Cranfield University

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SMALL-SCALE CONSTRUCTED WETLAND FOR ONSITE LIGHT GREY WATER TREATMENT AND RECYCLING

Centre for Water Sciences
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PhD Thesis

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Supervisors: Dr Paul JEFFREY and Dr Bruce JEFFERSON

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ABSTRACT

This study focused on the investigation of the impact of household cleaning and personal care products on the quality of grey water and the assessment and optimisation of grey water treatment by a novel constructed wetland design. The prototype wetland design which comprised three-stage cascading beds (0.27 m^2 by 0.20 m deep) with sand media, (d_{10}: 1.0 mm and d_{90}: 4.0 mm) was tested for treatment performance to meet non-potable reuse standards in three versions, unplanted open beds, unplanted covered beds, and planted beds (comprising mixtures of *Iris pseudacorus, Iris chrysographes, Carex elata Aurea* and *Mentha aquatica*). The prototypes were benchmarked against a standard single-pass wetland (6 m^2 by 0.7 m) planted with *Phragmites australis*. Performance was measured in terms of removal of conventional water quality determinant parameters, as well as Total coliforms and *E coli*, and surfactants. Microbial dynamics were also monitored during the study by looking at variations in microbial compositions with time for the different wetlands. All the wetland versions effectively removed more than 98 % turbidity and organics meeting the most stringent reuse wastewater reuse standards of < 2.0 NTU and < 10 mg BOD_5/L respectively. The influent grey water had low BOD:COD ratio ranging from 0.27 – 0.45, which is indicative of low biodegradability. The comparison of the cascade wetland performances showed the following: open beds > planted = covered, with the open beds version meeting reuse standards virtually throughout the monitoring period, despite recurrence of *schmutsdecke* in the top bed. All wetland technologies supported viable populations of microorganisms. Only phospholipid fatty acids (PLFAs) of lower carbon chain length (< C_{20}) had concentrations greater than 1 mol %, in all the wetlands beds, confirming that the majority of the PLFAs in the media were from contribution of microbial organisms and not plant organic matter. Characterisation of microbial organisms was carried out to understand the constructed wetlands functioning and thus the treatment processes. The household products showed nutrient deficiency signifying low treatability. Product branding did not show correlation with any water quality parameters. In terms of toxicity, laundry and cleaning products were more inhibiting to soil microorganisms than were personal care products.

Keywords: grey water, cleaning products, vertical flow wetland, water reuse, water quality

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And to my father, in heaven above, El Shaddai, "...you make all things possible, in your time".

It is only when you realise that
you have nothing to lose,that
you have everything to gain

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ABBREVIATIONS AND NOTATION

APEO – Alkylphenol ethoxylate

BAF – Biological Aerated Filter

BOD – Biochemical Oxygen Demand

CFU – Colony Forming Units

CIRIA - Construction Industry Research and Information Association

COD – Chemical Oxygen Demand

CTAS – Cobalt Thiocynate Active Substance

CTMAB - Cetyl trimethylammonium bromide

CW – Constructed Wetland

DBAS – Disulfine Blue Active Substance

DGGE – Denaturing Gradient Gel Electrophoresis

DO – Dissolved Oxygen

DTDMAC - Ditallow-dimethyl-ammonium-chloride

EC – Effective Concentration

EL-FAMEs - Ester-linked fatty acids methyl esters

FAME - Fatty acid methyl esters

$F{:}_{tot}B$ – fungal PLFA:bacterial PLFA ratio

$G^-{:}G^+$ - Gram negative bacteria to Gram positive bacteria ratio

GN – Gram-negative bacteria

GP – Gram-positive bacteria

GW – Grey Water

HF – Horizontal Flow

HLR – Hydraulic Loading Rate

LAS – Liner alkylbenzene sulfonate

MBAS – Methyl Blue Active Substance

MBR – Membrane Bio-Reactor

MPIP – Max Planck Institute Process

MPN – Most Probable Number

MSIR – Multiple Substrate Induced Respiration

MTBE- Methyl Tert-Butyl ether

NTU – Nephelometric Turbidity Units

OLR – Organic Loading Rate

OTR – Oxygen Transfer Capacity

PAA – Per-acetic acid

PCP – Personal Care Product

PLFA – Phospholipid Fatty Acid

PPCP – Pharmaceutical and Personal Care Product

PVC – Poly vinyl chloride

RABIT – Rapid Automated Bacterial Impedance Technique

RBC – Rotatory Bio-Contactor

SIR – Substrate Induced Respiration

SSF – Sub-surface Flow

TN – Total Nitrogen

TP – Total Phosphorus

TOC – Total Organic Carbon

TSS – Total Suspended Solids

VF – Vertical Flow

VFCW – Vertical Flow Constructed Wetland

VFPCW – Vertical Flow Planted Constructed Wetland

WWTP – Wastewater Treatment Plant

CHAPTER 1: INTRODUCTION

INTRODUCTION

1.1. PROJECT BACKGROUND

Urban development, the world over, depends on a sustainable supply of fresh water. However pressures on water resource availability have continued to increase due to increasing per capita demand and climate change. Although 100% of the water supplied to households in developed countries is of potable quality, only 3% is used for drinking and cooking and 35% ends up as black (toilet) water; the rest (65%) ends up as grey water. Grey water is defined as domestic wastewater which does not contain waste from toilets (*i.e.* wastewater from sinks, baths, showers, and domestic appliances) (Leggert *et al.*, 2001). In many cities facing water resource challenges, water reuse is becoming the natural next step in the struggle to improve resource security and grey water recycling is playing an increasingly important role as an alternative water source. The philosophy behind conventional wastewater treatment systems which are designed to collect liquid waste in a hygienic way and treat it off-site is shifting towards a more holistic model of resource management involving water recycling (GWI, 2010).

The potential for grey water recycling is increasing because recycling provides an alternative resource to meet increasing water demand and ease the pressure on conventional water supplies and wastewater treatment facilities. However, the application of low-cost technologies has not been extensively investigated for grey water treatment and recycling. Grey water recycling is currently practiced for non potable household uses, particularly for toilet flushing and landscape irrigation. Constructed wetland (CW) technology for grey water treatment for reuse is now attracting growing interest because of its applicability at small or single household scales. A constructed wetland is a wetland that is purposely constructed in a non-wetland area (International Association on Water Quality, 2000). There are advantages associated with use of constructed wetlands because the technology protects the environment and economics through the avoidance of the costly central disposal infrastructure and also provision of service water in water-scarce areas (Otterpohl *et al.*, 2003). Various studies have already shown that grey water reuse is feasible both economically and technologically for toilet flushing (March *et al.*, 2004) and landscape irrigation (Gross *et al.*, 2005). Different technologies such as membrane bioreactors (MBRs) or rotating biocontactors (RBCs), and biological aerated filters (BAFs) have been studied for grey water treatment and they achieve high removal efficiencies for most of the parameters of concern in wastewater, such as microbial pathogen, chemicals and drugs (Surendran and Wheatley, 1998; Toze, 2006; Jefferson *et al.*, 2001; Friedler *et al.*, 2005; Nolde, 2000). However, these technologies are high-tech and therefore not economically suited for small-scale applications, such as individual houses. Technical innovations are required to simplify the treatment processes so as to increase grey water recycling at small scale level and encourage a “circular society”.

Grey water provides an alternative source of non-potable water as well as a reduction of sewage flow rates and thus the cost of treating sewage. However, the contribution to pollutant loads and xenobiotics originating from the different cleaning and personal care products, which are found in grey water, are not well understood. In addition, their contribution to biochemical and microbial dynamics in treatment beds have so far not been included in most performance assessments of constructed wetlands.

1.2 MOTIVATION FOR THE WORK

The design of constructed wetland technologies is traditionally based on biochemical oxygen removal and concentrates on the removal of nutrients, nitrogen and phosphorus, and general water quality parameters. Hence, information on the suitability of wetland treated grey water for non-potable household uses is inadequate because of the biased focus on nutrients and public health parameters. Other important design issues, such as the dynamics of pollutant removal processes in the media of the treatment wetlands (the black-box) have not been adequately explored for constructed wetland. Instead design equations have borrowed some features, like retention time and surface area, from conventional treatment processes such as waste stabilisation ponds and sand filtration systems (Kadlec and Wallace, 2009). Consequently, the wetland technology has only been applied at large scale leading to low uptake of this technology at small-scale where its use for onsite treatment of grey water is easier. Other aspects of the technology such as, the role of plants in the wetland technology, choice of media, operation modes, are not well understood, thereby restricting design consideration to just higher plants, for instance. On the aspect of bed-depth, the typical depth for vertical flow SSF wetlands is 0.6 – 1.2 m yet the main degradation of substances takes part in the upper 0.2 m of the reactor-beds regardless of the bed depth (Platzer, 1999; Felde and Kunst, 1997). This study, therefore, was aimed at understanding this aspect as well as other factors (from grey water quality to flow design, and operation) that contribute to the treatment processes and, the role of plants (both the well known treatment wetland plants and other common wetland plants) in treatment wetlands. Performance of the wetland technology in removing surfactants was also investigated suitability of the treated grey water and implications for irrigation reuse.

1.3 SCOPE AND RESEARCH HYPOTHESIS

1.3.1 Scope

The overall goal of this research is to contribute to the design of the constructed wetland technology for on-site grey water treatment at small scale. The research explores the characteristics of products that make up the chemical component of the grey water and their impact on the resultant characteristics of the grey water. Further, the research monitors the performance of a prototype constructed wetland, designed with shallow beds which were arranged in a slanting cascading format to investigate aspects of the technology that contribute to treatment processes that occur in the wetlands.

1.3.2 Research hypothesis

It was hypothesised, firstly, that the quality of grey water can be predicted from the knowledge of the household cleaning and personal care products that are used by the generating household and secondly, that the wetland treatment technology can be optimised for grey water treatment. Accordingly, the following specific research aims were investigated, to:

- o Establish the predictability of physico-chemical characteristics and toxicity of grey water based category and branding of personal hygiene/cleaning products ("down-the-drain" chemicals)
- o Assess the treatment performance of the prototype cascading constructed wetland, to establish aspects of the wetland technology that can be optimised to generate treated grey water that meets published wastewater reuse standards.

1.4 STRUCTURE AND OVERVIEW OF THE THESIS

The thesis is presented in the form of a series of chapters formatted as journal papers, with all supporting information and references provided at the end of each chapter. One Chapter (5) has been accepted for publication in Water Science and Technology and the other Chapters are ready for submission to various journals (Table 1.1). All papers were written by the first author, Wilfred Kadewa, and have been edited by Dr Bruce Jefferson and Dr Paul Jeffrey. All experimental work was undertaken by Wilfred Kadewa at Cranfield University main campus. The prototype cascade wetland was designed and constructed by WPL Ltd in Portsmouth and the standard Vertical Flow Constructed Wetland was inherited form a previous PhD project under the EPSRC funded Water Cycle Management for New Development (WaND) Project.

The first paper (Chapter 2) is a *literature review* which discusses constructed wetland technology design, design criteria and treatment processes. The paper explores the implications of these aspects on grey water treatment and how they do not account for removal of xenobiotics which are important in grey water reuse, particularly, irrigation. The need for effective designs that are based on a more robust understanding of the bio-physical and biochemical processes that take place in vertical flow constructed wetland systems is discussed.

The technical content of the thesis is incorporated into Chapters 3 to 7. Chapter 3, *Impact of consumer product types on grey water characteristics,* explores the link between different types of consumer cleaning/personal care products and grey water characteristics. Wetland treatment technology is affected by the characteristics of influent grey water (Jefferson *et al.*, 2004; Eriksson *et al.*, 2006) which in turn depends on different categories and brands of cleaning and personal care products and their ingredients, as well as consumer behaviour. Understanding these aspects is important in technology design and optimisation of its performance. The paper explores the relationships (or lack) between the cleaning and personal-care products and grey water characteristics.

Chapter 4, *Bench scale filtration studies,* looks at bench scale filtration and investigates the media specifications and operational parameters which are suitable for vertical flow wetland configurations for grey water treatment. The paper focuses on the removal of suspended solids and COD and their clogging propensity, of different grain sizes, and for different media depths. The study also investigates the disinfection potential of silver coated sand for removal of Total coliform and E coli. Results are discussed with reference to suitability for cascade wetland.

Chapters 5, *Cascading shallow bed constructed sand filter,* and 6, *Effects of design of constructed wetland on treatment performance,* both look at performance of a novel cascading vertical flow wetland prototype. Three versions of the same design were tested, where the main distinguishing features were presence or absence of plants. The first paper (Chapter 5) looks at performance of an unplanted and open prototype which was benchmarked against a standard vertical flow wetland (6 m^2 by 0.7 m) planted with *Phragmites australis.* The second paper (Chapter 6) looks at two versions of a similar design as in the first case where one version was planted with mixtures of *Mentha aquatica, Carex elata, Iris pseudocorus and Iris chrysographes* and the other version was without plants and the treatment beds were covered except for small windows to ensure air access. Both versions were benchmarked against the standard (0.7 m deep) vertical flow planted wetland planted with *Phragmites australis*. Treatment performance is discussed for different operation modes, loading rates, environmental conditions, and design aspects. Correlations were made for different design aspects to determine circumstances that led to ‘good performance’.

Chapter 7, *Microbial composition in treatment wetland beds*, investigates the microbial community composition in the beds of all the three versions of prototype and discusses the relationship to treatment performance. In addition this chapter looks at the impact of grey water strength, weather conditions and vegetation regimes and discusses how they contributed to the microbial structure and thus treatment performance.

Chapter 8, *Discussion and conclusions*, is a summary discussion of all the technical findings from the research project. Chapter 8 links the outcomes of all the papers and discusses the impact of household products on grey water quality and in turn links this to wetland design and treatment. The main discussion focuses specifically on the design of the cascade wetland prototype and compared its performance to a standard vertical flow constructed wetland. The paper discusses implications of the design for grey water treatment and reuse applications as well as impact on microbial dynamics in the wetland beds. The chapter evaluates and further analyses the implications of cleaning and personal care products on grey water quality.

Finally, *Conclusions and Future Work*, Chapter 9, lists the key results of the study and makes recommendations how future investigations can expand current knowledge of small-scale constructed wetland technology for onsite grey water treatment and recycling.

Table 1.1: Thesis structure and objectives of each chapter

Chapter	Main objective	Contents	Status
Chapter 1	Introduction	General introduction and thesis outline	
Chapter 2	Literature review and Project aims and hypothesis	State of art and research gaps and sets out the aims and objectives of the study	To be submitted to *Ecological Engineering*
Chapter 3	Impact of consumer product type on grey water characteristics	Establishes the link between consumer products and grey water quality	To be submitted to *Science of the Total Environment*
Chapter 4	Bench scale filtration studies: Choice of media size and depth	Discusses wetland media specifications and operational parameters	To be submitted to *Water Science and Technology*
Chapter 5	Evaluation of a novel cascading shallow bed constructed sand filter for grey water treatment	Discusses performance of the unplanted cascade wetland	Accepted for publication in: *Water Science and Technology*
Chapter 6	Constructed wetland: Effect of design and vegetation on treatment of grey water	Discusses performance of the planted and covered versions of the cascade wetland	To be submitted to *Water Research*
Chapter 7	Characterisation of microbial diversity in vertical flow constructed wetlands: Relationship to wetland design and age	Discusses the microbial structure, and contribution to treatment, in the three beds of the cascade wetlands	To be submitted to *Applied and Environmental Microbiology*
Chapter 8	General Discussion and synthesis	Summarises all the papers and considers the impact of plants and bed depth in the cascade wetland design for GW treatment and reuse	
Chapter 9	Conclusion and recommendations	Conclusions and recommendations for future work	

Conference papers:

Kadewa, W.; Le Corre, K.; Pidou, M.; Jeffrey, P. J.; Jefferson, B. (2009). "Evaluation of a novel cascading shallow bed constructed sand filter for grey water treatment" *IWA Water Reuse Conference*, 20 – 25 September 2009, Brisbane, Australia.

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CHAPTER 2: LITERATURE REVIEW

DESIGN CONSIDERATIONS OF CONSTRUCTED WETLANDS FOR ENHANCED GREY WATER TREATMENT AND RECYCLING

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2.1. ABSTRACT

Internal recycling of grey water, whereby grey water is taken from single houses that generate it, requires only the use of low-tech treatment technologies which meet the requirements for use at small scale. Vertical flow constructed wetland technology is suitable for grey water internal recycling, and has been shown to be feasible for grey water treatment meeting existing reuse standards around the world. However, the challenge with low-tech treatment systems is the removal of xenobiotic organic compounds. Constructed wetlands are fast becoming the preferred treatment for small-scale treatment of grey water. The constructed wetland design has evolved from simple designs based on waste stabilizing ponds and sand filters, which were intended for wastewater treatment not reuse. The models are also based on variants of first-order kinetics based on BOD removal which does not always correlate with removal of xenobiotics found in grey water. Design principles for grey water treatment and reuse systems are currently poorly evidenced. Therefore effective designs need to be based on a more robust understanding of the bio-physical and biochemical processes that take place in vertical flow constructed wetland systems. This review discusses design considerations for grey water treatment focussing on aspects of the technology and their perceived role(s) in various treatment processes that take place.

2.2 INTRODUCTION

Different treatment technologies have been developed to treat wastewater from human dwellings and activities. Technology development continues to grow and diversify in order to provide some or all functions of treatment at different levels, *i.e.* primary, secondary or higher. Constructed wetland technology is fast becoming the preffered treatment for small-scale treatment of grey water because of its low capital and operation costs (Gross *et al.*, 2008; IWA, 2000) and its proven ability to remove suspended and dissolved particles, organics and pathogenic organisms (Kadam *et al.*, 2008; Toze, 2006; Dallas *et al.*, 2004). This makes it attractive for small-scale application, however, design considerations for constructed wetlands mainly focus on the removal of organics, nutrients and pathogens, which might be adequate for treatment of combined domestic wastewater. Treatment of grey water, which contains low levels of nutrients and pathogens and higher levels of xenobiotic organic compounds (Ericksson *et al*., 2003) requires a deeper understanding of the wetland ecosystem processes that take place in constructed wetlands, beyond the current knowledge which is based on slow sand filtration. Further, the contributions of plants and other wetland aspects, *e.g.*bed depth and media grain size, need to be well understood so that constructed wetland designs can be best suited to specific scenarios. The ability to predetermine the physico-chemical nature of grey water from the household cleaning and personal hygiene activities would also be very useful in wetland design and operation considerations, inorder to achieve desired treatment goals. This review, therefore, looks at grey water characteristics and the design considerations of constructed wetlands for grey water treatment and recycling, focussing on the roles of various components of the technology that can be optimised for grey water treatment.

2.2.1 Grey water characteristics

Grey water is defined as domestic wastewater which does not contain waste from toilets (Leggert *et al.*, 2001). It comprises the wastewater from bathtubs, showers, hand basins, laundry machines and domestic appliances with precise composition depending on the sources and installations from where the water is drawn (Eriksson *et al.*, 2002). Grey water is the most promising of the sources of urban water for recycling because its characterisation reveals a source water that is comparable to a low-medium strength municipal sewage influent, thereby making it relatively easier to treat, compared to other domestic wastewaters (Jefferson *et al.*, 2004). In terms of pollution loads, grey water is divided into two groups: light grey water, from personal washing, and heavily polluted grey water from washing machines, dish washers and kitchen sinks (Jefferson *et al.*, 1999). Light grey water forms the bulk of grey water because hand-washing, followed by teeth brushing and showering constitute the highest water-consuming activities in a household (Eriksson *et al.*, 2003). For a given geographical area, with fairly similar lifestyles, similar piping installations and water consumption levels, the main source of variability in grey water quality is likely to be the type and quantities of household cleaning and personal care products (ingredients) used. However, to date, direct risk assessment of the chemicals present in household cleaning and personal care products has been difficult because of the limited information provided on most lists of contents. Manufacturers tend to provide the bare minimum of information required by law and in most cases they use trade names instead of actual IUPAC (International Union of Pure and Applied Chemistry) chemical names for trade and other reasons. It is important to establish the relationship between grey water toxicity and the different chemical ingredients found in the household chemical products. This would make it easier to predict grey water properties and treatment requirements.

In the absence of data from manufacturers, another way of characterising grey water would be to measure its gross chemical (organics and nutrients), physical (suspended solids and particulates) and biological (microorganisms) constituents as done for grey water (Table 2.1). Variability across these categories is an overriding factor which greatly impacts on grey water quality and the potential effectiveness of treatment processes. To illustrate, grey water from baths shows the lowest turbidity (53 NTU) compared to the other types of grey water (131-254 NTU). Comparison of grey water organic strength with that of sewage wastewater (100-350 mg BOD.L^{-1}) (Metcalf and Eddy Inc., 2003) shows that bath, shower and wash basin grey water lie on the lower (organic strength) end whilst laundry grey water falls at the higher end. Kitchen grey water is even stronger and actually falls outside the range for wastewater. However, in terms of COD:BOD ratio (Table 2.2), the different grey water types have high ratios and nutrient defieciancy which indicates that grey water would be difficult to treat using biological processes (Jefferson *et al.*, 2004). Typical average value for

COD:BOD ratio of domestic sewage is 2.2 and the ratio for final effluent range from 3.0 to 10.0 (Metcalf and Eddy Inc., 2003). The observed variations in grey water characteristics have been linked to differences in types of products (Knops *et al.*, 2007) and behaviours (lifestyles, water consumption, socio-economic *etc.*) of the people generating the grey water (Eriksson *et al.*, 2002). In terms of toxicity, Eriksson *et al.* (2006) reported in their study that laundry and kitchen waters were the sources that contribute the most to general toxicity of grey water as a reflection of the chemical ingredients used. Major compounds used in household chemicals are categorised as surfactants, fragrances and flavours, solvents and preservatives. All these product categories, singly or in combination, give rise to toxicity which presents an environmental hazard especially for some grey water recycling applications. This knowledge gap makes it difficult to make any conclusions about the passage and fate of potentially hazardous chemical species through the treatment process (Knops *et al.*, 2007; Eriksson *et al.*, 2002). Surfactants in soil systems, for instance, have been studied extensively, but the medium of application in those studies was mostly sewage sludge and not grey water. However the effects other xenobiotic organic chemicals on both the treatment processes and the environment, as a whole, have not been explored extensively (Jefferson *et al.*, 2004; Eriksson *et al.*, 2002). Surfactants are the largest group of compounds in grey water and have the potential to cause changes in rhizosphere communities and capillary rise of water in soils affecting plants' uptake of water from the soil. Yet, their fate in grey water treatment and recycling has not been studied extensively. Furthermore, for grey water recycling, the reliability and efficiency of the treatment process/technology in removing these chemicals becomes more important. However, to date most treatment studies have not considered removal of surfactants and other xenobiotics. The importance of xenobiotics in treatment processes, as discussed by Volskay *et al.* (1990), is through their impact on substrate where they:

1. Cause substrate inhibition, preventing their own biodegradation (uncompetitive inhibition),
2. Influence the rate of substrate utilisation by competition (competitive inhibition) and
3. Inhibit substrate utilisation by micro-organisms that are not capable of the degrading xenobiotic organic compounds (non competitive inhibition).

Table 2.1: Grey water characteristics by source (*range for each parameter shown in parenthesis)*

Parameter	Bath	Shower	Wash basin	Laundry	Kitchen	Mixed
Turbidity (NTU)	53 (46-60)	131 (21-375)	133 (102-164)	254 (50-444)		84 (33-240)
SS ($mg.L^{-1}$)	53 (47-304)	173 (15-353)	183 (36-505)	238 (68-465)	528 (235-720)	113 (25-304)
pH	7.6 (7.5-7.6)	7.5 (7.2-7.6)	7.3 (7.1-8.1)	8.9 (8.1-10)		7.2(6.5-8.6)
BOD ($mg.L^{-1}$)	161 (129-192)	155 (99-212)	138 (33-252)	276 (48-472)	891 (536-1460)	154 (5-466)
COD ($mg.L^{-1}$)	210	170 (109-501)	280 (263-298)			240(23-1583)
TOC ($mg.L^{-1}$)	81 (46-104)	74 (65-83)	70 (40-99)	175 (110-251)		41 (9-254)
NH_4-N ($mg.L^{-1}$)	1.3 (1-3.6)	1.2 (0.4-12)		2 (0.7-11)	3.2 (0.3-5.3)	4 (0.1-17)
NO_3-N ($mg.L^{-1}$)	0.4 (0-4.1)	6.3 (0-29)		1.4 (0-26)	0.5 (0-6)	1.7 (0-9.6)
TKN ($mg.L^{-1}$)	8.7 (7-11)	15.2	6.8 (4-9.6)	29 (18-40)		4.6 (0-27)
TP ($mg.L^{-1}$)	2.7 (1-4.5)	1.8 (1.6-20	2.6	52 (42-60)	29 (23-35)	7.4 (0.4-31)
PO_4-P ($mg.L^{-1}$)	1.3 (0.4-10)	1 (0.3-19)	29 (0.4-49)	61 (13-171)	14 (10-26)	2.3 (0.4-10)
TC ($cfu.100mL^{-1}$)	10^3 (10^2-10^4)	10^4 (10^1-10^4)	10^5 (10^3-10^6)	10^4 (10^3-10^6)		10^4 (10^2-10^8)
FC ($cfu.100mL^{-1}$)	10^2	10^5 (10^2-10^6)	10^2 (10^1-10^2)	10^2 (10^1-10^3)		10^4 (10^2-10^8)
COD:BOD*	1.30	1.10	2.03			1.56
COD:N:P	1:0.04:0.01*	1:0.09:0.01*	1:0.02:0.01*	1:0.02:0.15		1:0.02:0.01

adapted from Metcalf and Eddy (2003), Jefferson et al. (2004), Frazer-Williams et al. (2007), Pidou et al. (2008), Birks and Hills (2007): * calculated from literature data presented in the table.

Table 2.2: Typical ratios for waste/grey water

Type of wastewater	**COD:BOD**	**COD:TOC**	**BOD:TOC**	**Reference**
Raw sewage	1.30 – 4.22	3.32 – 4.68	1.31 – 1.88	(Droste, 1997)
	1.25 – 3.33	n.a.	1.20 – 2.00	Metcalf and Eddy, 2003
Grey water	3.10	7.80	2.80	(Laine, 2002)
	2.40	3.00	1.30	Calculated*
Tertiary effluent	3.85 – 8.64	2.02 – 2.58	0.20 – 0.69	(Droste, 1997)
	3.33 - 10	n.a.	0.20 – 0.50	Metcalf and Eddy, 2003

The complex interaction between xenobiotics, micro-organisms and available substrate has unpredictable negative effects on the performance of treatment processes for domestic wastewater (Palmquist and Hanaeus, 2005). For instance the presence of xenobiotic compounds results in increased production of soluble microbial products (SMP) by micro-organisms, which in turn can have several effects on the treatment process including being toxic and affecting kinetic activity or altering sludge characteristics (Barker and Stuckey, 1999). For grey water treatment systems to be considered safe therefore, they must also be able to cope with xenobiotics and the worst conceivable contamination from the known sources within the household. The key here is to have knowledge of what is contained in the grey water and subsequently of the biochemical properties that are imparted to the grey water.

Surfactants in average domestic wastewaters account for the highest concentration of organic chemicals (Shafran *et al.*, 2005). Anionic surfactants which together with non-ionic surfactants comprise the largest groups of compounds in grey water are known to cause changes in rhizosphere communities and to inhibit the growth of some plants like lettuces (Eriksson *et al.*, 2006). Other essential chemicals for plants found in grey water, *e.g.* boron and sodium are toxic when present in high concentrations. Roesner *et al.* (2006) reported slight accumulation of boron in plants irrigated with grey water for two years where, however, no salt accumulation and increased Soil Adsorption Ratio (SAR) and sodium ions (Na^+), were observed. Longer term irrigation with grey water rich in surfactants might result in their accumulation in the soil and in the formation of water repellent soils (Eriksson *et al.*, 2006). Some studies on general wastewater reuse have shown that soil micro organisms generally benefit from organic matter and nutrients in wastewater and/or their by-products *e.g.* sewage sludge, despite there also being heavy metals in the wastewater (Kloepper-Sams *et al.*, 1996). Surfactants, on the other hand, have been shown to have both beneficial and detrimental effects on plants. To illustrate, microbial growth and/or stimulation effects may occur at low concentrations while phytotoxic effects may occur at high levels and long exposure. The differences are linked to potential hazards of long-term surfactant exposure, which result in the interaction of the surfactants with structural proteins and enzymes, interaction with cytomembranes, increasing their permeability, enhanced auxin absorption, chlorosis, solubilisation of chlorophyll protein complex, and altered nutrient uptake (Rinallo *et al.*, 1988).

2.2.2 Grey water reuse

The water reuse market has registered growth and migration of grey water reuse from lower value applications, such as agricultural use, to higher value applications such as industrial and domestic use due to increased availability of funding and sustainability credentials of reuse solutions (Global Water Intelligence, 2010). Water scarcity is a worldwide problem which is growing in importance in the whole world today. Unsustainable consumption, urban pollution, and effects of climate change continue to diminish and degrade water resources. Addressing pressures of high water demand and water-scarcity requires approaches (technologies) that offer economical use and reuse of water wherever possible (Bieker *et al.*, 2009). In many cities facing water resource challenges, grey water reuse is becoming the natural next step in securing reliable water supplies (Environment Agency, 2001). Wastewater recycling in general has, for the past decade, emerged as an integral part of integrated water resource management, promoting the preservation of fresh water supplies and also reducing pollution. The conventional wastewater treatment system was originally designed for sanitation reasons, to collect liquid waste in a hygienic way and treat it off-site centrally. However, due to high water demand which natural resources can no longer meet, water recycling, especially within the generating process loop *i.e.* within a household (hereafter referred to as internal recycling), is changing the philosophy and technology designs of wastewater treatment works. Internal recycling helps to minimise capital and operational costs on centralised systems, because water is retained in a small process loop (Jefferson *et al.*, 1999). Internal recycling is also preferred psychologically as most people feel comfortable dealing with their own wastewater (Jeffrey and Jefferson, 2001). Hence internally recycled water provides an alternative source of service water for both low and high quality applications even at small-scale. Local wastewater treatment and reuse is sensible in terms of reducing transportation pipe networks, energy, hazards from leakages and other infrastructural costs. The separation of water flows from different sources (grey water and black water) in the house is the first step towards the adequate treatment of domestic wastewater for treatment and reuse. Separating the two types of wastewater enables the subsequent use of only grey waters which exhibit low levels of pollution (Jefferson *et al.* 2004). This way the expensive recycling set up, where water is sourced from sewage works, is avoided. In addition, the need for high-tech treatment technologies is reduced.

Traditionally all water supplied to houses is of drinking quality, yet only small proportion, less than 3% in the developed world (EA, 2001) and less than 1% in the developing world (Ahmad and El-Dessouky, 2008) (Table 2.3) is used for drinking and/or cooking. In the UK for example, 12 % of the water supply is used for washing, 35 % for toilets, 20 % for showers and bath, and 6 % for outdoor use and other non-potable uses within the household (EA, 2001). Therefore grey water typically represents approximately 65 % of the total household water flux. The economic value of grey water, both in terms of quantity and as a resource is greatly underestimated. In areas of scarcity (Gross *et al.*, 2008; Otterpohl *et al.*, 2003) and/or poor water quality supply services, grey water reuse for agriculture and other purposes is very important. Reuse of grey water for non-potable needs around the household also provides significant water and financial savings to the people and also to the environment. For the developing world, this also means increased food security and improvement of public health (Madungwe and Sakuringwa, 2007; Morel and Diener, 2006).

Table 2.3 Grey water percentages (as a percentage of potable water supplied) in different parts of the world

Country/region	Potable use	Grey water outflow	Reference
U.K. (Europe)	3 %	65 %	EA, 2001
Pakistan (Asia)	1%		Ahmad and El-Dessouky, 2008
Zimbabwe (Africa)	-	60 %	Madungwe and Sakuringwa, 2007
South Africa (Africa)	-	75 %	(Carden *et al.*, 2007)
Costa Rica (C. America)	-	75 %	(Dallas *et al.*, 2004)

The principle challenge with treatment of grey water at household level arises from its pollutant load and variability nature. Grey water is subject to variations mainly arising from variations in consumer product choice (Knops *et al.*, 2007) and user habits (Eriksson *et al.*, 2003). These lead to unpredictability of the grey water characteristics which impact on the treatability of the grey water (Prathapar *et al.*, 2005; Royal Society of Chemistry, 2008) and treatment consistency to meet stringent reuse standards (US Environmental Protection Agency, USEPA, 2004). The challenge therefore is to design low-tech small-scale technologies which would be able to handle grey water in the absence of various buffering advantages observed in centralised systems which deal with large volumes of wastewater.

2.2.3 Public perception of grey water reuse

There are barriers to usage of recycled water in public water supply systems ranging legislation, cultural beliefs, ethics, income *etc*. Current trends worldwide show that where water resources are diminishing, water reuse is increasing, resulting in the breaking down of these barriers and vice versa. Generally the key to improving public perception of water reuse lays in higher confidence in the treatment technologies, whereby assurance of the safety of the reclaimed water, for both potable and non-potable uses, takes centre stage (Global Water Intelligence, 2010). A study by Jeffrey and Jefferson (2001) confirms that public perception of grey water reuse is positive if quality standards are assured. A number of studies have also shown examples where grey water reuse technologies are accepted, for instance, toilet flushing in hotels (March *et al.*, 2004) multi-residential single buildings, dormitories (Surendran and Wheatley, 1998) and landscape irrigation (Gross *et al.*, 2005). However from Figure 2.1 it is shown that from the current usage of reclamation water, agricultural and industrial uses are greater than the domestic uses which perhaps entails that the barriers for domestic reuse of grey water still exist. Further, although irrigation is the second most popular reuse type, the knowledge of the impact of xenobiotic organic compounds (XOCs), such as surfactants, in soil systems irrigated with treated or untreated grey water is limited (Eriksson *et al.*, 2006). Hence, more work is required to break down the existing barriers to usage of recycled grey water for all types of uses.

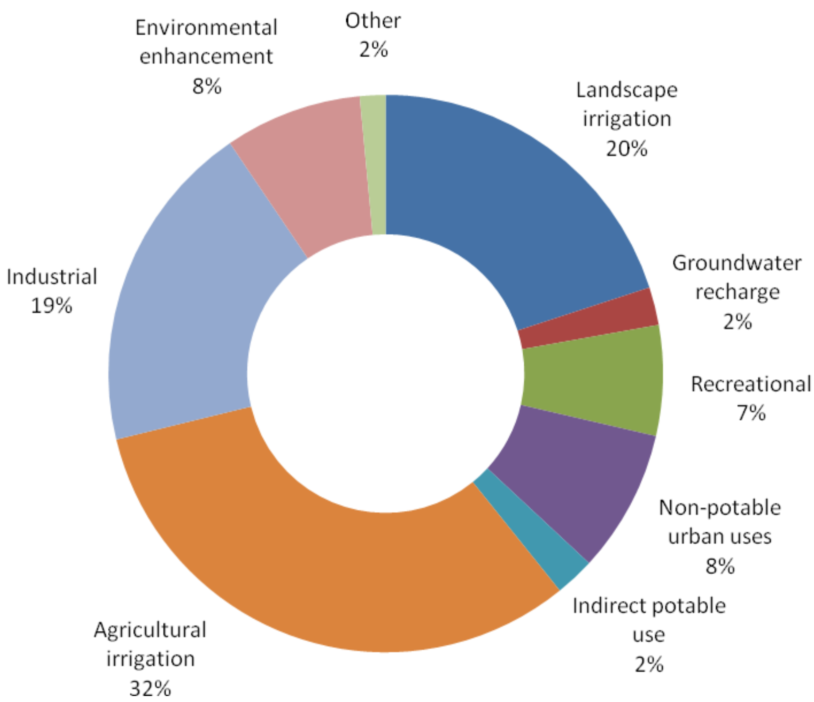

Figure 2.1: Water reuse: Market share by application, *GWI, 2010*

2.2.4 Grey water treatment technologies

In the past few years there has been a shift, world-over, in the use of wastewater treatment technologies from merely improving sanitation levels through removal of pathogenic and organic constituents and returning the water to surface water bodies to providing water for non-potable service use (Gikas and Tchobanoglous, 2009) and in some cases potable use (Lahnsteiner and Lempert, 2007). Despite this, the available technologies for wastewater reuse are still designed principally for the removal of pathogenic and organic constituents and so typically give low priority to proper removal or reduction of chemical species such as those found in grey water. Nonetheless, treated grey water is expected to meet set regulations and different levels of treatment for different types of reuse. Adoption of a technology for water reuse, therefore, has to be based on a number of criteria which incorporate both these aspects, which have been summarized in various literatures as follows (Metcalf and Eddy Inc., 2003; Bieker *et al.*, 2009; Ponce-Ortega *et al.*, 2010; Jokerst *et al.*, 2009).

- The type of water reuse application
- The type of influent wastewater
- Reuse water quality requirements
- Compatibility with existing conditions
- Process flexibility
- Operational and maintenance requirements
- Energy requirements
- Chemical requirements
- Personal requirements
- Environmental constraints

Treatment technologies for wastewater include physical treatment options such as filtration or membranes; biological based technologies such as, rotating biological contactors (RBC), biological aerated filters (BAF), membrane bioreactors (MBR) and reed beds; and chemical treatments such as electro-coagulation, photo catalysts, and membrane chemical reactors (Pidou *et al.*, 2007). However, most of these treatment technologies have high operation and investment costs especially when viewed from a customer expenditure point of view (GWI, 2010), which makes them unattractive for small-scale local treatment and non-potable reuse. Generally household expenditure for technologies that meet secondary and/or tertiary treatment standards at lower expenditure would be attractive. Constructed wetland (CW) technologies (*e.g.* reed beds) are being promoted as low technology (low-cost, low-maintenance) systems for treatment of domestic wastewater, because they meet this expenditure criterion (IWA, 2000). The technology combines two very old treatment methods of depth filtration (which is good for removal of particulate matter resulting in disinfection and removal of suspended and dissolved particles by means of filtration and adsorption) and microbial action (resulting in reduction of BOD). Use of constructed wetlands for grey water treatment has increased in recent years especially in areas where there are problems with conventional treatment systems. Although physical treatment systems for grey water, such as sand filtration, are the simplest (Kadlec and Knight, 1996), biological treatment systems are generally the most popular followed by extensive and physical treatment systems (Figure 2.2). However, the combination of these two processes, such as in constructed wetlands, enables removal of both particles and organics that are associated with particles.

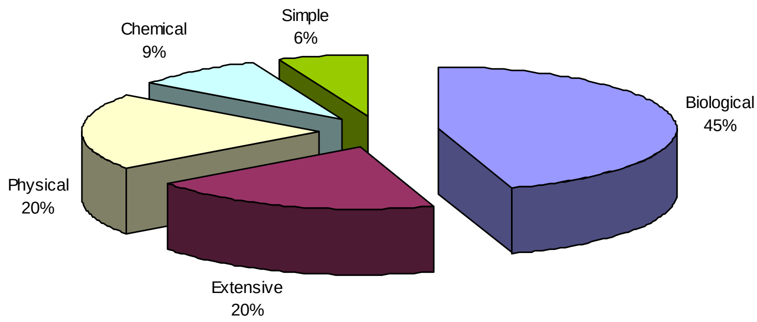

Figure 2.2: Prevalence of technologies for grey water recycling by process type *(Pidou et al., 2007)*

The efficacy of a filtration based technology, such as that provided by a constructed wetland, in removing cysts and other particles and dissolved chemicals in wastewater has been well documented (Kadam *et al.*, 2008; Heistad *et al.*, 2006; Kern and Idler, 1999). However, there is little information on the appropriate engineering design, construction and operation of constructed wetlands, to optimise the bio-physical and chemical processes, to meet the level of treatment and published reuse standards. Indeed, design considerations for CWs are still complicated and non-generic for the existing types of constructed wetland technologies (International Association on Water Quality, 2000). Clear treatment goals and the desired extent of removal of various (organic) chemical compounds of concern for different types of wastewater are the main criteria for defining appropriate design and operational parameters. The challenge that exists now is to optimise constructed wetland design, and the treatment of different types of wastewater (*e.g.* grey water) for sustainable and reliable treatment performance. This is because evidence from constructed wetland studies shows that although the technology can be used under different scenarios, the processes occurring within the wetland system are still not well understood.

2.3 CONSTRUCTED WETLANDS

2.3.1 Constructed wetlands for wastewater treatment

Constructed wetlands fall into two major types; Free Water Systems (FWS) also known as Surface Flow (SF) and Subsurface Flow systems (SSF). FWS have a permanent water depth with water flowing horizontally and treated across the wetland bed surface. Constructed wetland technology with sub-surface flow was first used as a technology for general wastewater treatment in Germany by Kathe Seidel in the 1960's (International Association on Water Quality, 2000). SSF systems used to be called the Root Zone Method because the water was treated in the root zone of a planted porous media. SSF systems are further sub-categorised into horizontal flow (HF) and vertical flow (VF) depending on how the wastewater flows through the bed. In HF systems water is introduced continuously at the inlet at one end close to the surface and flows through the media horizontally to the outlet at the opposite end. In VF systems, water is fed intermittently (in batches) from the top surface and flows vertically through the media. For the VF the unsaturated conditions that prevail between batches, allow for oxygen to circulate through the media voids thereby creating aerobic conditions in the VF system (Cooper *et al.*, 1996). These two designs have different dominating processes taking place which result in different treatment performance levels and these have been extensively investigated since the inception of wetland applications. Pollutant removal in CWs is a function of several physical, chemical and biological processes, with biological microbial processes driving the removal of organic matter and nitrogen (N). In terms of treatment efficiency, CWs have shown high BOD and COD removals of up to 98%, pathogenic indicator reduction of up to 99% (Vymazal, 2009; Vymazal, 2005; Frazer-Williams *et al.*, 2008). Hence constructed wetlands (CWs) provide a natural way for simple, inexpensive, and robust treatment of primary effluent wastewater. Constructed wetlands, unlike conventional treatment systems such as activated sludge, are able to treat wastewater with low organic concentration (< 50 – 80 BOD_5 mg/L) (Vymazal, 2009) in the HF variant and high organic concentrations in the VF variant (Frazer-Williams *et al.*, 2008; Langergraber *et al.*, 2009). This makes the constructed wetland technology suitable for grey water treatment, which naturally has a wide range of organic concentrations depending on the source. Despite the demonstrated effectiveness of CWs in handling wastewaters of different chemical characteristics, most grey water reuse projects have so far favoured advanced (state-of-the-art) treatment technologies such as membranes (MBRs) and BAFs (Jefferson *et al.*, 2001; Surendran and Wheatley, 1998) or RBCs (Friedler *et al.*, 2005; Nolde, 2000). Nonetheless, constructed wetland technology is becoming more common as a treatment option for grey water recycling. Knowledge of how CWs function, which are commonly viewed as "black box systems" (Langergraber, 2008), is on the increase because of the growing interest in the technology either as standalone processes or as a part of an integrated treatment system. However, there remains a paucity of knowledge on the critical factors required in designing CW designs, such as, the role of plants (Maltais-Landry *et al.*, 2009; Brix, 1999), and processes (Sirianuntapiboon *et al.*, 2007; Ramon *et al.*, 2004).

2.3.2 Design considerations

Constructed wetlands are referred to as a simple technology, providing limited removal of contaminants and targeting coarse solids, which might imply that they can be described using simple equations. However, simple treatment systems, such as, sand filtration systems, which only provide a limited removal of contaminants and target coarse solids, would not be expected to remove small particles and dissolved species in wastewater (Parsons and Jefferson, 2006). Constructed wetlands effectively function as eco-reactors (Kadlec and Knight, 1996), with many processes taking place and having the ability to remove small particles (depending on the porosity of the substrate filter media) and dissolved species. Therefore they cannot be put in the category of simple technologies. In fact constructed wetlands have been classified as extensive treatment systems (Pidou *et al.*, 2007) capable of performing both physical (filtration) and biological treatment of grey water. The pollutant removal performance of extensive systems is generally good with average removal levels of up to 99 % in terms of BOD, COD, turbidity and suspended solids (Dallas *et al.*, 2004; Frazer-Williams *et al.*, 2008).

Unlike horizontal flow wetland which run continuously, vertical flow wetlands exist in four variations described below (Gross *et al.*, 2007; Crites and Tchobanoglous, 1998):

1. Intermittent flow - periodic flooding of water at the top of the bed. This mode enhances oxygen transfer into the bed. When no plants are used, this design is effectively an intermittent sand filter.
2. Unsaturated down flow – water distributed across the top of a granular media trickles by means of unsaturated flow.
3. Saturated flow – employs continuous flow of water through the plant root zone. These can either be up or down-flow with the down-flow configuration being termed ‘anaerobic wetlands’.
4. Fill and drain (tidal) – cyclical filling and draining of granular bed where water is fed from the bottom until the bed is flooded. The wastewater is held in the bed in contact with the bacteria growing on the media for periods of up to 2 hours. Draining is mostly vertically downward.

Many studies have been conducted and many forms of design have been tested for horizontal SSF developed over the years since their development by Kathe Seidel in the 1960’s (IWA, 2000). However design considerations for wetlands have evolved along the simplistic lines adopted by civil and sanitary engineers using waste stabilisation pond models (Marais, 1974; Thirumurthi, 1974). Wetlands designs are typically based on variants of first order models but these are still based on BOD removal (Reed *et al.*, 1995; U.S. EPA, 1983) and are used for both the FWS and HF-SSF. Therefore they do not adequately address the operation and internal dynamics of the VF-SSF constructed wetland design. Other factors that have generally been difficult to model for the whole technology are the effects of hydraulics, meteorology, interactions of biota and treatment, wastewater type and source variations, and other stochastic variability (Kadlec and Reddy, 2001; Kadlec and Knight, 2000). The treatment performance of HF-SSF is based upon loading specification (population equivalents), but for the VF-SSF technology there is poor knowledge of design and operational factors. This has somewhat hindered the evolution of VF design models beyond the empirical stage.

Despite this, first-order models have still been used in the design of VF-SSF wetlands (Campbell and Ogden, 1999; Crites, 1994).

