	35	35	41	30	49	35	30	35	ND	25
B2	14	14	14	8	6	8	14	17	26	25	7
B5	9	9	9	ND	2	4	9	9	18	16	5
B6	15	15	15	4	5	4	15	15	38	33	8

* = mean flow values used as flow not measured or fault with flow meter

Table II- 2 Flow volume (l/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	60	452			
D5	46	292			
B2		117	92	135	282
B6		122	79	126	412

Table II- 3 BOD_5 loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	503.2	204.0	ND	120.0	108.0	136.0	182.0	ND	ND	364.0
D2	563.2	473.6	243.2	308.0	69.6	ND	217.6	140.0	ND	ND	162.4
D3	606.8	710.4	310.8	1142.4	29.9	142.8	44.4	258.4	ND	ND	190.4
D4	507.7	614.6	213.8	198.0	26.4	147.2	253.8	224.0	ND	ND	198.4
D5	616.0	616.0	294.0	541.2	48.0	19.6	140.0	132.0	ND	ND	140.0
B2	255.0	260.5	182.9	73.6	12.0	64.0	33.3	122.4	104.0	50.0	56.0
B5	194.4	133.2	115.2	ND	12.0	25.6	21.6	111.6	21.6	64.0	20.0
B6	250.7	226.2	116.2	36.8	16.0	25.6	73.4	180.0	60.8	158.4	41.6

Table II- 4 Suspended solids loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	1088.0	ND	ND	1596.0	ND	ND	12257.0	2536.4	ND	639.6
D2	2137.6	1696.0	153.6	805.0	2586.8	ND	358.4	4571.0	5132.8	ND	777.2
D3	2775.0	1968.4	118.4	1621.2	ND	ND	ND	775.2	1909.2	ND	788.8
D4	2464.9	2284.6	180.4	ND	1034.0	ND	ND	2688.0	113.6	ND	1233.8
D5	2625.0	1260.0	378.0	434.6	1350.0	ND	451.5	438.0	854.0	ND	565.0
B2	936.7	720.6	ND	360.0	110.4	ND	ND	482.8	3816.8	835.0	322.0
B5	550.8	498.6	257.4	ND	ND	ND	62.5	261.0	1011.6	163.2	417.0
B6	1158.7	687.9	85.6	261.6	ND	ND	41.3	396.0	2713.2	138.6	355.2

Table II- 5 Total copper loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	173.06	127.16	ND	180.00	1040.40	201.11	703.50	487.90	ND	150.80
D2	162.40	167.36	307.65	800.55	ND	396.96	1039.50	420.80	ND	227.65
D3	396.64	94.91	155.19	253.00	770.87	265.48	635.80	519.85	ND	550.80
D4	182.20	67.13	110.39	348.26	749.80	406.31	614.25	462.59	ND	241.80
D5	227.68	195.30	257.28	1405.20	1610.88	341.78	832.50	1022.00	ND	122.50
B2	68.66	36.72	39.12	74.64	361.28	194.00	177.65	137.80	460.00	81.20
B5	16.25	22.64	ND	5.63	129.72	67.14	34.20	158.40	277.60	25.75
B6	69.09	30.27	20.84	1.05	87.74	162.26	198.00	172.90	580.80	40.40

Table II- 6 Total cadmium loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	6.12	6.12	ND	197.25	43.88	6.12	21.00	25.50	ND	6.50
D2	5.76	95.68	49.18	268.40	ND	5.76	43.75	27.20	ND	8.70
D3	6.66	6.66	49.35	110.06	33.15	6.66	32.30	24.05	ND	39.10
D4	9.69	6.01	22.28	203.17	97.06	9.52	29.75	23.38	ND	20.15
D5	11.38	22.40	46.95	5.40	64.19	6.30	27.00	47.25	ND	4.50
B2	3.05	2.56	13.04	35.94	11.40	26.95	4.08	4.68	6.00	2.03
B5	1.62	2.25	ND	2.68	4.68	16.83	1.62	4.32	18.40	2.00
B6	58.24	3.82	12.72	27.95	4.30	79.79	11.25	6.84	67.65	2.00