The long term success of the Max Planck Institute Process (MPIP) systems where VF-SSF systems were developed (Kadlec and Wallace, 2009) has led to a more widespread application of vertical flow systems based on intermittent flow designs. Literature has shown that the existing design techniques, when used in isolation are not satisfactory and that the more useful attributes of the prevalent approaches are: i) areal loading specification and ii) exponential decline modelling. However, modelling the internal dynamics of the beds entails that both approaches be considered simultaneously in the performance of the vertical flow wetland system. This is because loading specifications, for instance, presently refer only to actual influent and effluent concentrations and do not relate to influent areal loading and the desired outlet concentration. Using areal loading ensures that the size of the wetland and quantity of the wastewater treated are both taken into consideration. First-order models base the design of the wetland on the rate constant (for BOD) (Kadlec and Knight, 2000) which separates the effect of flow rate from that of loading concentration. This tends to be misleading in terms of performance of different water flows, for example in a water-scarce situation or where water is plentiful, or indeed for scenarios where water use habits are different. As shown by Kadlec and Wallace (2009), the rate approach is dependent on concentration while the loading method is not but the outlet concentration for both depends on the inlet pollutant loading as well as the concentration (*Equation 2.1*)

$$C_o = C_i \exp[-k/q] = C_i \{\exp[-kC_i/qC_i]\} \qquad \textit{Equation 2.1}$$

Where C_i = inlet concentration (mg.L^{-1})

C_o = outlet concentration (mg L^{-1})

k = rate constant (d^{-1})

q = hydraulic loading (m^3 d^{-1})

and

qC_i = pollutant loading (g m^{-2} d^{-1})

Therefore for a desired outlet concentration, given the rate constant and inlet concentration, the corresponding pollutant loading rate can be easily obtained from Equation 2.1. However because of the periodic nature of the processes in VFs, first-order models are inappropriate unlike in other types of wetlands (Platzer, 1999). Modifications, which can be divided into sizing calculations and physical specifications, and requiring involvement of the dynamics of the filling and draining cycle which result in complex models have been studied (Sun *et al.*, 2002).

The principal designs for CW subsurface systems are based on the assumption of plug-and-flow movement of water through the wetland (Kadlec and Knight, 1996). Therefore the wetland system is considered as an attached growth biological reactor whose performance is described using first order plug-and-flow kinetics (IWA, 2000). The simplest design cases are based on known flow and inlet concentration of a single pollutant, which in most cases tends to be biochemical oxygen demand, BOD. BOD removal in wetlands has been described a by first-order model, also known as the Kickuth equation (Kadlec and Knight, 1996), as follows:

$$A = Q_d (\ln C_i - \ln C_e) / k_{BOD} \qquad \textit{Equation 2.2.}$$

Where A (m^2) = the surface area of the bed,
Q_d ($m^3 d^{-1}$) = the average flow,
C_i (mg L^{-1}) = the influent BOD_5,
C_e (mg L^{-1}) = the effluent BOD_5 and
k_{BOD} (m d^{-1}) = the BOD area-based rate constant.

Hydraulic, organic and nutrient loading values differ from one treatment system to another as well as by location. Hence different areas, and even countries, will have different values for these parameters. The *K*-values for instance, imply kinetic parameters (maximum growth bacteria), design parameters (substrate and bed depth) and operational parameters (temperature and oxygen supply), thus each treatment wetland will have its own *K*-values (Platzer, 1999). To date there are no design equations for other pollutants such as xenobiotics, microbial organisms and suspended solids. Further, the Kickuth model provides little or no information on vertical flow systems (IWA, 2000) because it was really based on the HF-SSF and not the VF-SSF and the two variants are fundamentally different. However, it is clear from the numerous studies that have been conducted that treatment wetlands cannot be represented by a plug-and-flow model. As a result, other models, such as tank-in-series

(TIS) have been explored and shown to capture important features of wetland performance (Keefe *et al.*, 2004; Henrichs *et al.*, 2009).

2.3.3 Design basis

Literature continues to show plug-and-flow models being used (*e.g.* Crites, 1994; Rousseau *et al.*, 2004). In plug-and-flow, design considerations are based on expected inlet concentrations and flows, target outlet concentrations and temperature ranges for the treatment site among others. However, this use of empirical equations to predict system performance whereby a mathematical relationship from pre-existing data is used, leads to inappropriate designs because the relationships are based only of statistical relationship and provide no explanation of internal dynamics (Silviya and Bogdana, 2010; Stefanakis and Tsihrintzis, 2009; Toscano *et al.*, 2009).

2.3.3.1 Design hydraulics

The sizing and description of treatment wetlands involves two principal features: hydraulics (related to volumetric through-put per unit time) and pollutant removal (required treatment efficiency) (Paulo *et al.*, 2009) (Equation 2.2). Some researchers have looked at the effect of number of influent batches (flushes) per unit time (Forquet *et al.*, 2009; Sklarz *et al.*, 2009). Equation 2.2 is a first order equation which is area specific and thus determines the necessary wetland area (A_h). On the other hand, first order equations can also be volume specific (IWA, 2000). With this kind of design, performance can be improved by increasing the water depth, hence increasing the nominal retention time. In design mode, the area specific design, as described by Equation 2.2, is used to calculate hydraulic loading (Q_d) and detention time (τ) (Equation 2.3) that would produce the required effluent concentration (C_e).

$$\tau = \varepsilon \, h / Q_d \qquad \textit{Equation 2.3}$$

Where τ (d) = the hydraulic retention time and
ε = the bed media porosity and
h (m) is the depth of the wetland.

Design of horizontal flow systems is commonly based on BOD removal, whereas in the case of vertical flow systems, design is based on a combination of BOD removal and nitrification (Vymazal, 2009). It has been established, however, that the unit of volume per person equivalent (m^3/pe) used for HF designs is not suitable for VF design purposes because values for hydraulic, nutrient and organic loads vary from place to place (Platzer, 1999). Dallas *et al.* (2004) further showed that designing wetland systems based on organic (BOD) removal alone is not adequate when the treated water is recycled for food crop irrigation where the important factor is pathogen removal followed by nutrient removal or residual concentration. In spite of this, other researchers (Kadam *et al.,* 2008) reported faecal coliform removal to be a function of BOD removal. They established that increased aeration made bacteria more susceptible to die-off. Increased aeration also lowers methane production by inhibiting methanogesis (Maltais-Landry *et al.*, 2009). This suggests that use of BOD in the design considerations serves the purpose of sizing the CW and volumetric through-put per day, but that CW's treatment efficiency is dependent on operation and maintenance of the system. But as shown by Forquet *et al.*, (2009), Frazer-Williams (2008) and Geller (1997), in cases where the raw wastewater has a low concentration of organic matter, treatment efficiency is good regardless of the type of CW design. For such strengths of wastewater inexpensive bench scale columns can be used to predict performance. However, when the influent water strength becomes very unsteady (*i.e.* increases), the treatment efficiency is more likely to go down (Toscano *et al.*, 2009). One of the explanations for this is that increase in influent loading strength would result in increases in pollutant deposition in the substrate media layers. If hydraulic loading rate (Q_d) is not adjusted downward accordingly, then using Equation 2.1, given that the size (A_h) of the CW is fixed, the strength of the effluent also increases. The changes in loading strength also have an impact on the distribution and composition of microfauna (Pigagut *et al.*, 2007).

Operational factors can be used to determine treatment efficiency and this is important because they can be changed even after the system has been built. These considerations are air entrapment and hydraulic loading parameters as described by Forquet *et al.* (2009). Given two scenarios, firstly where flooding occurs and secondly, where flooding does not occur, flooding was found to increase the downward velocity of air as the wetland is being filled up with wastewater. Vertical flow designs are normally operated as flood-and-drain systems because of the added advantage of increased oxygen transfer capacity (International Association on Water Quality, 2000; Vymazal, 2005; Cooper, 1999). Air also becomes trapped and stored by compression during flooding. When flooding stops, decompression occurs which leads to a redistribution of air to occupy empty spaces left by the drained water. Where flooding does not occur, limited compression results, and no air is trapped within the system. As the air is being forced downward, it penetrates into the deeper layers of the porous media more. In a system with multi-media substrate, the wastewater accumulates at interfaces (*e.g.* sand – gravel interface) which therefore results in percolation of the water through the lower media. While hydraulic loading does not actually affect the amount of air/oxygen entering the treatment bed, increasing loading time may reduce oxygen intake if the loading time exceeds residence time as observed by Platzer (1999), which may also lead to increased methane production due to the establishment of anaerobic conditions. Increasing the number of flushes is therefore one of the obvious solutions to this. Sufficient loading rate is still necessary to ensure good distribution over the surface of the treatment bed thereby avoiding preferential passage through the filter. The other factor that will impact on the treatment efficiency is the organic strength of the influent wastewater, which will affect the amount of dissolved oxygen and hence microbial activity.

Consequently, for a given wetland design and set of hydraulic conditions, the actual treatment efficiency of a CW is dependent on the influent wastewater strength (Tanner *et al.*, 1998), hydraulic loading parameters and the extent of biofilm formation (Bishop, 2007). However, k_{BOD} varies with biodegradability (C:N:P ratio) of the influent wastewater and type of media (Kadlec and Knight, 2000). This makes it necessary to have specific design equations for grey water which has higher non-biodegradable organic fractions (*i.e.* low nutrient levels) (Jefferson *et al.*, 2004; Paulo *et al.*, 2009). By basing all CW designs on BOD removal, the assumption made for grey water for instance is that it is similar to less polluted or secondary wastewater although the chemical characteristics of grey water can vary significantly depending on the source and a whole host of other factors. Design equations, have not been adequately tested in terms of the type of wastewater being treated, which can probably be done by taking into consideration the C:N:P (biodegradability) and BOD:COD (treatability) ratios and specific chemicals of concern. This would also guide requirements for pre-treatment which would ensure that the type and strength of the wastewater influent to the wetland system is always considered within the design specifications.

2.3.3.2 Treatment bed media

Originally, the HF beds developed at MPIP utilised soil in the root system and sand in the lower layers (Kadlec, 2009). Presently different media materials are used in different countries, but generally finer materials result in better treatment, because of their greater surface area. However, finer materials are more prone to clogging and are associated with hydraulic problems (Blazejewski and Murat-Blazejewska, 1997). Hence development of the VF technologies has seen a layered approach being favoured with sand at the top of the wetland bed and progressively coarser material lower in the bed (IWA, 2000). The layering and choice of media sizes is critical (Cooper, 2003) such that water movement is made possible without clogging. A summary of bed materials used in VF wetlands is outlined in Table 2.4, which shows, how crucial media size is in bed layering in order to achieve good treatment performance. Other types of media such as silver coated sands (Mahmood *et al.*, 1993; Ding *et al.*, 2010) and alum sludge (Babatunde *et al.*, 2009) have also been considered for use in wetland systems because of their improved disinfection or adsorptive properties.

Size and shape: shapes of individual grains affect filter design and performance in the following ways:-

1. Grain diameter determined by sieve analysis for non-spherical media is smaller than diameter of an equivalent volume of spherical media.
2. Shapes affects the packing of filter grains in a treatment bed, which affects the bed porosity and hence the filtration effectiveness (see later about porosity). Typical specifications of slow (sand) filters are an effective size between 0.30 and 0.45 mm and a uniformity coefficient of less than 2.5
3. Hydraulics through spherical grains is different from grains with angular and sharp surfaces. Flow regime, which is an important aspect of hydraulic behaviour, is identified by Reynolds number.

Table 2.4: Media specifications used in vertical flow constructed wetlands applications

Wetland type	Layer	Thickness	Media size	Material
Pulse-fed	Surface layer	8 cm	-	Sharp sand
	Second layer	15 cm	6 mm	Pea gravel
	Third layer	10 cm	12 mm	Round gravel
	Drainage layer	15 cm	30-60 mm	Round gravel
Pulse-fed	Main layer	>80 cm	0.2-1.0 mm	Soil/sand
Pulse-fed (first stage)	Surface layer	>30 cm	2-8 mm	Fine gravel
	Second layer	10-20 cm	5-20 mm	Gravel
	Drainage layer	10-20 cm	20-40 mm	Coarse gravel
Pulse- fed (second stage)	Surface layer	>30 cm	$0.25<d_{10}<0.4$ mm	Sand
	Second layer	10-20 cm	3-10 mm	Gravel
	Drainage layer	10-20 cm	20-40 mm	Coarse gravel
Pulse-fed (three stages)	Main layer	75 cm		Alum sludge
Pulse-fed	Surface layer	15 cm	20-40 mm	Gravel
	Main layer	55 cm	-	Sand/compost/soil
Pulse-fed	Surface layer	10 cm	8-16 mm	Gravel
	Second layer	>50 cm	0-4 mm	Sand/gravel
	Third layer	5-10 cm	4-8 mm	Gravel
	Fourth layer	Separation fabric	-	2.5 mm mesh
	Drainage layer	20 cm	16-32 mm	Gravel
Recirculating	Surface layer	1 cm	25 mm	Pebbles (limestone) Plastic media
	Main layer	12 cm		peat
	Drainage layer	2 cm		
Fill and drain (stages 1 – 8)	Surface layer	10 cm	Cobbles	24.4 mm
	Second layer	15 cm	Fine gravel	6 mm
	Third layer	10 cm	Sand	0.5 mm
	Drainage layer	15 cm	cobbles	50 mm
Fill and drain (stage 9)	Surface layer	20 cm	Gravel	24.4 mm
	Second layer	50 cm	Fine gravel	6 mm
	Drainage layer	20 cm	cobbles	50 mm
Fill and drain (stage 10)	Surface layer	25 cm	Gravel	24.4 mm
	Second layer	10 cm	Gravel	6 mm
	Third layer	30 cm	Sand	0.5 mm
	Drainage layer	15 cm	cobbles	50 mm

Adapted from (Kadlec and Knight, 2000; Stefanakis and Tsihrintzis, 2009; Babatunde et al., 2009).

Despite grain shape being this important, it is difficult to really account for it in filter designs. However, hardness of the filter media needs to be checked in order to reduce the probability of clogging the system as a result on abrasions if softer grains were used.

2.3.4 Process considerations

Another challenge in successful implementation of constructed wetland technologies is the inadequate knowledge of the physicochemical and biogeochemical processes associated with the reduction of organic chemicals in the wastewater. In this regard, knowledge of the physicochemical properties of the organic chemical would help to determine the treatment processes that different chemical compounds will undergo. There are several elimination pathways for organic chemicals, which can be categorised as follows; physical (sedimentation, adsorption, sorption), chemical (volatilisation, precipitation, ion-exchange) and biochemical (microbial degradation, and plant uptake). There exist three possible routes for plant uptake, phytovolatilisation, phytoaccumulation, phytodegradation. However as discussed earlier, performance and efficiency of pollutant removal in constructed wetland is also strongly related to the size of the wetland and is modified by flow (operational) and thermal (weather) characteristics (Kadlec and Knight, 1996). Treatment processes in vertical flow constructed wetland systems are comparable to slow sand filtration and aquifer recharging systems which operate on the same principles as wetlands. The nature and performance of wetland systems actually places this technology in between intensive and extensive systems (Figure 2.3). The mechanisms of both intensive and extensive systems are well understood and have generic design and operation models. The same is not the case for wetland treatment systems.

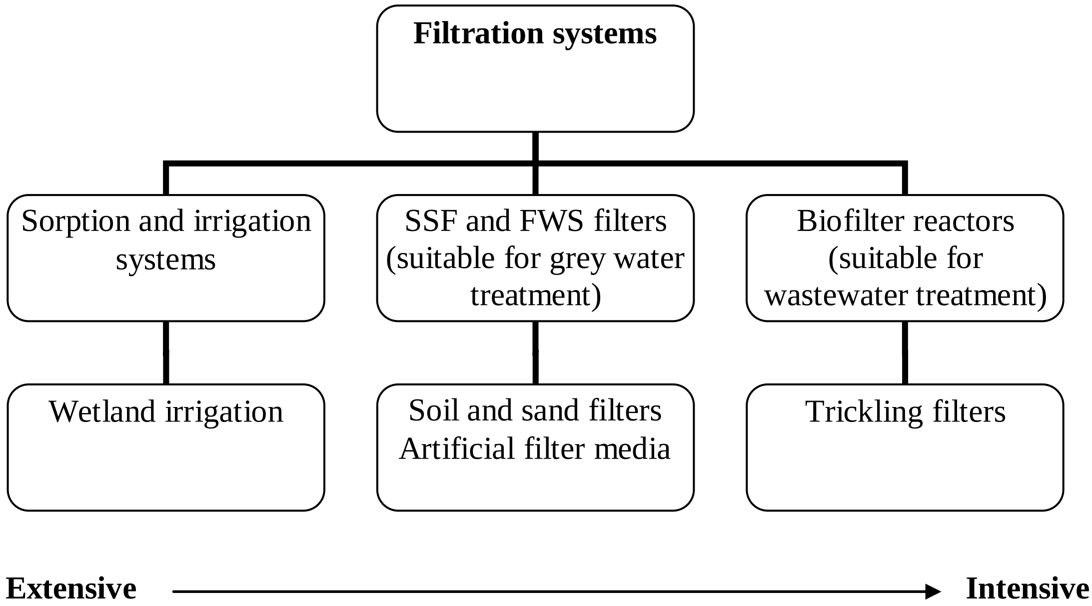

Figure 2.3: Range of filtration systems used for wastewater treatment

2.3.4.1 Physical mechanisms

The first reported technologies for grey water treatment were mainly physical treatment mechanisms, such as coarse filtration (Hypes *et al.*, 1975). Filtration is a process that has been used to clarify water for many years as it helps to remove algae, sediment, clay and other organic and inorganic particulate matter. Present day water treatment facilities use either granular media filters or membrane filters. Wetland treatment facilities use granular media and are operated at low filtration rates (slow filtration). Slow filtration systems are simple to construct and operate effectively because they do not require coagulation and backwashing. Suitability of these systems is proven for wastewater with turbidities below 50 NTU (Kadlec and Wallace, 2009). Variations of filtration systems in terms of direction of water flow exist and these are up-flow, horizontal and vertical. Treatment systems have been designed after these variations namely: Up-flow filtration, Horizontal flow (HF) and Vertical flow (VF). Up flow filtration systems do not clog as easily as do conventional (down flow/vertical) filters but have a higher energy requirement which is used to pump the water against gravity. The designs under discussion in this review are low tech system which among other things means that the energy requirements should be low (Brix, 1999). Therefore up-flow systems are excluded on this basis.

The main process in physical mechanisms is sedimentation whereby particulate materials are settled out/filtered mechanically under the influence of gravity as water passes through the substrate media. Particle removal efficiency in filtration systems with constant media characteristics is governed by the following equation:

$$dC/dy = \lambda C \qquad \textit{Equation 2.4.}$$

λ is the filter coefficient,

C, concentration and

dy, the filter area measured in the direction of the flow

At $t = 0$, $C = C_0$ and $\lambda = \lambda_0$

Hence:

$$C = C_{0.} \exp(-\lambda_0 L) \qquad \textit{Equation 2.5.}$$

The number of particles in suspension declines logarithmically with depth in accordance with *Equation 2.4*. However, because the number of particles retained in each layer as a deposit is not the same, the top layers will contain more particles than the bottom layers. Hence, the filter is expected to become uniformly clogged, throughout the depth profile, with the top layers performing most of the filtration requirement (IWA, 2000). The filter coefficient, λ, is less for larger grains, but larger grains are less efficient in removing particles because there is no straining force on the particles as they pass through the media. However, larger grains are more amenable to support aerobic processes because of higher porosity which gives more room for biofilm growth (enhanced biological treatment), as long as enough oxygen infiltrates through. When filtration efficiency is derived from the suspension characteristics and media type alone, the filtration coefficient becomes strongly dependant on the collector size and porosity of the bed (*i.e.* grain size). Hence type of media which in turn defines the porosity and hydraulic conductivity of the wetland becomes important (Table 2.4). As seen from the equations above ($\tau = \varepsilon$ h / Q_d), porosity is crucial in the design through determination of physical treatment mechanisms.

Choice of media size (porosity) affects performance of the filtration system and the effective filtration depth in several ways, amongst which are the need to balance filtration of smaller particles such as faecal bacteria and oxygen infiltration into the media. Kadam *et al.* (2008) showed that the fate of bacteria in wastewater is governed by filtration, adsorption and their die-off rate. However filtration performance is best described using phenomenological modelling (Parsons and Jefferson, 2006). Kadam *et al.* (2008) also showed that removal of bacteria correlates with turbidity and BOD removal. Removal efficiency of total coliform (indicator organisms) increases with decrease in grain size in artificially constructed columns (Tanik and Comakoglu, 1997). As a result treatment beds with fine media grains get clogged quickly because of their low media porosity if the wastewater being treated was high in total coliform, turbidity and BOD. Hence the media size and overall wetland design contribute to treatment processes and efficiency.

Although the filtration processes may be solely determined by particle and pore sizes, physical or chemical adhesion of molecules (sorption) to the surfaces of the media from partitioning of dissolved molecules still plays an important role. Sorption involves a mass transfer operation in which substances present in the liquid phase are adsorbed and accumulated on the solid phase and thus removed from the liquid-phase. These processes are used in drinking water processes for removal of taste and synthetic organic chemicals. Sorption is determined by the hydrophobicity of the compounds as well as the chemical structure and organic matter content of the substrate media (Imfeld *et al.*, 2009). Hence filtration efficiency (extent of sorption and filtration) changes as the constructed wetland ages due to decreased adsorption capacity (Omari *et al.*, 2003) and decreased porosity (Blazejewski and Murat-Blazejewska, 1997; Langergraber *et al.*, 2003). Use of activated carbon has been shown to improve removal of organics by adsorption meeting reuse standards when treating grey water with an initial strength of 186 mg BOD. L^{-1} (Pidou *et al.*, 2008). The effectiveness of activated carbon has been shown in bench-scale and pilot scale studies. However what remains is ascertaining the bed life and replacement frequencies required for practical application.

Sand filter clogging is described primarily as a surface phenomenon (Rodgers *et al.*, 2004) and decreases exponentially down the substrate profile. When filters are operated at low dosing rate, growth of microbial biomass occurs during and immediately after dosing with endogenous decay between doses (Mitchell and McNevin, 2001). Hence in order to operate filters for long periods without experiencing clogging, the endogenous decay period has to be sufficiently long to ensure filter porosity recovery. Some studies have suggested upper limits for the organic and hydraulic loading rates for grey water, 20-25 g BOD m^{-2} d^{-1} (Riddlestolpe, 2004), 25 g COD m^{-2} d^{-1} (Platzer and Mauch, 1997), and HLR of 5 – 10 cm d^{-1} (Fountoulakis *et al.*, 2009; Cooper, 2005) which are meant to control biofilm growth, oxygen infiltration and microbial activity, and prevent premature clogging. Media depth and velocity of the water through the system also determine the size of particles that are removed. However bio-film formation is important for effective treatment because it triggers essential biological processes in the system. A second operation used to prevent or slow down clogging is to implement multiple beds in a load-and-rest regime. The VF wetlands are designed to clog during the loading phase, with restoration of hydraulic conductivity occurring during the resting phase. Clogging and loading rate are related and specifying loading rate indirectly specifies the resting sequence associated with that loading rate. Clearly this entails that wetland sizing (which affects volumetric loading rate) is a very important part of the design process.

2.3.4.2 Chemical mechanisms

A large spectrum of chemical reactions takes place in wetland systems under various environmental conditions. Prevailing conditions determine both thermodynamic feasibility and enzymatic capability to achieve various biochemical reactions (Kadlec and Knight, 1996), with warmer temperature providing optimum conditions for the highest rates of biogeochemical process. Biogeochemical processes occurring in wetland systems involve the manipulation of the pollutant species in order to either convert them into oxidised products or increase their size and then remove them in a physical process (sedimentation). Chemical processes affecting contaminant removal in wetland systems depend on various other processes occurring within the wetland system (*e.g.* ion-exchange between the wastewater and the substrate media) on the surface of the bed (interface with the atmosphere) and at rhizosphere scale. Chemical processes, such as, coagulation have exhibited low removal of organic elements in grey water treatment. and they have not always able to meet the required treatment level for water recycling (Pidou *et al.*, 2008). Hence their suitability is for low strength grey water.

Wetland surface reactions

Volatilisation (direct contaminant emission from the water phase at the bed surface into the atmosphere) is an important contaminant removal route (Susarla *et al.*, 2002). Vapour pressure and Henry's constant are important factors in determining volatilisation and phytovolatilisatio behaviour of organic compounds (Imfeld *et al.*, 2009). Certain plants *e.g.* poplar trees when planted in hydroponic systems are able to phytovolatilise methyl tert-butyl ether (MTBE) (Hong *et al.*, 2001) and chlorinated organic compounds (Ma and Burken, 2003). Both volatilisation and phytovolatilisation processes are important for the removal of volatile hydrophobic compounds. However, phytovolatilisation becomes more relevant in subsurface systems because the slow diffusion rates of contaminants through the unsaturated zones and laminar flow in water saturated zones restrain direct volatilisation (Imfeld *et al.,* 2009). Therefore relatively low mass transfers occur through direct volatilisation. The density of plants in the wetland systems as well as the surface area of the wetland contributes to the rate and extent of volatilisation in the wetland system (Tunçsiper, 2009; Brix and Arias, 2005).

Substrate media-water interface reactions

Decomposition of organic chemical occurs for less stable compounds by phenomena such as oxidation and reduction. For instance, two aromatic surfactants found in grey water, Linear akylbenzene sulphonate (LAS) and Nonyl phenol ethoxylate (NPEO) are degraded faster under aerobic conditions. LAS undergoes ω-oxidation of the alkyl chain terminal carbon followed by β-oxidation while NPEO undergoes hydrolytic shortening of its polyethoxy chain (Di Corcia and Samperi, 1994; Kuhnt, 1993). Dynamic formation of oxic-anoxic interfaces in the wetlands which result from water table fluctuations, oxygen diffusion through the substrate media and transport via plant tissues creates oxygen gradient zones (Wießner *et al.*, 2005), which govern the electron donor-acceptor processes that regulate removal mechanisms. Some of the mechanisms that occur are ion-exchange between the solid (media) and liquid (water) phase, plant uptake (phytoextraction and phytoaccumulation), and photo-oxidation. However, phytoextraction with the use of high biomass crops is relatively more successful on soils with low and moderate concentration of chemicals (Ernst, 2005; Sebastiani *et al.*, 2004). Removal patterns vary for different contaminants with some (*e.g.* aromatic surfactants) having higher removal potential in predominantly oxic conditions. As the conditions change from predominantly oxic to anoxic and anaerobic, deeper into the wetland, chemical compound removal efficiency shift in favour of anaerobially degradable compounds *e.g.* highly chlorinated organic compounds (Matamoros *et al.*, 2007). Ion exchange and to some extent coagulation, interact with charged species and so will not remove uncharged components. The main contaminants that are affected by chemical mechanisms are nitrogen, phosphorus and heavy metals as well as bacteria and viruses. In drinking water, chemical mechanisms are used for water softening and demineralisation (removal of Ca, Mg, Na, Cl, SO_4 and NO_3 ions). But use of coagulants and/or specific media (for ion exchange) result in increasing cost of the constructed wetland technology. Recent studies by Zhao *et al.* (2009) have shown that the water treatment residual from use of aluminium sulphate as a coagulant known as 'alum sludge', is a valuable material in wastewater treatment. The aluminium ions in alum sludge enhance adsorption and chemical precipitation processes that remove various pollutants particularly phosphorus, which makes the alum sludge a suitable media for constructed wetlands.

The break down and removal of chemical species results in changes in dissolved oxygen carbon (DOC) concentrations. DOC performs several important functions in aquatic systems, such as, altering the bioavailability of toxic compounds (Driscoll *et al.*, 1995), and regulating ecosystem metabolism (Hanson *et al.*, 2003). Therefore the ability to predict or control DOC concentration in surface water could provide information for a whole suite of ecological processes in the constructed wetland. Hence DOC can also be used as a design criterion.

2.3.4.3 Biological mechanisms

Biological treatment mechanisms utilise a developed community of organisms to degrade the pollutants as part of their growth cycle. Such mechanisms mainly involve bacterial and plant metabolism. Microorganisms mediate many wetland processes and are mainly responsible for the transformation and mineralisation of degradable organic pollutants within constructed wetlands (Sleytr *et al.*, 2009). Bacterial metabolism is a predominant biochemical mechanism whereby colloidal solids and soluble organics are removed by benthic and plant supported bacteria. The main constituents that are removed include colloidal solids, BOD, nitrogen and refractory organics. Because of the differences in flow and the resultant oxygen transfer capacities in the HFCW and the VFCW, the two systems offer the extremes of biological systems in a natural format. Biological processes are the only destructive processes (phytodegradation and microbial degradation) that occur in the wetland system in that the organic contaminants are degraded or transformed into different organic compounds. However, there is still lack of knowledge concerning microbial community compositions within constructed wetlands. Phytodegradation (which includes rhizodegradation and phytotransformation) entails the breakdown of organic compound by plant enzymes or enzyme co-factors (Susarla *et al.*, 2002). These metabolic transformations however, depend on nature and properties of the organic compounds as well as, the type of plants in the treatment wetland. Some wetland plants are able to degrade some types of organic compounds better than others. To illustrate, poplar tree, have shown to have high degradation potential for chlorinated organic compounds (Newman *et al.*, 1997). In many cases phytodegradation, is a sub-process under combined phytoremediation processes which involve non-destructive (physical) extraction (phytoextraction and phytoaccumulation) whereby the contaminant taken up by the plants is not degraded rapidly or completely (Susarla *et al.*, 2002).

Microbial degradation of organic compounds also depends on the physico-chemical nature of the compounds in the wetland system. Removal of toxic and other organic compounds is believed to be largely a result of microbial mediated aerobic and anaerobic processes (Reddy and D'Angelo, 1997), which is a stepwise conversion of complex organic compounds to simple organic and inorganic compounds. The whole mechanism involves abiotic leaching and fragmentation (physical process), extracellular enzyme hydrolysis and catabolic activity (aerobic and anaerobic processes) of heterotrophic organisms which are microbially mediated. These processes are affected by environmental conditions such as pH, temperature and nutrient availability. To illustrate, Kadlec and Reddy (2001) pointed out that microbially mediated reactions are affected by temperature, with responses being typically greater for temperature changes below 15 ^{o}C than normal conditions of 20-35 ^{o}C.

Most treatment wetlands are effective at removing organics from wastewater because they are primarily designed to remove organic matter and solids (Vymazal, 2002). Indeed the ability of wetlands to remove BOD, COD TSS and coliforms from influent wastewater is greater (73 – 83%) than for nutrients (30 – 45%) (Vymazal, 2007; Neralla *et al.*, 2000; Gopal, 1999). The role of the media in wastewater treatment systems is principally to provide sufficient surface area for microbial growth to occur whilst maintaining a satisfactory hydraulic conductivity (Kadlec and Knight, 1996). Use of artificial media, such as absorbent plastic foam and non-woven textile chips, has also been reported and shown to be effective (Crites and Tchobanoglous, 1998) although at an elevated cost because they usually tend to be proprietary. As a result this has served as a driver for increasing costs in treatment systems.

2.3.5 Hybrid constructed wetland designs

A multi-stage CW design can have different designs for different stages, taking advantage of each specialised component to maximise overall treatment performance. In multi-stage systems careful management of the first stage containing a coarser substance for removal of suspended solids followed by a stage(s) containing fine deep media for removal of COD helps to avoid clogging (Morris and Herbert, 1997). Simple technologies used for grey water recycling are usually two-stage systems based on coarse filtration or sedimentation to remove the larger solids followed by a disinfection focussed stage. Removal levels across multiple and/or hybrid stages have been shown to be more effective than single stage systems producing effluent suitable for reuse. To illustrate, vertical (VF) and horizontal flow systems (HF) have different advantages which have been exploited through HF-VF or VF-HF hybrid designs. Two stage HF-VF hybrid systems achieve good removal of BOD, COD, SS, bacteria and ammonia but not total nitrogen because of low denitrification in the last VF stage (Vymazal, 2005; Cooper, 1999). Starting a hybrid CW with a horizontal bed system means that BOD will be removed and prevented from interfering with nitrification in the vertical flow stage. Cooper (1999) however recommends putting a VF at the front to remove BOD, COD and bacteria as well as oxidise all ammonical nitrogen to nitrate. This would also help to break down surfactants present in the grey water because they undergo aerobic degradation (Kuhnt, 1993). Surfactants in aqueous solutions tend to accumulate at the liquid/gas or solid/liquid interface (Shafran *et al.*, 2005), hence removal of suspended solids would play an important role in removal surfactants and general chemical toxicity removal (horizontal flow design of the constructed wetland). A two stage Recycled Vertical Flow Constructed Wetland (RVFCW) developed by Gross *et al.* (2007) achieved removal of 98% for TSS, 100%, COD - 81%, Total P - 71%, Anionic surfactants – 92% and Boron – 65%. However for grey water irrigation, a balance will have to be reached where surfactant removal will be optimised as well as reaching the required level of nitrogen and other nutrients. Obviously for irrigation purposes, lower removals of nutrients (50 - 70%) would be desirable, as have been observed in the multi-stage hybrid design, the GROW (Green Roof Water Recycling System) (Frazer-Williams *et al.*, 2008). A look at the percentage removals by the single, two-stage and multi-stage hybrid designs (Figure 2.4) shows the multi-stage hybrid design to be more suited to treatment of grey water for irrigation reuse, because of its high removal of BOD, COD, and turbidity. A multi-stage design offers a better design option where the VF can be put at the front and at the end, in order that all the positive attributes will be optimised. The treatment performance of a multi-stage VF system without and HF has not been reported as widely in literature. It is possible that having removed most of the BOD, COD, bacteria and ammonical nitrogen, the removal of these would continue (logarithmically) in subsequent VF beds which would take advantage of high oxygen transfer capacity especially for treatment of grey water.

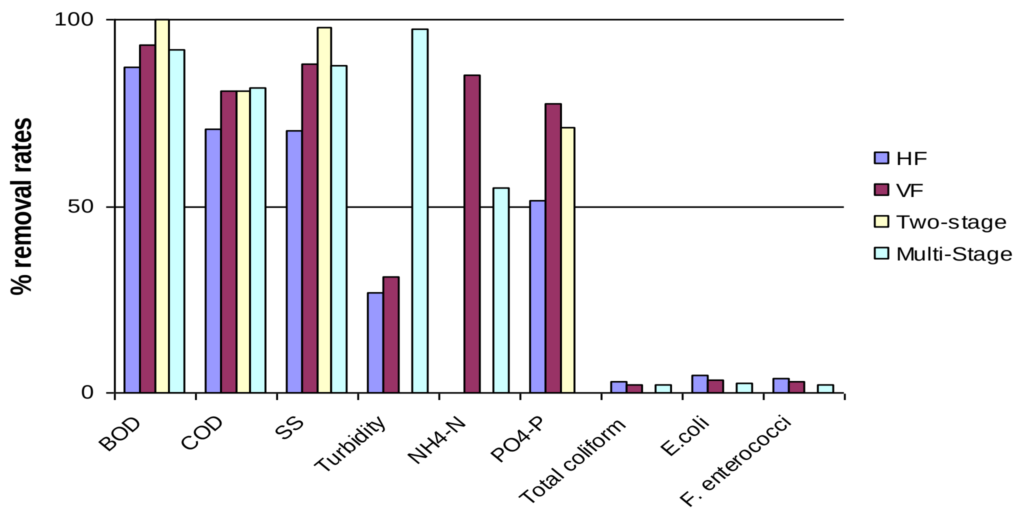

Figure 2.4: Performance of single, two-stage and multi-stage hybrid wetland systems for black and grey water (*Frazer-Williams et al., 2008; Gross et al., 2007; Vymazal, 2005; Cooper et al., 1999*)

2.3.6 Plants in treatment wetlands

Kadlec and Wallace (2009) defined wetlands as areas that are wet for long enough to exclude plant species that cannot grow in saturated soil conditions and to alter soil properties because of the chemical, physical and biological changes that occur during flood and drain cycles. Plants have many functions such as transpiration, flow control and particulate trapping, which are peculiar to plant type and density. The most used plants in wetland treatment are *Phragmites australis* (Fittschen and Niemczynowicz, 1997; Frazer-Williams, 2007; Shrestha *et al.*, 2001; Borin *et al.*, 2004) and *Schoenoplectus* (*Scirpus*) (Kadlec and Wallace, 2009). A tacit assumption that has been noted regarding wetlands and plants is that wetlands only work if green plants are present. On the other hand, wetlands have been shown to continue functioning in cold climates despite plants being brown and metabolising at low rates. This indicates that treatment does not necessarily depend on the presence of green plants.

Many studies have reported the different plant species that have been used in constructed wetlands, for example, *Iris pseudocorus, Veronica beccabunga, Glyceria variegates, Juncus effuses, Iris versicolor, Caltha palustris, Lobelia cardinalis* and *Mentha aquatica* (Frazer-Williams, 2007), *alisma*, *iris*, *typha*, *metha*, *canna*, *thalia*, *lysimachia*, *lytrum*, *ponyederia* and *preselia* (Borin *et al.*, 2004). However, the role of plants in removing pollutants from wastewater has been reported as perceivably low (Brix, 1999). Indeed, this has led to some researchers concluding that most ‘treatment wetland plant species’ are not critical as far as treatment performance is concerned. Nonetheless there are differences among plants behaviour towards some pollutants, notable heavy metals and trace chemicals. Hence consideration for plants, *e.g.* aesthetics, scent, hyperaccumulation of chemicals should probably be for specific chemical accumulation or tolerance with wastewaters of different chemical properties and for different wetland designs. Therefore treatment wetland studies on specific roles of plants and the different species in wetlands receiving different types of wastewater would be useful in improving the wetland technology.

The presence of plants in a wetland obviously provides a home to a variety of microbial species and other life forms. The resulting ecosystem diversities govern and act as indicators of the interactions that contribute to treatment of pollutants. This includes species diversities and concentrations for bacteria and fungi, two of the most important organisms in treatment wetlands because of their role in transforming, assimilating and degradation of pollutants in wastewaters. They are the first organisms to colonise and begin the breakdown of solids and access dissolved constituents in wastewaters (Gaur *et al.*, 1992). Such interactions have been studied extensively for soil ecosystems but inadequately so for treatment wetlands. Although functional and species diversities are limitless, they result in ability of the treatment wetlands to adapt to different types and strengths wastewater as well as environmental conditions and other factors like plant regimes. Knowledge of these interactions would advance the study of treatment wetlands greatly.

2.3.7 Design considerations for small-scale vertical flow treatment wetlands

Paulo *et al.*, (2009) and Toscano *et al.*, (2009) used the following design considerations to inform design criteria for wetland/filter treatments:-

1. Performance criteria, such as turbidity removal for domestic wastewater or metals and acidity for mine wastewater, filter run etc.
2. Process/mechanism design criteria such as required level of pre-treatment, filter media type, size and depth, filtration level and available head.
3. Flow distribution and control
4. Other major processes such as under drains, overflows, storage

As seen in the discussions above, the two most important mechanisms in determining the effectiveness of constructed wetlands for water treatment are biological processes and hydrological dynamics (Buchberger and Shaw, 1995). These processes are in turn linked temporally and spatially throughout the wetland via the hydrology of the system. Wetland media, soils or sands, have a high trapping efficiency for a variety of chemical constituents. Therefore, the design of constructed wetland systems which will appreciably reduce the impact of toxic chemicals, like surfactants, hinges on understanding and optimising these treatment mechanisms (Barber *et al.*, 2001) while keeping the energy requirements and other parameters constant.

The design criteria for treatment wetlands are dominated by empirically derived area-adjusted contaminant removal rates. The understanding of microbial structure and functions do not currently help towards a process driven basis to quantify and predict biological, chemical and physical interactions that control contaminant removal rates. Indeed the most important handicap of constructed wetland technology, is substrate bed clogging (Álvarez *et al.*, 2008), which is a result of both physical removal (sedimentation) and biological processes (biofilm growth). To illustrate, Austin *et al.*, (2007) used biological and chemical criteria by correlating the Damköhler number (Da) with COD/BOD loading to predict and control clogging in SSF systems at operation stage. The Damköhler numbers (*Equation 2.6*) are dimensionless numbers used in chemical engineering to relate chemical reaction timescale to other phenomena occurring in a system. However, attempts to biologically treat wastewater with high COD/BOD levels encounter performance and operational difficulties (Jefferson *et al.*, 2004; Metcalf and Eddy Inc., 2003). Although physical parameters have been used for design and operational parameters as discussed by Toscano *et al.* (2009), Paulo *et al.* (2009) and others, Langergraber *et al.* (2003) argue that clogging in SSF wetland systems correlates with biofilm growth. The suggested criterion (by Austin *et al.*, 2007) can therefore be used at design stage by predetermining the acceptable range of organic loading rates of the influent wastewater for specific filter (media) material depending on porosity (Platzer, 1999; Blazejewski and Murat-Blazejewska, 1997). Studies by Del Porto and Steinfeld (1999) show that powdered detergents and soaps as well as colloids are the main reasons for physical clogging. Hence for grey water, clogging propensity can be related to type and source of grey water as well as the design and operation parameters of the treatment system. Of the studies reported in literature, some have pre-treatment stages but the use of a pre-treatment stage would only help if it was possible to ensure adherence to a specific influent organic strength. This step would help to standardise the quality of influent grey water thereby enabling generic designs for defined treatment efficiency.

$Da = kC_0^{n-1}t$ *Equation 2.6*

Where k = kinetics reaction rate constant
C_0 = initial concentration
n = reaction order
t = time

2.4 CONCLUSIONS

Grey water characteristics fall into three main categories, organics and nutrient; physical and; microbiological. Design considerations for constructed wetland treatment technology mainly focus on the organics and nutrients category but for wastewater recycling, consideration for all categories is important. Removal of solids, organics and nutrients and pathogens from wastewaters is crucial. The design consideration in slow sand filtration could probably be based on filtration processes alone. However in constructed wetlands, where natural processes are simulated filtration alone is unlikely to achieve an appropriate level of treatment. Further, when dealing with grey water which is low in nutrients and pathogens, a deeper understanding of ecosystems processes is required. This includes every aspect of the technology that can be engineered, *i.e.* wetland size, location, choice of media, choice of plants. There are, as yet, no specific design considerations or equations for the VF design. General understanding in the vertical flow technology design and suitability for small-scale, such as, single and studio apartment, units demonstrating acceptable treatment performance and robustness at this scale of operation is missing. Further for single house and studio apartment units there are a number of other considerations that would suit a small-scale wetland design for grey water treatment and recycling such as:

1. Close proximity to the source is preferred reducing infrastructure costs
2. Ability to handle the solids present in grey water
3. Low capital and operation costs
4. Ease to maintain.

Finally, one of the main advantages of plants, for example hyper-accumulators, is their high tolerance to different chemicals. This means that constructed wetland plants can be chosen based on their hyper-accumulation potential. However, a greater understanding of the underlying bio-physical and biochemical processes taking place in order to utilise plants in treatment wetlands is required.

2.5 PROJECT AIMS AND OBJECTIVES

Main objective:

The overall aim of this research is to study the performance of a small-scale constructed wetland design with multiple beds (arranged in a cascading pattern) which were shallower than in the standard designs. Therefore shallower beds (0.2 m deep) were used in this study for grey water treatment. In support of this aim, the following main research objectives were formulated (as reported in Section 1.3.2):

- o Establish the predictability of physico-chemical characteristics and toxicity of grey water based category and branding of personal hygiene/cleaning products ("down-the-drain" chemicals)
- o Assess the treatment performance of the prototype cascading constructed wetland, to establish aspects of the wetland technology that can be optimised to generate treated grey water that meets published wastewater reuse standards.

Specific objectives:

In order to achieve the aims and objectives of this research the following research questions (specific objectives) needed to be fulfilled:

1. What is the impact of general chemical properties of the household cleaning and personal care products on grey water quality and treatability?
2. Can the toxicity of grey water be predicted from knowledge of cleaning and personal care products used in the household?
3. How would the performance of a shallow bed cascade wetland for grey water treatment compare with a standard constructed wetland?
4. What is the influence of wetland microbial community structure on grey water treatment performance?

The research questions were translated into a framework (Figure 2.5) which formed the project outline and boundaries of this study. The framework reflects the project phases, whereby, in the first instance analytical scoping work was carried out on the cleaning and personal-care (consumer) products in order to address Research questions 1 and 2. The second project phase sought to address Research question 3 and only focussed on testing the unplanted (open) version of the cascade wetland. The final third phase addressed Research questions 3 and 4, and focussed on the planted and unplanted (covered) cascade wetlands. All studies focussing on the performances of the cascade wetland were benchmarked against a standard single pass vertical flow planted constructed wetland in side-by-side studies.

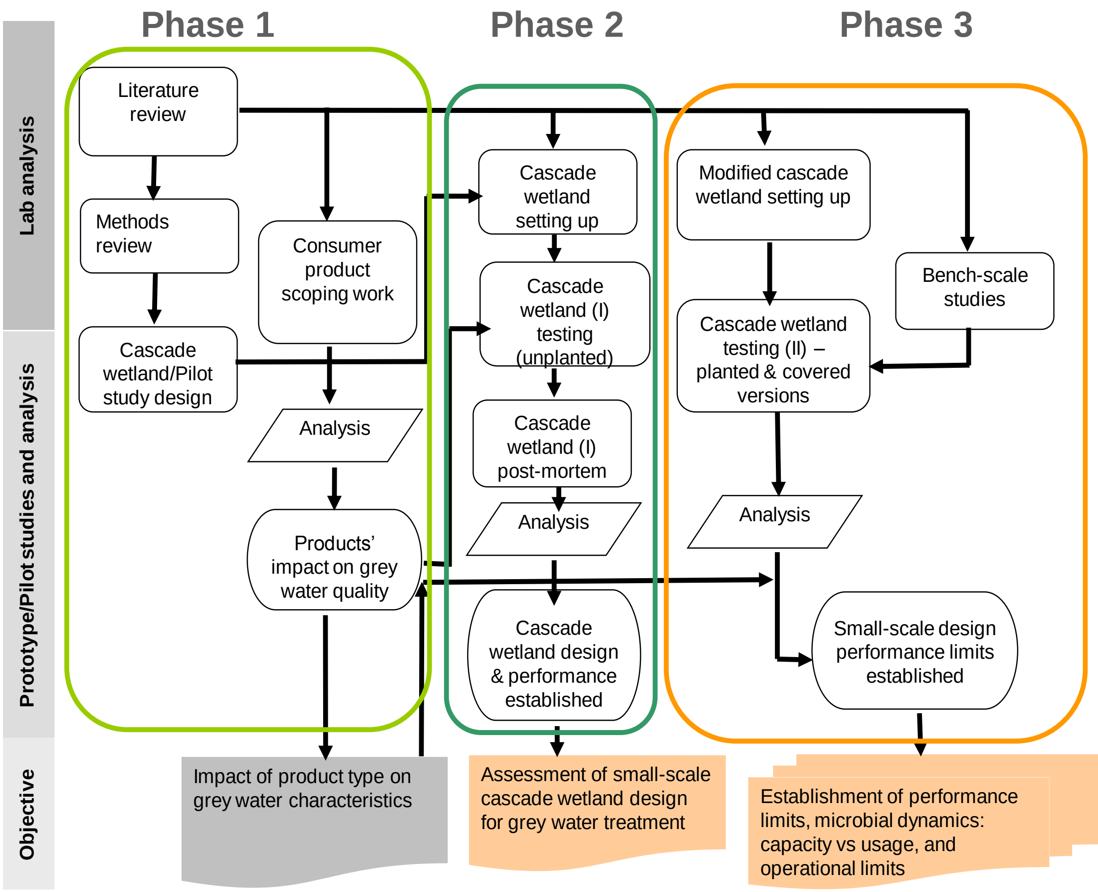

Figure 2.5: Project outline

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2.7 SUPPORTING INFORMATION

Appendix 2.1: Worldwide standards for wastewater reuse.

Location	Application	Parameters							Reference
		BOD_5*	**TSS***	**Turbidity****	**T-N***	**T-P***	**Faecal Coliforms~**	**Total Coliforms~**	
China	Toilet flushing	<10	<1500[1]	<5	-	-	3	-	Ernst *et al.*, 2005
	Irrigation of green	<20	<1000[1]	<20	-	-	3	-	
	Washing purpose	<10	<1000[1]	<5	-	-	3	-	
Japan	Toilet flushing	-	-	<2	-	-	-	ND[2]	Tajima, 2005
	Landscape	-	-	<2	-	-	-	<1000	
	Recreational	-	-	<2	-	-	-	ND[2]	
Taiwan	Toilet flushing	10	-	-	-	-	-	<10[2]	Lin *et al.*, 2005
Israel	Wastewater reuse	10	10	-	-	-	<1	-	Gross *et al.*, 2006
Germany	Wastewater reuse	<5[3]	-	-	-	-	<1000	<10000	Nolde, 2000
Spain, Canary Islands	Wastewater reuse	10	3	2	-	-	-	2.2	USEPA, 2004
Canada, British Columbia	Unrestricted urban reuse	10	5	2	-	-	2.2	-	CMHC, 2004
Costa Rica	Irrigation of food crops	<40	-	-	-	-	<1000	-	Dallas *et al.*, 2004
	Urban reuse	<40	-	-	-	-	<100	-	-

*: $mg.L^{-1}$, **: NTU, ~: $CFU.100mL^{-1}$, -: not applicable, ND: not detectable, [1]: as TDS, [2]: as *E. Coli*, [3]: as BOD_7.

Adapted from Pidou, M. (2006). Cranfield University

Appendix 2.2: Physical/phytotechnologies

Location	Design	Media	Type of process	Reuse type	HRT (flow rate, loading rate)	Performance: COD (mg.L^{-1})		BOD (mg.L^{-1})		Turbidity (NTU)		SS (mg.L^{-1})		Total coliforms (cfu/100mL)		Reference
						In	Out	In	Out	In	Out	In	Out	In	Out	
	Horizontal flow reed bed	Sand+soil +compost	Biological/physical		2.1 days	452	111	151	51	63	12	87	31	6.10^{7}	10^{4}	Frazer-Williams *et al.*, 2007
UK	Vertical flow reed bed		Biological/physical	Pilot scale	2 hours batch	452	27	151	5	63	2	87	9	6.10^{7}	2.10^{4}	
	Constructed wetland		Biological/physical		2.1 days	452	139	151	71	63	26	87	19	6.10^{7}	2.10^{6}	
Israel	Sedimentation + Vertical flow constructed wetland			Irrigation	8 -24 hours	839	157	466	0.7	-	-	158	3	5.10^{7+}	2.10^{5+}	Gross *et al.*, 2006
		PET media	Extensive biological/physical		100-104	-	-	155 - 290	4-26	103	ND			10^{6}-10^{8}	10^{3}	
Costa Rica		Crushed rock	Extensive biological/physical		60-122	-	-	155 - 290	7-26	103	ND			10^{6}	10^{3}-10^{5}	Dallas and Ho, 2005
		Rock filter	Biological/physical		58-110	-	-	155 - 290	9-28	103	ND			10^{6}	10^{4}-10^{5}	
Costa Rica	2 Reed beds + Pond			Irrigation	> 10 days (0.755 m^{3}/day)	-	-	167	3	96	5	-	-	2.10^{8+}	198^{+}	Dallas *et al.*, 2004
Costa Rica	Trench and plants			Pilot scale	4-5 days (0.01 m^{3}/day)	-	-	254	13	103	-	-	-	8.10^{7+}	2050^{+}	Dallas and Ho, 2004
Spain	Screening + Sedimentation			Toilet flushing	38 hours	171	78	-	-	20	17	44	19	-	-	March *et al.*, 2004

	+ Disinfection													
Italy	Reed beds	Domestic	7 days (0.09 m^3/day)	151	51	42	26	-	-	25	20	-	-	Borin *et al.*, 2004
Germany	Sedimentation + Constructed wetlands	Domestic	(70 L/p/day)	258-354	-	-	-	-	-	-	-	3.10^{5} *	10^{4} *	Li *et al.*, 2003
UK	Coarse filtration +Disinfection	Toilet flushing	-	-	166	-	40	-	40	-	35	-	ND +	Hills *et al.*, 2003
Nepal	Sedimentation + Reed bed	Toilet flushing, cleaning and garden watering	(0.5 m^3/day)	411	29	200	5	-	-	98	3	-	-	Shrestha *et al.*, 2001
Sweden	Gravel filter + 3 Ponds + Sand filter	Toilet flushing	~ 1 year	-	-	47#	0#	-	-	-	-	9.10^{4}	172	Gunther, 2000
UK	Filtration + Disinfection	Toilet flushing	-	157	47	-	-	21	7	-	-	2.10^{5}	13	Brewer *et al.*, 2000
UK	Filtration + Disinfection	Toilet flushing	-	74	11	-	-	2	1	-	-	TNTC	46	Brewer *et al.*, 2000
Switzerland	Sedimentation + Sand filter + Constructed wetland	Pilot scale	-	311	27	130	5	-	-	-	-	-	-	Schonborn *et al.*, 1997
Sweden	Sedimentation + Reed bed + Sand filter	Irrigation	4 days	361	56	165#	<5#	-	-	-	-	3.10^{6}	<20	Fittschen and Niemczynowicz 1997

* as *E. Coli*; + as Faecal Coliforms; # as BOD_7.

CHAPTER 3: IMPACT OF CONSUMER PRODUCT TYPES OF GREY WATER CHARACTERISTICS

IMPACT OF CONSUMER PRODUCTS TYPES ON GREY WATER CHARACTERISTICS

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3.1 ABSTRACT

A study was conducted to explore the potential impacts of 'down-the-drain' chemicals of the characteristics of grey water. Different household cleaning and personal hygiene products were analysed for toxicity, biodegradability, treatability and other parameters of interest in wastewater treatment. The products, which ranged from shower gels and bath crèmes (personal-care) to bath tub and shower cleaners (cleaning products), were further sub-categorised according to branding (variation within each category) which impact on consumers' choice, in order to study the how these factors relate to grey water quality. Microtox and MicroResp (soil respiration) techniques were used to study general aquatic toxicity of the products and toxicity on soil organisms respectively. Conventional wastewater/sewage treatment parameters were analysed for all the sample products. The personal-care products generally showed nutrient deficiency, unlike the cleaning products. This would impact on the treatability of the grey water where conventional treatment methods are used. Microtox toxicity results showed higher sensitivity of the test species (*Vibrio fischeri*), to the products than MicroResp toxicity results. MicroResp produced both metabolic stimulation and inhibition of the soil microorganisms which clearly shows the soil organisms' higher tolerance or ability to assimilate chemicals present in the products than the *Vibrio fischeri*. Product branding (and pricing) did not show any correlation with any of the chemical parameters, therefore they cannot be used to predict grey water characteristics easily. Overall, laundry and cleaning products were more inhibiting to soil microorganisms than were personal care products.

3.2 INTRODUCTION

Grey water recycling, as a water demand management option, is on the increase, providing water for non potable household and industrial reuse (EA, 2001). As a result, considerable attention has been directed towards grey water treatment in recent years. Grey water recycling reduces the pressure on water resources and wastewater treatment facilities resulting from climate change and other global pressures (Jefferson *et al.*, 1999). Grey water is generated by the use of personal care, cleaning products and their associated human interaction. The high concentration of xenobiotics in grey water, from the household cleaning and personal care products are a concern because most treatment systems are not designed for removing xenobiotics (Eriksson *et al.*, 2002). This study therefore explored the potential impacts of category of cleaning and personal care products on the characteristics, treatability and potential toxicity of grey water. Physico-chemical characteristics that are considered in sewage treatment were assessed for each product as well as toxicity to marine pure bacteria (*Vibrio fischeri*) and soil microorganisms. The sample products were grouped according to category (*i.e.* shampoos, washing powders, wash-up liquids, shower gels, bath crèmes *etc.*) and with each category sub-divided according to their branding (*i.e.* eco-friendly product, or supermarket's own brand or open brands by specialised companies), which is assumed to be linked to pricing.

A survey in Western Europe showed that people generally have a right shower attitude with respect to water conservation whereby 48% of the people in the UK are reported to spend less than 5 min in the shower (59 % in Germany, France and Spain). In order for this to have a meaningful impact on the resultant grey water quality (*i.e.*treatability or indeed overall chemical load), there is need for a similar response to usage of personal care products. However, the survey revealed that less than 50% of the people in the UK are concerned about contaminants from personal care products that are washed away into the water system. On average, the percentages for right attitude to usage of personal-care products were lower *i.e.* 37%, 59%, 44% and 63% for UK, Spain, Germany and France respectively than for water usage (Royal Society of Chemistry, 2008). In order to achieve meaningful sustainability in water treatment for reuse, people also need to be made aware of the quantities of contaminants that go into the water system from personal-care products. Two assumptions are made here,

1) that the quantity depends on the amount of product actually used, and 2) that the quantity depends on the brand used. This could then enable the treatability and toxicity of the grey water, especially in small scale systems treating grey water only, to be predicted. This study therefore only focussed on the potential physico-chemical impacts of the products on grey water characteristics without the human interaction factors which require a separate social study.

Existing grey water treatment technologies vary greatly both in complexity and performance (Pidou *et al.*, 2007). The technologies also differ as a result of differences in the characteristics and quantity of the grey water that can be treated (Gross *et al.*, 2007; Nolde, 2000). The characteristics of grey water also vary considerably and they are not well defined (Eriksson *et al.*, 2006). The main factors determining the characteristics of grey water are, the quality of the water supply, the type of fresh water treatment and distribution, and the activities of the household (Eriksson *et al.*, 2002). Activities of the household, as determined by types, quantities, *inter alia*, of cleaning and personal-care products used, are very important and have a significant impact on the ultimate characteristics of grey water. Although knowledge of such impacts is relatively low, it is nonetheless essential in order to understand the impacts on toxicity and treatability of grey water. Knowledge of toxicity of grey water, in addition to treatability, is crucial, for water reuse planning and management. The impacts of the difference chemicals in grey water on human health, terrestrial and aquatic ecosystems will depend on what is actually contained in the grey water, which is itself dependent upon the nature (qualitative) and amounts of the products that were used in the generating household(s). Bioactive chemicals are continually being introduced into the environment as complex mixtures via domestic water (Daughton and Ternes, 1999). The recent increased drive and motivation for grey water reuse also means increased introduction of such chemicals into the water environment.

Information available on grey water quality mainly concerns the content of organic matter (BOD/COD), nutrients (N, P, K) and indicator pathogens for the different sources of grey water *i.e.* bath/shower water, kitchen water etc. (Droste, 1997; Gray, 2004). However, for each source of grey water, there is a whole range of varieties of products, by different manufacturers (*i.e.* containing different ingredients in differing concentrations), which interact differently depending on different permutations of mixtures and a whole host of different factors. In terms of treatment in conventional WWTPs, generally systems with high efficiencies in the removal of conventional wastewater constituents (BOD_5 and NH_4^+) also provide good removal of cleaning and personal care products (PCPs). Some studies have been conducted on pollutants *e.g.* Pharmaceutical and Personal Care Products (PPCPs) in conventional treatment systems but studies of the same in small scale engineered systems have not been extensively documented (Matamoros *et al.*, 2009). With small scale systems becoming more popular for grey water treatment, adequate knowledge of contributions by individual and group (product type) to the chemical quality of the grey water needs to be well understood because it would greatly contribute to adequate treatment designs and operation of such systems. It is therefore important to investigate the differences in grey water quality for different categories and within product categories (*i.e.* different brands). Whole product analysis is essential for grey water because it brings out the un-buffered potential influence of the individual ingredients in the different chemical products which would provide useful information for small-scale treatment design and operation. To illustrate, sodium dodecylsulfate, the main ingredient, by volume, found in most cleaning and personal care products, and its biodegradability and toxicity in surface waters have been extensively studied. However, the biodegradability and toxicity of the household products that contain this chemical are different. Toxicity of whole (household) products would therefore give an indication of grey water's toxicity, because all the other ingredients present in lower concentrations are also included. Any relationship that might emerge between toxicity and conventional wastewater quality parameters would also provide quick pointers to the quality of any grey water and the necessary treatment requirements. Therefore, it is of utmost importance to have some characterisation/typology of grey water that could, if possible, be linked to parameters that are easy to measure since different types of grey water will have different impacts on the terrestrial, aquatic and agricultural environments as well as human health. The objective of this study was to identify the impact of general chemical properties of the household cleaning and personal care products on grey water quality and treatability, and explore whether toxicity of grey water can be predicted from knowledge of cleaning and personal care products used in the household.

3.3 MATERIALS AND METHODS

3.3.1 Sampling and product characterisation

A total of 84 cleaning and personal-care products representative of the various household product categories on the market were analysed (Table 3.1). The products were purchased from local supermarkets and were chosen on the basis of their brand, which as discussed in Chapter 2, is assumed to link to cost of the products. This was done to explore whether branding can be used to predict the impact on the physico-chemical characteristics of a product on the resultant grey water. For each of the products, general physical and chemical parameters were analysed according to appropriate Standard methods (APHA, 1998).

Table 3.1: Test products categorisation

Shampoos (SH)	Washing Powders (WP)
Hair conditioner (CON)	All Purpose Cleaner (APC)
Shower Gels (SG)	Toothpaste (TP)
Bath Crèmes (BC)	Mouthwash (MW)
Washing up liquids (WU)	Baby Products (BP) – such as bath crèmes, soaps
Fabric Conditioners (FC)	Shaving Foam

3.3.2 Analytical techniques

Conventional water quality parameters

The parameters investigated were conductivity, pH, turbidity, Total organic carbon, chemical oxygen demand, total phosphorus and total nitrogen. Total organic carbon (TOC) (mg.L^{-1}), was analysed using a total organic carbon analyser Shimadzu TOC-5000A (Shimadzu, UK); Turbidity (NTU), using a Turbidimeter Hach 2100N; pH and conductivity, using the Jenway 3540 pH and conductivity meter; and Merck cell tests (Merck, VWR International), Poole, UK) for chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP). Solutions of 20ml L^{-1} (for liquid products) and 10 mg L (for powedered products) were prepared and used for analysis. Further dilutions were made where necessary to obtain optimum results.

Aquatic and soil toxicity analysis

The toxicity of each product was assessed in relation to the response of specific bacteria, *Vibrio fischeri,* and to active soil micro-flora in a standard soil. Specific bacterial toxicity was measured using Microtox® technique (Microbics M500 toxicity analyser, Azur Environmental, UK) to determine the median effective concentration EC_{50}. Microtox® uses standard tests on a pure culture of marine bacteria *Vibrio fischeri,* (NRRL B-11177) that luminesce as a by-product of their metabolism. All sampled products were ranked in relation to standard Phenol and Linear alkylbenzene sulfonate (LAS) an anionic surfactant. Microtox® testing was performed according to the standard procedure recommended by the manufacturer. A working solution of luminescent bacteria was prepared by reconstituting a vial of freeze-dried *Vibrio fischeri* which was usable for several hours when kept chilled. A dilution series of the samples to be analysed was prepared in sodium chloride solution (2% NaCl). Different concentration intervals were used for the tested chemicals depending on the expected EC_{50} values. A fixed amount of bacteria (10^6 cells) was added to the dilution vials. Luminescence was measured at time zero and after 5 min and 15 min and compared to the measured value of a bacterial control solution (sodium chloride 2%) lacking the tested compound. Toxicity (EC_{50}), obtained from the dose-response curves, was defined as the median concentration of the toxicant causing the 50% reduction of an activity function of the bacterial culture.

Soil microbial toxicity was measured using the MicroResp$^{(TM)}$ technique (Kaufmann *et al.*, 2006; Macaulay Enterprises Limited, 2006). This is a soil respiration technique which was used in this study to measure the effect of the sample/xenobiotic on the metabolic activity of soil microbial organisms and is an important method for determining short-term effects of a xenobiotic on soil micro-flora (Ritz *et al.*, 1994). A standard soil, sandy loam (Agriculture and Food Standards Policy Committee, 1994), alkaline (pH: 7.6), non-saline, stone free (74% sand, 18% silt and 8% clay) was used for the soil toxicity tests in this study. Median effective concentrations were also obtained from the MicroResp measurements. The Spearman rank correlation was used to test the relationships between toxicity, brand type, acidity, COD/TOC ratio and unit price (*i.e.* price per ml) for the tested products. Spearmann rank coefficient (r_s) is a non-parametric measure of statistical dependence between two variables. It assesses how well the relationship between two variables can be described using a monotonic function. If there are no repeated data values, a perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other. The significance of the r_s is then assessed to determine wherther or not there is any dependence between the compared variables.

3.4 RESULTS

Conventional water quality parameters

The tested products displayed considerable differences in concentrations in their physico-chemical parameters (Table 3.2). For instance COD levels in all the tested products were high (4 – 38 g COD L^{-1}). Products in the All Purpose Cleaner category exhibited the lowest levels of COD (4.15 ± 2.75 g COD L^{-1}) while all the other categories, including those used on a daily basis in the household, had COD levels in excess of 11 g COD L^{-1}. TOC levels ranged from 0.17 to 2.7 g TOC L^{-1}, with the highest levels (> 2.0 g TOC L^{-1}) reported for toothpastes, bath crèmes, and wash up liquids. TOC is an important parameter because it also acts as a measure of pollution characteristics of wastewater (Metcalf and Eddy Inc., 2003). In this study the importance of TOC and the relationship to COD was particularly important with regards to treatability and toxicity of the resultant grey water. Indeed the high levels of COD indicate the potential presence of a toxic component or that acclimated microorganisms would be required to treat these product solutions. Spearman Rank correlation (which was used to test the independence of the different parameter) showed significant correlation between COD and TOC ($r_s = 0.67$, $P < 0.05$, n = 84) and between TOC and pH ($r_s = 0.24$, $P < 0.05$, n = 84). Correlation tests were carried to explore if there were any predictive relationships that could be used to determine grey water quality from the products.

Table 3.2: Mean (± std dev) values for different parameters by product category; ranges given in parenthesis

Product group		Number of products tested	Turbidity (NTU)	Conductivity (mS)	pH	COD (g. L^{-1})	TOC (g. L^{-1})	COD:TOC	COD:TN:TP
Shampoos	SH	14	2.6 (< 1.0 – 24)	37.5 ± 15.6 (20.3 – 76.7)	5.9 ± 0.7	20.5 ± 13.4 (5.6 – 53.7)	1.9 ± 1.03 (0.4 – 3.6)	12 : 1	100 : 0.010 : 0.002
Conditioners	Con	7	32.5 (7.5 – 64.2)	0.4 ± 0.5 (0.1 – 1.5)	4.5 ± 0.5	20.2 ± 20.6 (5.9 – 65.7)	0.17 ± 0.05 (0.1 – 0.23)	107 : 1	100 : 0.020 : 0.001
Shower gels	SG	8	4.2 (< 1.0 – 19.6)	25.2 ± 20.2 (0.04 – 68.0)	5.4 ± 0.8	29.4 ± 27.5 (4.5 – 75.4)	1.6 ± 0.9 (0.8 – 3.5)	16 : 1	100 : 0.010 : 0.007
Bath crèmes	BC	5	3.5 (< 1.0 – 12.5)	28.6 ± 16.4 (4.9 – 40.2)	5.6 ± 0.8	50.2 ± 28.2 (11.5 – 73.2)	2.18 ± 1.9 (0.6 – 4.7)	42 : 1	100 : 0.009 : 0.001
Wash up liquids	WU	8	1.0 (0.02 – 7.5)	24.3 ± 12.2 (2.2 – 44.8)	6.7 ± 2.0	38.2 ± 23.0 (6.8 – 76.9)	2.5 ± 1.9 (0.5 – 6.6)	17 : 1	100 : 0.010 : 0.003
Fabric conditioners	FC	7	23.5 (< 1.0 – 48.4)	1.18 ± 0.26 (0.9 – 1.65)	2.5 ± 0.1	21.1 ± 12.9 (14.3 – 50.2)	0.74 ±0.50 (0.45 – 1.74)	31 : 1	100 : 0.005 : 0.003
Washing powders	WP	9	87.2 (1.1 – 266)	13.6 ± 1.8 (9.9 – 15.7)	10.8 ± 0.3	27.7 ± 15.2 (14.0 – 49.8)	1.31 ±0.50 (0.81- 2.03)	20 : 1	100 : 0.020 : 0.050
All Purpose Cleaners	APC	7	5.3 (< 1.0 – 29.9)	17.7 ± 18.0 (4.6 – 53.7)	8.3 ± 2.9	4.15 ± 2.74 (0.2 – 6.9)	0.42 ± 0.31 (0.21 – 0.84)	8 : 1	100 : 0.240 : 0.010
Baby products	BP	5	190 (<1.0 – 949)	14.5 ± 21.1 (0.01 – 48)	6.5 ± 1.3	23.2 ± 33.3 (5.5 – 82.7)	0.79 ± 0.75 (0.15 – 2.10)	28 : 1	100 : 0.022 : 0.001
Toothpaste	TP	4	115 (2.74 – 349)	2.18 ± 1.35 (0.98 – 3.71)	7.2 ± 0.5	23.1 ± 9.7 (16.7 – 37.2)	2.7 ±1.0 (2.0 – 4.2)	9 : 1	100 : 0.020 : 0.020
Mouth wash	MW	6	0.02 (0.01 – 0.04)	2.51 ± 0.01 (0.13 – 12.11)	6.1 ± 1.2	11.3 ± 6.1 (1.20 – 20.6)	0.92 ± 0.40 (0.40 – 1.60)	11 : 1	100 : 0.050 : 0.005

Assessment COD:TN:TP ratios (Table 3.2) in the test sample products showed that the levels of nutrients (N and P) were low in all the product categories except for washing powders and toothpaste products (Figures 3.1 and 3.2). Literature values for COD:TN:TP are 100:2.25:0.06, 100:2.91:0.05 and 100:1.77:0.06 for bath, shower and hand washing basins respectively (Pidou *et al.*, 2007) and 100:20:1 for domestic wastewater (Metcalf and Eddy, 2003). The very high COD concentrations in all the products resulted in very high C:N ratios (>> 1000:1) indicating serious nutrient deficiency in the products. All the products in this study fell far below the optimum ratio for biological treatment of 100:5:1 (Gray, 2004). Ratios below this value potentially reduce the efficiency of biological processes (Jefferson *et al.*, 2004). This entails that N and P additions would be required to correct the imbalance and improve the efficacy of biological systems in treating the resultant grey water as shown by Jefferson *et al.* (2001) and also increase the treatability potential when using extensive technologies.

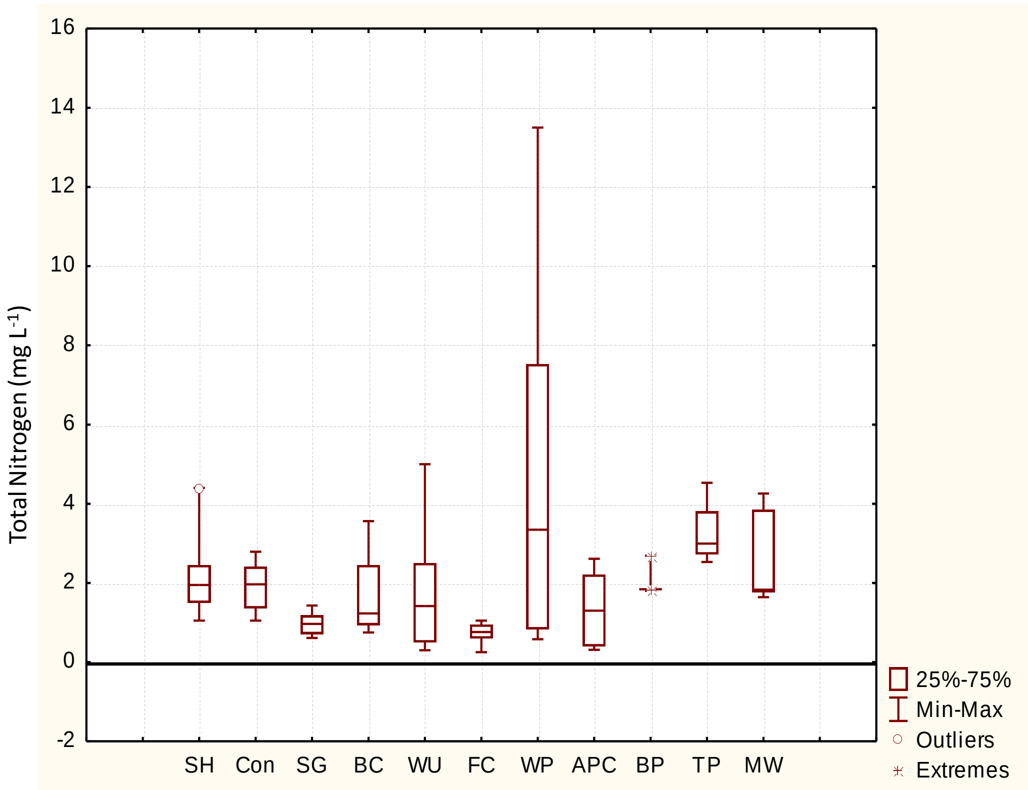

Figure 3.1: Total Nitrogen by product type.

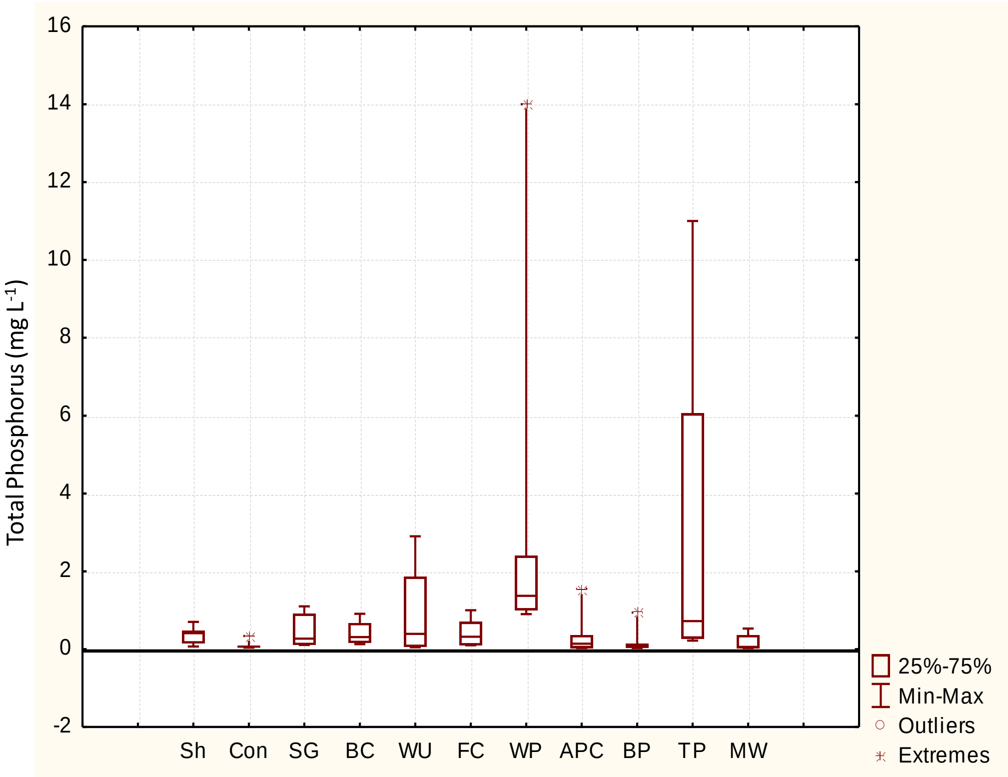

Figure 3.2: Total Phosphorus by product type.

The pH range for all the tested sample cleaning and personal care products was from 2.33 to 11.7. Fabric conditioners (FC) exhibited the lowest pH levels (2.49 ± 0.14) and washing powders (WP) had the highest pH levels (10.8 ± 0.34). Only one-third of the products fell in the pH range 6 to 9, which is important for determining the existence or survival of most forms of life in soil. This is crucial for grey water reuse because of the potential hazardous effects that grey water outside this pH range might cause on soil health if reused for irrigation. The pH values for personal care products (shampoos, shower gels, bath crèmes and conditioners) largely fell in the range 5 – 7 while pH for laundry products were above 10 (Figure 3.3). Interestingly the pH range for personal care products is slightly below the pH range reported for bathroom wastewater (pH 6.4 – 8.1) while that of laundry products is higher than in laundry wastewater (pH 9.8) (Eriksson *et al.*, 2002). This indicates that pH of the products shifts after use to the circum-neutral pH range of 6 to 8. However, use quantities and water quality are contributing factors that play a part. Washing powders, baby products and toothpaste showed the highest average turbidity levels (> 85 NTU) equivalent to turbidity levels reported for high strength (BOD > 300 mg L^{-1}) grey water (Frazer-Williams *et al.*, 2008).

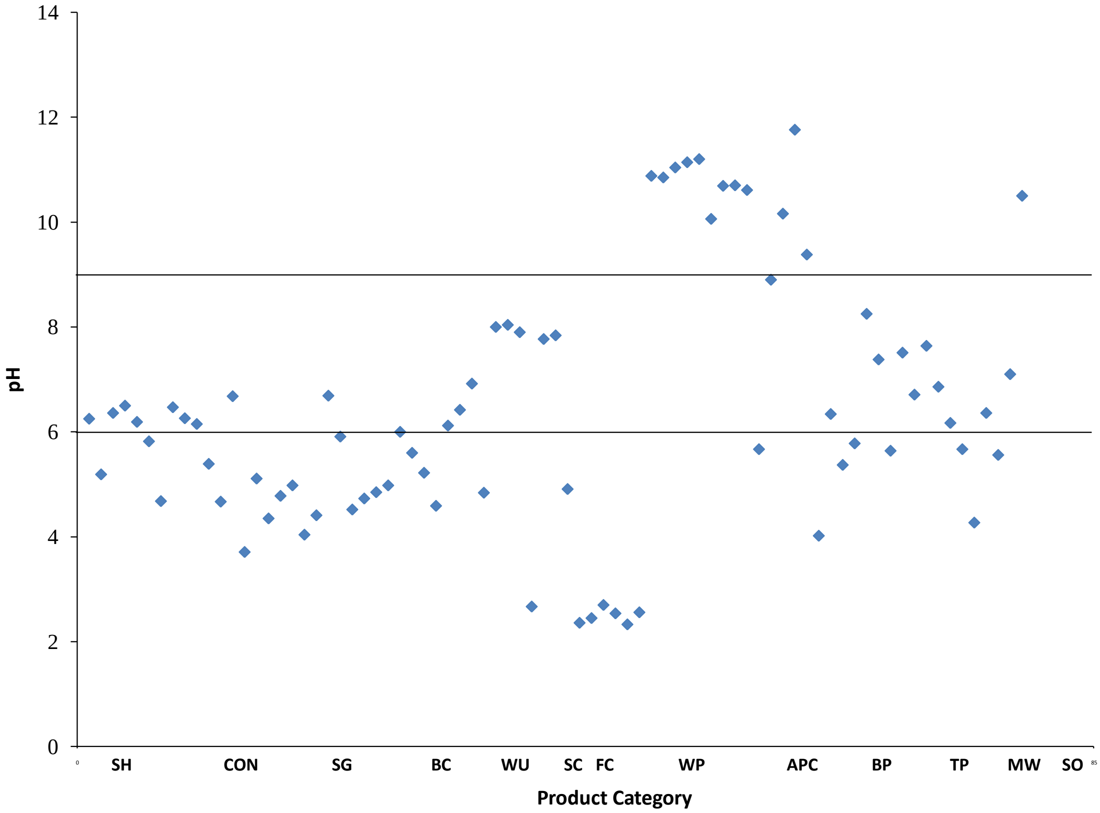

Figure 3.3: pH measurements of sample product by product category

Microtox® Toxicity

The Microtox results were compared to pure linear alkylbenzene sulfonate (LAS) and cetyl trimethylammonium bromide (CTMAB). The EC_{50} values ranged from the most toxic, 0.37 ± 0.32 µl. l^{-1}, (for a shampoo product) to the least toxic product 1841 ± 45 µl. l^{-1}, (a conditioner product). The toxicity ranking by category shows the shampoo category to be the most toxic and the All Purpose Cleaner and Mouth Washes as the least toxic product categories (Figure 3.4). Shampoos were the more toxic ($\log_{10}$ EC_{50} values below 1.0) than even the standard surfactant, linear alkyl benzene sulfonate (LAS) which is the main surfactant that is used in detergent and personal care products (Eriksson *et al.*, 2002; del Olmo *et al.*, 2004). Details of the toxicities of individual sample products for each category are shown in Appendix 3.2. Interestingly, Eco-labelled products were found to be no less toxic compared to the other brands. Spearman Rank correlation analysis showed no significant correlations between toxicity and brand type (branded product - B, supermarket's own brand - OB and eco-friendly brand - EB) as well as between toxicity and product price (Table

3.3). The orders of toxicity in terms of branding were different across the different product categories. However, the correlation between TOC and the Microtox EC_{50} (5 min and 15 min) values were significant (r_s = -0.43 and -0.45 respectively, $p < 0.05$, n = 84). Indeed this is in agreement with literature (Metcalf and Eddy, 2003) that increase in TOC concentrations, is related to increasing toxicity (indicated as decreasing EC_{50} values hence the negative correlation). Some of the EC_{50} values for the eco-labelled products in this study were much more toxic than other non-eco products, a case in point being for All Purpose Cleaners and Wash Up liquids. Interestingly, the Shampoo category which was on average, the most toxic also had the least toxic product from the total sample population. The analysis also showed that branding was not necessarily related to pricing of the products. This therefore shows that toxicity of a product or the resultant grey water cannot be predicted based on the brand type, labelling or the price.

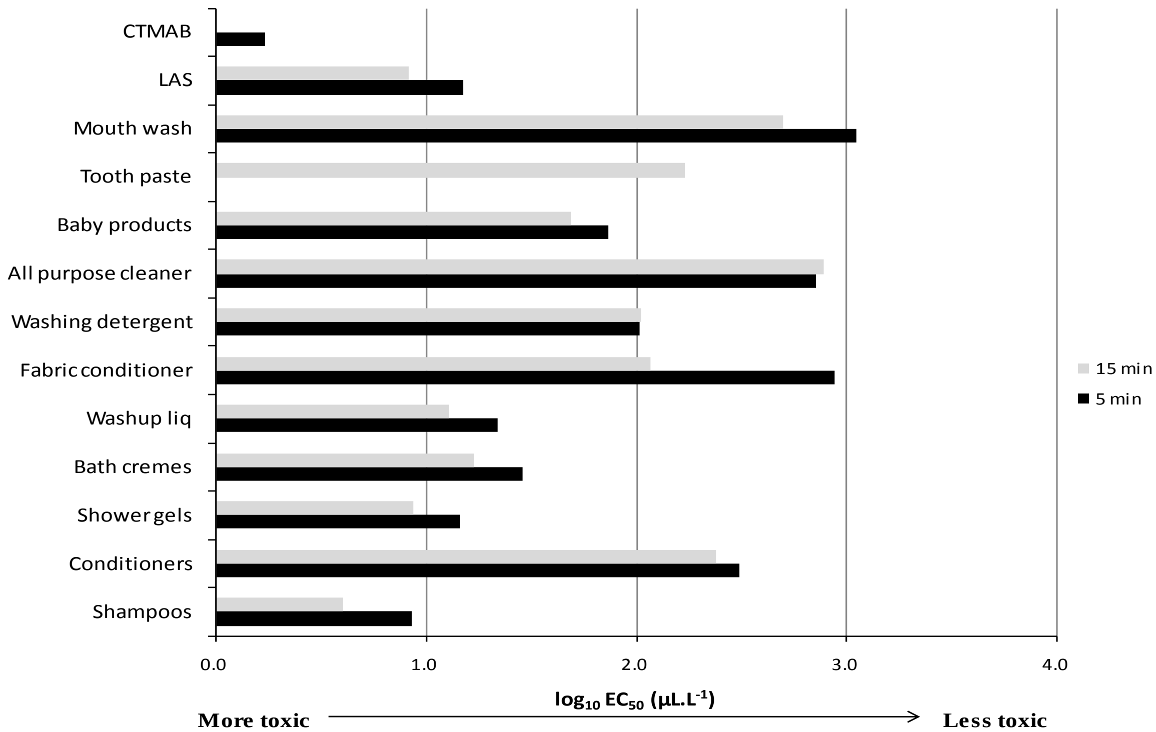

Figure 3.4: Microtox toxicities by product category

(Note: LAS is linear alkylbenzene sulfonate and and CTMAB is cetyl trimethylammonium bromide).

Table 3.3: Spearman Rank Order Correlation for all tested products

	Brand type	EC_{50} (5 min)	EC_{50} (15 min)	EC_{50} (basal)	pH	tNitrogen	tPhosphorus	COD	TOC	COD:TOC	Price
Brand type	1.000										
EC_{50} (5 min)	0.031	1.000									
EC_{50} (15 min)	0.053	***0.980***	1.000								
EC_{50} (basal)	-0.003	-0.119	-0.097	1.000							
pH	***0.367***	-0.024	0.001	-0.029	1.000						
tNitrogen	-0.084	0.025	0.055	0.132	0.194	1.000					
tPhosphorus	0.099	-0.040	-0.063	0.010	***0.389***	-0.135	1.000				
COD	-0.023	-0.221	-0.234	0.139	0.000	-0.019	***0.372***	1.000			
TOC	-0.011	***-0.427***	***-0.445***	-0.038	***0.241***	0.038	***0.385***	***0.673***	1.000		
COD:TOC	0.072	0.205	0.217	***0.248***	***-0.306***	-0.156	0.051	***0.394***	***-0.331***	1.000	
Price	***-0.654***	-0.145	-0.136	-0.002	-0.157	0.155	-0.104	-0.056	0.117	***-0.252***	1.000

Italicised and bold correlations are significant at $p < 0.0500$
Brand type: 1-B, 2-OB and 3-EB

Assessment of the product ingredients revealed surfactants as the main ingredient (conventionally listed at the top end of the ingredients list). However no detailed information of the exact nature and quantities of the surfactants is provided on the packaging and this proved to be difficult to investigate for each product. Oral products (Toothpaste and Mouth Wash products), had no surfactant ingredient listed on the packaging and were not least toxic category (Figures 3.4 and 3.5). Therefore there is no prominent ingredient group that can be associated with toxicity, and no reliable indicator other than using the COD and/or TOC values as well as degradation tests (these were not conducted in this study). Nevertheless, in human washing products such as Shampoos and Shower Gels, ingredients such as sodium lauryl sulphate (SLS), sodium hypochlorite, and essential oils are key and are likely to be the most toxic as pointed out by Knops *et al.* (2007).

MicroResp$^{(TM)}$ Toxicity

All product categories contained products that caused metabolic stimulation (measured as increased basal activity) of the soil microorganisms, and some product categories had products that inhibited microbial metabolism (Figure 3.5). The Chi-Square test (Appendix 3.1) for test of distribution of products that caused stimulation and those that caused inhibition in the soil microorganisms showed that stimulating and inhibiting products were not evenly spread ($\chi^2 = 26.1098$, df = 2, p = 0.000) about the no effect point. There were more products that caused a stimulation response from the soil microorganisms. The bias favouring this effect is probably due to availability of easily degradable carbon because the 6 hr MicroResp analysis measures activity rather than cellular growth (Anderson and Domsch, 1978). Hence the increased respiration of the soil microorganisms that were live at t = 0 was more likely to have resulted from availability of more resource substrate from the test products. Average (median) overall inhibition values were observed for the following laundry and general cleaning product categories (Wash Up, (2.5 µL L^{-1}), Fabric Conditioner (3.6 µL L^{-1}) and All Purpose Cleaners (4.9 µL L^{-1}). Inhibition values were also obtained for some personal care products in the following categories: Conditioners, Shower Gels, Baby Product (bath crème) and Mouth Wash, although overall group averages showed stimulation response. There was no significant relationship between Microtox and MicroResp toxicities, which confirms the different responses by the respective test organisms used in the two methods. Hence, Microtox toxicity test is not a suitable test to predict potential toxicity effects on soil ecology. To illustrate, all shampoo products notably showed increased microbial activity, despite shampoos being the most toxic category according to the Microtox assessment. The least toxic product as determined by MicroResp was an Own Brand product which had stimulation EC_{50} of 433 ppm and an inhibition Microtox EC_{50} of 23 ppm (not shown in Figures 3.5 and 3.6.) making it one of the slightly more toxic products.

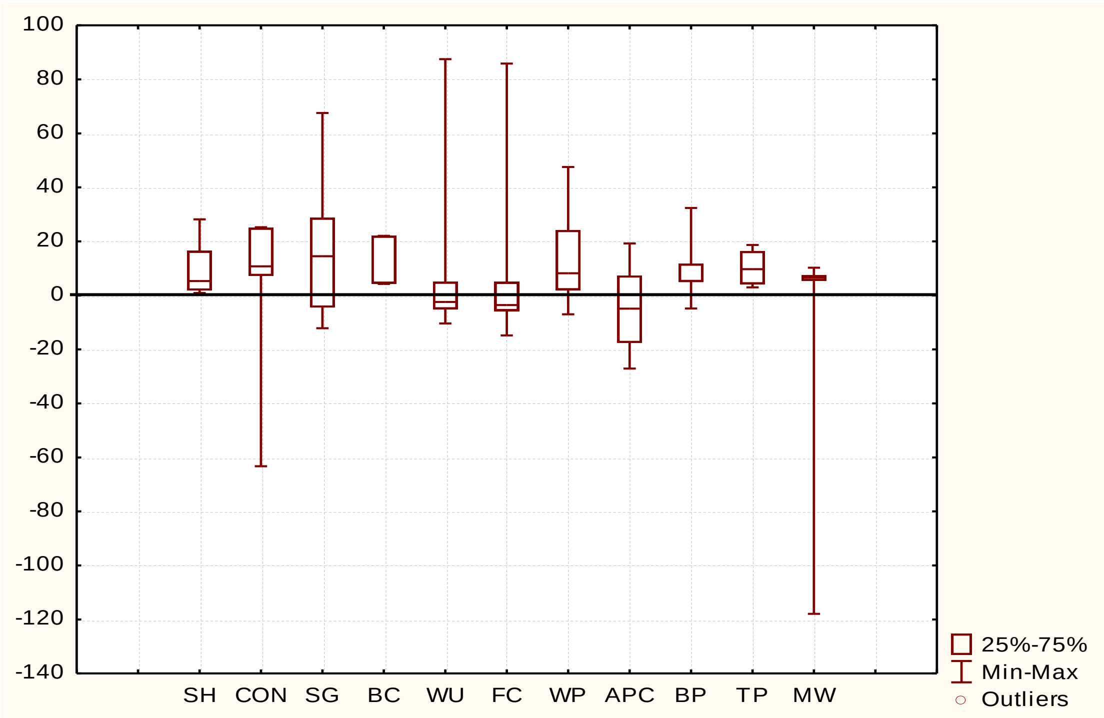

Figure 3.5: MicroResp EC_{50} values for product categories showing the spread of products producing stimulation (positive values) and inhibition (negative values) to soil microbial metabolic activities. Note: The maximum EC_{50} for shampoo category taken as an outlier was 433 ppm

The variations in MicroResp toxicity which resulted in both stimulation and inhibition within product categories suggest that there is a parameter that affects toxicity. The Spearman rank correlation showed significant positive correlation between MicroResp EC_{50} values and the COD/TOC ratio ($r_s = 0.25$, $p < 0.05$, n = 84) (Table 3.3). This suggests that stimulation of soil microorganisms was associated with decrease in TOC, which signifies reduced concentration of toxic ingredients. Surfactant exhibit reduced toxicity with reduced chain length (Garland *et al.*, 2004) which also results in reduced TOC. This probably means that the main factor affecting the response on the soil microorganisms in the MicroResp analysis is TOC associated with different surfactant chain homologues. Hence removal of TOC during treatment is important in order to reduce the probability of residual toxicity which would negatively affect soil microorganisms in soils receiving treated grey water. The MicroResp results also showed more negative response for substrate induced conditions where readily available carbon was added in the form of glucose to activate dormant microorganisms. This indeed means that available carbon (or organic matter) in the soil increases the bioavailability of a xenobiotic and its toxicity as also shown by Leita *et al.* (1999).