Table II- 7 Total chromium loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	77.18	42.67	ND	117.45	174.38	65.28	71.75	329.80	ND	55.90
D2	72.48	19.36	71.75	81.93	ND	37.28	343.00	171.20	ND	85.55
D3	57.54	9.99	11.34	23.12	233.33	31.27	108.80	135.05	ND	125.80
D4	92.35	9.02	8.91	48.84	599.15	53.77	49.00	48.43	ND	31.00
D5	57.75	11.20	11.07	62.85	246.23	29.05	103.50	47.25	ND	17.50
B2	3.74	3.74	3.96	1.62	30.28	11.64	5.95	7.02	41.25	9.10
B5	2.43	2.43	ND	0.54	14.70	15.17	2.43	5.13	17.60	1.68
B6	4.13	4.13	1.36	6.75	10.00	13.45	5.25	10.26	28.05	5.60

Table II- 8 Total nickel loadings (µg/s) recorded at identified sampling point s at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	1015.41	5883.02	ND	1593.00	1062.45	554.88	960.75	2223.60	ND	516.10
D2	995.84	3560.96	2112.25	826.21	ND	559.36	1620.50	606.40	ND	693.10
D3	515.78	1461.32	1197.00	507.04	1682.24	710.96	1864.90	331.15	ND	717.40
D4	548.60	1769.70	673.20	503.58	1992.49	846.86	1179.50	292.25	ND	356.50
D5	452.38	1177.75	543.25	571.50	2296.88	430.50	1054.50	325.50	ND	293.75
B2	186.17	74.14	81.20	36.21	125.20	83.56	272.00	96.20	56.25	17.85
B5	55.85	86.49	ND	11.33	64.80	58.28	85.05	3.15	45.60	12.00
B6	107.15	180.14	23.60	31.70	44.98	102.11	179.25	5.70	49.50	10.40

Table II- 9 Total lead loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	26/06/1997	28/08/1997	30/10/1997
D1	15.30	93.84	ND	342.45	195.08	50.15	32.38	74.80	ND	40.30
D2	23.36	120.16	211.23	588.41	ND	252.16	1522.50	120.00	ND	45.68
D3	32.01	159.84	178.50	277.50	311.61	16.65	81.60	260.85	ND	477.70
D4	93.19	149.63	123.26	324.94	239.66	24.22	115.50	29.23	ND	56.58
D5	288.23	72.10	200.29	934.20	140.14	39.38	171.00	60.38	ND	11.25
B2	8.80	32.98	11.64	45.21	73.08	14.27	120.70	11.70	391.25	45.50
B5	4.05	20.61	ND	3.11	29.34	4.05	4.05	8.10	236.80	2.25
B6	36.15	29.12	2.28	7.05	13.44	36.15	6.75	17.10	315.15	34.40

Table II- 10 Total zinc loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	476	1292	ND	5025	675	408	2310	1088	ND	1365
D2	512	10256	2030	2596	ND	544	5635	1712	ND	1813
D3	1443	1739	546	391	2448	444	4114	1018	ND	2142
D4	668	718	396	660	782	518	1820	1019	ND	1287
D5	2555	578	1333	1110	1029	438	1485	700	ND	775
B2	651	243	288	144	96	166	3978	1430	1088	333
B5	108	108	ND	24	48	108	108	216	928	130
B6	841	183	88	1548	48	497	413	456	2048	128

Table II- 11 Chloride loadings (mg/s) recorded at identified sampling point s at the Dagenham and Brentwood wetlands during dry weather.

	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1998	28/08/1997	30/10/1997
D1	NS	2169.20	3604.00	ND	2693.10	4246.20	4503.30	4144.35	3840.30	ND	2785.41
D2	2689.95	1965.12	3219.84	2346.75	2801.11	ND	3619.52	3976.00	3752.96	ND	3091.63
D3	3159.43	3119.47	3981.57	2864.82	2013.88	4316.64	3991.19	4243.20	4693.82	ND	3693.08
D4	2743.91	2017.69	3524.37	2111.67	2155.12	3826.28	5342.66	4069.10	3487.29	ND	3706.86
D5	2749.88	2168.78	4343.85	3091.40	3056.70	4177.74	5162.50	3353.10	4087.30	ND	2775.08
B2	1584.40	1002.36	1330.15	484.96	621.00	682.80	1529.69	1422.90	2222.48	1490.50	1036.83
B5	1160.56	666.09	840.15	ND	210.20	306.80	1739.52	740.88	1584.18	1002.88	430.66
B6	1982.04	1106.30	1359.21	242.76	534.35	301.76	2511.44	1249.65	3256.98	2172.72	611.66