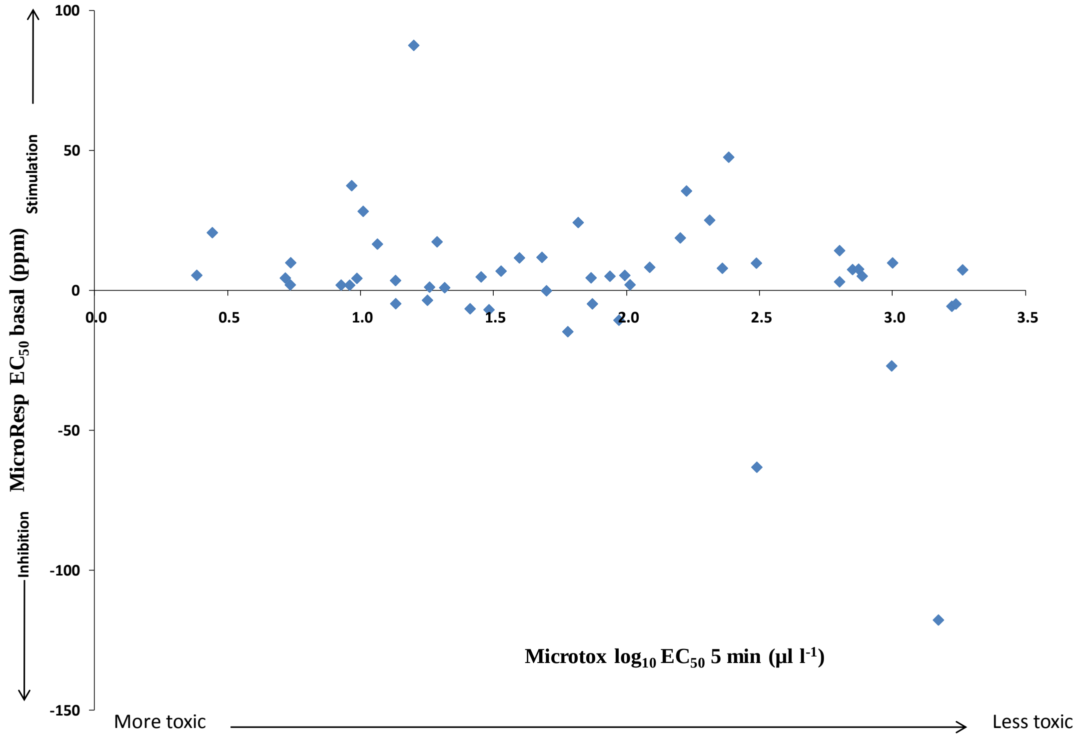

Figure 3.6: Relationship between Microtox and MicroResp toxicity

Measured COD values had very wide variations (Table 3.2), commensurate with variations reported in previous studies (Frazer-Williams *et al.*, 2008; Birks and Hills, 2007). These variations arise from categorisation of the products, as shown in this study, but could also arise from user's water consumption (Friedler and Butler, 1996) and washing habits (Knops *et al.*, 2007) which result in different dilution effects. Comparison of toxicity results obtained using Microtox and MicroResp showed no correlation (Figure 3.6). The results show Microtox to be a more sensitive measure of toxicity than MicroResp. Therefore the Microtox method, which is quick and relatively easier to execute, cannot be used to predict potential toxicity effects of grey water on soil ecology. Microtox has been reported to be slightly more sensitive towards non-ionic surfactants (Tzoris and Hall, 2006). In this study this may have served two purposes, firstly indicating ranking of products by toxicity due to the presence of this group of surfactants and secondly, improving the general understanding of chemical characteristics of household products (xenobiotics). As has been suggested by other researchers, (*e.g.* Knops *et al.*, 2007), *Vibrio fischeri* do not exist in a habitat that is similar to soil microorganisms and thus would be expected to respond differently. The Microtox method has indeed been seen to produce more sensitive results compared to other forms of respirometry (Ricco *et al.*, 2004; Reynolds *et al.*, 1987). Hence the MicroResp results in this study present the most meaningful toxicity result with regards to the subsequent use of grey water for irrigation. Interestingly though, the order of decreasing (Microtox) toxicity for some personal care products (Shampoos > Shower Gels > Bath Crèmes), is a mirror of the toxicity pattern in real grey water (washing and shower > kitchen > bathtub and hand basin) as reported by Eriksson *et al.* (2006).

3.5 DISCUSSION

Characterisation of the household products showed a general nutrient deficiency in their synthetic solutions which would affect their biodegradability in grey water. This observation has also been made for grey water from different sources within the household as well as on a larger spatial scale (Eriksson *et al.*, 2002; Pidou *et al.*, 2008). Laundry products in this study were the only group that had significantly higher nutrient levels. This highlights the benefit of including some laundry water and/or kitchen water to bathroom/shower grey water to improve the treatability of the grey water without compromising on other factors, especially in cases where treatment was by extensive technologies. Alternatively washing some clothes by hand in the bath/shower tubs would help to improve the treatability of the grey water from these sources, because washing products have significantly higher nitrogen and phosphorus levels as also shown by Jefferson *et al.* (1999).

MicroResp analysis of the products showed that some caused an increase in soil microbial activity or stimulation (positive EC_{50} values) and others a decrease (negative EC_{50} values). Initial increase in microbial activity was observed at low concentrations for all products, which is expected because at low xenobiotic concentrations, microbial cell membranes are usually still intact and can block passage of xenobiotic. However, it may also mean that micro organisms are able to resist and/or sequestrate the organic compounds present in the household products at low concentrations (Rinallo *et al.*, 1988). In some cases, microbial activity started to decrease as the concentrations were increased. This could have resulted from destabilisation of the cell membranes at high concentrations resulting in decreased microbial activity. The implication of this might be that long term use of grey water or presence of higher concentrations of some chemicals might result in microbial cell wall permeability and cell lysis.

Thefore there are two responses expected from increased product concentration, metabolic stimulation or inhibition. The effect of activation from xenobiotics is usually observed at low concentration when the cell membranes are still intact and inhibition is observed at high concentrations when the cell membranes are destabilised, which results in increased xenobiotic influence. In terms of grey water recycling, critical concentrations of xenobiotics from the down-the-drain products in irrigated soil, for example, can be reached either in the short or long term. Interestingly from the results in this study, going by brand types, the own brand products showed much lower toxicities for MicroResp although there was no significant relationship in terms of brand type and price. Despite this, some of the own branded value products were generally the cheapest, though as shown above not always the least toxic. Chemical interactions between these ingredients and other physico-chemical factors would have to be studied in order to determine the link to toxicity.

Relevance of this work in grey water treatment

The assessment of ecological risk of household products by category or brand is not meaningful unless linked to actual usage of water for cleaning and bathing or washing. Average water volume usage in a household is 150 litres per person per day (EA, 2001). Table 3.4 gives estimated levels of the products that are likely in domestic wastewater discharge over a 24 hour period. These values are based on the products being used once in a day and at average usage quantities. Usage rates would of course be different from person to person and also vary as a function of local potable quality (*e.g.* pH). This study has again affirmed that Microtox results are more sensitive than respirometry as indicated by the very low quantities of the pure products required to cause an effect in the test species (*Vibrio fischeri*) (Table 3.4). On the other hand, MicroResp results showed that much higher volumes would be required in order to obtain an effect in the soil microorganisms. To illustrate, the MicroResp results showed that only Fabric Conditioners (432 ml) and All Purpose Cleaners (734 ml) pose the greater concern because of the lower volumes required to reach the inhibiting EC_{50} for soil microorganisms. Pettersson *et al.* (2000) and Eriksson *et al.* (2006) also reported these product categories as imparting a relatively high toxicity to laundry and wash water. The likelihood of 432 ml Fabric Conditioner and 734 ml All Purpose Cleaner, (which is essentially more than half bottle if a 750 ml volume is considered the standard size) respectively, being used per person per day is very low. Laundry discharges are diluted between three and five times in a total grey wastewater collection system, higher toxicity of this grey water source can still be expected. It should be noted that the risks described above relate to untreated grey water, treatment will lower the risks associated with any specific pollutant. These observations are significant as they show the importance of MicroResp toxicity test over Microtox toxicity for grey water to be used for irrigation.

Table 3.4: Daily volumes needed to be discharged to reach product EC_{50}; italicised values for MicroResp denote activity inhibition.

Category	Water usage of 150 l p^{-1} d^{-1}	
	Volume to reach Microtox EC_{50} (in ml)	Volume to reach MicroResp EC_{50} (in ml)*
Shampoos	3.7	798
Conditioners	57.1	1268
Shower Gels	2.5	1430
Bath Crèmes	5.9	634
Wash Up Liquids	1.9	596
Fabric Conditioners	9.1	*432*
Washing Detergents	30.4	1227
All Purpose Cleaners	11.2	*734*
Baby Products	28	773
Toothpaste	53.6	2121
Mouth Wash	-	*8280*

*volume per kg of soil

A comparison of surfactant concentrations in products from the surfactant rich product categories shows that All Purpose Cleaners have higher concentrations of anionic and non-ionic surfactants (15 – 30 %) compared to Fabric Conditioners (5 -15 %), which instead have higher cationic surfactant concentrations. The rest of the products result in little change in microbial activity and would only cause an increase in activation (*i.e.* stimulation) anyway if more than 750 ml was used in one day.

3.6 CONCLUSION

Factors that normally determine consumer choice of product are branding and price but these do not have a significant correlation with the conventional physical and chemical quality parameters and toxicity of the grey water. The study showed that combinations of product type and usage patterns may affect the characteristics of grey water such as treatability and toxicity and impact the receiving ecosystem negatively. Differences were generally observed between laundry/cleaning product categories (such as Wash Up liquids, All Purpose Cleaners and Fabric Conditioners) and personal care product categories (such as Shower Gels, Bath Crèmes and Shampoos) due to the concentrations of surfactants. However, other factors like product usage patterns or lifestyle choices are still influential but are perhaps of secondary importance to water usage and dilution effects.

Microtox was found to be a generally more sensitive toxicity analytical tool than the MicroResp method and so cannot be used reliably to predict grey water toxicity to soil microorganisms if treated grey water is to used in irrigation. Although the variability of Microtox toxicity values and COD of the product categories dominated the principal components, TOC and the ratio between COD and TOC were found to be the best predictors of the chemical and toxic characteristics of the products. However most of the products are unlikely to be used in sufficient quantities within a household to cause significant impact on grey water quality. This study has shown that there is potential to predict physico-chemical and toxicity of grey water based on product use type although detailed information of the ingredients of the cleaning and personal care products is still necessary. Parameters such as COD:TOC ratio and TOC can also be used to predict potential toxicity of grey water.

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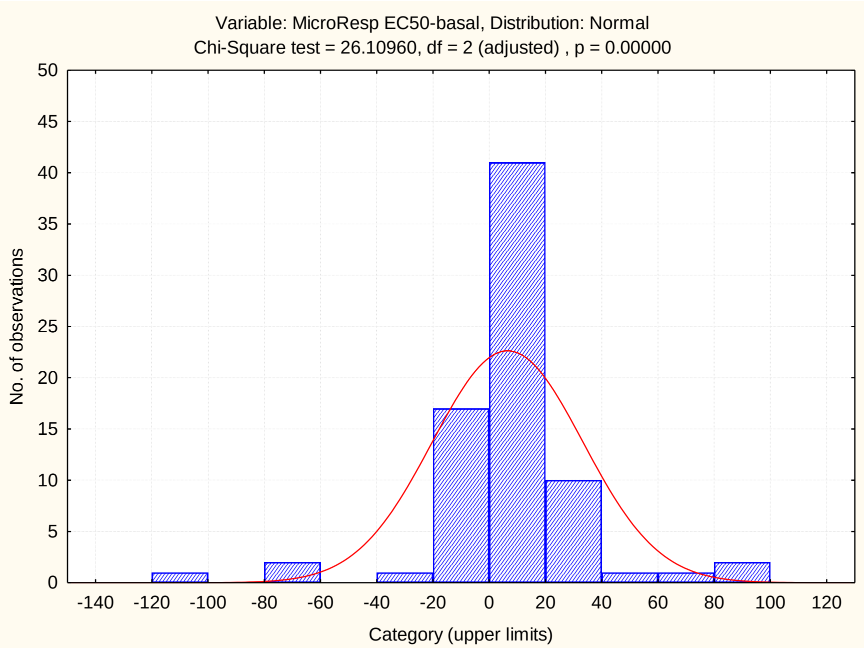
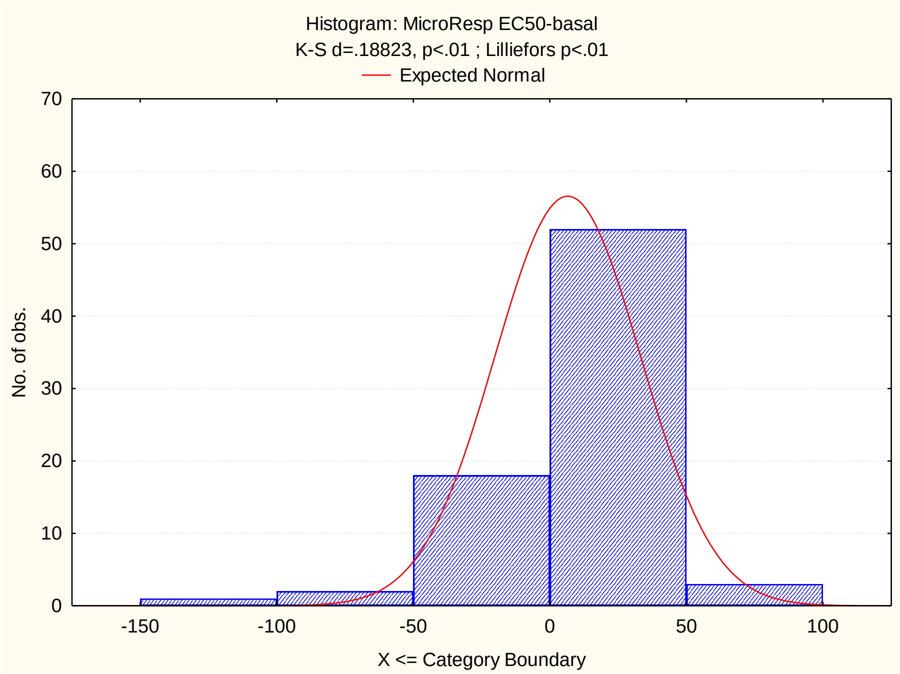
3.8 SUPPORTING INFORMATION

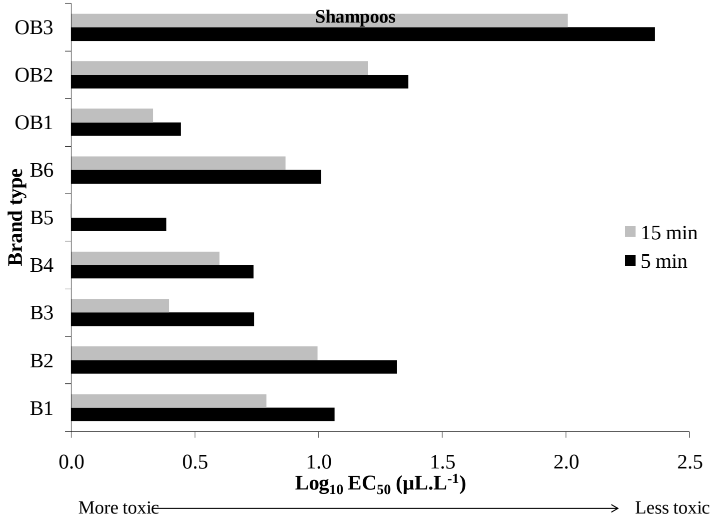
Appendix 3.1: Chi-square test and histograms for MicroResp EC_{50} values

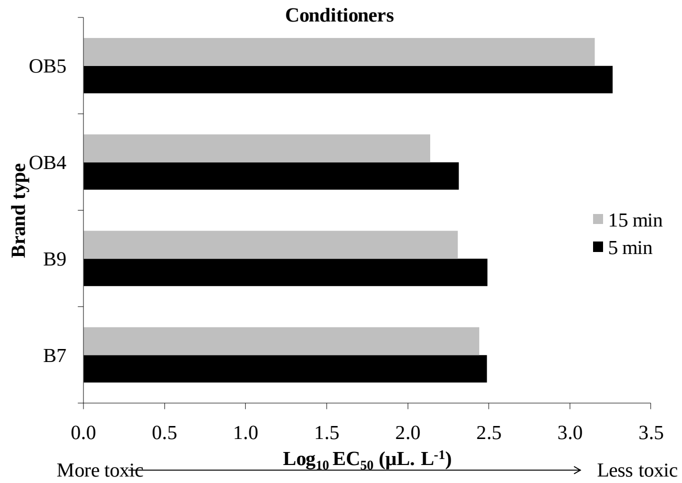


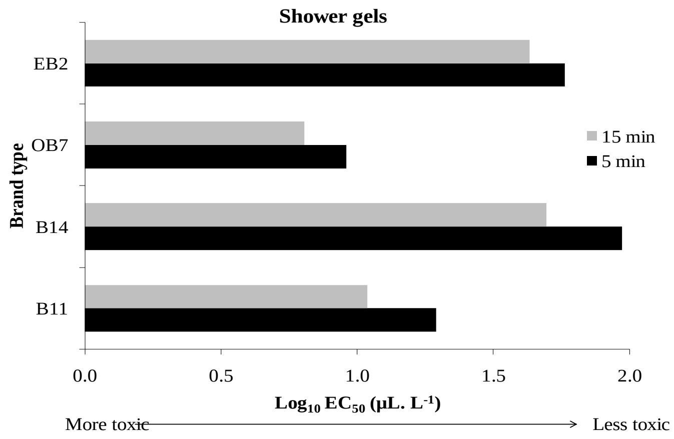


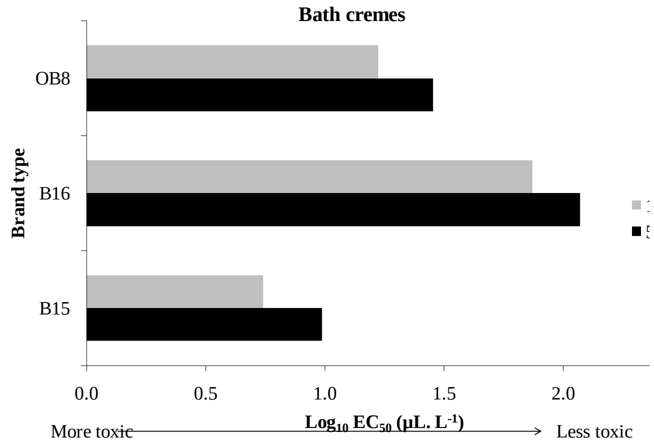
The H_o that the products were binomially distributed between those that cause stimulation (positive EC_{50} values) and those that cause inhibition (negative EC_{50} values) about the no effect value (*i.e.* $EC_{50} = 0$) is rejected as the calculated χ^2 (26.1098) is greater than the critical one $\chi^2_{0.001, 2}$ (13.82) at p = 0.000.

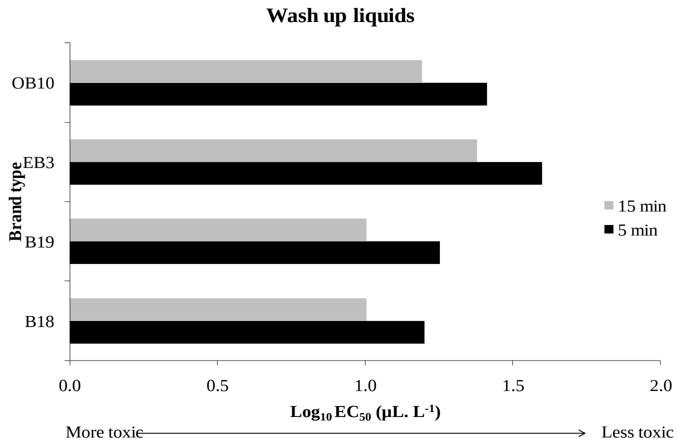
Appendix 3.2: Microtox toxicities for tested products by product category. (Note: OB is supermarkets' Own Brand, EC is Eco-friendly brand and B is for all other brands)

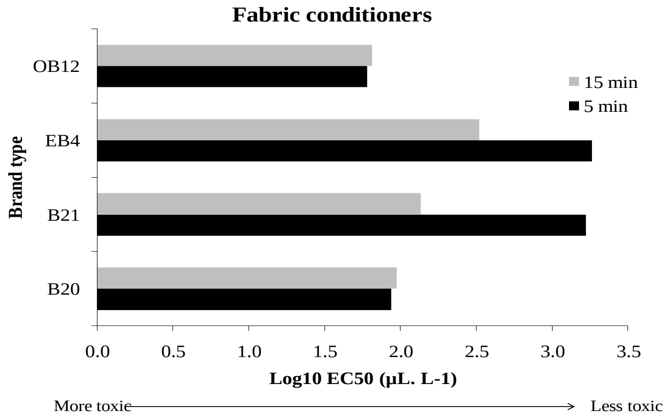


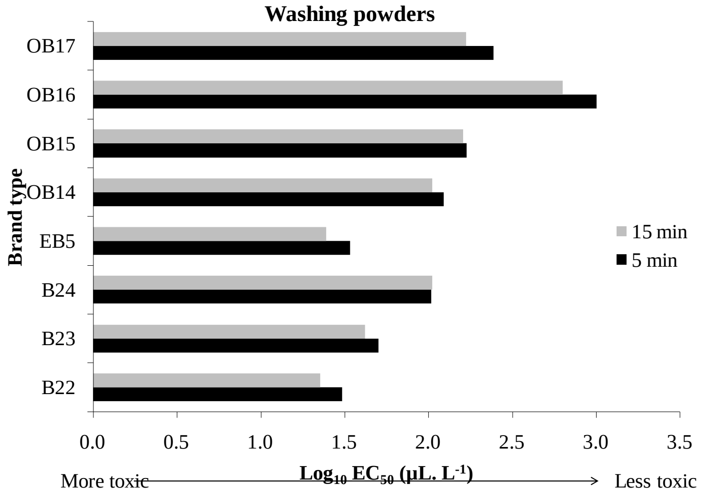


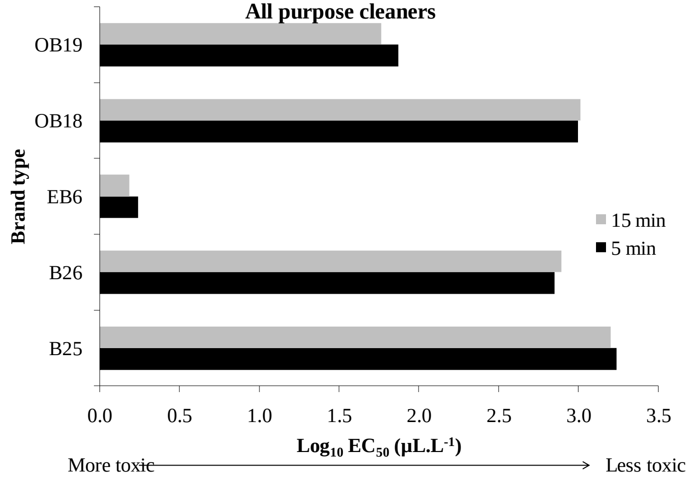

Shower gels
EB2
OB7
B14
B11
Brand type
15 min
5 min
0.0
0.5
1.0
1.5
2.0
Log_{10} EC_{50} (μL. L^{-1})
More toxic
Less toxic

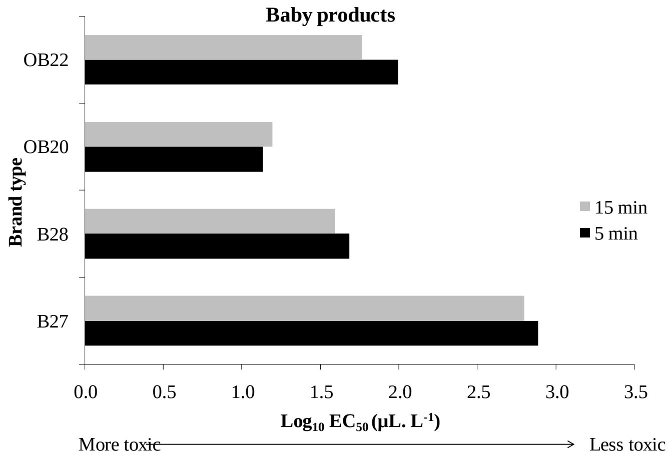

Bath cremes
OB8
B16
B15
Brand type
0.0
0.5
1.0
1.5
2.0
Log_{10} EC_{50} (μL. L^{-1})
More toxic
Less toxic


Wash up liquids
OB10
EB3
B19
B18
Brand type
15 min
5 min
0.0
0.5
1.0
1.5
2.0
Log_{10} EC_{50} (μL. L^{-1})
More toxic
Less toxic


Fabric conditioners
OB12
EB4
B21
B20
Brand type
15 min
5 min
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Log10 EC50 (μL. L-1)
More toxic
Less toxic


Washing powders
OB17
OB16
OB15
OB14
EB5
B24
B23
B22
Brand type
15 min
5 min
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Log_{10} EC_{50} (µL. L^{-1})
More toxic
Less toxic


All purpose cleaners
OB19
OB18
EB6
B26
B25
Brand type
15 min
5 min
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
Log_{10} EC_{50} (µL.L^{-1})
More toxic
Less toxic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Log_{10} EC_{50} (µL. L^{-1})
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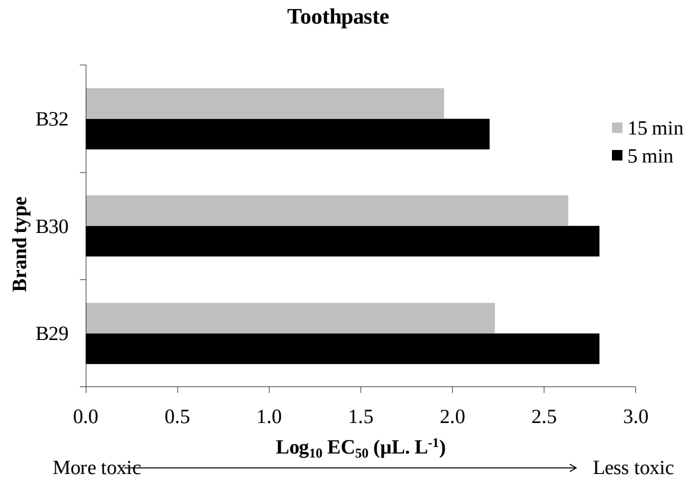
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Log_{10} EC_{50} (µL. L^{-1})
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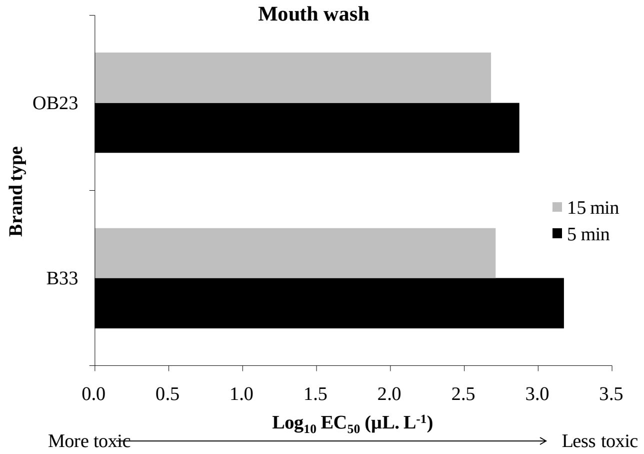
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Log10 EC50 (µL. L-1)
More toxic
Less toxic

CHAPTER 4: BENCH SCALE FILTRATION STUDIES

BENCH-SCALE FILTRATION STUDIES: CHOICE OF MEDIA SIZE AND DEPTH FOR CONSTRUCTED WETLANDS

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4.1 ABSTRACT

Media grain size is an important design aspect of constructed wetland systems. The combination of vertical flow design and the use of sand medium is a fairly recent innovation in sub-surface flow constructed wetlands. Sand however, has been widely used in conventional filtration systems. The treatment efficiency of sand filters depends on a number of parameters, such as media size used and dimensions of the filter (*i.e.* depth), which have been studied for slow-sand filtration applications. In most sand filters, back filtration is part of the operational process which helps to prolong the life span of the filter but this is not the case for constructed wetlands. A bench-scale study was conducted to look at sand specifications for grey water treatment in a constructed wetland set-up, focussing on treatment performance and clogging propensity. The study looked at different grain sizes and different media depths to observe the removal of suspended solids and COD. The study also investigated the potential of silver-coated sand for disinfection of the grey water. Grey water from student flats was used in columns (22.0 mm Ø) containing different sand media and for depths ranging from 5 to 20 cm. The results showed that the mid-range grain sizes, 1.0-4.0 mm were more suited to long term use, for media depths of 15 and 20 cm. The finest sizes (0.5-1.0 mm) clogged too soon and largest sand sizes (4.0-8.0 mm) did not achieve substantial removal of SS and COD. Coating the sands with silver also showed potential to improve disinfection of treated water as shown by the better removal of indicator organisms by the coated sands.

4.2 INTRODUCTION

The use of sand media in filter systems is well known for enhancing aerobic degradation of organics resulting in significant reduction of COD, total suspended solids and pathogenic microorganisms especially when the wastewater is also fed intermittently (Leverenz *et al.*, 2009). Wetland technology design variants include both horizontal flow sub-surface systems and vertical flow systems (VFs) with sand media (Vymazal *et al.*, 1998). Sand media is more porous compared to soil and this facilitates aerobic wastewater treatment through increased oxygen transfer into the bed between doses (Cooper, 2005). Filter clogging has been reported to be the biggest operational problem in constructed wetland (Platzer and Mauch, 1997; Blazejewski and Murat-Blazejewska, 1997) and is directly linked to media grain size. Despite grain size being important, treatment performance in vertical flow systems is associated more with operational factors such as loading rates, frequency of loading cycles, and the duration of resting periods between cycles (Kadlec and Wallace, 2009; Kadlec and Knight, 2000). The same factors that govern proper performance of a vertical flow system, also contribute to clogging propensity. Langagraber *et al.* (2003) attributes substrate clogging to reduced oxygen transfer capacity as a result of reduced free porosity due to accumulation of suspended solids, surplus sludge production and other minor factors. Implicitly, oxygen transfer rate is a combination of design and operation of the vertical flow wetland system. Platzer (1999) pointed out that in the design of VFs, the most critical factor is total oxygen input (oxygen added by water and that induced by air flow), which in terms of operation aspects of a single wetland bed relates to the design (substrate media size and depth), wastewater strength (dissolved oxygen concentration) and frequency of loading cycles.

The main pollutant removal mechanisms in wetlands are filtration (physical) and microbial degradation (biological). Physical mechanisms are dependent solely on design aspects while biological mechanisms depend of both physical and biological mechanisms. Platzer and Mauch (1997) have demonstrated that certain factors governing filtration can be utilised for the elimination of xenobiotics (*e.g.* pharmaceuticals and polychlorinated organic compounds) which cannot be removed by other means. The most critical of these factors is grain-size distribution, which determines the immobilization of surface area for biofilm growth and also influences root growth and hydraulic conductivity of the substrate media. Hence malfunctioning (*e.g.* clogging) of VF wetlands is a function of grain-size distribution (Stevenson, 1997). Alvarez *et al.* (2008) attributes substrate clogging to organic load and the suspended solid load. Organic loading is an indirect parameter which affects sludge production derived from bacterial growth while suspended solids are a more direct one. Little information is available for guidance on TSS loading rates. Available values are specific to wastewater type and are not generic. Despite the numerous claims in literature linking clogging occurrence to influent organic concentration and loading rates of TSS as the main contributing factors, data on TSS concentrations of flows entering CWs are scarce. Nonetheless, the higher clogging propensity in vertical flow wetlands is due to their more efficient TSS removal compared to horizontal flow (Kadlec and Wallace, 2009).

A review of the literature reveals that grey water is consistently contaminated with faecal material and can contain enteric pathogens of concern in significant numbers and indicates a particular risk of grey water reuse. Treatment of grey water using the wetland technology has not managed to reduce this level of risk to low enough levels particularly for reuse (Frazer-Williams *et al.*, 2008; Winward *et al.*, 2008). One possible alternative for disinfection of treated grey water is the use of metals such as silver (Ag) and copper (Cu). The disinfectant capacities of silver (Mahmood *et al.*, 1993), silver and copper mixture and peracetic acid (PAA) (Cassells *et al.*, 1995) have been studied. The application of Ag and Cu in ionic form, alone or in combination, has focused successfully towards the elimination of pathogenic microorganisms present in wastewater for reuse (Davies and Etris, 1997).

This paper presents a bench-scale study conducted to test the design considerations of bio-filters, especially at small-scale, focussing on grain size and depth of substrate (sand) media, in constructed wetlands for treatment of grey water. This study sought to establish the grain-size and depth that would provide effective filtration with the slowest diminishing effective pore volume due to solids accumulation within the pores. The findings of this study were incorporated in the setting up of pilot prototype cascade wetland studies in order to avoid problems with maintenance of prototype wetland. The study also looked at disinfection potential of silver coated sand grains.

4.3 MATERIALS AND METHODS

4.3.1 Experimental set-up

Bench-scale filtration columns were used to simulate pollutant removal from grey water by filters packed with sand of different grain sizes and at different depths (Figure 4.1) PVC columns (22.0 mm in diameter and 250 mm long) were set up and fed with grey water pumped up by a peristaltic pump (505Du, Watson-Marlow, UK), which also served to control the hydraulic loading rate. The grey water used in the study was real grey water collected from a block of student flats at Cranfield University campus in the UK.

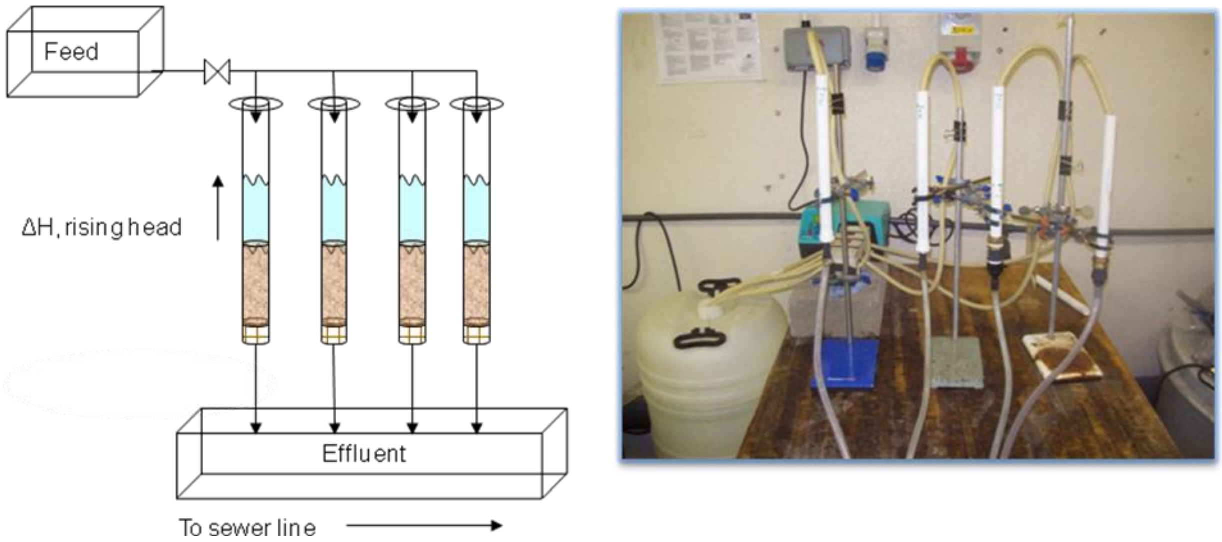

Figure: 4.1: Diagram of the experimental set-up

River sand bought from a garden shop (B&Q, Bedford, UK) was used in this study. The physico-chemical analysis of the sand was: pH 7.1, Organic matter 0.21%, Loss on ignition 16.8%. The sand was washed and dried, at 105^{o}C before use to improve its packing ability, and graded into different sizes using sieve analysis as described in British Standards (BS ISO 13765-5:2004). The four grain size classes were as follows:

1) 0.50 mm < d < 1.0 mm
2) 1.0 mm < d < 2.0 mm
3) 2.0 mm < d < 4.0 mm and
4) 4.0 mm < d < 8.0 mm, Where d is the diameter of the sand grains.

The effective grain sand size classifications were 0.5-1.0 mm, 1.0-2.0 mm, 2.0-4.0 mm and 4.0-8.0 mm sands (Figure 4.2). The grey water was fed intermittently as in VF wetlands. The feeding rate into each column was 105 ml for one minute every 5 minutes giving a feeding rate of 3.31 $m^3\ m^{-2}\ h^{-1}$. The influent tank was homogenised every 30 minutes by manual stirring. The study cycles were carried out for two days. To follow the filtrate performance, influent and effluent samples were collected from each column every 2 – 5 hours until significant head loss occurred. The samples were analysed for suspended solids, COD and pH. Performance of the silver coated sands for Total coliform and E coli removal was also carried out. The silver coated sands were prepared in the laboratory from the plain sand prepared as described above. Silver sand was coated by mixing graded sand with $AgNO_3$ (0.005 M). The ingredients were mixed thoroughly and allowed a maturing time of 1 hour. The mixture was then treated with NaOH (1.0 M) and mixed thoroughly. The sand was treated with 1:1 NH_4OH solution and 15 ml of reducing solution (9% sugar solution) respectively, mixed thoroughly and left for 1 hour between each addition. The treated sand was solar dried, washed with distilled water to pH 7 and finally dried to 100-110^oC.

Figure 4.2: Graded sand pictures

4.3.2 Experimental procedure

Grain size study

The four different grain sizes classes were randomly allocated to four columns and 10 cm deep filtration columns were prepared. During the monitoring period, the columns were fed with grey water at a loading rate of 3.31 m^3 m^{-2} h^{-1}. The feeding mode was intended to simulate intermittent feeding used in the prototype pilot studies (Chapter 5). The grey water was then let to filter through each column by gravity. The filtration runs continued until appreciable clogging (measured as head loss) occurred.

Depth study

The filtration experiments were repeated for all but the finest grain sizes, whereby different depths (5, 10, 15, and 20 cm) were used for each class of sand to assess the effect of depth of the filterability of grey water. Filtration runs were conducted for the same period as in the grain size study in order to ensure comparability of the two filtration studies.

Analytical procedures

Water samples were collected from each column for all column set-ups every 2-5 hours for analysis. Suspended solids measurement was by filtration method, turbidity (NTU), using a Turbidimeter Hach 2100N; pH using the Jenway 3540 pH and conductivity meter; and Merck cell tests (Merck, VWR International), Poole, UK) for chemical oxygen demand (COD). Total coliforms and *Escherichia coli* were enumerated by most probable number (MPN) method using IDEXX Colisure and Quanti-Tray (Maine, USA) according to standard methods (APHA, 1998).

4.4 RESULTS

4.4.1 Assessment of impact of grain size and media depth

The proportions of grain sizes in the original bag of sand used for this bench-scale study and the cascade wetland trials (Chapters 5 – 7) were 13 % (0.5-1.0 mm), 58 % (1.0-2.0 mm), 28 % (2.0-4.0 mm) and 1 % (4.0-8.0 mm) as determined by sieve analysis. The influent water quality of the grey water used in the bench scale study is summarised in Table 4.1. Filter lengths of 5 and 42 hours were observed for the size ranges of 0.5-1.0

mm and 1.0-2.0 mm sands respectively and > 65 hours for the higher grain sizes 2.0-4.0 mm and 4.0-8.0 mm. Extensive clogging was observed in the finer sizes (0.5-1.0 mm and 1.0-2.0 mm) at the end of the filter runs. Conversely, the finer sands produced better treatment which decreased with increase in grain size (Table 4.2, Figures 4.3 and 4.4). Percentage suspended solids removal were 69%, 58%, 55% and 50% for the different grain size range (1 to 4). This observation is in agreement with general filtration principles, whereby filtration efficiency is higher because of the smaller pore size which produces better solids entrapment. The removal rates in this study are lower than reported by Rolland *et al.* (2009) whose SS and COD removal rates for comparable HLR and media size range (0.5-2.0 mm and 1.0-4.0 mm) were > 90 % and > 85 % respectively. The main differentiating factor between the two studies is column height, whereby the shorter column (this study) resulted in lower removal efficiency. Interestingly, despite the column height in this study being one-fifth, they still exhibited higher removal, more than 50 % of rates reported by Rolland *et al.* (2009).

Table 4.1: Influent grey water characteristics

	BOD_5 ($mg.L^{-1}$)	**COD ($mg.L^{-1}$)**	**Turbidity (NTU)**	**Suspended solids ($mg.L^{-1}$)**	**Total coliform (log_{10} cfu 100 mL^{-1})**	***E* coli (log_{10} cfu 100 mL^{-1})**
Mean	156.4	327.0	17.6	25.4	6.2	4.0
Std dev	19.3	27.1	5.4	6.2	0.3	0.2

Table 4.2: Performance of different grain sizes at 10 cm media depth

Size range	**Grain size range**	**SS removal (%)**	**COD removal (%)**
1	0.5 < d < 1.0	68.9	42.9
2	1.0 < d < 2.0	57.6	33.3
3	2.0 < d < 4.0	54.5	26.3
4	4.0 < d < 8.0	50.2	19.9

The study also looked at variation of depth for each grain size (1.0-2.0 mm; 2.0-4.0 mm and 4.0-8.0 mm sands). The results of the depths studied, 5 cm, 10 cm, 15 cm and 20 cm showed that increase in media depth produced better filtration and COD reduction (Figures 4.4 and 4.5). This study did not include the finest gain size of 0.5-1.0 mm sand, because of the observed low volumetric throughput and high rate of diminishing pore volume in the constant depth studies which also resulted in short filter runs. For both suspended solids and COD, removal improved with increasing depth. Interestingly for suspended solids, the removal by the 1.0-2.0 mm sand class at 5 cm was the same for the 2.0-4.0 mm and 4.0-8.0 mm grains at depths of 15 and 20 cm respectively. Hence for any given depth the finer grain sizes showed better performance with the 1.0-2.0 and 2.0-4.0 mm grain sizes showing significantly better performance than the 4.0-8.0 mm sand at 20 cm depth. Removal performances were expected to be distinctly different for the sand classes, but the only real difference was between the finest class (0.5-1.0 mm) and the rest. The sand classes ranging from 1.0 to 8.0 mm are commonly used in filtration system and VFs but for longer depths (> 40 cm) where they show appreciable removals (approximately 90 %) (Rolland *et al.*, 2009; Kadlec and Wallace, 2009).

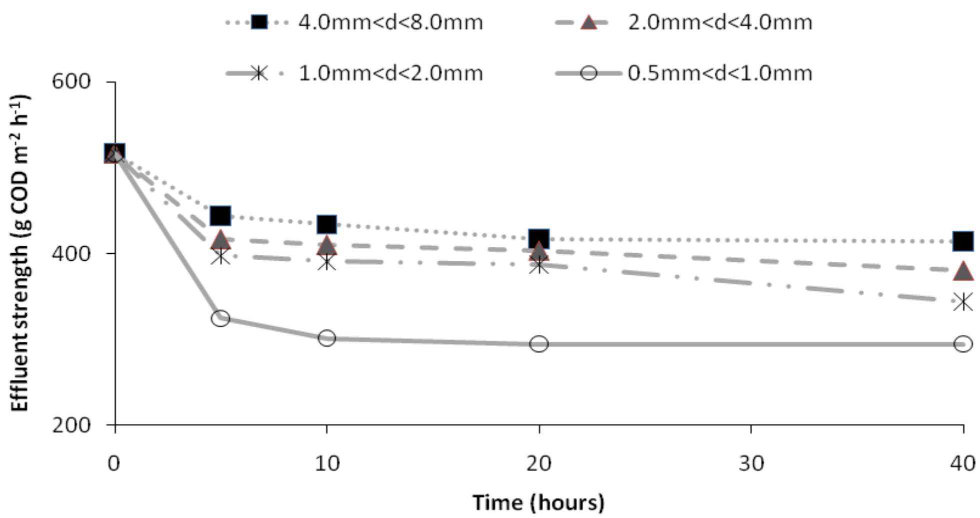

Figure 4.3: Measured effluent COD strength until clogging time

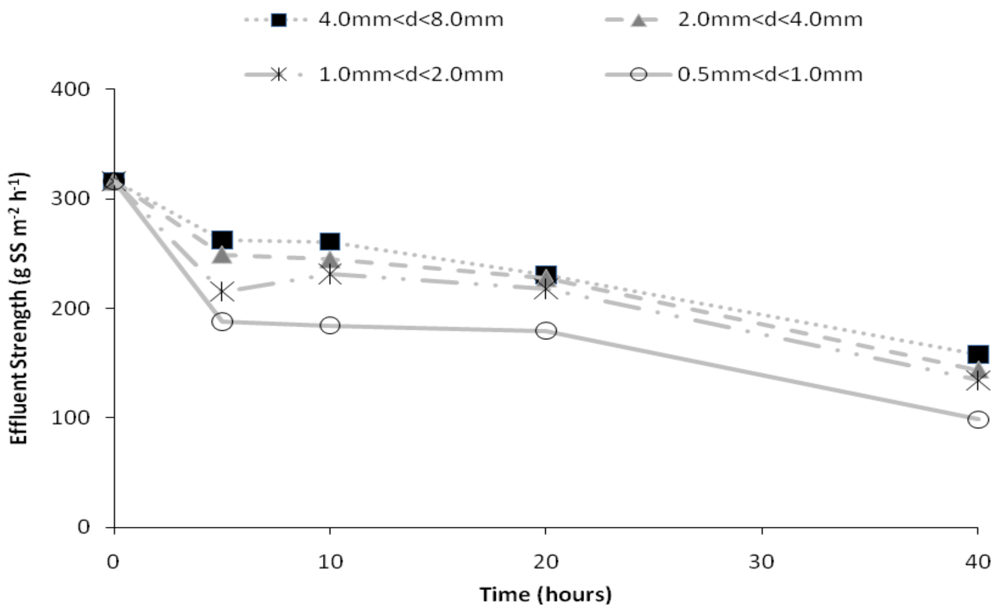

Figure 4.4: Measured effluent suspended solids strength until clogging time

Treatment for suspended solids and COD showed that sand medium depths below 15 cm have no influence except when coarse sand is used. Nevertheless treatment was observed to improve with increase in depth for all grain sizes (Figure 4.5 and 4.6). However, the 4.0-8.0 mm sand did not exhibit appreciable improvement in suspended solids removal for the 10 cm, 15 cm and 20 cm depths. While both the 1.0-2.0 mm and 2.0-4.0 mm had similar performances at depth of 20 cm, the improving trends in the 2.0-4.0 mm sand coupled with the large volumetric throughput before clogging signs started to become apparent. Despite claims that depth is not a crucial factor in filtration due to poor removal for the deeper columns, the combination of depth and grain size is shown to be critical, particularly when considering volumetric rate constant (Stevenson, 1997). This was observed in this study, for suspended solids removal particularly for the 15 and 20 cm depths where grain size was more important.