Table II- 12 Nitrate loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	NS	1273.30	1230.12	ND	862.80	2490.75	918.34	2013.55	3846.42	ND	522.11
D2	891.68	990.24	2059.84	694.40	1938.94	ND	813.12	1562.05	3752.96	ND	772.24
D3	1207.72	1670.37	1527.73	1042.86	750.72	1090.89	838.42	1231.14	1121.47	ND	790.43
D4	934.57	1049.93	1162.32	601.26	867.02	1673.48	1504.67	1776.60	864.73	ND	699.33
D5	1097.32	1194.73	1765.75	839.27	1118.10	1003.52	1171.10	942.90	946.40	ND	563.30
B2	303.24	253.31	351.00	158.72	192.30	164.32	266.47	379.78	498.68	423.50	127.20
B5	240.03	195.84	222.21	ND	260.10	63.88	299.70	197.10	261.18	312.64	59.75
B6	392.95	329.56	351.72	58.76	89.35	65.80	459.95	325.50	606.48	585.09	87.49

Table II- 13 Phosphate loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1998	28/08/1997	30/10/1997
D1	NS	37.47	51.68	-	105.60	98.78	70.50	78.40	-		83.17
D2	61.76	18.34	56.96	60.20	70.18	-	91.24	51.45	-	-	56.70
D3	63.97	30.86	62.53	92.82	44.85	118.22	66.49	99.28	41.07	-	66.37
D4	44.29	30.36	55.11	55.11	53.68	112.98	59.83	106.75	26.39	-	59.52
D5	43.79	32.13	54.25	67.24	54.90	114.51	61.39	90.00	47.25	-	48.00
B2	-	-	-	4.80	-	-	-	-	-	-	-
B5	-	2.61	-	-	-	-	-	-	-	-	-
B6	-	-	-	-	0.95	-	-	-	-	-	5.75

Table II- 14 Sulphate loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	4792.03	3580.20	ND	2830.20	3673.35	2969.04	3531.50	3324.86	ND	1672.55
D2	3841.47	2388.80	3137.28	2187.50	3046.16	ND	7629.78	3644.90	3217.60	ND	1941.58
D3	4740.26	5430.56	3830.61	2501.52	1992.72	4333.47	2937.28	3481.26	3329.63	ND	2345.97
D4	4052.39	2627.41	3295.24	1972.41	2073.50	3772.00	4005.58	3475.85	2401.79	ND	2227.91
D5	4796.09	2625.18	3903.55	2487.06	2969.70	3870.51	4106.26	3299.40	2896.60	ND	1683.40
B2	3968.73	1521.38	1952.47	707.60	833.04	1052.48	1838.20	3044.70	3477.24	2425.50	1047.73
B5	3176.62	957.65	1178.91	ND	288.04	486.72	2318.77	1346.31	2348.64	1612.64	550.72
B6	4950.80	1694.96	2088.95	360.20	734.25	486.64	3476.46	2211.00	5003.84	3244.56	812.06

Table II- 15 Trihalomethane loadings (µg/s) recorded at identified sampling points at the Dagenham wetland during dry weather.

Site	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/07/1997	28/08/1997	30/10/1998
D1	618.00	2119.50	2441.20	1634.50	1795.20	ND	573.60
D2	545.20	ND	2403.20	1001.00	1472.00	ND	533.60
D3	522.10	1565.70	2578.90	1319.20	1554.00	ND	418.20
D4	682.00	2686.40	2097.52	1169.00	1219.10	ND	412.30
D5	630.00	666.40	2901.50	972.00	1267.00	ND	240.00

Table II- 16 Total ammonia loadings (mgN/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

	18/10/1995	10/01/1996	06/03/1996	01/07/96	11/09/1996	05/11/96	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	11.322	15.878	ND	9.000	36.000	3.400	4.095	13.600	ND	20.800
D2	19.200	10.656	17.088	3.500	10.643	#VALUE!	3.200	3.500	11.744	ND	48.372
D3	24.679	12.321	40.108	7.014	3.059	6.783	3.071	14.756	14.800	ND	7.956
D4	20.040	8.918	26.720	3.300	2.574	107.364	3.908	4.095	11.122	ND	10.323
D5	18.690	3.500	25.690	4.797	3.990	29.400	4.095	3.510	11.655	ND	6.675
B2	2.771	2.314	6.014	0.800	7.002	0.936	1.621	1.700	13.884	2.075	0.700
B5	1.197	1.800	2.403	ND	3.736	0.800	0.747	0.900	2.394	1.600	0.835
B6	2.553	3.057	4.081	0.400	15.000	0.668	10.196	1.245	6.346	3.300	0.500

Table II- 17 Unionised ammonia loadings (µgN/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during dry weather.