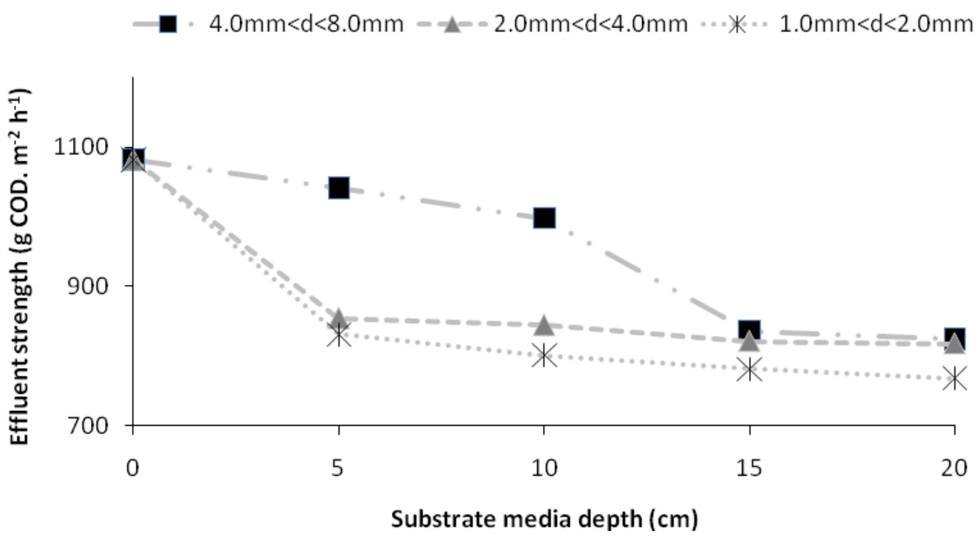

Figure 4.5: Removal of COD by media of different grain sizes at different depths

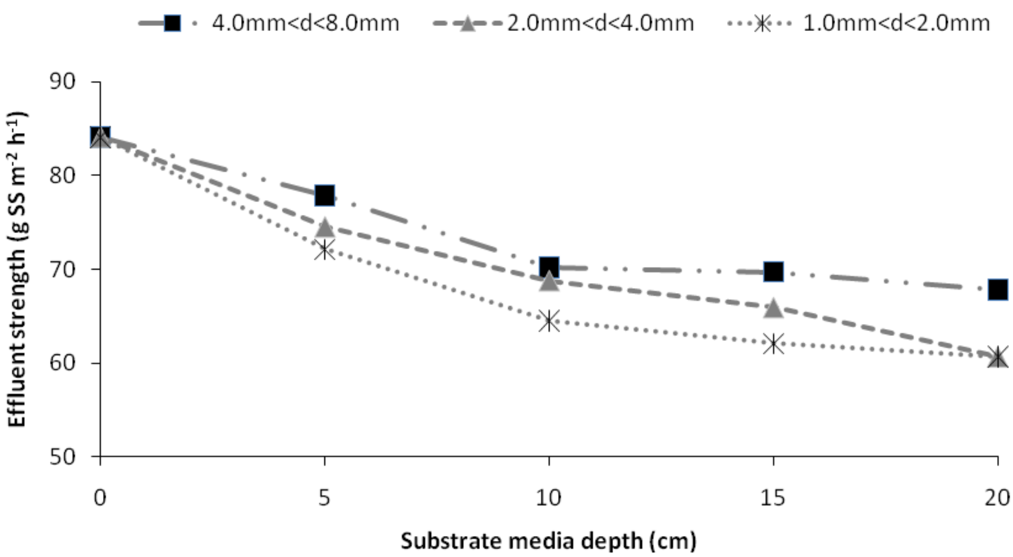

Figure 4.6: Removal of SS by media of different grain sizes at different depths

4.4.2 Assessment of grain coating on indicator organisms removal

Removal of indicator pathogens showed slightly better results for the silver coated sands than plain sands (Table 4.3). But even then, only a maximum of 1 log concentration removal was observed. Higher log-concentration removals were obtained (approx 1 log) than for the plain sands (< 0.4 log removals). These removals were better, given that the contact times were 2-5 min, than reported in literature *e.g.* Mahmood *et al.* (1993) who used 60 min contact times for mixtures of coated and plain sands in columns approximately 22 cm long. The effects of continued filtration using the silver coated sands were not studied because the silver coated filtration runs were only carried out for a short period. In this study all the media in the columns was silver coated however, Mahmood *et al.* (1993) observed that different mixtures, treated (silver coated) and plain (untreated) sands, performed differently, with a 1:1 mixture (*i.e.* 50% of the sand being coated) achieving greater pathogen removal. Grey water has the added property of media surface modification by cationic surfactants from the household washing products, particularly, laundry water due to its high levels of cationic surfactants in fabric softeners (Chapter 3). The surface modification of sand enhances the adsorptive capacity for organic solutes (Ding *et al.*, 2010), resulting in positive effects of removal of indicator pathogenic organisms. Cationic surfactants are known for their ability to enhance adsorbent's capacity for organic solutes (Ding *et al.*, 2010), which could possibly be a factor in the performance of the silver coated media. However, this was a preliminary bench study, which did not assess the effects of various other variables such as exposure time, silver concentration, pH, temperature, and other chemical constituents of grey water.

Table 4.3: Residuals of indicator pathogenic organisms (n = 3)

Grain size	**Total coliform**		**E coli**	
	Plain sand	Silver coated sand	Plain sand	Silver coated sand
1.0–2.0 mm	5.7 ± 0.2	5.0 ± 0.3	3.6 ± 0.2	2.0 ± 0.2
2.0-4.0 mm	6.0 ± 0.2	5.2 ± 0.2	3.7 ± 0.1	3.0 ± 0.1

According to Winward *et al.* (2008) and Lazarova *et al.* (1999) the quality of the water has an impact of the effectiveness of the disinfectants being used, with the presence of suspended solids, dissolved organic matter and pH as the main parameters to consider. The suspended particles act as protection for the microorganisms from disinfectants. Nevertheless, this study has shown that use of silver coated media as a disinfectant has the potential advantages and could be suitable to the treatment process on the back end of a wetland system to disinfect the treated grey water after removal of disinfection interferences such as suspended solids.

4.5 DISCUSSION

The study showed the 0.5-1.0 mm sands to be unsuitable for use in the cascade wetland on the basis on high rate of diminishing pore space, despite producing good removal for suspended solids and COD which is expected for fine sand media sizes in filtration systems (Moore *et al.,* 2001; Platzer and Mauch, 1997; Woodward and Ta, 1988). This though is not in line with literature that typical sand bed media in VF systems has to be relatively fine (d_{10} < 0.25 mm) (Kadlec and Wallace, 2009). The performances of the middle grain sizes (1.0-2.0 mm and 2.0-4.0 mm sands) were observed to be similar for longer filtration runs (more than 10 hours). Improvement of filtration with time, clearly results from diminishing pore size due to settling of solids in the pore spaces. Grain size is the principle filtering characteristic that affects the filtration operation. It affects both the clear-water head loss and the build up of head loss during the filter run.

The size range in bed media spans a scale that encompasses the three main filtration mechanisms documented in filtration textbooks (*e.g.* Metcalf and Eddy, 1993; Crites and Tchobanoglous, 1998) namely: i) inertial, ii) diffusion and iii) flow line interception. All mechanisms combine to remove incoming SS, however inertial and diffusion (random) deposition mechanisms predominate for fine media while flow line interception predominate for gravel media (d_{10} > 4 mm). Therefore media selection is an important aspect in bio-filters and wetland treatment beds which is based on the ability of the filter to produce the desired filtrate quality. Small grain sizes will obviously pack together and produce small void spaces. Hence, for larger granules porosity is higher which results in less resistance to flow for any given depth. This also lowers the bed pressure differential (head loss), but has the associated low removal of particles. Hence suspended solid and colloidal particle removal is low as well. Therefore, finer sand classes (approx. d_{10} = 0.25 mm) which are used in slow-sand filtration should also be used in multi-stage constructed wetland especially for stages that are solely meant for removal of pathogenic organisms because of their good performance shown in this study. Earlier stages with coarser sand grains but depths greater than 15 cm would still be suited for removal of physico-chemical parameter like COD, BOD and Suspended solids. This is so because this study showed that increasing media depth resulted in higher removal of SS and COD but the relationships were not mathematically related as would have been expected. Studies by Blazejewski and Blazejewska (1997) showed that finer grain sizes tend to clog shallowly (up to a few centimeters), while larger grain clog much deeper and further in the bed, which may explain the lack of a mathematical relationship. The effect of this is that for shallower beds finer grains exhibit better removal than larger grains. However, one drawback with the finer grains was the clogging problem which resulted in shorter filter runs. Cumulative experience with wetland indicates that deeper beds (> 40 cm) contain an upper zone, with plant roots and a lower zone without roots. The roots in the upper zone impede flow and also increase the pore size while at the same time also increasing available surface area for biofilm formation. Biosolids (from biofilms) are more effective in entrapping organic and inorganic solids (Kadlec and Wallace, 2009). Obviously, the combination of mechanisms occurring in the upper (root) and root zones govern overall performance of a wetland bed. However, where a shallower bed (< 40 cm) is to be used, grain size selection is crucial as shown by Rolland *et al.* (2009), and would depend on whether the bed will be planted or not. This is because, there would essentially only be one zone, and hence a balance between the prevailing filtration mechanisms and microbial (biosolids) contribution would be necessary.

Removal of indicator pathogenic organisms was better for the coated sands because of improvement of the overall adsorptive capacity of the coated sand media for organics. The combination of biosolids formation, fine grain sands and silver coating would therefore result in further improvement in removal of indicator organisms. Silver sands release silver ions into the water as it flows through the media which serve as a disinfectant (Mahmood *et al.*, 1993). However, the silver coating decreases steadily with time as this oxidation reaction is irreversible, which entails that the coated sand would require periodic replacement. The results in this study suggest that silver coated sand could be used as an effective disinfectant probably on the back end of a wetland bed. This finding also emphasises the observation that adsorptive filtration performance of natural organic matter (NOM) improves where modified (coated) media is used.

4.6 CONCLUSIONS

The bench-scale study has shown that clogging can be controlled by application of sufficiently porous bed materials large enough to produce appreciable filtration and small enough to achieve longer retention times of the wastewater. Overall depth was shown to have no influence for removal of suspended solids and COD except when coarse sand was used. The grain sizes of 2.0-4.0 mm, and depths of 15 and 20 cm, produced the desired combination of good filtration and reduced clogging propensity that augurs well with other considerations for the novel wetland design (Chapter 5, section 5.2). Else the design consideration for the prototype wetland system would have to incorporate a pre-treatment stage in cognisant of the fact that pre-treatment contributes to reduction of suspended solids. This study also demonstrated that silver treated sands remove coliform and faecal coliform more effectively than plain sands. Therefore coating wetland media with silver may be used as a disinfection medium in multi-stage wetland systems for grey water treatment. So the coarser grain size would be preferred for a planted treatment bed followed by an unplanted bed with probably a mixture of plain and coated fine sand grains.

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CHAPTER 5: CASCADING SHALLOW BED CONSTRUCTED SAND FILTER

COMPARISON OF GREY WATER TREATMENT PERFORMANCE BY A CASCADING SHALLOW BED SAND FILTER AND A CONSTRUCTED WETLAND

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5.1 ABSTRACT

A novel unplanted vertical flow subsurface constructed wetland prototype comprising three shallow beds (0.6 m length, 0.45 m width and 0.2 m depth) arranged in a cascading series and a standard single-pass Vertical Flow Planted Constructed Wetland (VFPCW, 6 m^2 and 0.7 m depth) were assessed for grey water treatment. Choice of media for the prototype beds was based on the bench-scale study findings. Particular focus of this study was to test the prototype cascade wetland and modify it so that the effluent meets consent for published wastewater reuse parameters and assess the prototype at different hydraulic (HLR) and organic (OLR) loading rates. Treatment performances for three hydraulic loading rates of 0.08, 0.11 and 0.17 m^3 m^{-2} d^{-1}, and organic loading strengths ranging from 13 to 29 g COD m^{-2} d^{-1}, were compared. Both technologies effectively removed more than 90 % turbidity and more than 96 % for organics with the prototype meeting the most stringent reuse standard of < 2 NTU and < 10 mg BOD /L consistently. The prototype performed better than the VFPCW except for surfactant removal (results shown in Chapter 6). Published reuse standards for all the tested loading rates were met and overall performance of the prototype was better compared to the standard single-pass vertical flow wetland (VFPCW). The influent flush rate and organic strength were important factors for both treatment performance and control of *schmutzdecke* formation. Treated effluent from the prototype had higher dissolved oxygen saturation (> 90 %) compared to the VFPCW (approx. 55 %). These observations confirm that, with appropriate media, shallow beds provide a more oxidised environment leading to higher BOD_5 and COD removals.

5.2 INTRODUCTION

Water recycling is moving from being just an environmental motivation to being both a financial and an environmental motivation. The increasing pressures on water resources that are affecting most parts of the world caused by inadequate water resources, scarce rainfall, rapid development of local economies, and climate change, highlight the importance of modification of traditional water generation activities. Internal grey water recycling, where water is retained within a local process loop (Jefferson *et al.*, 1999), has advantages in water-scarce areas and also where centralized disposal infrastructure coverage is poor (Otterpohl *et al.*, 2003). Raw water sources available for recycling include rain water, grey water and municipal wastewater (Dixon *et al.*, 1999). Technical innovations are required to simplify the treatment technologies so as to increase the attractiveness of grey water internal reuse at smaller scales. Different technologies such as membrane bioreactors (MBRs) or rotating biological contactors (RBCs), and biological aerated filters (BAFs) have been studied for grey water treatment and showed high removal efficiencies for most of the water quality parameters (Pidou *et al.*, 2007; Jefferson *et al.*, 2001; Surendran and Wheatley, 1998). However, these technologies are high-tech and therefore not economically suited for small-scale applications. Extensive treatment technologies for grey water treatment for reuse are now attracting a growing interest due to their applicability at small-scale and positive public perception.

Constructed wetlands (CW) are an extensive technology with the robustness (Vymazal, 2005) and treatment efficiency necessary to handle diverse types of wastewaters such as municipal wastewater (International Association on Water Quality, 2000; Cooper *et al.*, 1996), storm water (Lee *et al.*, 2006), industrial water (Kern and Idler, 1999) and grey water (Kern and Idler, 1999; Frazer-Williams *et al.*, 2008). CWs have shown high BOD and COD removals of up to 98%, pathogenic indicator reduction of up to 99% (Vymazal, 2009; Vymazal, 2005; Frazer-Williams *et al.*, 2008). Surfactant removal was shown to be generally good (Gross *et al.*, 2007a), albeit individual compounds behave differently depending on their isomeric forms and molecular weights (Kadlec and Wallace, 2009). The main pollutant removal processes in constructed wetlands are filtration and physical adsorption, (physisorption) (physical processes), ion exchange and adsorption (chemisorption) (physical processes) and microbial metabolism (biological processes). Additionally, plant uptake and phytovolatilization contributes to the transformation and attenuation of organic chemicals (Susarla *et al.*, 2002). The CW technology therefore is proven to be suitable for treating grey water, which usually has fewer pollutants than domestic wastewater, and its application at small scale would be more economical. Indeed there are a growing number of studies on the performance of different designs of constructed wetlands and different combinations of such designs in order to improve grey water treatment to meet published reuse standards (Table 5.1). Design parameters of subsurface flow wetlands (SSF) have also been extensively studied for different hydraulic and organic loading rates, and for nutrient removal (Vymazal, 2005). The focus of the studies in literature has been on influent and effluent characteristics. However, the effects of substrate media and vegetation have not been adequately studied (Maltais-Landry *et al.*, 2009; Brix, 1999) especially for grey water treatment.

Table 5.1: Standards for non-potable grey water uses and applications

Categories		**Treatment goals**	**Application**
Recreation impoundments, lakes	Unrestricted uses	BOD_5: ≤ 10 mg/L Detergent (anionic): ≤ 1 mg/L pH: 6 – 9 Faecal coliform: 500/100 mL	Lakes and ponds for swimming ornamental fountains; recreational impoundment
	Restricted uses	BOD_5: ≤ 30 mg/L Detergent (anionic): ≤ 1 mg/L Faecal coliform: ≤ 10000/100 mL	Recreation without body contact
Urban uses	Unrestricted uses	BOD_5 ≤ 10 mg/L Detergent (anionic): ≤ 1 mg/L Turbidity: ≤ 2 NTU pH: 6 – 9 Faecal coliform: 3/100 mL	Toilet flushing; laundry; air conditioning; process water; landscape irrigation; fire protection; construction; surface irrigation of food crops and vegetables (consumed uncooked); street washing
	Restricted uses	BOD_5: ≤ 30 mg/L Detergent (anionic): ≤ 1 mg/L TSS: 30 mg/L pH: 6 – 9 Faecal coliforms: ≤ 500/100 mL	Landscape irrigation where public access is infrequent and controlled, subsurface irrigation of non-food crops and food crops (consumed after processing)

(Adapted from Li *et al.*, 2009)

The study reported here investigated the performance of a full scale multiple–bed constructed wetland prototype with shallow beds (arranged in a cascading pattern) and compared its operation with a more typical vertical flow constructed wetland. Typically the depth for vertical flow SSF wetlands is 0.6 – 1.2 m, yet the main degradation of substances takes part in the upper 0.2 m of the reactor-beds regardless of the bed depth (Platzer, 1999; Felde and Kunst, 1997). Hence, shallower bed design (0.2 m deep) was used, informed by the understanding of vertical flow constructed wetlands for grey water treatment. In side-by-side studies, technology replication becomes important if the influent wastewater chemistry, flow rates and climatological factors such as, rainfall and temperature are different (Moore *et al.*, 1994). Therefore sources of variations were expected to result from differences due to vegetation (or lack thereof), system geometry, and hydraulic and organic loading rates. Variability caused by disturbance of the substrate media and vegetative communities, is reported in the next chapter (Chapter 6).

5.3 MATERIALS AND METHODS

5.3.1 Pilot plants

The tested pilot technologies were (i) a prototype small-scale rig (the prototype; WPL, UK) comprising three shallow beds (0.6 m length by 0.45 m width, 0.20 m depth) (Figure 5.1) and (ii) a single bed reactor (Vertical flow Planted Constructed Wetland: VFPCW, 6 m^2 surface area and 0.7 m deep) (Figure 5.2). The pipes were buried 150mm from base using 20 – 40mm∅ washed gravel, followed by ~700mm sand/compost/soil mix (ratios - 65/25/10). The wetlands were constructed using plastic troughs for the prototype and double skinned plastic container (3m x 2m x 1m) for the VFPCW. A header tank was incorporated for storage and to maintain adequate flow pressure into the top cascade bed thereby ensuring constant volume flow. All pipes were lagged in winter to prevent freezing. Solenoid controlled valves were placed between each unit (tank or bed) act as flow regulators, only opening for specific periods sequentially at programmed times, starting with the bottom valve going up. The prototype cascade wetland beds contained sand with grain sizes ranging 0.2 – 8.0 mm sand (where according to the sieve analysis, 60 % of the grains were in the range 1.0 – 4.0 mm) and thin gravel (8 – 10 mm) at the bottom in a 9:1 ratio. Each bed had a splash plate underneath the inlet pipe to help spread the influent grey water on the bed surface and avoid hydraulic short circuiting. The prototype was unplanted, consequently running as a constructed sand filter. The VFPCW was planted with *Phragmites australis* and contained a mixture of sand, soil and organic matter as media. Therefore the hypothesis for this study was that the shallow beds in prototype cascade wetland would achieve better treatment than the standard reactor.

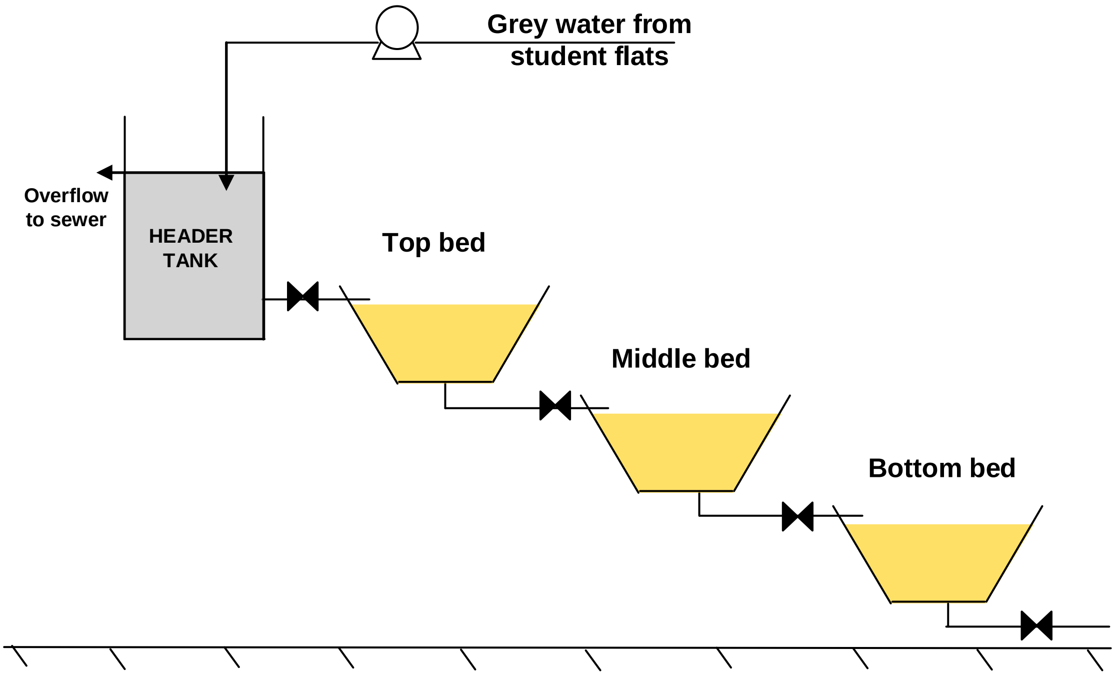

Figure 5.1: Schematic of the prototype constructed wetland - cascaded shallow bed vertical flow constructed wetland

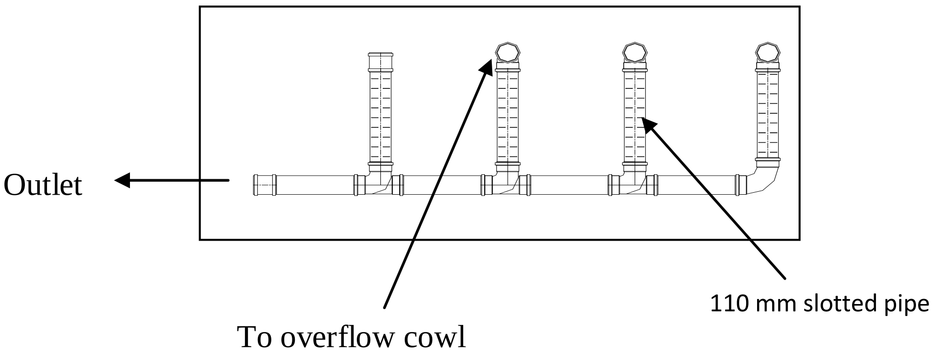

Figure 5.2: Schematic of the distribution and collection pipes for the Vertical Flow Planted Constructed Wetland

Influent grey water was collected from eighteen specially plumbed student flats at Fedden House, Cranfield University. Water from baths, showers and bathroom sinks was drained to an underground communal sump and then pumped to two inter-connected holding tanks. The grey water source was of low organic strength (BOD_5 30 ± 11 mg/L and BOD:COD ratio ranging from 0.29 – 0.45) which was thought to reflect dilution effect of high shower usage and degradation within the pipe works. The retention time in the pipes was second but pumping was intermittent which allowed for air inlet when pumping stopped and this contributed to the degradation in the pipes. To enable higher feed strengths to be tested a supplementary dosing system was used. The high strength supplementary solution was a 10% (v/v) mixture of a shampoo in tap water. The high strength supplementary solution and the real grey water were pumped, at a ratio of 1:55, into a second holding tank during monitoring periods 1 to 3 and a ratio of 2:55 during monitoring period 4 and 4:55 during monitoring period 5. The dosed grey water was then pumped to the pilot plants. Different hydraulic loading rates (HLR) for the cascade wetland indicated in Table 5.2, were ran over a study period of 11 months. The HLR for the VFPCW was not changed as it was used to benchmark the perfromance of the cascade wetland. Grey water feeding to the pilot plants was by flood-and-drain (pulse loading) such that the systems were analogous to intermittent dosing used in sand filters. The flood-and-drain cycles per day in the prototype were controlled by means of electric operated valves placed in the interconnecting pipes between the cascading beds. The studies were designed to test treatment variability arising from intra- and inter-system differences between the two wetland technologies. Typically the differences between the two would be expected to be more significant than differences within a each individual system due to design, loading rates, flow regimes, substrate media type and volume and vegetative differences. So in order to test these differences a side-by-side study approach was used. The two systems (cascade wetland and the VFPCW) were fed with the same grey water and, for each monitoring period, operation conditions were varied so as to monitor the effects of the differences that existed between the two. The outcomes were compared by looking at distribution of parameter values, such as BOD area-based rate constant, k_{BOD} and percentage removal of various pollution measures. The treatment units were monitored for 11 months.

Table 5.2 Mean values of hydraulic and organic loading rates during the study duration

Period	Weeks	Period	HLR (m^3 m^{-2} d^{-1})		Pollutant loading strength (g BOD_5. m^{-2} d^{-1})		Pollutant loading strength (g COD. m^{-2}. d^{-1})	
			*Cascade wetland	VFPCW	*Cascade wetland	VFPCW	*Cascade wetland	VFPCW
1	1 – 17	May – Jul	0.08	0.08	4.3±1.5	4.3±1.5	14.6±4.7	14.6±4.7
2	18 – 24	Aug – Sep	0.11	0.08	5.8±1.4	4.2±1.0	13.3±2.5	9.7±1.8
3	25 – 33	Oct – Nov	0.17	0.08	7.3±3.8	3.8±1.8	19.5±5.1	10.0±2.4
4	34 – 37	Nov – Dec	0.11	0.08	6.4±1.8	4.5±1.5	18.1±5.8	13.2±4.2
5	37 - 54	Jan – Mar	0.11	0.08	8.9±1.1	6.4±0.2	27.8±4.5	20.2±3.3

5.3.2 Sampling and analytical procedures

The treatment wetland were ran continuosly and influent and effluent samples were collected once a week between 08:00-10:00 am during Periods 1 and 2 and fortnightly for the rest of the monitoring period. Conventional water quality analyses were monitored as follows: Total organic carbon (TOC), using a total organic carbon analyser Shimadzu TOC-5000A (Shimadzu, UK); Biochemical Oxygen Demand (BOD), using the standard BOD_5 test; Turbidity (NTU), using a Turbidimeter Hach 2100N; pH and conductivity, using the Jenway 3540 pH and conductivity meter; and Merck cell tests ([Merck, VWR International], Poole, UK) for chemical oxygen demand (COD). Total coliforms and *Escherichia coli* were enumerated by most probable number (MPN) method using IDEXX Colisure and Quanti-Tray (Maine, USA) according to standard methods (APHA, 1998).

5.3.3 Determination of rate removal constant

The particle removal efficiency in filtration systems with constant media characteristics is governed by the following equation:

$$dC/dy = \lambda C \qquad \textit{Equation 5.1}$$

Where λ = the filter coefficient,
C = concentration and
dy = the filter area measured in the direction of the flow

At $t = 0$, $C = C_o$ and $\lambda = \lambda_o$

$$\text{Hence: } C = C_{o.} \exp(-\lambda_o L) \qquad \textit{Equation 5.2}$$

Removal kinetic parameters in constructed wetlands are generally described as first-order reactions (Kadlec and Knight, 1996), in which behaviour of water quality is explained by Equations 5.3 and 5.4.

$$C_e = C_{in.} \exp(-\kappa_T t) \quad \textit{Equation 5.3}$$

$$\ln(C_e / C_{in}) = -\kappa_T t \quad \textit{Equation 5.4}$$

Where C_e = effluent concentration,

C_{in} = influent concentration,

t = the HRT and

κ_T = the temperature dependent rate removal constant.

5.3.4 Statistical analysis

Statistica 9 (StatSoft) advanced was used to carry out comparisons. Significant differences between the prototype cascade wetland and the VFPCW were determined by analysis of variance (ANOVA). Comparisons were made for various inter-system factors (design and operation) as well as intra-system (seasonal and loading rates), to establish performance of the constructed sand filter.

5.4 RESULTS

5.4.1 Influent grey water

5.4.1.1 Biochemical quality

The detailed influent and effluent grey water quality for the two wetland systems in this study are presented in Table 5.3. Interestingly there was variation in pollutant parameters in the influent grey water, which is in line with site specific factors as well as differences in lifestyles of the generation community as observed in literature (Jefferson *et al.*, 2004; Eriksson *et al.*, 2002). To illustrate, influent BOD levels ranged from 42.6 ±22.2 to 80.1 ± 2.3 mg/L, turbidity levels from 14.4 ± 10.4 to 57.6 ±14.7 throughout the study period. This resulted in variations in influent loading rates in the VFPCW for instance, ranging from 3.0 to 6.6 g BOD_5 m^{-2} d^{-1}, which was maintained at a constant HLR throughout the study. The VFPCW, being a more established design (being in its 5th year of operation) was used as a benchmark to assess the performance of the novel design (the cascade wetland). Frazer-Williams *et al.* (2008) also noted that variability of grey water quality is more pronounced at smaller scales where the activities within one household have a proportionally greater impact on the grey water quality. In this study the grey water strength was determined by level of occupancy in the flats, during term-time and holiday time as well as seasonal effects and lifestyles. In summer, a lot of students were involved in sporting activities leading to more showering occurrences which resulted in higher but more dilute (Table 5.3) grey water volumes. This is perhaps a reflection of increased amount of time generally spent enjoying the shower and not necessarily washing. To illustrate, turbidity, BOD_5 and COD were generally lower in study Periods 2 and 3, although the loading rates (HLR) to the pilot wetland systems were higher during these periods than Period 1. Period 2 was during the summer months and Period 3 was at the end/beginning of an academic year. In between academic years, there is always change of occupancy in the student flats, the amount of grey water being generated changed a lot. This made it necessary to increase the dosing strength in order to maintain desired organic areal loading in the pilot wetlands but this probably resulted in higher variations in the influent levels as observed in the hight Sem values for COD (Table 5.3).

Table 5.3: Summary of influent characteristics and grey water residuals for the different technology designs (Prototype cascade wetland vs VFPCW). Removal percentages are given in parentheses. SEM is the standard error of the means

	Influent	Prototype unit	VFPCW	**Influent**	Prototype unit	VFPCW	**Influent**	Prototype unit	VFPCW	**Influent**	Prototype unit	VFPCW	**Influent**	Prototype unit	VFPCW
Period		**1**			**2**			**3**			**4**			**5**	
Turbidity (NTU)	**57.6**	1.0 (98.3)	10.4 (81.9)	**31.7**	1.7 (94.6)	10.4 (67.2)	**14.4**	2.7 (81.3)	10.9 (24.3)	**18.6**	1.7 (90.9)	4.0 (78.5)	**24.1**	2.4 (90.0)	4.3 (82.2)
SEM	**14.7**	0.4	7.2	**3.0**	0.6	1.9	**10.4**	0.9	2.8	**6.2**	0.3	1.6	**6.7**	1.1	0.3
Conductivity (mS)	**510**	662	710	**547**	702	711	**396.3**	421	417.8	**458**	608	475.5	**573.3**	875.3	572.8
SEM	**26**	61	70	**49**	21	42	**89.6**	52.7	22.9	**124.5**	41	67.2	**79.5**	76.7	94.4
pH	**7.3**	7.7	7	**7.17**	7.9	6.9	**7.3**	8.1	7.2	**7.1**	7.9	6.7	**7.6**	7.5	7.3
SEM	**0.4**	0.6	0.5	**0.1**	0.3	0.2	**0.4**	0.2	0.4	**0**	0.1	0	**0.2**	0.1	0.5
DO ($mg.L^{-1}$)	**0.9**	8.9 9	6.4	**0.9**	9.7	7.3	**0.9**	11.7	4.9	**0.5**	10.6	7.3	**1.0**	7.7	8.6
SEM	**0.6**	0.8	2.7	**0.5**	2.2	2.6	**0.4**	0.4	1.1	**0.1**	0.4	0.6	**0.4**	0.5	1
BOD ($mg.L^{-1}$)	**43.9**	0.2 (99.5)	1.7 (96.1)	**52.5**	0.1 (99.8)	1.5 (97.1)	**42.6**	0.15 (99.6)	1.2 (97.2)	**56.3**	0.2 (99.6)	0.8 (98.6)	**80.1**	0.9 (98.9)	1.1 (98.6)
SEM	**10.6**	0.4	1.5	**13**	0	1.4	**22.2**	0.05	1.4	**19.2**	0	0.2	**2.3**	0.1	0.1
COD ($mg.L^{-1}$)	**151**	7.9 (94.8)	10.1 (93.3)	**121**	2.5 (97.9)	3.0 (97.5)	**119.4**	21 (82.4)	12.9 (89.2)	**164.8**	3.8 (97.7)	2.4 (98.5)	**252.5**	32.1 (87.3)	6.2 (97.5)
SEM	**88**	12.4	7.3	**22.7**	1.4	1.4	**30.2**	18	5.2	**52.7**	1.3	2.3	**41.0**	8.3	1.1
TOC ($mg.L^{-1}$)	**10**	4.5 (55.0)	6.8 (26.5)	**ND**	**ND**	**ND**	**14.7**	8.5 (42.2)	7.5 (49.0)	**21.1**	4.4 (79.1)	3.4 (83.9)	**29.1**	11.3 (61.2)	7.9 (72.9)
SEM	**4.9**	3.4	3.6	**ND**	**ND**	**ND**	**8.8**	3.5	1.4	**14.2**	0.1	0.8	**7.3**	3.1	1.5

The BOD of the influent grey water (31.0 ± 10.7 mg/L), before spiking, was in the low end ranges of typical mixed grey water strength from similar sources (bath, hand basin and sink) which range from 5 – 252 mg/L (Pidou *et al.*, 2007; Al-Jayyousi, 2003) and domestic wastewater (Metcalf and Eddy Inc., 2003), typically around 30 mg/L and 200 mg/L respectively. The BOD:COD ratio of the influent grey water ranged from 0.20 – 0.29, which indicates that the influent raw grey water was less treatable by biological means and the need for acclimated microorganisms (Metcalf and Eddy Inc., 2003). This ratio was also lower than the range reported for grey water in literature (0.21 – 0.91) (Jefferson *et al.*, 2004) and domestic sewage (0.3 – 0.8) (Metcalf and Eddy, 2003). Worldwide loading rates range from 3 – 10 (with an average of 3.9 g BOD m^{-2} d^{-1}) (Cooper, 1999). One possible reason for this could have been due to appreciable degradation in the piping network from the flats to the underground sump and the receiving tank in the control container, just before dosing, as was alluded to by other studies that used the same grey water source (Pidou *et al.*, 2007; Winward *et al.*, 2008). Sedimentation in the header tank contributed to the treatment of grey water for the prototype system. To illustrate, 10 – 15 % decreases in BOD_5 and turbidity were observed in the header tank before the top bed of the prototype wetland system. The residence time of the water in the header tank was less than 24 hours, so it was assumed that anaerobic reactions, which alter the grey water characteristics if storage is for 24 – 48 hours or longer (Casanova *et al.*, 2001; Dixon *et al.*, 2000) were not initiated. However, the BOD:COD of the grey water rose to 0.30 - 0.46 after spiking, demonstrating that the spiked/dosed grey water had better biodegradability. The variability of the spiked grey water strength also enabled robustness of the wetland plants to be studied and this is indeed a reflection of the scenario of any typical household where such variations would be expected.

5.4.1.2 Physical quality

The main parameter used to measure the physical pollution in the grey water in this study was turbidity. The influent grey water turbidity was highly variable throughout the monitoring period ranging from 4 to 82 NTU. These levels are comparable to the ranges for bath (46-60 NTU), shower (21-375 NTU), and mixed grey water (33 – 240) (Jefferson *et al.*, 2004; Metcalf and Eddy Inc., 2003; Birks and Hills, 2007). Interestingly this turbidity range is outside the range reported for hand washing grey water alone (102-164 NTU), which perhaps suggests that shower grey water comprised the largest fraction of grey water in this study. Both the highest and lowest turbidity levels were measured in the summer period towards the end of academic years when the occupants of Fedden flats were very peripatetic. This wide range of turbidity levels showed that generally peoples washing habits and/or water usage in summer change considerably which impact on the physical qualities of the grey water. This may in turn have significance on the treatment efficiency of the constrcted wetlands.

5.4.1.3 Microbial quality

Microbial analysis only focussed on indicator organisms and no attempt was made to quantify specific indicator microorganisms. Total coliform and *E coli* concentrations in the influent grey water were 6.1 ± 0.5 and 3.6 ± 0.6 $\log_{10}$ respectively. Overall the concentration of these indicator microbial organisms were at the higher end of grey water from similar sources (bath, shower and sink), which range from 1 to 8 $\log_{10}$ cfu 100 ml^{-1} (Eriksson *et al.*, 2003; Nolde, 2000). There was no correlation between the total coliform concentrations and environmental conditions but for *E* coli there was a decrease of approximately 1 $\log_{10}$ between the samples collected in summer and the winter samples. Hence levels were high in summer and lower in winter, which may have been a result of either user behaviour and/or abiotic conditions.

5.4.2 Treatment performance

5.4.2.1 Chemical/Physical performance

Both wetland technologies provided BOD removal of 96 % or greater throughout the monitoring period with final residue effluent concentrations of 2 mg/L or lower (Figure 5.3) which exhibits very good treatment against the reuse standard of 10 mg/L (US EPA, 2004). Dissolved oxygen (DO) levels in the prototype effluent were greater than 90% and in some cases were even over 100 % (Figure 5.4) implying occurrence of supersaturation. Performance of the prototype was better than the VFPCW throughout the study as shown by the lower residual levels (Table 5.3). Residual effluent of the VFPCW was comparable to effluent from the middle bed of the prototype, hence effective depth of the prototype required to produce the same effluent quality as the 60 cm deep VFPCW was approximately 40 cm. COD removal was also high (> 82 %) for both technologies, but both technologies showed more variations in treatment efficiency of COD (ranging 82 – 98 %) compared to BOD. The lowest removals (82.5 % and 89.2 % for the cascade wetland and the VFPCW respectively) were observed during the period of highest HLR, which shows that the best performance is obtained when the influent grey water is of low strength. Despite this, loading rates (hydraulic and organic) were not significant factors when considering performance of the individual beds in the unplanted prototype, as much as was the position of the beds in the prototype (Figure 5.5 and 5.6). The hydraulic and organic loading rates in this study far exceeded the recommended range for constructed wetlands for avoidance of clogging (Platzer, 1999). However, the very low BOD:COD ratios (< 0.1) of the treated effluent clearly show very good BOD removal by both wetland systems (Metcalf and Eddy Inc., 2003). For turbidity removal, the two technologies produced more varied removals (81.3 – 98.3 % for the prototype and 24.3 – 81.9 % for the VFPCW). Again the lowest removal percentages for the prototype were observed during the highest HLR (Period 3, Table 5.3) and conversely the highest removals correspond to the lowest HLR. Both wetland technologies exhibited lower removals for chemical parameters with the VFPCW generally achieving much higher (but not significantly different) removals. Removal of TOC was generally lower compared to other parameters, 42 – 61% in the prototype and 26 – 84 % in the VFPCW. Effluent pH of both pilot plants was found to circum-neutral showing buffering capacity of the media in both technologies.

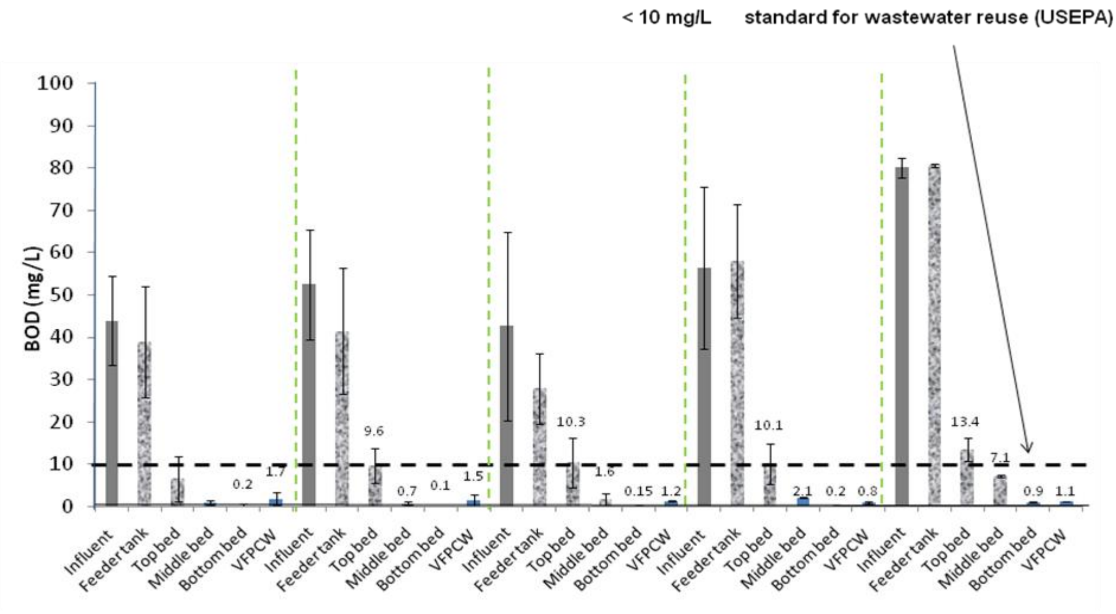

Figure 5.3: BOD removal residuals throughout the monitoring period

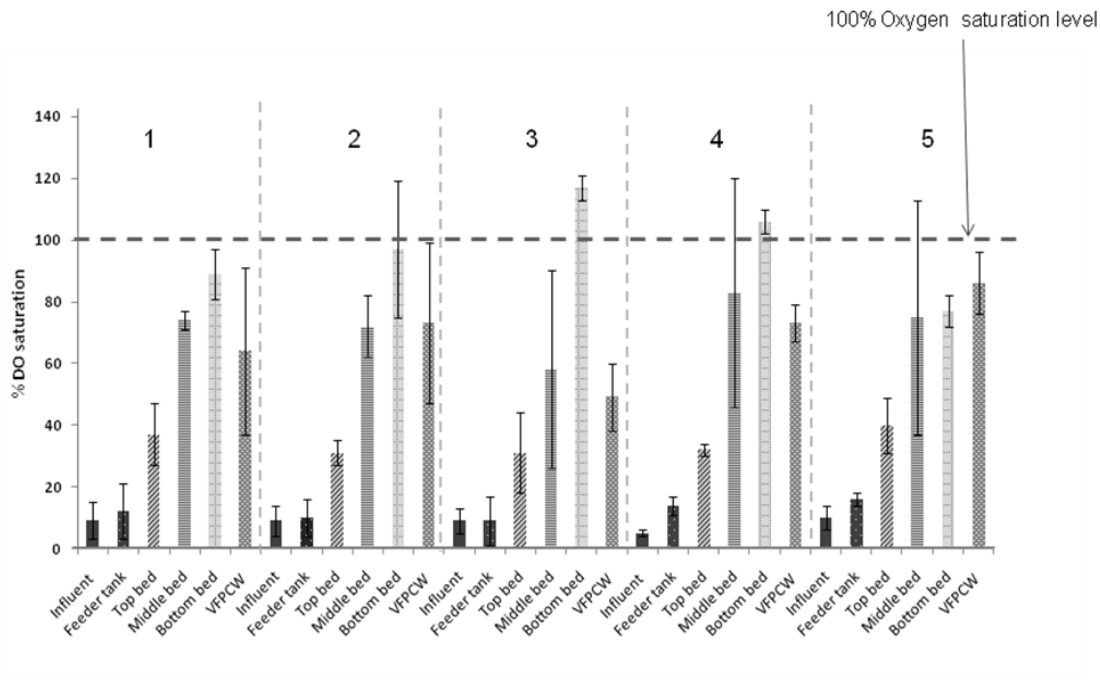

Figure 5.4: Oxygen saturation levels throughout the monitoring period

Increased conductivity and pH of the effluent probably indicates release of alkali metals from surfactant salts resulting in slightly basic effluent solutions. Surfactant removal was high (> 90% for both systems) (details in Chapter 6) suggesting the breaking down of long molecular weight (MW) surfactant molecules into low molecular weight organic molecules. These low MW molecules may not have appreciably degraded, as shown by the high TOC residuals in the effluent water. This possibly suggests that complete molecular decomposition of the organic fractions was not achieved. aAlthough higher removal were observed for BOD, the same was not true for COD (Figure 5.5 and 5.6), which suggests that the available oxygen in the treatment beds may not have been sufficient for breakdown of complex organic molecules (*e.g.* surfactants).

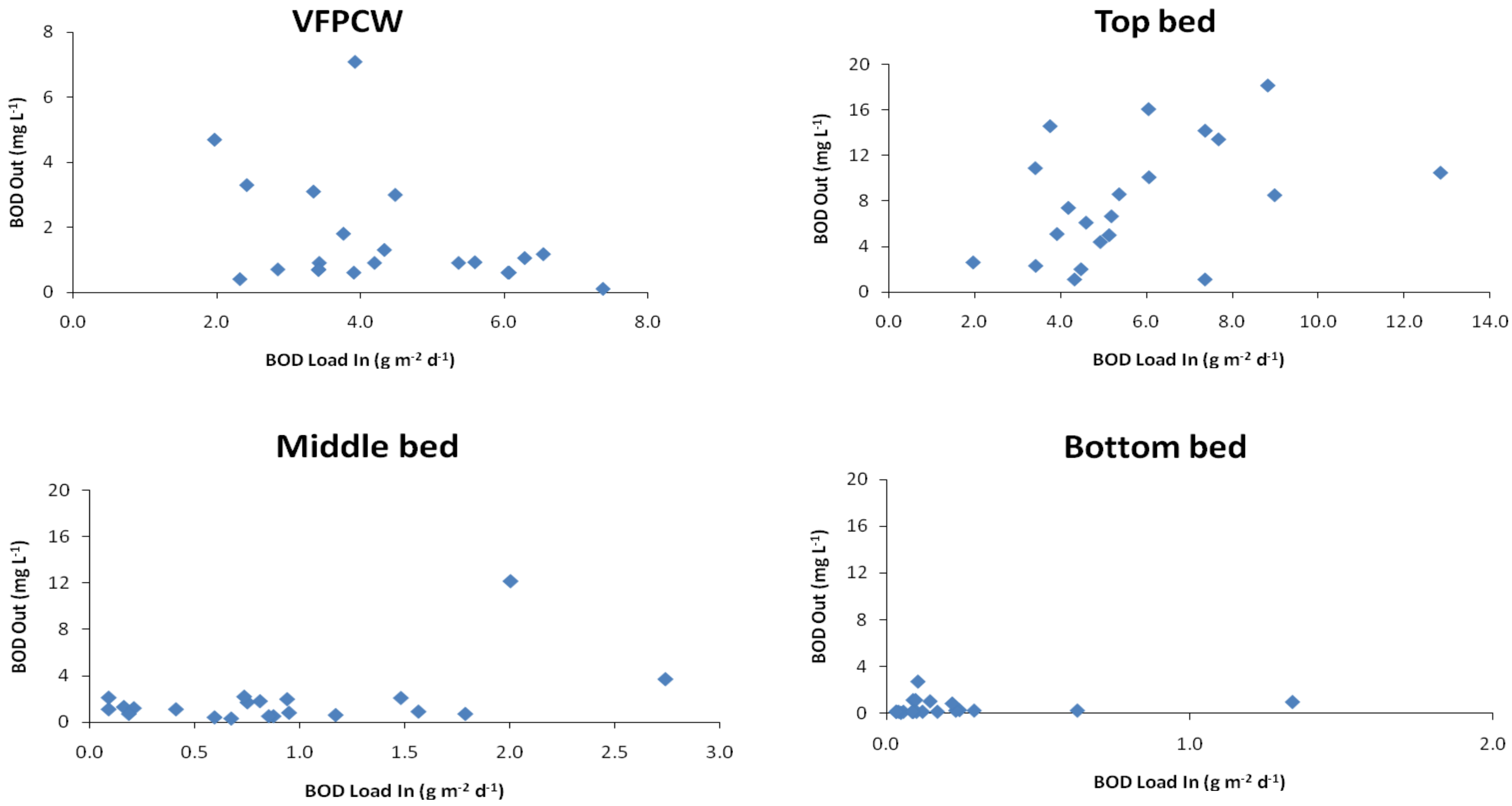

Figure 5.5: Effect of BOD influent organic loading on treatment performance

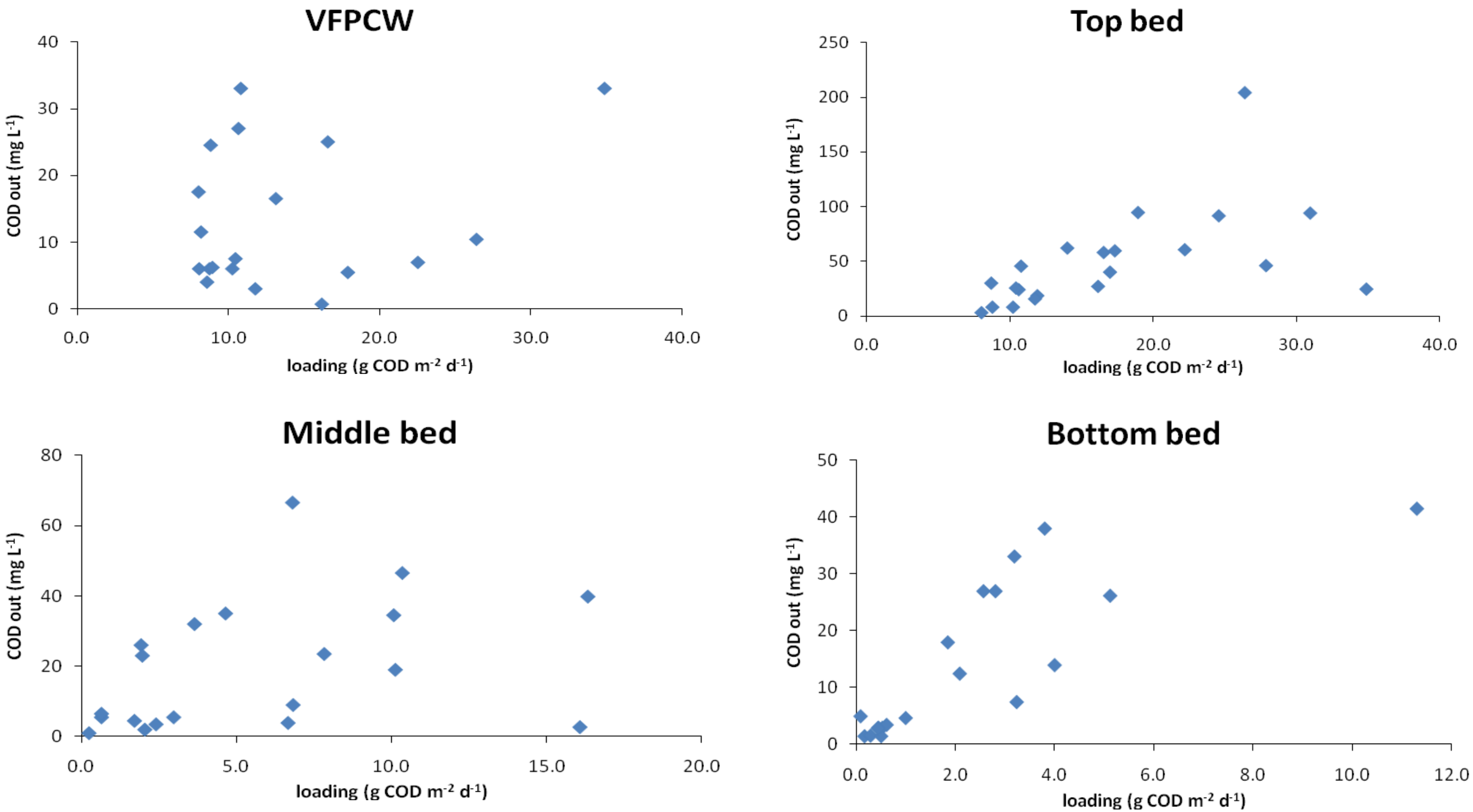

Figure 5.6: Effect of COD influent loading on treatment performance

5.4.2.2 Microbiological treatment

The prototype produced the better removals for both total coliform and *E coli* than the VFPCW. To illustrate, Total coliform removal in the prototype was 3.1 $\log_{10}$ concentrations compared to a 2.3 $\log_{10}$ concentrations removal by the VFPCW. The same was the case for *E coli* removal, whereby the prototype gave a 2.6 $\log_{10}$ removal compared to a 1.7 $\log_{10}$ removal by the VFPCW (Figure 5.7). Each of the beds in the prototype produced on average 0.7 to 1.4 $\log_{10}$ removals for total coliform and 0.6 to 1.1 $\log_{10}$ removals for *E coli*, with the middle bed producing the highest removals for both indicator organisms. Interestingly, the highest removal of physico-chemical parameter was observed in the top bed while for biological pollutants, the highest removal occurred in the middle bed (Figure 5.7). This confirms the observation that chemical components hinder the disinfection processes (Winward *et al.*, 2008). Again the microbial quality of the effluent from the middle bed of the prototype was not significantly different from the effluent quality of the VFPCW. These removal levels are comparable to removal observed in standard single pass vertical flow CW (Frazer-Williams *et al.*, 2008) and an extensive system comprising 2 reed beds and a pond (Dallas and Ho, 2005) of 3.0 – 3.8 $\log_{10}$ removals of Total coliforms for instance.

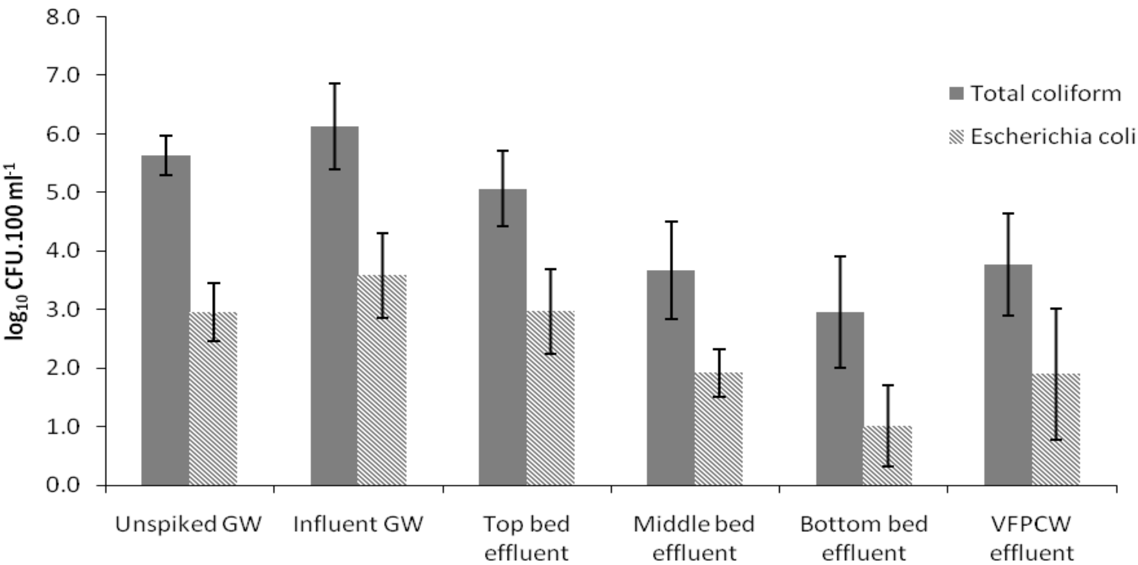

Figure 5.7: Indicator bacteria in raw (unspiked), influent and treated effluent from the wetland beds from April 2008 to March 2009. Data are mean values with standard deviations, n = 35.

The influent levels were independent of loading strength (for the prototype) as shown in Figures 5.8, and both wetland technologies did not meet the USEPA reuse standard of 2.0 $\log_{10}$ CFU 100 mL^{-1} for total coliform and 1.0 log g_{10} CFU 100 mL^{-1} for *E* coli and the effluent quality did not meet the less stringent Australian reuse standard of 4.0 $\log_{10}$, for the higher organic loading strengths (study Period 4 and 5). The two wetland technologies showed significant variations in their removals of the indicator bacteria over the study period. Total coliform removal in both wetlands did not show any obvious seasonal variation as was also observed by Winward *et al.* (2008), but there was a general increase in removal percentages of Total coliform by the VFPCW in the warmer months (Period 3). The cascade wetland showed correlation between influent strength and removal percentage, with better total coliform removals being obtained for the periods of low organic strength influent. Seasonality may have also contributed to the performance because as shown in Chapter 6, microbial composition and formation of *Schmutsdecke* was higher in warmer conditions. Removals of *E coli* also showed similar trends as for total coliform and consent for waste water reuse of 1.0 $\log_{10}$ (USEPA, 2004) was only met at low influent organic strength in the prototype (Figure 5.8).

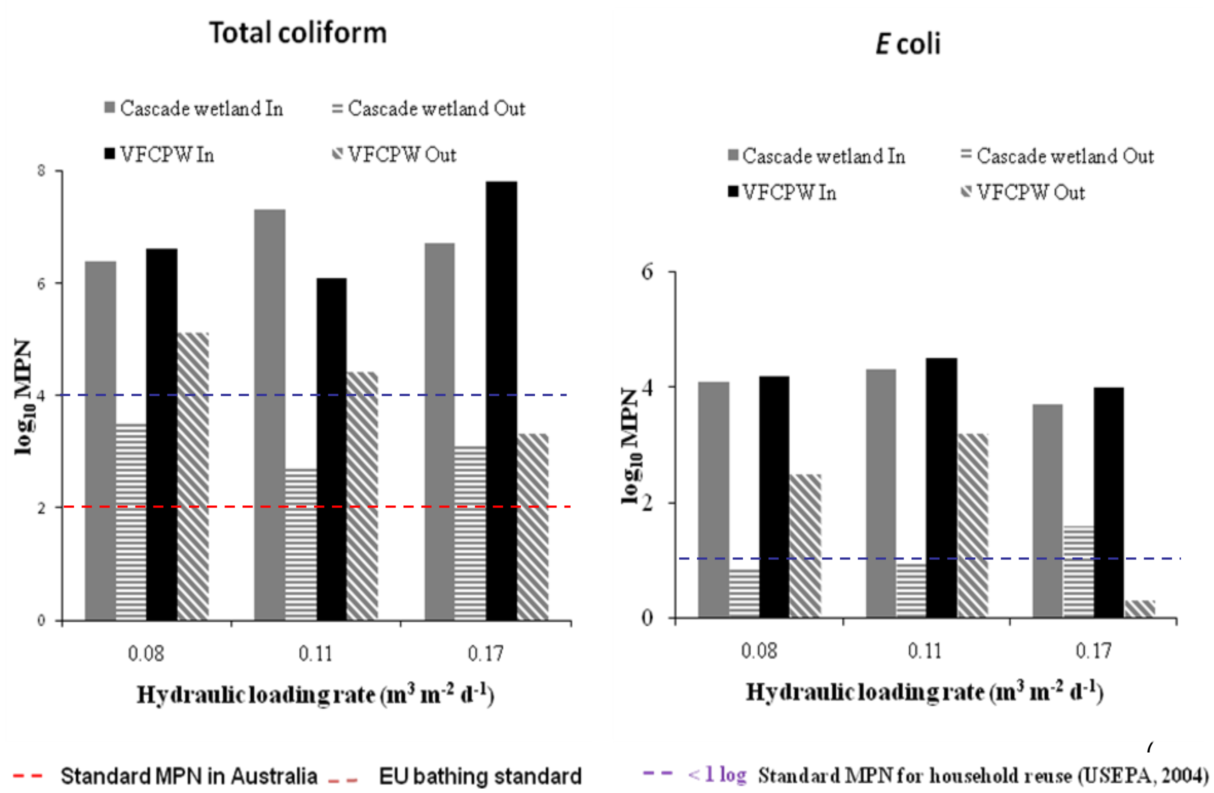

Figure 5.8: Pathogenic indicator removal performances by study pilot wetland technologies for total coliform and *E* coli. Note: HLR of the VFPCW was fixed at 0.08 m^3 m^{-2} d^{-1} throughout the study

As noted by other researchers, faecal contamination of grey water is common, which was also evident in the levels of indicator bacteria in the influent grey water in this study. There was a re-growth of bacteria in grey water during storage which was also stimulated by increase in organic strength between the undosed and wetland influent grey water. Total coliform and *E* coli increased by 0.5 and 0.6 log_{10} units respectively, with the addition of the synthetic supplement to create the grey water with a BOD:COD greater than 0.3. The influent grey water to the cascade wetland was fed via a header tank where it was stored on average for up to 1 day, and this resulted in increased in total coliform and *E* coli between 0.2 to 1.4 and 0.1 to 1.1 log_{10} units respectively. Similar changes in indicator bacteria levels resulting from storage were also observed by Winward *et al.* (2008), who reported 1 to 2 log_{10} unit increase in total coliform in grey water stored for up to 48 hours and Dixon *et al.* (1999) who reported increase of over 2.0 log_{10} units, for 24 hour grey water storage. In contrast, Ottoson and Stenström (2003) pointed out that re-growth does not occur in grey water because bacterial indicators, particularly of the coliform group, overestimate the faecal load of grey water substantially. They suggest that such a growth is based on the presence of easily degradable organic carbon and that therefore faecal enterococci, are the most appropriate to use because they do not overestimate the faecal load in grey water.

5.4.2.3 Microbial activity

Microbial growth was also reflected in the levels of microbial activities in each bed (Figure 5.9) whereby higher microbial activity was observed in the top few centimetres of each bed confirming the fact that higher attenuation was achieved in the top layer of the media bed. Hence pollutant parameters were removed or degraded more in the upper layers of the beds. The highest microbial activity, expectedly, was observed for the top bed. Indeed the top bed experienced reduced infiltration after every 3-4 months of operation, however, no appreciable clogging was observed until after operating for approximately one year. Removing the biologically active layer (*Schmutzdecke*) from the top bed and replacing it with fresh sand restored infiltration rates for the top bed. Low microbial activities of the inner layers (>10 cm depth) probably result from low oxygen infiltration and low organic matter in the deeper parts of the beds at this stage of the monitoring. Further work is required to model the exponential decay and also use model to determine the number of beds or combined media vertical depth to achieve stipulated effluent concentrations.

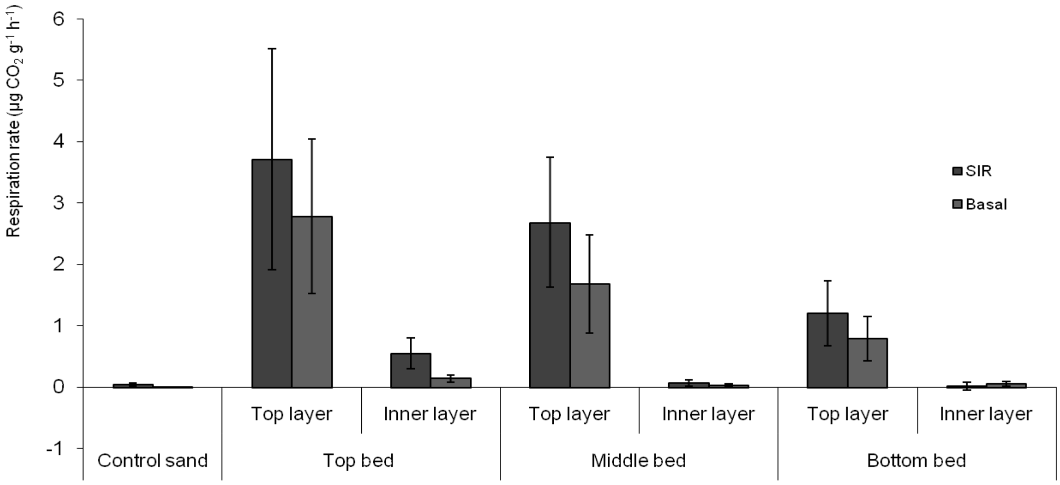

Figure 5.9: Microbial activities for surface and inner media samples from the three beds

5.5 DISCUSSION

5.5.1 Chemical/physical treatment performance

In order to understand the different sources of variability, the analysis of the performances took into account differences in designs which give rise to scatter performances for the two systems and comparison of these central tendencies (inter-system variability). As the two wetland technologies differed in configurations and presence/absence of vegetation. The cascade wetland was designed and operated as a constructed wetland and had granular media but was ran without vegetation essentially making it comparable to a slow sand filtration system. The consideration to have 20 cm bed depth in the cascade wetland was a result of the demonstration in literature that the majority (more than two-thirds) of microbial biomass in standard vertical flow wetlands (0.6 – 1.2 m deep) is located in the top 20 – 30 cm of the standard 0.6 – 1.2 m deep vertical flow bed (Akratos and Tsihrintzis, 2007;

Langergraber *et al.*, 2003). Sand filtration systems have been shown to be suitable for wastewater with turbidities below 50 NTU (Kadlec and Wallace, 2009). Despite, the influent grey water turbidity ranging from low turbidity levels to well over 50 NTU, the cascade wetland was able to treat the grey water to meet even the most stringent reuse standard of < 2 NTU. In most extensive systems, clogging is by far the biggest concern affecting performance. This is due to the reduced adsorption efficiency with age and decreased bed media porosity (Langergraber *et al.*, 2003; Blazejewski and Murat-Blazejewska, 1997) and increased microbial growth and sludge production against degradation of trapped organics. However, in this study clogging was not experienced, which, perhaps indicates that, firstly, biological degradation or indeed other complementary processes were responsible for treatment (Ottova *et al.*, 1997) and secondly, that clogging prevention cannot be solely attributed to presence of plants as has been alluded to by some researchers (*e.g.* Brix and Arias, 2005). The rate of microbial growth and sludge production did not affect the media porosity. The effluent water from the cascade wetland achieved more than 90% (approx 10 mg/L) dissolved oxygen (DO) saturation. DO concentration of 7 mg/L, such as the one achieved by the VFPCW, is usually indicative of good quality effluent. Hence the cascade wetland shows better treatment, and this without the contribution expected from plants. The DO level in the cascade wetland effluent was higher than 100 % (the highest levels of 11.7 mg/L at mean temperature of 17.6 ^{o}C were observed during Period 3) than total measured saturation, which is indicative of the occurrence of supersaturation. This could have been a result of increased oxygen production by phytoplankton in the shallow beds due to the availability of solar energy (Marks, 2008). This phenomenon usually occurs where nutrient levels are high. However, in this study the levels of nutrients were not measured systematically to confirm this. The lower DO concentration in the VFPCW effluent (compared to the cascade wetland) is thought to be a result of oxygen usage to deal with nitrogen removal. A one off measurement of nitrogen (as total nitrogen) showed residual concentrations in the two pilot systems of 3.30 ± 1.1 mg/L and 2.65 ± 0.35 mg/L for the cascade wetland and the VFPCW respectively against an influent concentration of 6.20 ± 0.57 mg/L.

Throughout the monitoring period, performance of the cascade wetland in removing BOD_5 was greater than 99%. Fluctuations observed for other tested chemical parameters were dependent on the HLR. To illustrate, COD removal performances were 94.8 % and 93.3 % at low loading and 82.4% and 89.2 % at higher loading for the cascade wetland and the VFPCW respectively. Nevertheless, both technologies were able to considerably reduce varying COD loading throughout the study period despite this being presented as one of the major difficulties in grey water treatment (Al-Jayyousi, 2003) due to recalcitrance nature of some chemicals. The high strength spiked grey water in this study was of decreased biodegradability because it essentially had higher COD (COD:TN:TP) levels. Data from individual beds indicate that most of the treatment occurred in the top bed, while the middle and bottom beds were effectively polishing steps. The increased removal of indicator microbial organisms in the middle and bottom beds shows the importance of these subsequent stages for disinfection purposes. The cascade wetland unit met the most stringent reuse standards (US Environmental Protection Agency, USEPA, 2004) for $turbidity_{out}$ < 2.0 NTU, BOD_{out} < 10 mg/L and the usual range for irrigation water (Ayres and Westcot, 1985) of pH_{out} 6 – 9. This performance is consistent with reported results for vertical flow constructed wetlands (Frazer-Williams *et al.*, 2008; Dallas *et al.*, 2004; Shrestha *et al.*, 2001; Gross *et al.*, 2007b). Hence the unplanted cascade wetland was as effective at removing pollutants as a fully-fledged constructed (planted) wetland (Vymazal, 2002) and other advanced technologies reviewed by Pidou *et al.* (2007).

The pH trend showed slight increase from acidic to mild alkali (pH: 6.9 – 7.6) with passage through the cascade wetland due to interactions between the substrate and biofilm (Kadlec and Knight, 1996), which is in line with the observation that treatment wetlands operate in circum-neutral pH conditions for influents that are not strong acid or bases due to the natural buffering the substrate media (International Association on Water Quality, 2000). The interaction with the substrate also results in salt formation from the media to water explaining the slight increase in the conductivity with passage through the unit. However, a slight decrease in conductivity was observed for the last (bottom) bed, which may be attributed to low biofilm formation due to low organic loading strength water reaching the bottom bed.

The VFPCW was designed based on the hydraulic loading rate of 0.08 m^3 m^{-2} d^{-1}. This is in line with standard design numbers for standard vertical flow wetlands (Cooper, 1999), which is equivalent to 2 $m^2.PE^{-1}$. The cascade wetland was not necessarily designed to any specific loading rate because the main considerations were size and flow design, but it was tested at different loading rates. The BOD_5 loading rates throughout the study period ranged from 3.8 to 7.4 g BOD m^{-2} d^{-1} for the VFPCW, and 2.0 to 12.9 g BOD m^{-2} d^{-1} (Figure 5.5) for the cascade wetland (whole system) and the COD loading rates ranged from 8.0 to 34.9 g COD m^{-2} d^{-1} for both systems (Figure 5.6). However influent strength to the bottom bed of the cascade wetland was below 1.5 g BOD m^{-2} d^{-1}, which equates to a tertiary treatment in municipal sewage works. The performance of the VFPCW shows dependence on influent loading for loading rates above 4 g BOD m^{-2} d^{-1}. This is a departure from expected trends in wetlands where better performance generally was obtained at all loading rates below 15 g BOD m^{-2} d^{-1} (Frazer-Williams *et al.*, 2008; Langergraber *et al.*, 2007). The VFPCW had been in operation for 5 years and the erratic performance for loading rates below 4 g BOD m^{-2} d^{-1} may be attributed to internal release of particulate and dissolved biomass from the wetland into treated water (background BOD) which may have been increasing with age. The performance of the cascade wetland beds, however, showed weak dependence on influent concentration in the top bed, but for the middle and bottom beds the dependence on influent concentration was strong and this was also true for COD removal performance. Influent concentrations to the middle and bottom beds were very low anyway because most of the treatment occurred in the top bed. The background concentrations in all the cascade wetland beds was very close to zero which may be linked to low biomass perhaps due to the type of media used and the absence of plants.

Organics play a major role in the biogeochemical process in wetland and influence the characteristics, chemical interactions and biological availability of trace elements and synthetic organic compounds. Natural wetlands assimilate and produce organic matter, but the nature of the organic matter is not well known. However, the biological, chemical and physical functions that take place are linked to the influent concentrations, environmental factors and hydraulic retention time (Barber *et al.*, 2001). Organics are known to reduce bacterial adsorption in porous media by competing for adsorption sites (Stevik *et al.*, 2004) and reducing the affinity of bacterial surfaces for adsorption. Having multiple shallow beds/reactors as opposed to one deeper bed, improves oxygen infiltration into the wetland system and oxygen penetration by convection is higher in multiple bed system. In turn removals of organics, SS, COD *etc.* are improved, and this is important particularly for high hydraulic loads because natural infiltration of oxygen in each bed in the multiple bed system is akin to artificial aeration of a large single bed. Hence, energy requirements needed for aeration are avoided and exchange of gases between the beds and the atmosphere (volatile organics and oxygen) is improved.

5.5.2 BOD_5 rate constant

The range of overall κ_{BOD} values for removal kinetics of the cascade wetland (1.60 – 3.38 m d^{-1}) was higher than for the VFPCW (0.96 – 1.75 m d^{-1}) (Table 5.4). Both these rate constant values were higher than the values (0.16 – 1.38 m d^{-1}) reported in other studies for vertical flow CWs treating grey water (Frazer-Williams *et al.*, 2008; Gross *et al.*, 2007b), implying better BOD_5 removal by the whole cascade wetland. The κ_{BOD} values for the cascade wetland were clearly much higher than the reported range for domestic wastewater (Frazer-Williams *et al.*, 2008). However, assessment of individual cascade wetland beds shows that the BOD rate constants were lower than for the VFPCW. As both technologies were operated at the same HLR in the first instance, the implication of this is that higher retention time in the VFPCW was key, as alluded to in literature (Kadlec and Knight, 2000), hence structural (*i.e.* wetland size) contributes to the higher removal rate constant.

Table 5.4: Comparison of BOD rate removal constants

Period	HLR	Temp (^{o}C)	Rate constant (κ_{BOD}) (m d^{-1})				
			Top bed	Middle bed	Bottom bed	Overall cascade wetland	VFPCW
1	0.08	19.2	0.57	0.59	0.45	1.60	0.96
2	0.11	17.3	0.69	1.07	0.79	2.55	1.05
3	0.17	17.6	0.89	1.17	1.31	3.38	1.06
4	0.11	11.4	0.71	0.64	0.96	2.31	1.73
5	0.11	14.5	0.73	0.26	0.84	1.83	1.75

For the cascade wetland, increasing HLR (Periods 1, 2 and 3) was achieved by decreasing retention time (according *Equation 2.3*) which resulted increase in κ_{BOD} (*Equation 2.2*) in the individual cascade wetland beds. However, increasing the organic loading strength (Periods 2, 4 and 5), only produced κ_{BOD} increase in the top bed and the overall performance of the cascade wetland was decreasing as shown by the decreasing κ_{BOD} of the whole cascade wetland system (Table 5.4). These observations suggest that HLR was not the limiting factor in the performance of the cascade wetland beds. However, organic strength was observed to be important especially in the top bed. Therefore, the increase in κ_{BOD} with increased HLR suggests that the resource quality (*i.e.* organic strength of the grey water) was still low and did not result in very high increase in microbial growth in the beds. This may also have been the reason for non occurrence of clogging in the beds. The overall BOD rate constant for the cascade wetland was still higher than literature values for standard CWs (Frazer-Williams *et al.*, 2008) throughout the study period. The BOD rate constant is temperature dependent, yet in this study, there was no obvious relationship between κ_{BOD} and temperature because κ_{BOD} increased despite an overall decrease in average temperatures. This suggests that temperature dependence was not as significant as was indicated by Steinmann *et al.* (2003). The implication of this is that removal of organics in small scale wetlands is not a result of temperature dependent factors only (*e.g.* microbial activity) but probably the wetland design and size as well. Microorganisms function even at temperatures as low as 5^{o}C and in winter porous media helps to keep the water temperature higher than air temperature by 2 – 3^{o}C, which allows the microorganisms to continue functioning (Akratos and Tsihrintzis, 2007). Therefore, it can be said that in small scale wetlands κ_{BOD} is influenced by organic pollutant concentration (Tanner *et al.*, 1998), and it decreases as the biodegradability of the feed is reduced. The high strength spiked grey water in this study was of decreased biodegradability because it essentially had higher COD (COD:TN:TP) levels from the supplement chemical (COD) rich shampoo product used to spike the grey water.

5.5.3 Influence of hydraulic and organic loading rates

5.5.3.1 Chemical quality of the treated effluent

Increases in hydraulic and organic loading rate throughout the study resulted in effective increase in BOD and COD loading from 4.3 to 8.9 g BOD_5 m^{-2} d^{-1} and 14.6 to 28 g COD m^{-2} d^{-1} for the cascade wetland. As such the cascade wetland was operated just within the recommended loading rates for wetlands. If loading rate for the first stage (top bed) alone is considered, the loading rates ranged from 12.4 to 26.7 g BOD_5 m^{-2} d^{-1} and 43.8 to 83.4 g COD m^{-2} d^{-1}. Recommended loading rates for vertical flow systems are 20 g BOD_5 m^{-2} d^{-1} and 25 - 27 g COD m^{-2} d^{-1} (Platzer, 1999; Langergraber *et al.*, 2007). Therefore the top bed which received the same grey water strength as the VFPCW operated at much higher loading rates than the limiting design loading rate. The VFPCW, on the other hand, was operated below the COD limiting loading rate throughout the study period. Both the cascade wetland and the VFPCW were operated as intermittent flow systems because of the added advantage of increased oxygen transfer capacity (Vymazal, 2005; IWA, 2000). Random small fluctuations in organic strength in the influent grey water did not result in significant changes in overall treatment performance. However, for BOD_5 removal, very high organic strength (BOD > 80 mg L^{-1}) resulted in reduced treatment efficiency. This is in agreement with other studies on this technology (Frazer-Williams *et al.*, 2008; Akratos and Tsihrintzis, 2007).

5.5.3.2 Microbial quality of the treated grey water

Performance of the prototype in removing *E* coli was lowest at the highest studied loading rate of 0.17 m^3 m^{-2} d^{-1}. This could be associated with reduced porosity in the media, due to increased biofilm growth in the media resulting for increased microbial activity. Removal of pathogens in sand or soil media is governed by retention (filtration and/or adsorption) and die-off (Reddy and D'Angelo, 1997). Indicator pathogen removal efficiency was lower (approximately 1-log_{10} units) in each of the cascade wetland beds compared to the VFPCW which showed average removals of 2.3-log concentration for total coliform. Higher removals for *E* coli were also observed for the VFPCW compared to the individual cascade wetland beds, which clearly shows that removal increases with substrate depth (*i.e.* higher HRT). Nonetheless, the combined depth of the cascade wetland beds still produced appreciably higher indicator pathogen removals. Increased retention times, such as are achieved in extended and recycling wetland technologies (Kadam *et al.*, 2008), achieve higher pathogenic removals. This means that effective substrate media depth also contributes to the removal efficiency, as does the grain media size where smaller grains (*e.g.* the soil substrate used in the VFPCW) result in higher removals of indicator pathogens (Tanik and Comakoglu, 1997). In this study reduced removal levels were also observed at higher organic loading strength, where the grey water was supplemented with an organic- and surfactant-rich shampoo. This is attributed to reduction in the affinity of bacterial surfaces for adsorption, because organics and surfactants are known to compete for adsorption sites in porous media (Stevik *et al.*, 2004). Therefore in order to achieve optimum removals for both organic pollutants (surfactants) and indicator pathogen in the cascade wetland, smaller size grains would have to be used for the last (bottom) bed where influent organic strength was much lower. These lower/subsequent beds should have lower microbial growth due to reduced levels of organic carbon and surfactants in the influent water implying lower competition for adsorption sites. Smaller grain size would improve the filtration efficiency (of bacteria), and provide more adsorption sites due to increased surface area.

Overall, the data shows that the cascade wetland, which had the same total effective media depth as the standard single reactor VFPCW, achieved better removals than the VFPCW under all conditions. This confirms that aerobic unsaturated removal pathways or higher oxygen transfer systems are more appropriate for grey water treatment. Despite high removal rates observed, both systems did not reach the reuse standards of “no presence of coliform bacteria/100 ml”. This confirms the need for disinfection if human contact is envisaged as was pointed out by Winward *et al.*, (2008). But in terms of irrigation reuse, the mode of irrigation becomes crucial (Ayers and Westcot, 1985). Despite all this, the actual long-term risks of treated grey water to humans would be expected to be lower because the levels in treated grey water in this study are well below the infectious doses ($>10^6$) (Metcalf and Eddy Inc., 2003). Indeed the degree of biological contamination depends on the source of the grey water, the household age structure, grey water storage time (Toze, 2006; Gross *et al.*, 2005) and irrigation management and reuse setup (Ayers and Westcot, 1985).

5.5.4 Operation aspects, management and evaluation of the design

The design considerations of the cascade wetland took into account the need for extensive and yet compact treatment systems that can be used in space constrained urban areas. The study has shown the viability of this novel small-scale vertical flow constructed wetland design, which has a smaller footprint and relatively low hydraulic residence time compared to standard VFCWs. The unplanted cascade wetland was effective at removing organics meeting the objectives of a fully-fledged constructed (planted) wetland (Vymazal, 2002). The cascade wetland design ensures that water can flow through the system under gravity, thereby reducing the need for pumping, which ensures low capital and operating costs. Ample space is available underneath the beds, for storage of treated water until needed. The shallow beds (0.2 m) ensure higher oxygen infiltration. Influent water lands onto a splash plate which aids the spreading of the water across the surface of the beds and increases mixing with oxygen and infiltration into the substrate media through convection. This contributes to maintaining aerobic conditions in the treatment beds and generally increasing oxygen transfer capacity which is crucial for VF wetland systems.

The cascade wetland performed very well for the hydraulic rates at which it was tested. It demonstrated robust performance in the face of occasional fluctuations in influent grey water strength, but overall performance decreased slightly at higher HLR. As observed from the removal rate constant values, increasing organic strength also had a significant impact on treatment performance (Tables 5.2 and 5.4). Therefore raw grey water storage may have to be considered for periods of higher grey water supply to avoid having to increase the HLR and also to dilute high strength influent water (Gross *et al.*, 2007a) if recirculation was feasible. The benefits of this would have to be weighed against the compromise on the size of the actual treatment system itself and probably increased energy requirements that could be necessitated due to recirculation pumping. The residence time of the water in the header tank was less than 24 hours, so it was assumed that anaerobic reactions, which alter the grey water characteristics if storage is for 24 – 48 hours or longer (Casanova *et al.*, 2001; Dixon *et al.*, 2000) were not initiated. Another significant drawback was significantly reduced performance during cold spells. Unlike the VFPCW, which was still operating, albeit, at low rate and reduced efficiency, the cascade wetland just stopped functioning completely at low temperatures (0 - 5 ^{o}C) resulting from ice formation which, perhaps was accelerated due to absence of vegetation shielding and insulation from varying temperature conditions. Similarly at higher temperatures where it was observed that formation of the biologically active layer, *Schmutzdecke*, was more frequent, which resulted in reduced daily flow, as evidenced by development of gradual ponding in the top bed. To remedy this, the volumetric flow rate was reduced, however, replacing the *Schmutzdecke* with clean sand layer was found to be a more lasting solution.

It is expected that uptake of pollutants by plants, activates the enzymatic machinery of the plants in order to degrade the pollutants (Arias *et al.*, 2001), which would speed up microbial degradation of pollutants. However, in this study, treatment performance of the unplanted shallow bed cascade wetland was better than the planted standard VFPCW. This therefore indicates that the crucial factors in removal of organic substances in grey water are related to oxygen transfer rates which are dependent of the design and operation of the treatment wetland (Weedon, 2003). This also indicates that non plant related elimination pathways; *e.g.* volatilisation, photochemical oxidation, sedimentation and sorption (Susarla *et al.*, 2002; Kadlec and Knight, 1996), are indeed significant. The study has shown that operation parameters such as HLR and flush frequency can be controlled to achieve acceptable performance and prolong life cycle of 'wetland' systems for grey water treatment.

The treatment wetland was inspected at least three times a week. More time input was spent on the top unplanted bed which required replacing of the *Schmutzdecke* layer. This was not the case for the middle and bottom beds which did not develop the biological active layer. Influent pipes and the header tank were flushed and cleaned once a week. The connecting pipe between the top and middle beds blocked more often than the rest of the system (on average, twice a month) and required to disconnected and flushed with clean water in order to dislodge any particles stuck in the solenoid controlled valves in between each unit (tank or bed) acting as flow regulators (Figure 5.1).

5.6 CONCLUSION

Performance of the shallow bed cascade wetland shows that sufficient treatment of grey water below the recommended loading rates for wetland plants was achieved. High oxygen infiltration was also evident and is attributed to the design configuration (cascading vertical flow beds) and the porous (sand) media. This study has shown that small-scale wetland technologies are applicable for grey water treatment and recycling. The following conclusions are drawn from this study:

1. Operational parameters such as hydraulic loading rate and number of flushes were crucial in the overall performance of both technologies. Good performance was exhibited by the cascade wetland meeting stringent world-wide reuse standards.
2. The small-scale constructed wetland with multiple cascaded shallow bed depth (≈ 0.2 m) design provide higher pollutant removal rates for conventional water quality parameters especially in the first stage and disinfection in the later stages after appreciable removal of interfering chemical components than a single-pass standard CW.
3. The cascade wetland is compact, easy to run and service and can be located in one's backyard or garden. Most importantly, it is a "green" grey water treatment unit.

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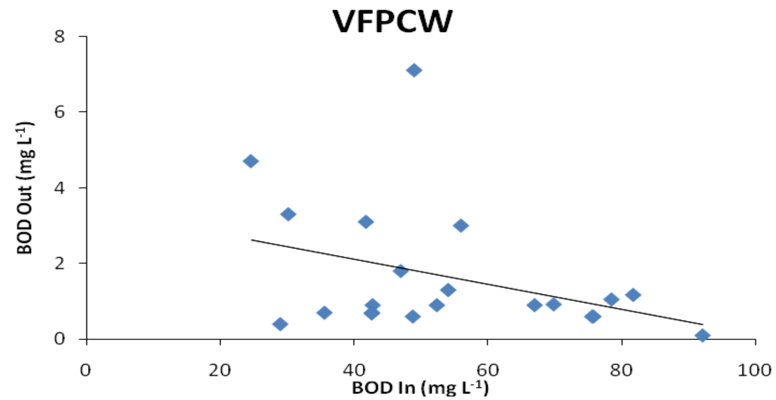
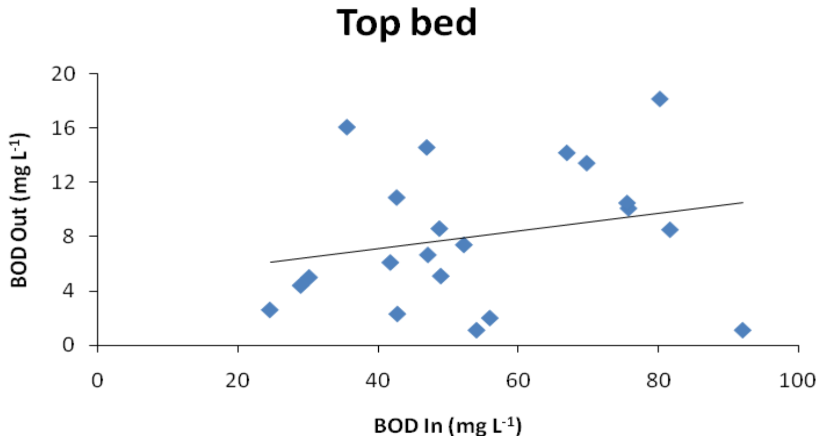
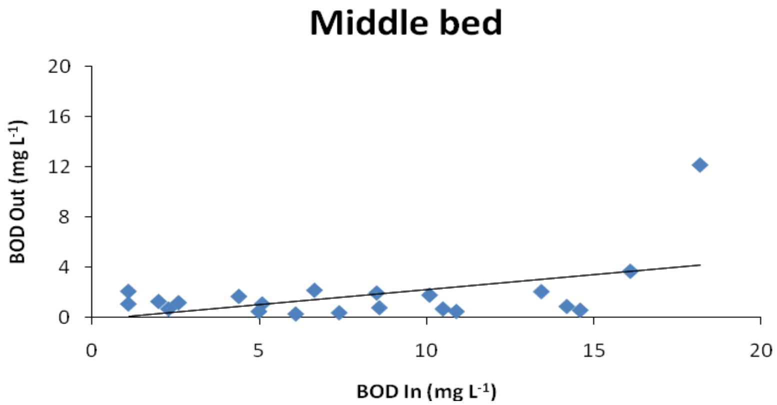
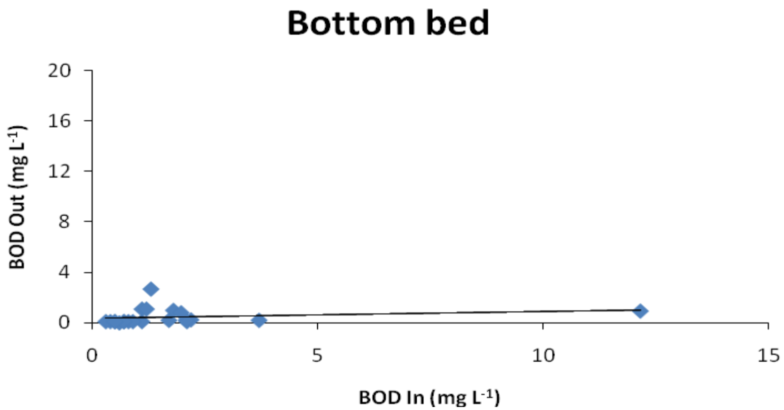
5.8 SUPPORTING INFORMATION

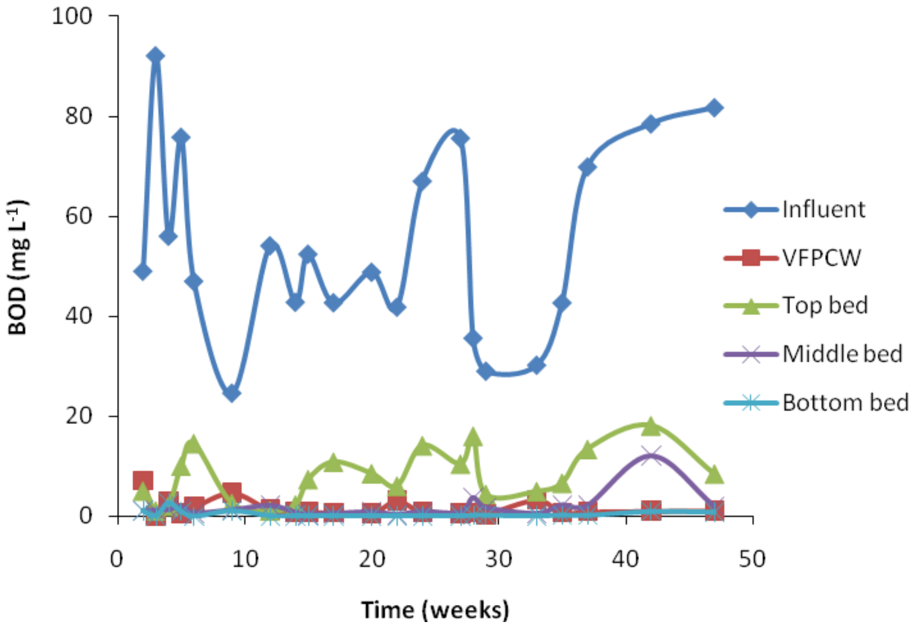








Appendix 5.1: Effect of influent organic loading on wetlands residual BOD concentration



Appendix 5.2: Influent BOD to the two technologies and effluent levels from the different wetland beds

Appendix 5.3: Statistical analysis

Anova: Single Factor (Unplanted prototype vs. Planted Std VF)

Factor: Wetland type

H_o: BOD effluent levels are the same in the wetland (*i.e.* $\mu_{\text{unplanted prototype}} = \mu_{\text{VPFCW}}$)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
VFPCW	21	34.52	1.64381	2.931295
Bottom bed	21	9.49	0.451905	0.411936

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
Between Groups	14.91669	1	14.91669	8.923516	0.004791	4.084746	Reject H_o
Within Groups	66.86462	40	1.671615				
Total	81.78131	41					

$\mu_{\text{unplanted prototype}} \neq \mu_{\text{VPFCW}}$

Anova: Single Factor (Unplanted prototype vs. Planted Std VF)

Factor: Bed type

H_o: BOD effluent levels from every bed are the same regardless of the wetland technology (*i.e.* $\mu_{\text{top bed}} = \mu_{\text{middle bed}} = \mu_{\text{bottom bed}} = \mu_{\text{VPFCW}}$).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
VFPCW	21	34.52	1.64381	2.931295
Top bed	21	168.9	8.042857	26.47517
Middle bed	21	37.8	1.8	6.30745
Bottom bed	21	9.49	0.451905	0.411936

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
Between Groups	739.2316	3	246.4105	27.28357	2.98E-12	2.718785	Reject H_o
Within Groups	722.517	80	9.031463				
Total	1461.749	83					

$\mu_{\text{top bed}} \neq \mu_{\text{middle bed}} \neq \mu_{\text{bottom bed}} \neq \mu_{\text{VPFCW}}$

Anova: Single Factor (Unplanted prototype vs. Planted Std VFPCW)

Factor: Bed type

H_o: BOD effluent levels from the top two beds in the prototype is the same as the std VFPCW (*i.e.* $\mu_{\text{middle bed}} = \mu_{\text{VFPCW}}$).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
VFPCW	21	34.52	1.64381	2.931295
Middle bed	21	37.8	1.8	6.30745

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
Between Groups	0.256152	1	0.256152	0.055452	0.815036	4.084746	Accept H_o
Within Groups	184.7749	40	4.619372				
Total	185.031	41					

$\mu_{\text{middle bed}} = \mu_{\text{VFPCW}}$

Anova: Single Factor (Unplanted prototype vs. Planted Std VF)

Factor: Bed type, to test whether the bottom bed makes a significant difference to the treatment performance since middle bed meets the BOD reuse consent.

H_o: The bottom bed does not significantly improve the BOD removal of the effluent water from the middle bed (*i.e.* $\mu_{\text{middle bed}} = \mu_{\text{bottom bed}}$).

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Middle bed	21	37.8	1.8	6.30745
Bottom bed	21	9.49	0.451905	0.411936

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
Between Groups	19.08229	1	19.08229	5.679771	0.022	4.084746	Reject H_o
Within Groups	134.3877	40	3.359693				
Total	153.47	41					

$\mu_{\text{middle bed}} \neq \mu_{\text{bottom bed}}$ in fact the bottom bed does significantly polish up the effluent from the middle bed (*i.e.* $\mu_{\text{bottom bed}} < \mu_{\text{middle bed}}$)

Anova: Two Factor (Hydraulic loading rate, HLR, and bed type)

H_o: Treatment performance in the unplanted prototype was independent of the HLR and the bed type (*i.e.* $\mu_{\text{top bed}} = \mu_{\text{middle bed}} = \mu_{\text{bottom bed}}$).

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
5.1	2	2.2	1.1	0
1.1	2	1.2	0.6	0.5
2	2	4	2	0.98
10.1	2	2.8	1.4	0.32
14.6	2	0.6	0.3	0.18
2.6	2	2.3	1.15	0.005
1.1	2	2.2	1.1	2
2.3	2	0.8	0.4	0.18
7.4	2	0.5	0.25	0.045
10.9	2	0.6	0.3	0.08
8.6	2	0.9	0.45	0.245
6.1	2	0.4	0.2	0.02
14.2	2	1	0.5	0.32
10.5	2	0.8	0.4	0.18
16.1	2	3.9	1.95	6.125
4.4	2	1.9	0.95	1.125
5	2	0.6	0.3	0.08
Middle bed	17	19.4	1.141176	0.698824
Bottom bed	17	7.3	0.429412	0.484706

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
HLR	10.85765	16	0.678603	1.343964	0.280604	2.333484	Accept H_o
Bed type	4.306176	1	4.306176	8.528324	0.010009	4.493998	Reject H_o
Error	8.078824	16	0.504926				
Total	23.24265	33					

Hence, performance of the prototype beds was independent of the loading rate, but the performance was dependent on the type of bed.