Site	18/10/1995	10/01/1996	06/03/1996	01/07/1996	11/09/1996	05/11/1996	21/01/1997	04/04/1997	24/06/1997	28/08/1997	30/10/1997
D1	ND	164.56	259.08	ND	56.70	242.10	11.22	30.80	58.48	ND	136.76
D2	51.52	154.88	275.20	2.45	43.79	ND	10.56	20.30	25.28	ND	649.60
D3	69.19	113.59	650.46	53.34	16.79	47.94	12.58	89.76	40.33	ND	85.68
D4	39.41	65.46	393.79	48.51	28.82	604.44	12.36	29.05	22.71	ND	177.94
D5	28.35	20.30	170.10	35.67	130.50	214.62	16.45	38.70	42.70	ND	9.00
B2	6.37	0.83	94.09	5.12	92.76	48.64	13.44	3.57	80.08	16.25	1.40
B5	2.43	5.67	37.62	ND	31.38	22.04	6.21	7.20	7.56	7.84	4.65
B6	25.99	30.72	64.66	4.48	235.05	35.48	86.67	7.35	41.04	8.25	6.96

Table II- 18 BOD_5 loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	1056.0	2350.4			
D5	772.8	1868.8			
B2		187.2	809.6	378.0	6768.0
B6		97.6	758.4	252.0	8569.6

Table II- 19 Suspended solids loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	SE 28/11/97	26/05/1998
D1	240.0	5695.2			
D5	644.0	7416.8			
B2		3018.6	6191.6	20142.0	45167.9
B6		2244.8	9788.1	16203.6	47470.6

Table II- 20 Zinc loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	37920	52432			
D5	552	29784			
B2		6025.5	18584	9585	62322
B6		15372	8374	10395	44084

Table II- 21 Cadmium loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	575.40	2892.80			
D5	8.28	1576.80			
B2		538.20	276.00	87.75	310.00
B6		591.70	284.40	22.68	453.00

Table II- 22 Lead loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	1911.00	13243.60			
D5	325.22	5825.40			
B2		2316.60	10101.60	4347.00	17343.00
B6		1884.90	3792.00	2627.10	26945.00

Table II- 23 Chromium loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	322.20	6237.60			
D5	12.42	2090.72			
B2		322.92	1435.20	749.25	2200.00
B6		294.02	537.20	441.00	2637.00

Table II- 24 Nickel loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	1873.50	26419.40			
D5	1292.83	16527.20			
B2		1895.40	1398.40	270.00	2651.00
B6		2769.40	323.90	75.60	3049.00

Table II- 25 Copper loadings (µg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	2100.90	19345.60			
D5	140.76	11709.20			
B2		1690.65	3896.10	3543.75	8714.00
B6		1994.70	584.60	2066.40	15409.00

Table II- 26 Chloride loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/96	25/08/97	08/10/97	28/11/97	26/5/98
D1	5599.80	8899.88			
D5	2962.86	7329.20			
B2		4992.39	2665.24	25014.15	5964.30
B6		3143.94	2770.53	13573.98	7972.20

Table II- 27 Nitrate loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/96	25/08/97	08/10/97	28/11/97	26/5/98
D1	1981.20	7132.56			
D5	839.96	4984.44			
B2		1909.44	500.48	2818.80	1869.10
B6		1378.60	679.40	3180.24	2715.08

Table II- 28 Phosphate loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/96	25/08/97	08/10/97	28/11/97	26/5/98
D1	84.60	81.36			
D5	69.00	110.96			
B2		25.74	ND	ND	ND
B6		13.42	ND	ND	135.96

Table II- 29 Sulphate loadings (mg/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/96	25/08/97	08/10/97	28/11/97	26/5/98
D1	4222.8	11675.16			
D5	2783.00	8710.36			
B2		4145.31	4102.28	10739.25	8643.86
B6		4509.12	4672.85	12869.64	12557.76

Table II- 30 Total ammonia loadings (mgN/s) recorded at identified sampling points at the Dagenham and Brentwood wetlands during storm events.

	01/05/1996	25/08/1997	08/10/1997	28/11/1997	26/05/1998
D1	36.0	150.5			
D5	7.7	155.9			
B2		19.5	10.8	36.0	37.5
B6		24.4	31.6	25.2	54.8