Anova: Two Factor (Organic loading strength, OLR, and bed type)

H_o: Treatment performance in the unplanted prototype was independent of the OLR and the bed type (*i.e.* $\mu_{\text{top bed}} = \mu_{\text{middle bed}}$ and $\mu_{\text{top bed}} = \mu_{\text{middle bed}} = \mu_{\text{bottom bed}}$). Note: this analysis only considered the two higher strength loading rates.

Factors: Organic loading strength and bed type

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Row 1	3	9.09	3.03	10.8333
Row 2	3	15.72	5.24	51.3136
Row 3	3	31.28	10.42667	76.55773
Row 4	3	11.3	3.766667	17.28203
Column 1	4	46.8	11.7	26.844
Column 2	4	18.4	4.6	25.40967
Column 3	4	2.19	0.5475	0.146092

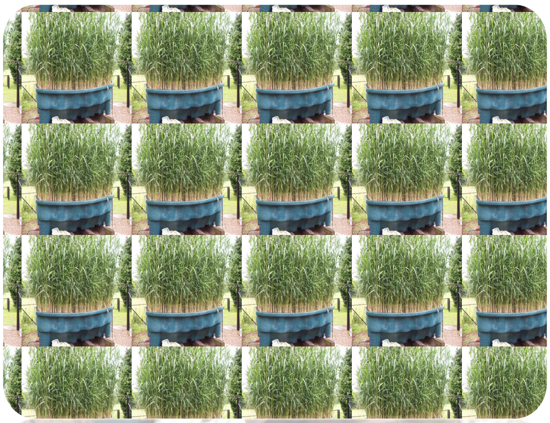
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
OLR	100.174	3	33.39132	3.513315	0.088993	4.757063	Accept H_o
Bed type	254.948	2	127.474	13.41236	0.006107	5.143253	Reject H_o
Error	57.02532	6	9.504219				
Total	412.1473	11					

Hence, performance of the prototype beds was independent of the organic strength, but the performance was dependent on the type of bed.

Appendix 5.4: Pictures of the study wetland technologies

A5.4.1: The prototype – cascaded vertical flow CW



A5.4.2: Standard CW – VFPCW

A5.4.3: The prototype – bed flooding

A5.4.4: The prototype – in low temperature conditions

CHAPTER 6: EFFECTS OF DESIGN ASPECTS OF CONSTRUCTED WETLANDS ON TREATMENT PERFORMANCE

CONSTRUCTED WETLANDS: EFFECT OF DESIGN AND VEGETATION ON TREATMENT OF GREY WATER

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6.1 ABSTRACT

The constructed wetland technology has been proven to handle different types of wastewater with treatment efficiency that meets various published reuse standards. However, there remains controversy on the actual role of plants in constructed wetlands. To understand the significance of plants in these systems, this study looked at the performance of two variants of the cascade wetland, one with a mixture of plants and the other with covers instead of plants. Performance was monitored for the conventional quality parameters and surfactants removal from grey water. In addition, catabolic diversity (which is a measure of the response of microorganisms in the treatment beds to different complex molecules) was measured in all the wetland treatment beds. Interestingly high removals were obtained for conventional parameters for both cascade wetland versions. BOD and COD removals were over 99% and 96% respectively. There was no significant difference between the performances of the two cascade wetland versions, however the removal of BOD by the standard VFPCW was significantly higher than the both of the cascade wetlands versions. Removal of surfactants was higher in the planted CW systems and was dependent on age and bed depth as shown by the positive correlation of surfactant removal with organic matter content and residence time. Catabolic evenness and species richness was initially different between the surface and inner layers of the cascade wetland beds but became more balanced with time (age) indicative equal ability to breakdown different complex molecules. This resulted in improvement of the overall performance of the individual beds as the inner layer microorganisms became more active with time. These findings show that shallow bed depths used in the cascade wetland generally suit removal of conventional wastewater treatment analytes, while removal of surfactants, correlated with presence of additional surfaces, *i.e.* plant roots and increased retention, *i.e.* depth.

Therefore both planted and the covered cascade wetlands can be useful depending on the treatment objectives.

6.2 INTRODUCTION

The applicability of the constructed wetland technology for treatment of diverse types of wastewater has been on the increase. Recent studies have been focusing on understanding and developing the vertical flow subsurface systems which are considered as the latest generation for this technology for wastewater treatment (Vymazal *et al.*, 1998). These systems can fulfil stringent effluent standards and guarantee low effluent concentrations regarding ammonia nitrogen and have shown high oxygen transfer capacities compared to the other types of constructed wetlands. This technology is capable of removing more than 60% of the concentration based hydrocarbons in different types of wastewater (Keefe *et al.*, 2004). The robustness of the vertical flow wetlands and overall performance beyond, those typically accomplished by secondary treatment, have made it the preferred design for removal of xenobiotics (Tang *et al.*, 2009). Use of the vertical flow constructed wetland (VF-CW) design for treatment of grey water is good for irrigation reuse. Surfactants in grey water pose the greatest threat for irrigation due to the potential problems that surfactant may cause such as, reduced capillary rise in the receiving soils (Shafran *et al.*, 2005), and toxicity to important soil micro-organisms (Eriksson *et al.*, 2006). Vertical constructed wetland leads to more effective reduction of organic pollutants, and this makes the technology more suited to treatment of grey water. However, as it still is a relatively new form of constructed wetland, the design of this system has so far been based on dimensions, *i.e.* a specified unit area for a given organic loading, typically expressed as population equivalents (Kadlec and Wallace, 2009).

Surfactants represent a broad group of chemicals that are important and vital in a variety of fields including laundry and personal cleaning but pose environmental risks when present in surface water and soil. Problems of strong foam formation, in river water receiving sewer discharge (Weedon, 2003), toxicity to aquatic life and plant (Sutterlin *et al.*, 2008) have been reported to be caused by surfactants. Surfactants in average domestic wastewater account for the highest concentration of organic chemicals (Shafran *et al.*, 2005). Very few data about the occurrence of surfactants in wastewaters and their elimination in WWTP are available with the exception of a few compounds such as linear alkyl benzenesulfonate (LAS) and alkylphenol ethoxylate (APEO), which have been frequently and systematically analysed. As for constructed wetland, the scarcity of data is even greater. Some work has been reported by Gross *et al.* (2007) on anionic surfactants removal in constructed wetlands. Monitoring studies conducted to assess the removal of LAS in wastewater treatment illustrate the capacity of treatment CWs to efficiently reduce the LAS loads in sewage (Huang *et al.*, 2004). This needs to be shown for other forms of surfactants in CWs as well, especially where the technology is being used to treat grey water for irrigation reuse in view of the higher concentration of surfactants coming from laundry and washing activities.

In order to produce treated water that is suitable for irrigation and other uses, it is necessary to match the water requirements and the treatment efficiency of the wetland system. Literature reveals a number of constituents in typical grey water that are potentially harmful to plants singly or in combination. A case in point is the surfactant group of chemicals, whose effects in soil systems are known from other sources (*e.g.* sewage sludge and wastewater). Published analyses of grey water are generally focussed on conventional parameters that are relevant to sewage treatment plant operation. Although different xenobiotics in sludge amended and wastewater irrigated soil systems have been studied extensively, the medium of application in those studies was not necessarily grey water. Further, information regarding the content of xenobiotics in grey water is limited (Eriksson *et al.*, 2002). Therefore a gap exists in knowledge of both short- and long term effects of grey water irrigation on soil and plant health.

The microbial activity of soil is used as an indicator of soil quality or the health of the soil ecosystem. Different indices of this activity have been widely included in the assessment of soil quality (microbial biomass, microbial respiration and diverse enzymatic activities such as dehydrogenase and protease as representatives of the system's activity). These parameters are useful in monitoring changes in soils' health. This can be adapted to study microbial activity in the wetland media and to characterise internal decomposition processes of organic compounds and conventional treatment parameters as shown in literature (Morán and Gil, 2004) which would aid the design and operation criteria of vertical flow constructed wetlands. The functioning of the microbial community is central to understanding ecosystem-level processes such as decomposition and nutrient cycling as well as defining microbial community structure and functions, though this has been problematic in soil studies (Zak *et al.*, 1994). Genetic composition of microbial communities (*e.g.* fungi to bacteria ratios) can potentially be used to study the various processes in wetland environment with different vegetations, and operations, as is done for soil ecosystems (Bardgett *et al.*, 1996). This is because the chemical nature of organic matter in different wetlands differs with plant genera which can be captured by assessing the microbial compositions. In addition the study of catabolic response profiles can be used because differences in land uses and cropping intensity (Sparling *et al.*, 2000), soil organic carbon status (Degens *et al.*, 2000), and stress or disturbance to the soil (Degens *et al.*, 2001), produced different catabolic profiles. Therefore, the technique can be adapted to treatment wetland studies in order to characterise or gain an insight into processes taking place in the wetland ecosystems. Hence this study focussed on the internal dynamics of the cascade wetland, in its planted and unplanted versions using the study of catabolic diversity in the wetland beds in order to get some insight of the structural and functional changes between and within the beds in the planted and unplanted prototypes. A part of the study also looked at removal of surfactants, ionic and non-ionic forms.

6.3 MATERIALS AND METHODS

6.3.1 Grey water source and pilot study description

Grey water was collected from bathroom sinks, baths and showers of 18 specially plumbed student flats on Cranfield campus. Collection was by gravity in an underground sump and then continuously pumped into a holding tank, where the grey water strength was increased by supplementing with a shampoo solution, to enable higher feed strengths to be tested. The higher strength grey water was pumped to the pilot wetlands and fed by flood-and-drain (pulse loading). The pilot technologies were (i) two cascade wetland rigs (the prototype; WPL, UK) comprising three shallow beds (0.6 m length by 0.45 m width, 0.20 m depth) and (ii) a single bed reactor (Vertical flow Planted Constructed Wetland: VFPCW, 6 m^2 surface area and 0.7 m deep). One of the cascade wetland was planted with a mixture of *Iris pseudacorus, Iris chrysographes, Carex elata Aurea* and *Mentha aquatic* (Figure 6.1[A]), the second cascade wetland was unplanted but had all its beds covered, essentially operated as a covered constructed sand filter. The covers had small vents to enable exchange of air (Figure 6.1 [B]). Plant selection was based on a review of potential plants (Appendix 6.1) and final selection was based on scent (*Mentha aquatica*), structure (*Carex elata Aurea*), and beauty (*Iris species*). The VFPCW was planted with *Phragmites australis* (Figure 6.1 [C]) and contained a mixture of sand, soil and organic matter as media. The consideration to have 20 cm bed depth in the prototype system was a result of the demonstration in literature that the majority of microbial biomass in standard vertical flow wetlands (60 – 120 cm deep) is located in the top 20 - 30 cm of the VF bed (Akratos and Tsihrintzis, 2007; Langergraber *et al.*, 2003). The test hypothesis in this study was that: "*shallow beds as in the prototype achieve better treatment than the standard 0.6 – 1.2 m beds and that, a planted constructed wetland system performs better than an unplanted one*". The covered cascade wetland was tested as an alternative for the unplanted version whereby oxygen transfer and incident sunlight are controlled to slow down *schmutsdecke* formation.

All the wetland technologies were fed grey water from the same source. The cascade wetlands were operated at a slightly higher hydraulic loading rate (0.11 $m^3\ m^{-2}\ d^{-1}$) (for the whole system) compared to the standard VFPCW (0.08 $m^3\ m^{-2}\ d^{-1}$). Treatment performance for the different technologies was assessed by looking at loading rates vs. effluent concentration graphs and by looking at distribution of parameter values, such as k_{BOD} and performance graphs of various pollution residuals. The study was conducted for 6 months.

A B C

Figure 6.1: Pictures of the three wetland technologies that were tested in this study. A and B are the planted and unplanted prototypes; C is the standard VFPCW

6.3.2 Sampling and analytical procedures

Influent and effluent samples were collected between 08:00-10:00 am. Conventional water quality analyses were monitored as follows: Total organic carbon (TOC) ($mg.L^{-1}$), using a total organic carbon analyser Shimadzu TOC-5000A (Shimadzu, UK); Biochemical Oxygen Demand (BOD), using the standard BOD_5 test; Turbidity (NTU), using a Turbidimeter Hach 2100N; pH and conductivity, using the Jenway 3540 pH and conductivity meter; and Merck cell tests (Merck (VWR International), Poole, UK) for chemical oxygen demand (COD). Analysis for anionic surfactants was carried out for the unplanted open prototype and the VFPCW during the first part of the study (reported in Chapter 5). From month 13 of the whole project, the analysis was extended to cationic and non-ionic surfactants but focussed on the planted and covered cascade wetlands. Samples for surfactants analysis were filtered through microfiber filter paper and sublated (isolated from non-surfactant substances) by bubbling nitrogen gas through a column containing the sample into a layer of ethyl acetate (Figure 6.2). The solvent was separated, dehydrated, and evaporated leaving the surfactant as a residue suitable for analysis (APHA, 1998). Analysis was done as follows: a) anionic surfactants using the Methyl Blue Active substances (MBAS) method. Amberlyst A-26 anion exchange resin (^{-}OH form) was used for ion exchange, b) Analysis for cationic surfactants using the Disulfine Blue Active Substances (DBAS). An anion-exchange resin (Cl^{-} form of Bio-Rad AG1-X2) was used to remove non-surfactant cationic interferences, and c) Non-ionic surfactants using Cobalt thiocyanate Active Substance (CTAS). An ion-exchange column containing an anion-exchange (OH^- form of Bio Rad AG1-X2 anion resin) and cation exchange resins (50-100 mesh H^+ form of Bio Rad 50W-X8 cation exchange resin) was used to remove cationic and anionic interferences (APHA, 1998).

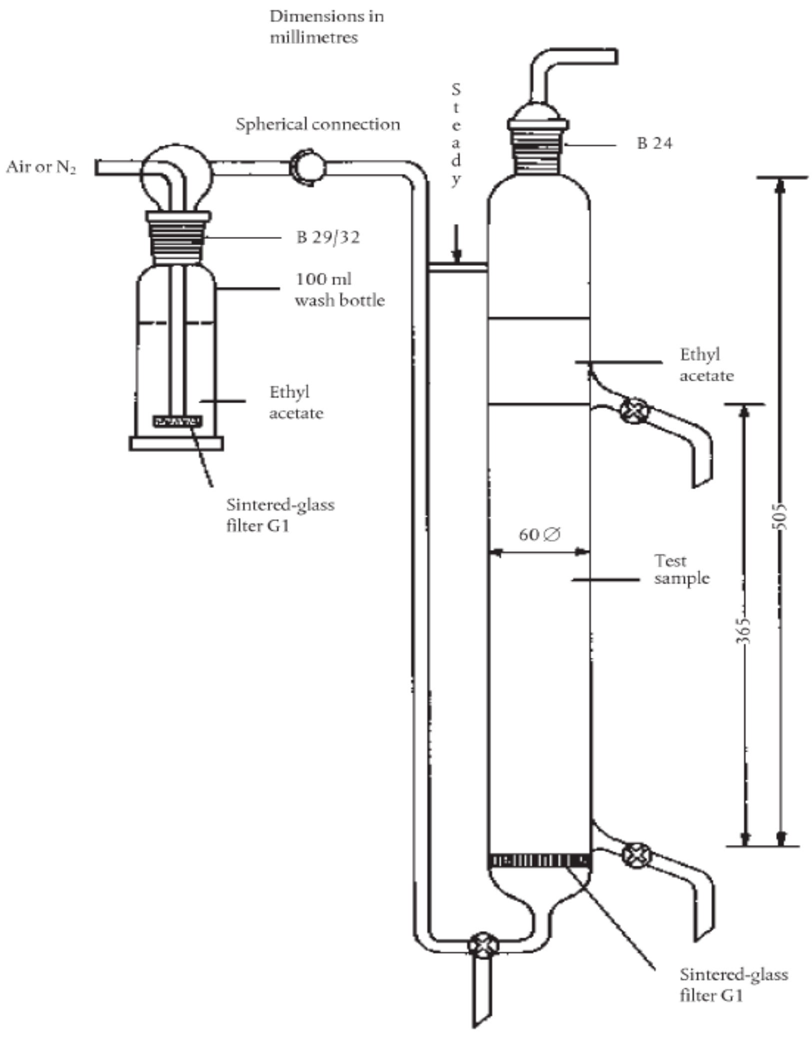


Figure 6.2: Sublation set up. (Ref: *Standards Methods for Water and Wastewater, 20th ed.*)

6.3.3 Microbial biomass in the wetland beds

Microbial biomass was measured using the soil respiration technique, substrate induced respiration (SIR), where increase in respiration before cellular growth is related to substrate-responsive microbial biomass (Anderson and Domsch, 1978).

Basal and SIR (glucose) were measured at the same time as catabolic diversity using the multiple substrate induced respiration (MSIR). MSIR allows use of several substrates, and is used to assess the catabolic responses to specific substrates which can be used to differentiate microbial communities in a soil ecosystem (Degens *et al.*, 2000; Stevenson *et al.*, 2004). The Rapid Automated Bacterial Impedance Technique (RABIT) (developed by Don Whitley Scientific Ltd), technique was used in this study. This technique uses measurement of impedance to monitor respiration parameters continually over a much greater time frame than the standard 4 – 6 hours used in most respiration studies. Measurements were taken every six minutes, which allowed observations of respiration kinetics, rather than a single reading at the end of 6 hours (Figure 6.3).

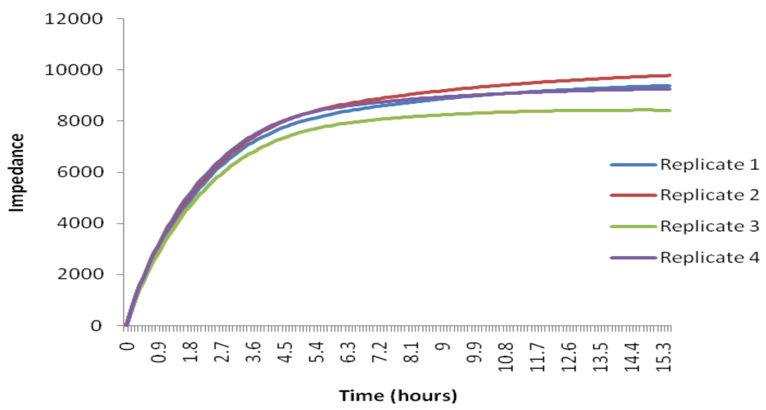


Figure 6.3: A typical graph obtained from RABIT showing a slowdown in respiration rate (measured indirectly as impedance) after approximately 6 hours.

6.3.4 Measurement of microbial catabolic diversity in the prototype beds

Microbial catabolic diversity was assessed using the MSIR data for the following substrates: glucose (carbohydrate), α-ketogluteric acid (a carboxylic acid) and L-arginine (amino acid). Concentration of the carbohydrate solution was 75 mM, carboxylic acid solution was 100 mM and amino acid was 15 mM. All solutions were adjusted to between pH 5.8–6.0 before addition to 1 g (dry weight equivalent) of the moist wetland media samples. Deionised water was added to additional samples to determine basal respiration. Respiration was measured for 16 hours, using a time resolution of 6 minutes, and temperature of 25^{o}C and a detection criterion of 10 microsiemens. Laboratory conditions were set at 20^{o}C while individual incubator modules were set at temperature of 25^{o}C but were controlled within the range 4 – 50^{o}C.

Respiration data for each media sample were standardized by dividing by the mean of the 3 substrates sampled. The standardized catabolic response represents a relative measure of the contribution of a particular substrate to the activity of all substrates. Catabolic diversity uses the substrates to measure both the richness (number of substrates metabolised by the microbial community) and evenness (variability in substrate use). Catabolic evenness (E) was calculated using the Simpson-Yule index: $E = 1/\sum p^{2}_{i}$ (Schipper *et al.*, 2001; Magurran, 1988) where p_i is summed for all substrates and $r_i / \sum r_i$ = (defined as the respiration response of each substrate (r_i) as a proportion of total respiration responses summed over all substrates ($\sum r_i$). The results of an ecosystem's richness and evenness depend of the number and variety of substrate used. This study used one amino acid, one carboxylic acid and one carbohydrate. Ideally, use of more substrates would have been preferred but the substrates chosen for this study have given the greatest responses for pasture and forest soil studies (Graham and Haynes, 2005; Bardgett *et al.*, 2001) and were used in order to get an insight of what was happening in the wetland beds. Phospholipid fatty acid (PLFA) profiles were used (Chapter 7) to study microbial compositions and variations in the wetland beds in more detail with regard to bed type (*i.e.* top or middle or bottom bed) and wetland regime (*e.g.* presence or absence of vegetation cover).

6.3.5 Statistical analysis

The main effects of wetland type differences were compared in terms of outflow quality from the three technologies using StatSoft. Inc. (2009) STATISTICA (Data Analysis software system) version 9.0. One way analysis of variance, pair wise comparisons of means and independence sample *t*-tests were performed for significant differences, at 5% level, in the treatment performances of the wetland technology used in this study and also to compare the catabolic functional (richness and evenness) differences. Principal component analysis (PCA) was performed on media samples from the unplanted cascade wetland, to check for differences in microbial biomass levels and ANOVA to check for catabolic differences between the cascade wetland beds.

6.4 RESULTS

6.4.1 Influent grey water quality

BOD and COD concentrations for the influent (spiked) grey water ranged from 28 to 185 mg/L and 74 to 279 mg/L respectively. Total coliform and E coli levels were 5.0 to 6.5 $\log_{10}$ concentrations and 3.4 to 4.0 $\log_{10}$ concentrations respectively (Table 6.1). These levels are within the mid-range of the high strength load reported for grey waters in literature, 59 to 155 mg/L (BOD), 158 to 587 mg/L (COD) (Frazer-Williams *et al.*, 2008; Friedler *et al.*, 2005; Friedel *et al.*, 1999), 1.8 to 7.4 $\log_{10}$ CFU. 100 mL^{-1} total coliforms (Ottoson and Stenström, 2003). Turbidity and pH levels averaged 24.9 NTU and 7.0 pH units respectively. This turbidity lies on the lower end of reported turbidities in literature and the pH lies mid-way of most reported ranges for grey water.

Anionic surfactant levels ranged from 3.3 to 27.5 mg/L which is below the levels reported elsewhere (40 to 90 mg/L) in grey water (Gross *et al.*, 2008; Kantawanichkul and Wara-Aswapati, 2005; Eriksson *et al.*, 2003). Non-ionic surfactants ranged from 1.45 to 1.96 mg/L which is also below the estimated levels (10 mg/L) in grey water but similar to levels reported in municipal wastewater (1.0 mg/L) by Síma *et al.* (2009) and cationic surfactants ranged from 4 to 5 mg /L. This confirms observations that substantial degradation of surfactants occurs in the sewer system before the grey water reaches the wetland treatment pilot systems, as shown by the detection of the decomposition metabolites, sulfophenyl carboxylates (SPC's), in the influent samples of WWTPs (Knepper and Berna, 2003). Influent surfactant concentrations showed anionic surfactants levels to be the highest, which is in agreement with literature indicating anionic surfactants as the most widely used in the laundry and personal-care products (Eriksson *et al.*, 2002). The cationic surfactants in the grey water in this study are thought to be predominantly from hair conditioners and not fabric conditioners because wash water from the laundrette was not included in the raw grey water collected from the Fedden flats.

Table 6.1: Influent grey water characteristics based on conventional parameters in sewage treatment

	BOD (mg/L)	COD (mg/L)	TOC (mg/L)	Turbidity (NTU)	pH	Total coliform (log_{10} CFU $100ml^{-1}$)	E coli (log_{10} CFU $100ml^{-1}$)
Mean	87.5	169.8	8.95	24.9	7.0	6.2	3.7
Std dev	58.3	79.8	15.81	11.6	0.5	0.3	0.2
Min	28.2	74.0	8.8	8.9	6.1	5.9	3.4
Max	185.3	278.5	42.85	41.0	7.8	6.5	4.0

(n = 8)

6.4.2 Treatment performance

With respect to conventional water quality parameters

All three constructed wetland technologies achieved good removal of organics from the grey water (Table 6.2). Influent loading rates were similar for all the technologies throughout the monitoring period, and all the technologies, on average, met the USEPA BOD reuse standard of 10 mg/L. The greater removal in all three cases amongst the various constituents was observed for BOD and COD with over 99% and 96% removals respectively. The effluent concentrations (residuals) from the VFPCW were much lower showing better performance by the VFPCW for BOD, COD and TOC removals. The difference was significant ($P < 0.05$) for BOD removal only. The effluent COD and TOC concentrations (2.5 to 11.2 mg/L and 4.3 to 8.4 mg/L respectively) fell within the lower end of the range reported in literature for various treatment technologies (Dallas *et al.*, 2004) and were comparable to performance of an upflow anaerobic-aerobic grey water treatment system (Abu Ghunmi *et al.*, 2010). Removal of BOD was better than COD in all wetland technologies, which is indicative of presence of recalcitrant chemical pollutants (Gray, 2004) in the grey water. Although the planted cascade wetland also produced effluent with lower BOD concentration than the unplanted one, the performance was statistically no better than that of the unplanted version ($P < 0.05$, d.f. = 12). Comparison of treatment performance was carried out for BOD, COD and TOC, in corresponding beds for the planted and unplanted cascade wetlands beds (*i.e.* top planted bed vs. top unplanted bed *etc.*). This sought to check if treatment in the planted beds was better than in the unplanted beds (*i.e.* H_o: The mean concentrations for each parameter under consideration for corresponding beds are lower in the planted beds compared to the unplanted beds ($\mu_1 < \mu_2$), where Group 1 represents planted beds and Group 2 represents unplanted beds). The analysis showed no significant differences between the two systems. However, a two-factor ANOVA (factor 1: vegetation structure and factor 2: wetland design), indicated that that vegetated wetland had better BOD removal, probably due to the added influence microbial activity resulting from additional surface provided by the root system. The VFPCW, performed better than the other two (mixed vegetation and no vegetation).

Table 6.2: Treatment performance of the wetland technologies

	VFPCW		Planted cascade wetland		Covered cascade wetland	
	Influent load ($g\ m^{-2}\ d^{-1}$)	Effluent (mg/L)	Influent load ($g\ m^{-2}\ d^{-1}$)	Effluent (mg/L)	Influent load ($g\ m^{-2}\ d^{-1}$)	Effluent (mg/L)
BOD	7.0 ± 4.7	0.3 ± 0.3	9.6 ± 6.4	0.9 ± 0.9	9.6 ± 6.4	1.3 ± 0.9
COD	13.6 ± 6.4	6.5 ± 4.0	18.7 ± 8.8	7.2 ± 4.0	18.7 ± 8.8	6.5 ± 3.9
TOC	1.5 ± 1.3	5.5 ± 1.2	2.1 ± 1.8	7.0 ± 1.4	2.1 ± 1.8	6.5 ± 1.4

Dissolved oxygen levels in the influent water ranged from 0.7 to 3.8 mg/L and in the effluent water from the three wetlands, 46.0 to 56.0 mg/L, 78.4 to 86.3 mg/L and 78.2 to 89.1 mg/L in the VFPCW, planted and unplanted cascade wetlands respectively. Higher DO concentrations which were observed in both cascade wetlands indicate that aerobic conditions were dominant in these systems. The overall performance of the pilot systems suggests that the designs had more influence in how they performed than did the presence/absence of vegetation. The cascading design, having multiple shallow beds which are operated in series maximises oxygen influence into the bed media by convection (Chapter 5) and other factors that help to create aerobic conditions throughout the wetland beds. This is also important in wastewater treatment in a number of ways and saves having to feed oxygen to the system artificially. Performances of the planted and unplanted versions of the cascade wetlands in removing organic matter and other parameters were similar (Figure 6.4) and both systems met most stringent published reuse standards for treated wastewater. This is consistent with observations that plants only provide small improvements in BOD and COD removal (Tanner, 2001). Higher dissolved oxygen levels in the effluent from the planted and unplanted version, compared to the VFPCW, indeed confirm that the cascade wetland design improved oxygen transfer into the treatment beds. Removal of organics (BOD, COD and TOC) was comparable for the cascade wetlands and the VFPCW despite differences in effluent DO concentrations. This suggests that there were different degradation processes going on in the two wetland designs (Cascade wetland and VFPCW). On the other hand similarities between the planted and unplanted cascade wetlands suggest that the influence of plants in removing organics was not significant.

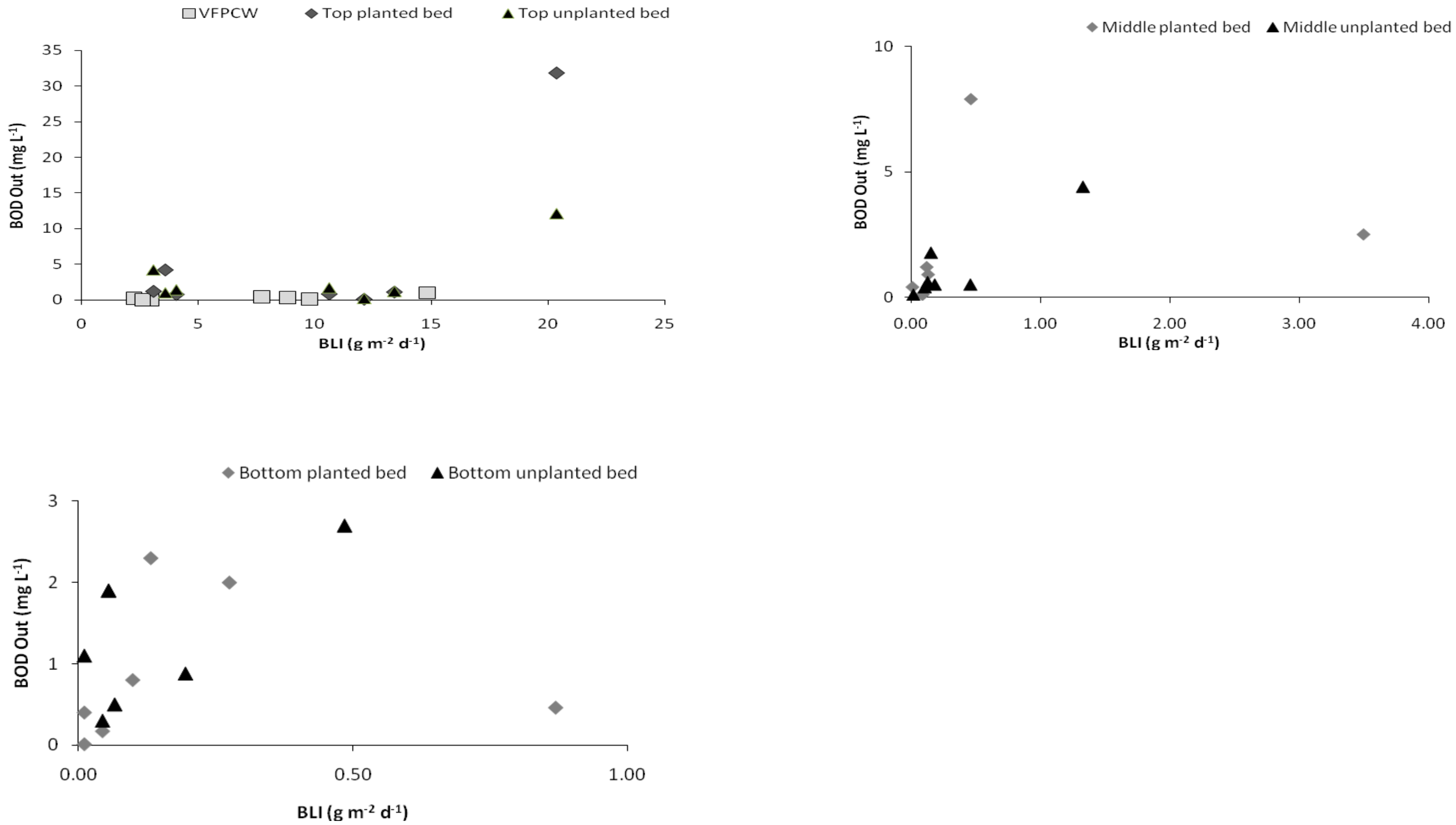


Figure 6.4: Correlation charts of effluent concentration and pollutant load for the planted and unplanted cascade wetland beds

Removal of surfactants

The order of anionic surfactant removal was: planted prototype > VFPCW > covered cascade wetland (83 ± 9%, 80 ± 11%, and 60 ± 23% respectively). The unplanted cascade wetland exhibited lower (< 50%) anionic surfactant removals initially, probably because the filter bed was still ripening and so the microbial community was probably not yet acclimated (Garland *et al.*, 2004) and organic matter content was probably still low. It was initially suspected that the type of analytical methods used in this study contributed to the low surfactant residuals. Measurement of surfactant using liquid-liquid and spectrometry analytical methods is suitable as it indicates the loss of surface-activity (Madsen *et al.*, 2001) and aquatic toxicity (EC_{50}), approximately 12 mg/L for C_{12} AS (García *et al.*, 2009). However, these methods only measure forms of intact surfactants molecules (*i.e.* degradation intermediates do not contribute to the overall value) (OECD, 2000). This probably confirms the degradation of intact surfactant into intermediates in the constructed wetlands. The planted cascade wetland, which started operating in month 13 of study, was inoculated with 'acclimated' media from the unplanted prototype and so it did not exhibit the low surfactant removal percentages which were observed in the case of the unplanted prototype (Figure 6.5). Non-ionic surfactant removals were similar in all the wetland technologies but for cationic surfactants, the VFPCW performed significantly better than the cascade wetlands (Figure 6.6). These observations are in agreement with studies by McAvoy *et al.* (1994) and Zoller *et al.* (1994), who showed that sorption and transport of an anionic surfactant (LAS) and a cationic surfactant (DTDMAC, Ditallow-dimethyl-ammonium-chloride) in sand filters and aquifers with > 90% sand and up to 0.5 % C_{org}, produced 88% reduction of LAS (from 13,850 to 1,640 µg L^{-1}) and 96% reduction of DTMAC (from 4,570 to 195 µg L^{-1}) within 0.5 m. They also showed higher sorption in upper 7.5 cm which also correlated with organic matter content. The VFPCW media had higher organic matter content originally (at the beginning of this study because it had been operated for 4 years prior to this study) from deposition after 5 years of operation compared to the cascade wetlands which were started-up with clean river sand and had only been in operation for about a year. However, the presence of plants seems to have improved surfactant removal, particularly anionic surfactants in the planted cascade wetland. The results obtained in this study are similar to comparative studies by Huang *et al.* (2004) and Thomas *et al.*

(2003) which showed high LAS (anionic surfactant) removals (71 and 57 % respectively) in wetlands. Studies by Gross *et al.* (2007) on a recycled vertical flow constructed wetland and Síma *et al.* (2009) on a horizontal flow constructed wetland, also reported higher anionic and non-ionic surfactant removals (> 95%). The main factor for the slightly lower removals in this study seems to be the residence time of the water in the treatment system. Hence higher retention times augur well with adsorption processes which improves removal of anionic surfactants. A similar observation was also made by Síma *et al.* (2009) and Barber *et al.* (2001).

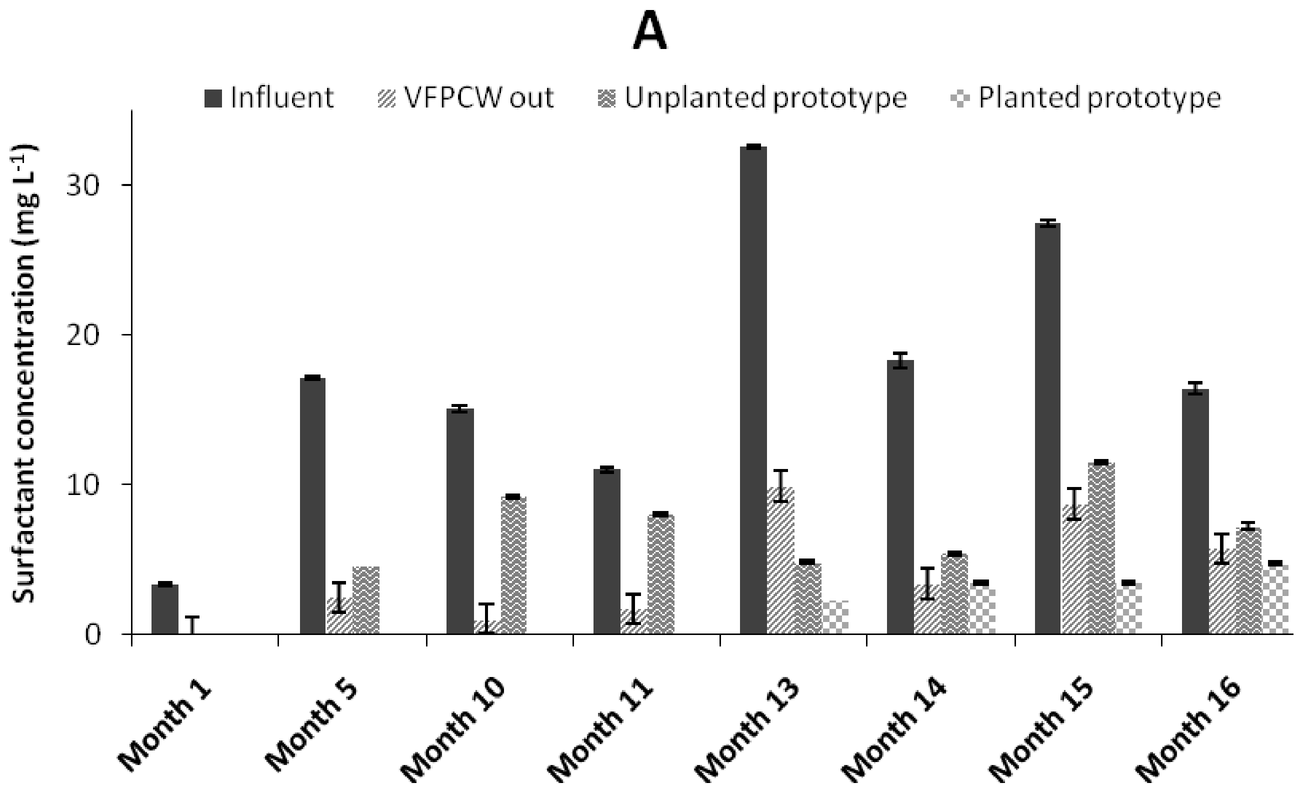
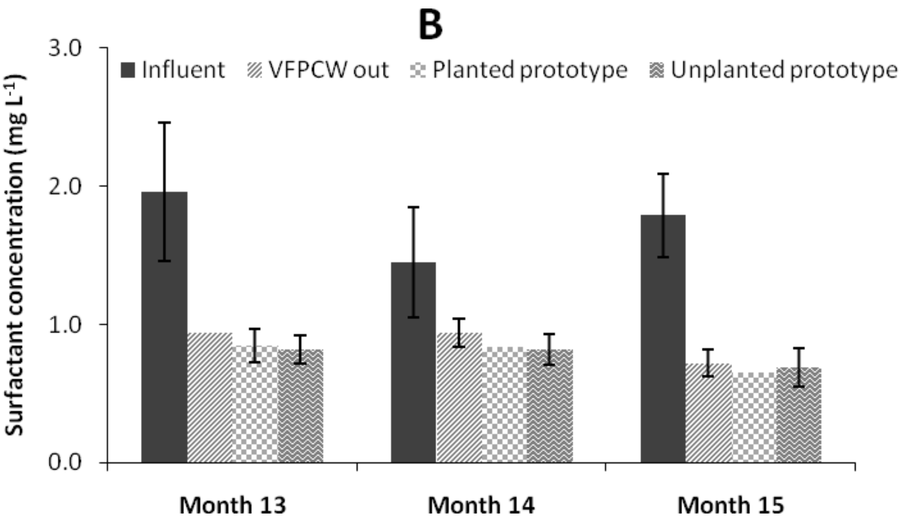


Figure 6.5: Influent and effluent concentration of anionic surfactants from the three wetland technologies



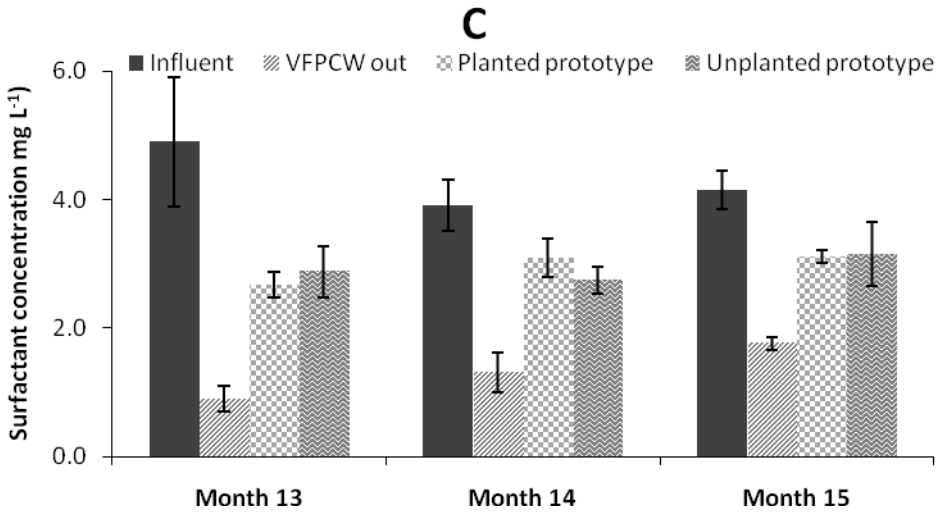


Figure 6.6: Influent and effluent concentration of non-ionic (B) and cationic (C) surfactants from three wetland technologies

Microbial activities in the treatment beds

Basal microbial activities were similar and higher in the top (23.3 ± 2.7 µg CO_2 g^{-1}) and middle bed (22.9 ± 5.9 µg CO_2 g^{-1}) compared to the bottom bed (2.6 ± 0.4 µg CO_2 g^{-1}) initially (batch 1, sampled after 30 weeks). But later on (batch 2, after 50 weeks) the basal activity in the bottom bed (23.6 ± 1.5 µg CO_2 g^{-1}) also increased (Figure 6.7) while the top and middle beds registered no change with time. This may mean that most of the microorganisms in the bottom bed that were dormant initially (Jenkinson and Ladd, 1981) had become active by the time the second batch sampling. Biomass levels (as measured by substrate, glucose) were not significantly different ($P < 0.05$) within individual beds (*i.e.* between surface, 1 – 5 cm samples, and inner-layer, 5 – 10 cm, samples) and between beds. The respiration response to the other substrates (α-Ketoglutaric acid and L-arginine) also indicates that species richness was no different between the beds (Figure 6.8) as shown by the similarities in utilisation levels. The general decrease in both basal and substrate induced respiration (SIR) between the two sampling periods (batch 1 and batch 2) is thought to be a result of abiotic factors.

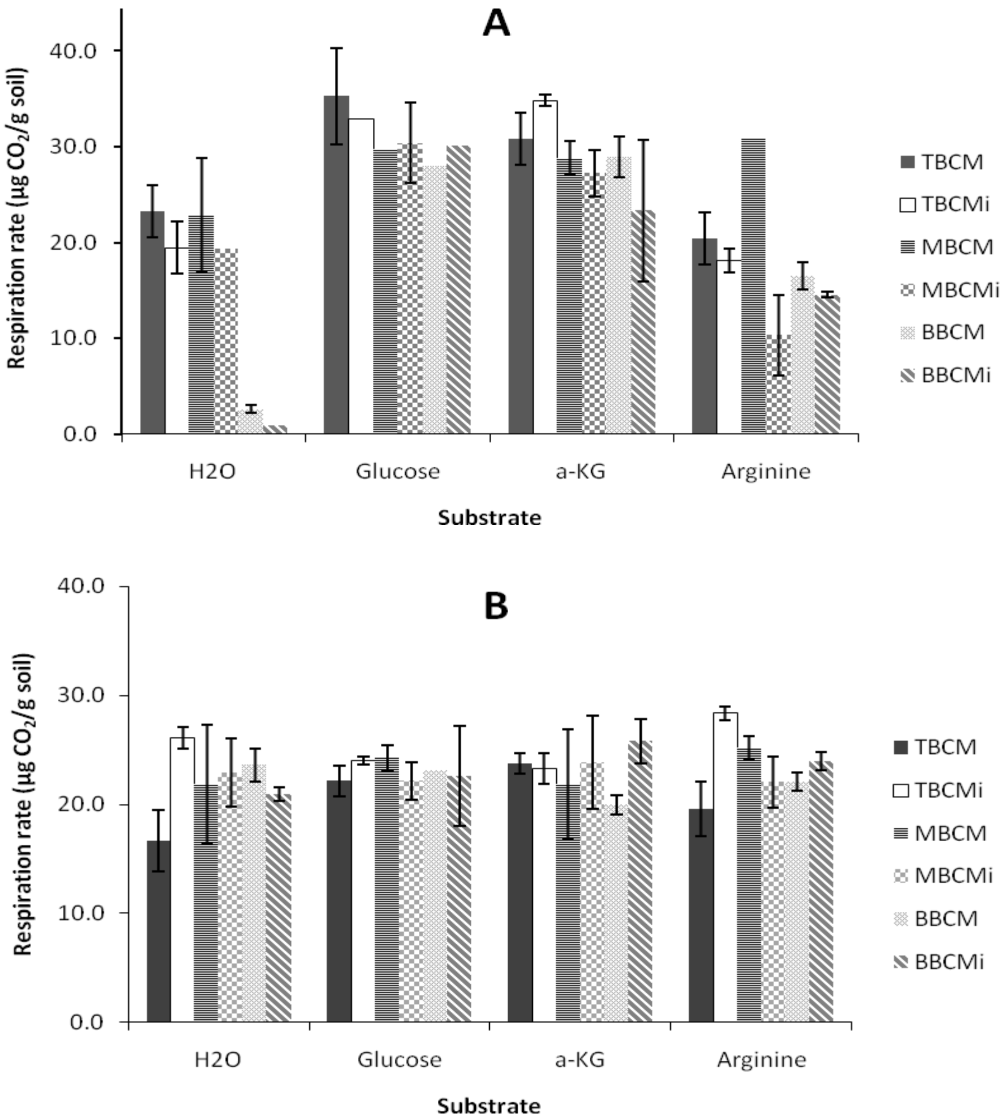


Figure 6.7: Respiration responses of wetland media samples to difference substrates. Samples were taken from surface and inner-bed layers (denoted with 'i' bed layers). TBCM: Top bed cascade media, MBCM: Middle bed cascade media and BCBM: Middle bed cascade media. A and B represent sample batches collected after 30 and 50 weeks of prototype operation.

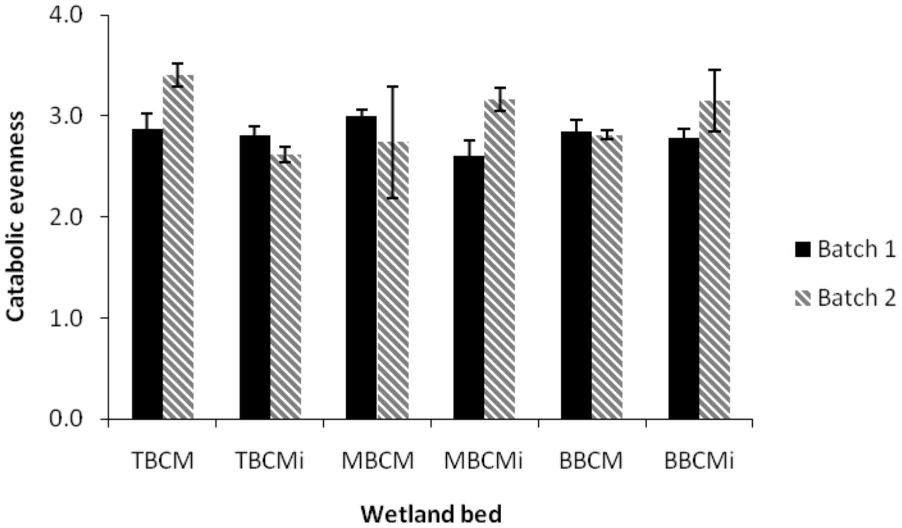
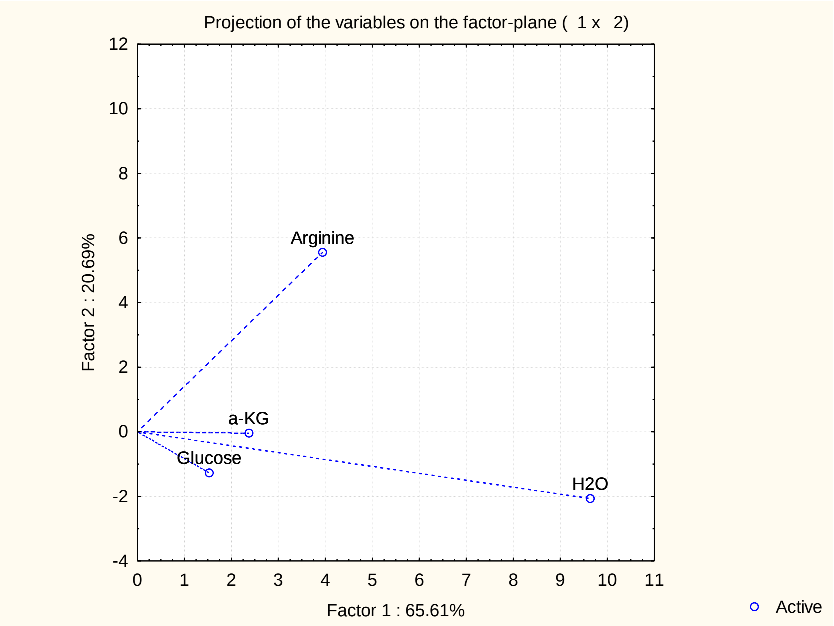
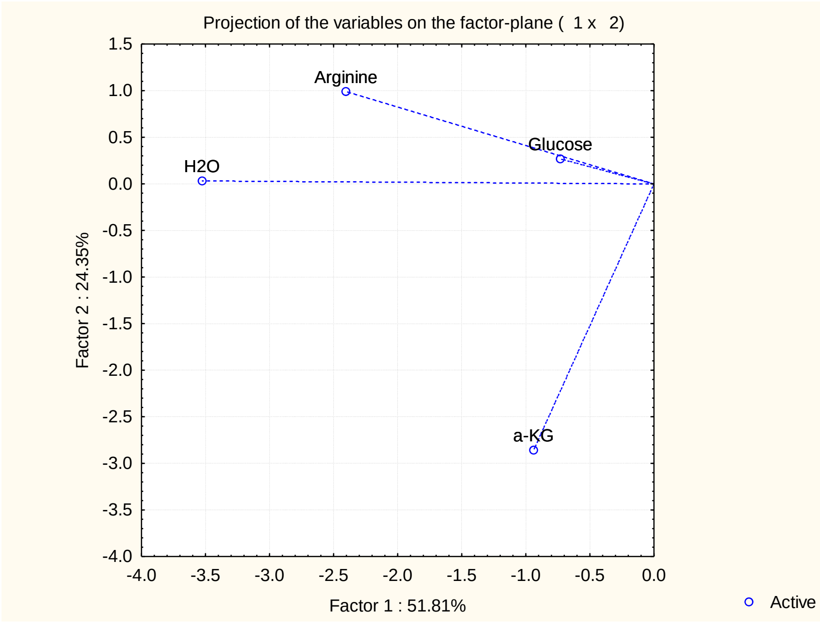


Figure 6.8: Catabolic evenness in the unplanted prototype beds. (n = 8, error bars represent standard error of the mean)

The greatest variations in respiration responses between the beds were observed for basal activity (H_2O) and L-arginine samples for both sample batches, as indicated by PC 1 in principle component analysis). L-arginine and α-Ketoglutaric acid respiration response dominated the variation in PC 2 in sample batches 1 and 2 respectively (Figure 6.9). All the substrates were positively correlated in both batches, showing that all substrates affected the microbial respiration in the same way. Nonetheless, respiration responses for all the substrate were high and independent of the bed or layer in the bed (Figure 6.7). This is an indication of similarities in mineralisable organic matter in all the beds, which is also shown by similarities in levels of catabolic richness (*i.e.* the ability by microbial communities to metabolise the different substrates).



Batch 1



Batch 2

Figure 6.9: Principal component analysis results for substrates used in the RABIT analysis

6.5 DISCUSSION

6.5.1 Performance in removing organics

In subsurface constructed wetland systems, organic matter is removed by aerobic bacteria attached to porous media and plant roots (Vymazal, 2002). The expected role of the plants in the planted cascade wetland, therefore, is to create aerobic conditions and support aerobic bacteria in the sand media (Breen, 1997). However, for the cascade wetland design, the contribution of the plants in this regard, appears to have been minimal and the hypothesis that led to this design was upheld other than the aesthetic beauty, from the *Iris* spp. and scent from the *Mentha aquatica*. Analysis of the data generated across the trial period (Figure 6.4) indicated that the top bed of the planted cascade wetland deviated from a high treatment level for loading rate > 15 g BOD m^{-2} d^{-1} (which corresponds to the highest BOD and COD influent of approximately 185 mg/L and 260 mg/L respectively (approximately 30 g COD m^{-2} d^{-1}) (Table 6.2). This result is in agreement with findings by Frazer-Williams *et al.* (2008), who reported an upper influent BOD_5 limit of approximately 200 mg/L for vertical flow constructed wetlands. The decreased treatment performance was also seen in the unplanted cascade wetland system, but the unplanted bed still produced effluent that met the 10 mg/L reuse standard. The top planted bed experienced increased BOD as a result of high plant growth and corresponding increased bacterial activity as shown in very high increase in gram-positive PLFA concentrations (discussed in Chapter 7). However, this was made good by the subsequent treatment stages (middle and bottom beds) which improved the quality of the effluent from the top beds. The strength equates to tertiary treatment in municipal treatment works. This data enable loading rate comparison to be done for the cascade wetland and the VFPCW which showed deviation at approximately 15 g BOD m^{-2} d^{-1}. However, effluent concentration in the VFPCW was below 1 mg BOD L^{-1} while as in the prototype the effluent concentration fell between 2 – 3 mg BOD L^{-1} range. The findings of the effect of organic loading on treatment performance are consistent with literature. To illustrate, Langergraber *et al.* (2005) claims that once steady state has been reached, performance of a VF wetland stays constant until after 6 years (all things being constant *e.g.* operation and maintenance). The VFPCW has maintained its performance even after 5 years of operation (during this study). This was probably achieved through maintenance of steady state biomass levels. The performance of the planted and unplanted (covered) cascade wetland however was better than expected. Although loading rates in the cascade wetlands reached 30 g COD m^{-2} d^{-1}, the reuse standards, for instance BOD_5, were always met, contrary to claims by Platzer (1999) and Langergraber *et al.* (2007) who stated that for that vertical flow wetlands receiving wastewater of more than 25 -27 g COD m^{-2} d^{-1} do not always meet effluent standards for reuse. Comparison of the pilot constructed wetlands showed that treatment performance was depended of the design, in particular, the size (bed depth) and the number of treatment stages. However, the role of vegetation for general wastewater pollutant removal in constructed wetland was observed to be minimal.

6.5.2 Performance in removing surfactants

The performance of the unplanted cascade wetland for surfactants removal was generally lower than the VFPCW. This lower performance, though, was against expectations considering that the shallow beds provided a more oxidised environment leading to higher BOD_5 removal and higher saturated DO and that surfactant degradation improves in more aerobic conditions (Kuhnt, 1993). This reduced performance may be due to alkyl chain shortening of the surfactants, resulting from aerobic oxidation, which corresponds to lower surfactant removals (Thomas *et al.*, 2003; Inaba, 1992). The low residence time at which the cascade wetlands were operating (approximately 0.54 hrs) compared to the VFPCW (2 hrs) may also have contributed to this. Surfactant behaviour depends largely on the molecular properties and residence time of the constituent chemical species in the medium into which they are discharged. Major processes governing transformations of surfactants and other synthetic compounds in wetlands include bio-uptake, sorption and photolysis (Kadlec and Wallace, 2009).

The performance of the cascade wetland shows that highly oxidised conditions alone are not sufficient for higher surfactant removal. Indeed, the significantly higher removal rates achieved by the VFPCW indicate the added influence of the presence of the plants with extensive root system and a large media depth (*i.e.* longer retention) which enhance adsorption processes. Indeed, the ability of plants to detoxify xenobiotics is recognised, however, compared to microorganisms they only play a secondary role in the direct degradation of organic chemicals in wastewater treatment systems as described by Stottmeister *et al.* (2003). But the combination of degradation and sorption is very important, as shown during soil-aquifer studies where more than 80% of the PPCPs were removed (Ternes and Joss, 2006). Plants' roots provide surfaces for additional associated biodegradation microbial communities which were not present in the unplanted cascade wetland (especially) leading to lower microbial contribution to surfactant degradation (Huang *et al.*, 2004) in that system. In addition, the plants in the planted system contributed to the organic matter pool which probably enhanced adsorption of the surfactants so that free concentration in the water is reduced (García *et al.*, 2001; Di Corcia and Samperi, 1994). Hence, both bed depth and plants were important factors contributing to the removal of surfactants from grey water by the wetland technologies.

Microbial functional diversity

In the cascade wetlands bacterial activity was initially more pronounced in the upper parts of the filter bed and gradually decreased with depth (as reported in Chapter 5). But activity became uniform throughout the bed with time (Figure 6.7) as a result of development of uniform catabolic diversity (*i.e.* catabolic evenness). Catabolic diversity gives an indication of the functional variability of microbial community in an ecosystem (*i.e.* their ability to metabolise different xenobiotics). Its use in this study provided a measure of the microbial response (activities) to different substrates (*i.e.* nutrient transformations and decomposition) (Geller, 1997; Wardle *et al.*, 1999) for the different stages of the cascade wetland. Long term field studies in soil irrigated with treated wastewater indicate that the benefits of organic matter and nutrients in wastewater outweigh the detrimental effects, thus leading to an overall increase in total biomass and microbial activity (Filip *et al.*, 2000). Results in this study showed that with time (age), the differences in microbial activity across the depth of the wetland beds decreased resulting in an even distribution of active microorganisms throughout the whole depth of the treatment beds develops. This is evidence of occurrence of acclimatisation, a process that all the beds went through in succession and based on their position in the treatment train. The bottom bed was the slowest to adapt because of the weaker grey water strength reaching it, which contained low resource material for the microorganisms compared to the top and middle beds. Microbial activity increased with time throughout the depth of all the cascade wetland beds, which suggests increased adsorption of surfactants thereby increasing availability of hydrolysable carbon within the beds. This is in agreement with observations by Friedel *et al.* (1999) that organic constituents, such as surfactants, may be a source of easily degradable carbon substrate for many microbial populations. Availability of readily hydrolysable carbon stimulates growth and overall activity of micro organisms. Inputs of N and P via grey water application also do stimulate soil micro organisms but in the wetlands these are present in low concentrations. However microbial PLFAs results (discussed in Chapter 8), showed a decreasing trend in biomass across the system (*i.e.* from top bed to bottom bed). Therefore this increased activity probably relates to all other dormant microorganisms, which do not contribute to basal respiration. These dormant organisms may have become active as a result of increased carbon availability from the grey water's chemical constituents such as surfactants.

6.5.2 Impact of technology design on treatment performance

The shallow vertical depth and increased aeration in the cascade wetland contributed to the treatment performance of this system with regards to conventional parameters normally measured in sewage treatment (BOD, COD, TOC, and turbidity). Hence the objective of having a small-scale wetland technology to cater for space constrained areas was met. The role of plants has been questioned (Brix, 1999) because, as shown in chapter 6, wetland systems without vegetation have performed just as good. This shows that the major influencing parameters in treatment wetlands are influent concentration of the wastewater and the loading rates, as suggested recently in literature (Kadlec and Wallace, 2009). Several studies had concluded that treatment efficiency is only a function of the influent pollution load (Frazer-Williams *et al.*, 2008; Platzer, 1999) however this study has highlighted the impact of plants for removal of surfactants as observed in the removal percentages of surfactants in the planted systems. The increased microbial contribution in the planted systems was attributed to availability of additional surface that supports the micro-organisms. This therefore provides flexibility in designs depending on the need of the treated water (type of reuse), availability of space and other considerations.

Various studies on catabolic diversity in soils have shown that history (crop, pasture or forest) of a soil is significant. In treatment wetland terms, when comparing performance of a planted and an unplanted wetland, the same (history or length of existence) also is shown to be significant as observed in the performance of the VFPCW. Performances of the planted and unplanted cascade wetland were found to be similar to, but statistically different from the planted VFPCW. Statistical analysis showed that the difference was not due to wetland designs but plant regime. To illustrate, the VFPCW had the lowest BOD effluent levels (mean: < 1.0 mg/L) followed by the planted cascade wetland (mean: ≈ 1.0 mg/L) and lastly the unplanted cascade wetland (mean: > 1.0 mg/L). The root structure of the plants and the period that the plants have been in the wetlands was a significant factor in removal of surfactants. The *Phragmites australis* in the VFPCW have extensive root system and were in the VFPCW for the five years that it was in operation while the planted cascade wetland had only operated for less than 6 months.

All the beds in the cascade wetland were similar in terms of size and substrate media. However, as seen in the results above most of treatment was occurring in the top bed, this means the middle and bottom beds were operating below their capacities. Modifications to the design would be necessary by either allowing for periodic rotation of the beds so that biomass growth is controlled in the beds receiving higher strength grey water, or having two beds running in parallel (to cater for high influent rate) and the third bed in series.

6.6 CONCLUSION

The performances of the planted and unplanted cascade wetlands were similar and also comparable to a standard constructed wetland. Treated effluent from both systems met published wastewater reuse standards. Therefore the combination of using multiple shallow beds (arranged in a cascading series) and porous media (*e.g.* sand) does indeed improve treatment performance of such a design. The presence of vegetation in one did not significantly improve its performance with regards to removal of conventional analytes, other than the aesthetic beauty, from the *Iris* spp. and scent from the *Mentha aquatica.* Better performance in the planted wetlands was related more to increased microbial activity contribution from the plant as oppoed to direct addition of oxygen by the plants. The unplanted prototype cascade wetland also has the advantage that it can operate with or without covered beds with obvious advantages in terms of management for the covered version. Additionally the arrangement of beds can be altered to fit available space, which is advantageous for situations with space constraints. In terms of microbial processes, all the beds showed the capacity to handle different loading rates and organic strengths, through their similar catabolic (species') richness. Constant variations of organic strength, expected in raw grey water from residencies, would therefore not affect the catabolic functioning of the cascade wetland given that the organic strength is below 15 g BOD m^{-2} d^{-1}.

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6.8 SUPPORTING INFORMATION

Appendix 6.1: Cascade wetland potential plants

Plant	variations	H (cm)	Spread (cm)	Sun	Hardy	Scent	Growth	Toxic	pH	Root density	Notes
Shallow marginals											
Acorus gramineus	variegates	25	15	FS	**		SE, P				Grass like
Alisma	plantago-aquatica	75	45	FS	***		D, P				Pink/whiteflowers in summer
	var. parvisflorum										
Calla palustris		25	30	FS	***		D/E, P	Y			Large white spathes in spring followed by red/orange fruit
Caltha leptosepala	c.palustris	30	30	FS	***		D, P				White flowers in spring
Houttuynia cordata	H. cordata'Chameleon'	10	∞	PS	***		D, P				Small white flowers in summer
Lysichition americanus	L. camtschatcensis	75	60	FS	***		D, P				Pure white spathes in spring. RHS merit award
Mentha aquatica		90				Mint	P		acidic		Tiny pinkish to lilac flowers, appearing from mid-late summer
Menyanthes trifoliata	23	23	30	FS	***		D, P				White flowers in spring
Myosotis scorpioides	Myosotis scorpioides	15	30	FS	***		D, P				Small blue flowers throughout summer

Plant	variations	H (cm)	Spread (cm)	Sun	Hardy	Scent	Growth	Toxic	pH	Root density	Notes
	'Mermaid'										
Sagittaria latifolia	S. sagittifolia,	150	60	FS	***		D, P				White flowers in summer
Typha minima		45-60	30				D, ,P				Rust brown spikes, late summer followed by seed heads l
Zizana aqatica					-						
Deep marginals											
Acorus calamus 'Argenteostriatus'		75	60	FS	***	tangerine	SE, P				
Butomus umbellatus		100	45	FS	***		D, P				Pink/rose flowers in summer. Flowering rush
Saururus cernuus		23	30	FS	***		D, P				Swamp lily. Cream flowers in summer
Sparaganium erectum		100	60	FS	***		D-SE, P				Vigorous, Small green brown burs in summer
Iris pseudocurus		120	100	FS, PS	* (fully)		P		low pH		Has large lumps of green leaves and produces yellow flowers. It can become evasive. Phyto extracts heavy metals
Glyceria		75	75	FS	*				Acidic,		A vigorous,

Plant	variations	H (cm)	Spread (cm)	Sun	Hardy	Scent	Growth	Toxic	pH	Root density	Notes
variegates									alkaline		strongly variegated grass with green and cream striped foliage which is tinged pink when young
Bog and moisture loving plants											
Alnus glutinosa		20-30	12				D, P				Flowering, sticky, symbiotic relationship with *Frankia alni*, nitrogen fixing
	A. incana	30		PS			D, SE				
Anemone rivularis		30-60		PS, S	***		P		Acid, neutral and basic soils		Flowers with startling blue anthers, challenging germination, prefers light sandy to loamy soils, antiemetic and vermifuge
Aruncus dioicus 'Kneiffii'		91	60	FS, PS			P, E		neutral		Cream coloured flowers, not suited to hot and humid conditions, slow to establish
Astilbe x arendsii		100	90	FS,	***		P				Produces lush

Plant	**variations**	**H (cm)**	**Spread (cm)**	**Sun**	**Hardy**	**Scent**	**Growth**	**Toxic**	**pH**	**Root density**	**Notes**
				PS							filigree foliage and feathery spikes of lilac-pink flowers
	A. chinensis	25	20	FS, PS	***		P		5.5-7		Dwarf variety
	S. simplicifolia	40	40	S, PS, FS					acidic		Pale pink flowers, Requires cutting back after flowering, 2 – 5 to mature
Cardamine pratensis		20	30	FS, PS	***		P		Acidic to basic soils		Lilac pink flowers, soft coloured and dainty, family of Brassicaceae
Carex elata ‘Aurea’		70	50	FS, PS			P		acidic		Dwarf sedge, compact ornamental grass
	C. pendula			PS, FS	***		P, E			Acidic to alkaline	Grassy with red-brown flowers in summer
Cornus alba		300	300	FS	***						Beautiful variegated foliage. Flowers turn stunning shade of red before falling.
Darmera peltata		120	60	PS, S			P		Acidic to basic		Grows in sandy, loam or clay soils

Plant	variations	H (cm)	Spread (cm)	Sun	Hardy	Scent	Growth	Toxic	pH	Root density	Notes
									soils		
Eupatorium purpureum		250	100	FS	***		P		chalky alkaline shell		

Hardy: - not hardy, * tolerates down to 0°C, ** tolerates down to -5 °C, *** tolerates down to -15°C
Sun: FS full sun, PS partial shade, S shade
Growth: D deciduous, P Perennial. A annual, E evergreen, SE semi evergreen

Four species were chosen for the test rig (prototype) based on the following: -
Mentha Aquatica – for scent
Iris chrysographes (black flowered Siberian iris)
Iris pseudacorus – variegate (both for aesthetics, the flowers) and
carex elata – aurea – for structure

Appendix 6.2: Pair wise t-test for wetland treatment performances

Using one-tail *t*-test for independent samples to test if the planted prototype beds performed better than the unplanted prototype beds:

H_o: The mean concentrations for each parameter under consideration for corresponding beds are lower in the planted beds compared to the unplanted beds (*i.e.* $\mu_1 < \mu_2$ or $\mu_1 - \mu_2 < 0$), where Group 1 represents planted beds and Group 2 represents unplanted beds.

H_A: The planted beds perform no better than the unplanted beds (*i.e.* $\mu_1 > \mu_2$ or $\mu_1 = \mu_2$)

BOD removal comparisons for planted (Group 1) vs. Unplanted (Group 2) beds

Group comparisons	Mean Group 1	Mean Group 2	t-value	df	p	Std Dev Group 1	Std Dev Group 2	F-ratio variances	P Variances	Confidence -95.0%	Confidence +95.0%
Top beds	5.71	3.11	0.55	12	0.587	11.58	4.15	7.78	0.024	-7.53	12.72
Middle beds	1.87	1.18	0.57	12	0.575	2.79	1.51	3.39	0.163	-1.92	3.30
Bottom beds	0.88	1.32	-0.94	12	0.363	0.91	0.87	1.09	0.918	-1.48	0.57

$t_{0.05\ (1),\ 12} = 1.782$

We reject the null hypothesis in all three cases as the mean BOD effluent concentrations in the planted prototype are not significantly less than the mean effluent concentrations in the unplanted prototype.

COD removal comparisons for planted (Group 1) vs. Unplanted (Group 2) beds

Group comparisons	Mean Group 1	Mean Group 2	t-value	df	p	Std Dev Group 1	Std Dev Group 2	F-ratio variances	P Variances	Confidence -95.0%	Confidence +95.0%
Top beds	20.91	12.66	0.967	12	0.353	19.95	10.56	3.57	0.147	-10.34	26.84
Middle beds	8.28	6.71	0.542	12	0.597	5.56	5.23	1.13	0.855	-4.72	7.85
Bottom beds	7.21	6.46	0.355	12	0.729	4.00	3.90	1.05	0.954	-3.85	5.35

$t_{0.05\,(1),\,12} = 1.782$

We reject the null hypothesis in all three cases as the mean COD effluent concentrations in the planted prototype are not significantly less than the mean effluent concentrations in the unplanted prototype.

TOC removal comparisons for planted (Group 1) vs. Unplanted (Group 2) beds

Group comparisons	Mean Group 1	Mean Group 2	t-value	df	p	Std Dev Group 1	Std Dev Group 2	F-ratio variances	P Variances	Confidence -95.0%	Confidence +95.0%
Top beds	6.08	6.39	-0.294	6	0.779	1.841	1.103	2.79	0.423	-2.94	2.31
Middle beds	6.78	6.55	0.304	6	0.771	0.504	1.372	7.40	0.134	-1.57	2.01
Bottom beds	7.01	6.53	0.486	6	0.644	1.359	1.448	1.14	0.919	-1.95	2.91

$t_{0.05\,(1),\,6} = 1.943$

We reject the null hypothesis in all three cases as the mean TOC effluent concentrations in the planted prototype are not significantly less than the mean effluent concentrations in the unplanted prototype.

Points to note:

- BOD removal for the bottom planted bed was better than the bottom unplanted bed, though there were no significant difference in the performance
- TOC removal in the Top planted bed was better than in the Top unplanted bed, though again the performance was not significantly difference.

Appendix 6.3: Univariate results for one way ANOVA: Type of wetland technology and presence of absence of vegetation (No distinction was made between the types of vegetation)

The test were merely designed to test for differences between the there technologies in BOD removal throughout the monitoring period

Test hypotheses:
1. H_o: BOD effluent levels are the same in the wetland technologies (*i.e.* $\mu_{\text{mixed plants}} = \mu_{\text{unplanted}} = \mu_{P.\ australis}$).
2. H_o: BOD effluent levels are the lower (better performance) in the prototype technologies compared to the standard VF wetland (*i.e.* $\mu_{\text{prototype}} < \mu_{\text{Std VF}}$).
3. H_o: Differences in BOD effluent concentrations independent of the type of (*i.e.* are the same for different) type of technology or presence of vegetation, A x B interaction.

	Effect	*Degrees of freedom*	*BOD Effluent SS*	*BOD Effluent MS*	*Fcrit $_{0.05}$ (1)*	*Calculated F*	*P*	*Conclusion*
1.	Vegetation (factor A)	2	4.365	2.182	3.49	3.863	0.041	Reject H_o
2.	Wetland design (factor B)	1	0.013	0.013	4.35	0.023	0.880	Accept H_o
3.	A x B	0						
	Error	17	9.604	0.565				
	Total	20	13.982					

Note: 2-factor ANOVA showed that there were variations in the BOD effluent concentrations

Anova: Single Factor (Planted prototype vs Planted Std VF)

Factor: type of design because the unplanted and planted prototype performed the same

H_o: BOD effluent levels are the same in the wetland technologies (*i.e.* $\mu_{\text{planted prototype}} = \mu_{\text{planted VPFCW}}$).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Wetland type	14	21	1.5	0.269231
BOD Out	14	8.16	0.582857	0.528822

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit 0.05 (1)*	*Conclusion*
Between Groups	5.888057	1	5.888057	14.75606	0.000706	4.225201	Reject H_o
Within Groups	10.37469	26	0.399026				
Total	16.26274	27					

Conclusion: $\mu_1 \neq \mu_2$

Anova: Single Factor.

H_o: BOD effluent levels are the same in the wetland technologies (*i.e.* $\mu_{\text{unplanted prototype}} = \mu_{\textit{planted VFPCW}}$).

Groups	*Count*	*Sum*	*Average*	*Variance*
Wetland type	14	21	1.5	0.269231
BOD Out	14	11.3	0.807143	0.693376

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit 0.05 (1)*	*Conclusion*
Between Groups	3.360357	1	3.360357	6.981787	0.013758	4.225201	Reject H_o
Within Groups	12.51389	26	0.481303				
Total	15.87424	27					

Conclusion: $\mu_{\text{unplanted prototype}} \neq \mu_{\textit{planted VFPCW}}$)

Anova: Single Factor

H_o: BOD effluent levels are the same in the wetland technologies (*i.e.* $\mu_{\text{planted prototype}} = \mu_{\text{unplanted prototype}}$).

Groups	*Count*	*Sum*	*Average*	*Variance*
Vegetation	14	21	1.5	0.269231
BOD Out	14	15.42	1.101429	0.783152

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit 0.05 (1)*	*Conclusion*
Between Groups	1.112014	1	1.112014	2.113327	0.157987	4.225201	
Within Groups	13.68097	26	0.526191				Accept H_o
Total	14.79299	27					

Conclusion: $\mu_{\text{planted prototype}} = \mu_{\text{unplanted prototype}}$

We conclude that the main source of variation is the type of design, since the performance of prototype treatment technologies did not differ significant but when an ANOVA looking at mixed vegetation, single spp. and no vegetation showed that there were significant variations. Therefore these may only be attributed to the combination of design and mode of vegetation.

CHAPTER 7: MICROBIAL COMPOSITION IN THE WETLAND TREATMENT BEDS

CHARACTERISATION OF MICROBIAL COMPOSITION IN VERTICAL FLOW CONSTRUCTED WETLANDS: RELATIONSHIP TO WETLAND DESIGN AND AGE

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7.1 ABSTRACT

Microorganisms are responsible for the degradation of environmental pollutants in constructed wetlands. They take part in all the processes that are integral to attenuation of organic chemicals in wetland systems. Microbiological structure and dynamics is crucial to the understanding of processes that govern treatment in wetland systems. Further understanding of their compositions and dynamics is necessary in order to elicudate their roles in the processes in the wetlands. This would help to improve the design and operation of wetland systems for various wastewater treatment needs. This study set out to understand the influence of wetland microbial community structure on grey water treatment performance in constructed wetland systems. Phospholipid fatty acid profiling, PLFA, was used to determine microbial composition and diversity in the pilot wetland beds and also for temporal variations of individual wetland beds receiving grey water of different strengths. The study showed that the shallow and porous unplanted wetland beds allowed ample oxygen penetration and maintained the right balance of bacterial and fungal communities needed to achieve sustainability of wetland treatment performance for grey water.

7.2 INTRODUCTION

Wetlands provide suitable environmental conditions for growth and reproduction of microbial organisms. Two important groups of these microbial communities are bacteria and fungi. Bacteria and fungi are typically the first to colonise and begin the sequential decomposition of solids and also have the first access to dissolved constituents in wastewaters (Zhang *et al.*, 2010; Calheiros *et al.*, 2009; Ibekwe *et al.*, 2003; Gaur *et al.*, 1992). Their genetic and functional responses mediate physical, chemical and biological transformation of pollutants, which can be managed in engineered environments (*e.g.* constructed wetlands), to achieve desired transformations in wastewater treatment. Microbial metabolism depends, among others, on environmental conditions such as temperature, dissolved oxygen (DO), pH, and concentration of the chemical substrate undergoing transformation. The study of diversity, distribution, function and behaviour of microbial communities in treatment wetland media is essential for a broad understanding of treatment wetland media ecology.

Phospholipid Fatty Acids (PLFAs) are essential structural components of living cell membranes which have been used to assess microbial biomass, community composition and physiological status in environmental samples. Fatty acid (FA) profiling is an analytical method that has become popular for determining microbial community structure in soils. Changes in microbial communities in soils media under different systems, environmental conditions and operation regimes occur rapidly (Buyer and Kaufman, 1997; Tunlid and White, 1992) which makes microorganisms an efficient indicator of rapidly changing environmental conditions (Drenovsky *et al.*, 2004). PLFAs represent viable microbial community because they rapidly decompose after microorganism death. Therefore, PLFA extracts, fatty acid methyl esters (FAMEs) produced after methylation of the phospholipids fraction can be used to characterise spatial and temporal changes to microbial community structure in any environment. Numerous studies have been carried out in soils treated with sewage sludge and therefore containing different levels and types of chemicals that ‘stress’ the micro-organisms in the soils and also soil under different management regimes. In stressed environments, the conversion of substrate Carbon to biomass is less efficient. This has been explained in terms of either differences in size of biomass or in the microbial community structure (Chander and Brookes, 1991) or influence of the xenobiotics present in the substance being introduced. PLFA analysis therefore helps to detect changes occurring to the overall microbial community which are then linked to the level or type of stress that a system might be facing. In terms of wetland treatment, PLFA analysis would provide valuable information on how the influent strength of the wastewater affects the microbial community structure in the wetland media and probably treatment performance. This use of PLFA analysis in treatment wetland studies has not been fully utilised to provide information about microbial levels and structure in wetlands but it can be very useful to achieve good and sustainable wetland treatment performance. Phospholipid fatty acids decompose rapidly on cell death (through hydrolysis of the phosphate group by cellular enzymes), and this gives the assurance that only the living cells in the sample media were studied.

Removal of organic chemicals in wetland systems is largely attributed to microbial action (Reddy and D'Angelo, 1997) but the impact of organic strength and the type of wastewater on the microbial population has not been extensively studied. It is necessary to understand microbial degradation pathways in constructed wetlands and quantify organic chemical degradation potentials (Imfeld *et al.*, 2009). Knowledge of the microbial structure and dynamics in the wetland systems can improve our understanding of the microbial contributions to the treatment processes in wetland systems. The variation in composition of microbial (Bossio *et al.*, 1998) and non-microbial (Jandl *et al.*, 2005) lipids with vegetation, soil management and amount of plant biomass input (Drenovsky *et al.*, 2004) has been well established. Using this background knowledge, the hypothesis that “for constructed wetlands, media management, and hydraulic operation of the system would result in different PLFA profiles which can then be used to explain the processes in the constructed wetland beds (planted and unplanted)” was formulated for this study. Hence, the PLFA information obtained from each bed would help to determine the status of bioremediation of the grey water as it passed through the treatment wetland and using the different ratios (*e.g.* Fungi to total bacteria or gram negative to gram positive bacteria) also to monitor changes in aerobic/anaerobic conditions throughout the system as shown in literature (Guckert *et al.*, 1986). So, the objective of this paper was to compare microbial structure for the cascade wetland beds as well as looking at temporal changes for each individual bed and relate these to treatment performance of the wetlands.

7.3 MATERIALS AND METHODS

Temporal and spatial characterisation of microbial composition of the cascade wetland beds was undertaken. The focus was on understanding the factors that lead to consistency in the levels of microorganisms, which could then be linked to performance of the wetlands. The main objective was to establish how the levels change with changing biotic and abiotic factors. The secondary objective was to know if there are any significant changes in microbial physiological profile in shallow beds, which are related to overall performance of the wetland beds.

7.3.1 Pilot treatment wetlands

The pilot technologies were a cascade wetland rig (the prototype; WPL, UK) comprising three shallow beds (0.6 m length by 0.45 m width, 0.20 m depth) and a single bed reactor (Vertical flow Planted Constructed Wetland: VFPCW, 6 m^2 surface area and 0.7 m deep). Two 'versions' of the cascade wetland were used, as described before, one was planted with different marginal bog plants, *Mentha aquatica*, *Iris pseudacorus*, *Iris chrysophages* and *Carex elata Aurea*. The second version was operated with covers and no plants, consequently running as a covered constructed sand filter (Figure 5.1: Chapter 5). Sand media in the range 0.15 – 4.0 mm (d_{10} of 1.0 – 2.0 mm and d_{90} of 4.0 – 8.0 mm) was used in the cascade wetland. The sand was stratified for in the top 16 cm of the beds (following observations from the bench-scale study, Chapter 4) with the smallest grains at the top and a 2 cm gravel layer (8 – 10 mm) at the very bottom. The VFPCW was planted with *Phragmites australis* and contained a mixture of sand, soil and organic matter as media. The planted cascade wetland beds were inoculated with acclimated media (25 % v/v) from the corresponding unplanted cascade wetland beds and run for four weeks before planting.

The covered cascade wetland was set-up in the same way with the covers put in place at the time the planted cascade wetland was being planted.

7.3.2 Microbial analysis

7.3.2.1 Sampling

Sampling for PLFA analysis started after the development of the filter-media in the cascade wetlands was deemed to have reached optimum level of performance judging from the conventional effluent water quality results. Wetland media samples were collected from the top two strata; 1 – 5 cm and 5 – 10 cm for the cascade wetland beds while as for the VFPCW, sampling was only done for the top 1 – 5 cm layer. One sampling episode collected samples from the full depth of the cascade wetland beds, 1 – 5 cm, 5 – 10 cm, 10 – 16 cm and 16 – 20 cm for the top bed in order to capture greater details of the microbial profiles and possible changes with depth. Samples were collected from four positions within each layer of the treatment beds and then bulked and homogenised to form a composite sample. The samples were stored at -80 ^{o}C for 24 hours before being freeze dried and stored at -20 ^{o}C until ready for analysis. On the day of analysis, a small sample (5 g), obtained by corning and quartering for analysis.

7.3.2.2 Microbial community structure

The PLFA profiles were determined using a modification of the method described by Frostegård *et al.* (1991), based on the modifications described by Bligh and Dyer (1959) and White *et al.* (1979). The PLFA technique analyses for ester-linked fatty acids methyl esters (EL-FAMEs), which are separated by the capillary gas chromatography. The different retention times of the resulting fatty acid methyl esters depend on the length of the fatty acid chain. These were used to identify the main PLFAs in the wetland media samples and their relative concentrations (measured against standard mixtures of known PLFAs (SUPELCO) of each PLFA. All the solvents and chemicals used were of analytical-HPLC grade. PLFA concentrations (expressed as Mol %) were calculated as the sum of all identifiable and unidentifiable PLFAs.

An Agilent Technology G.C. 6890N with Agilent G2070 ChemStation software for G.C. systems was used for the PLFA analyses. The GC was fitted with a split/splitless injector and a HP-5 (Agilent Technologies) capillary column (30 m length, 0.32 mm ID, 0.25 µm film) which is 5% phenylmethyl siloxane to separate the PLFAs. Helium was used as the carrier gas (1 mL per min) and the FAMEs separated by using a temperature programme. FAMEs were detected using a FID operating at 320^{o}C. The microbial community structure was characterised according to the following PLFAs:

1) Ester-linked branched chain fatty acids, indicative of Gram-positive bacteria: *i*15:0, *a*15:0, *i*16:0, *i*17:0and *ai*17:0 (O'Leary and Wilkinson, 1988).
2) Ester-linked monounsaturated fatty acids, such as, 16:1ω5, 16:1ω7, 18:1ω9c (O'Leary and Wilkinson, 1988) and 18:1ω9t and ester-linked hydroxy fatty acids *e.g.* 2-OH 16:0, and ester linked cyclo propane fatty acids *e.g. cyc* 17:0 (Zeller *et al.*, 2001), all indicative of Gram-negative bacteria.
3) Ester-linked polyunsaturated 18:2ω6 and 18:2ω9, were used as an indicators of fungal biomass (Bååth and Anderson, 2003), which were reported as fungal biomarker as well (Olsson, 1999).

7.3.3 Statistical analysis

PLFA concentrations were calculated, as mole percentages (mol %) expressed in terms of the total PLFA weights. Similarly the estimated concentrations of Gram-positive, Gram-negative bacterial and fungal PLFAs were also calculated as their percentages and expressed as described above. Individual PLFAs were subjected to PCA analysis to elucidate major variations and covariate patterns within the wetland beds. Differences in the concentration between and within beds (at different depths/layers) were compared by analysis of variance (ANOVA). For each of these analyses, the statistical package, StatSoft. Inc. (2009) STATISTICA (Data Analysis software system) version 9.0 was used. All the beds in the study (the three cascade wetland beds and the VFPCW bed) were be taken as different treatments and compared against each other. The PLFA profiles of the media from the treatment beds were thus used to provide information on microbial community composition and relative species abundance as well are changes due to different factors.

7.4 RESULTS

Only peaks in the region between tetradecanoic methyl ester (14:0) and arachidonic acid 20:0 eicosanoate were included in the analysis although longer chain PLFAs > C_{20} (20:0) were also obtained. This range contains the main ester-linked FAMEs used as microbial biomarkers but excludes several shorter and longer chain FAMEs reportedly associated to plants and organic matter in soil (Frostegard *et al.*, 1993). Higher PLFA concentrations (> 1.0 %) were only observed for peaks corresponding carbon chain lengths between C_{14} (14:0) and C_{20} (20:0).

PLFA patterns in the top 0 – 5 cm of the wetland beds

Both the cascade wetland and the standard vertical flow planted constructed wetland (VFPCW) contained a variety of saturated and monounsaturated and polyunsaturated PLFA's. A total of 39 PLFA's with chain lengths up to C_{20} were detected by GC-FID. The peaks were identified on the basis of comparison to peaks obtained after running the standard of known PLFA's (SUPELCO) of each PLFA. As there are no specific references in literature to microbial studies (PLFA) in sand filters and wetland media, the findings in this study were compared to soil studies that used PLFA analysis to explain the effects of various biotic and abiotic factors affecting micro-organisms in subsurface wetland media.

The composition of PLFAs in the cascade wetland was different compared to the standard vertical flow wetland (VFPCW). Total bacterial PLFA proportions were generally higher in the cascade wetland beds than the VFPCW. As expected, the trend in total bacterial PLFAs proportions for the cascade wetland was as follows: top bed > middle bed > bottom bed. The VFPCW PLFA levels were somewhere between the middle and bottom beds PLFA levels with the proportions of the saturated PLFAs, representing presence of gram-positive bacteria showing the greatest fluctuations throughout the monitoring period (Figure 7.1). The changes in PLFA for gram-positive bacteria, interestingly, followed a similar pattern in all the beds of the unplanted cascade wetland. This may be explained in terms of influence of similar factors, most probably abiotic, as the patterns changed correspondingly with changes in environmental conditions. Hence the environmental conditions were a major factor affecting the concentration of gram-positive bacteria in the beds. On the other hand, there was higher consistency (less variation) for gram-negative bacteria in the top bed of the unplanted cascade wetland and the VFPCW. The middle and bottom beds of the unplanted cascade wetland showed increasing gram-negative concentration throughout the monitoring period. Fungi proportions (measured as polyunsaturated PLFA, 18:2ω6) were considerably low (< 0.70 mol %) in all the wetland beds throughout the monitoring period. These levels were also lower compared to levels reported for forest soils (4.65 mol %) (Frostegård *et al.*, 1993).

A sharp increase in the gram-positive PLFA concentration was observed in the planted cascade wetland top bed, between weeks 64 and 69 of the study (Figure 7.1). This occurred when plant growth was rapid in the top bed. The growth rate of the plants in the top bed was very fast, evidenced by the *Iris chrysophages* flowering a month before the same occurred in the middle and bottom beds. The spread of the *Mentha aquatica* was also very fast such that pruning in the top bed was being done fortnightly while no pruning was done in the middle and bottom beds at any time during the study. *Mentha aquatica* encroached on every available space in the top bed which made it unsuitable for that bed despite producing a good scent in the vicinity of the treatment wetlands.

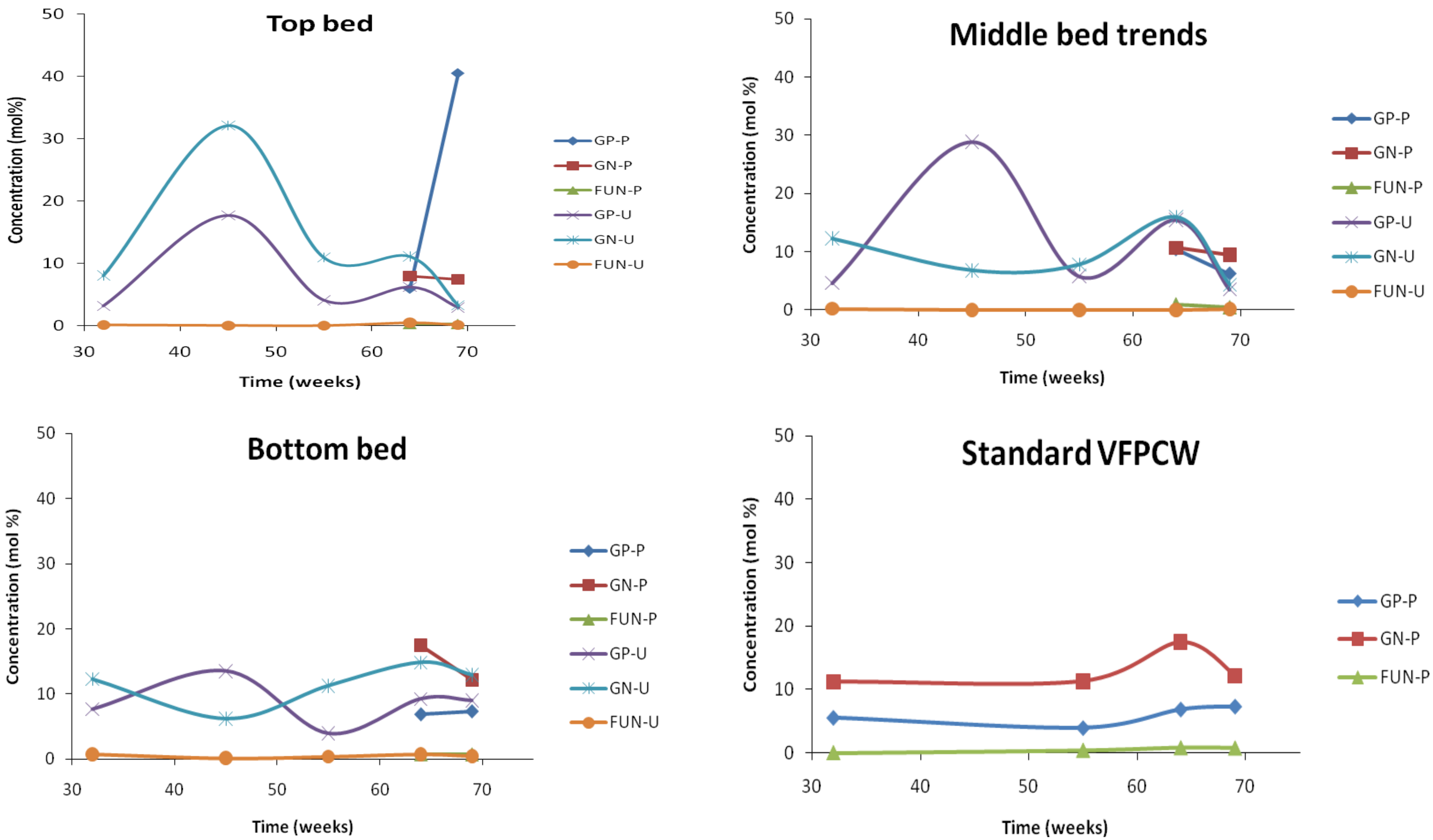

Figure 7.1: General trend of PLFAs in top 0-5 cm of the cascade wetland beds and the VFPCW
(Note: GP = gram-positive, GN = gram-negative, FUN = fungi, P = planted bed, U = unplanted bed)

The PLFA variations shown by the PCA loading plots for all the beds (Figure 7.2) indicate that gram-positive PLFAs (16:0 2-OH, 18:0 (isomer), 20:0 and Me17:0) dominated factor loadings for the first principal component. The highest levels in the unplanted (open beds) were observed after 30 weeks and for the unplanted (covered beds) and planted cascade wetlands after 64 weeks of operation. The lowest levels were seen after 45 weeks, which was during the winter season of February 2009. The trend was the exact opposite for the 18:0 (isomer), which showed the highest levels after 45 weeks. Interestingly, week 30 was in November 2008, when the temperatures in the study area were in descent as conditions changed from autumn to winter conditions (< 15^{o}C); week 45 fell in July 2009, when warmer summer weather conditions were settling in.

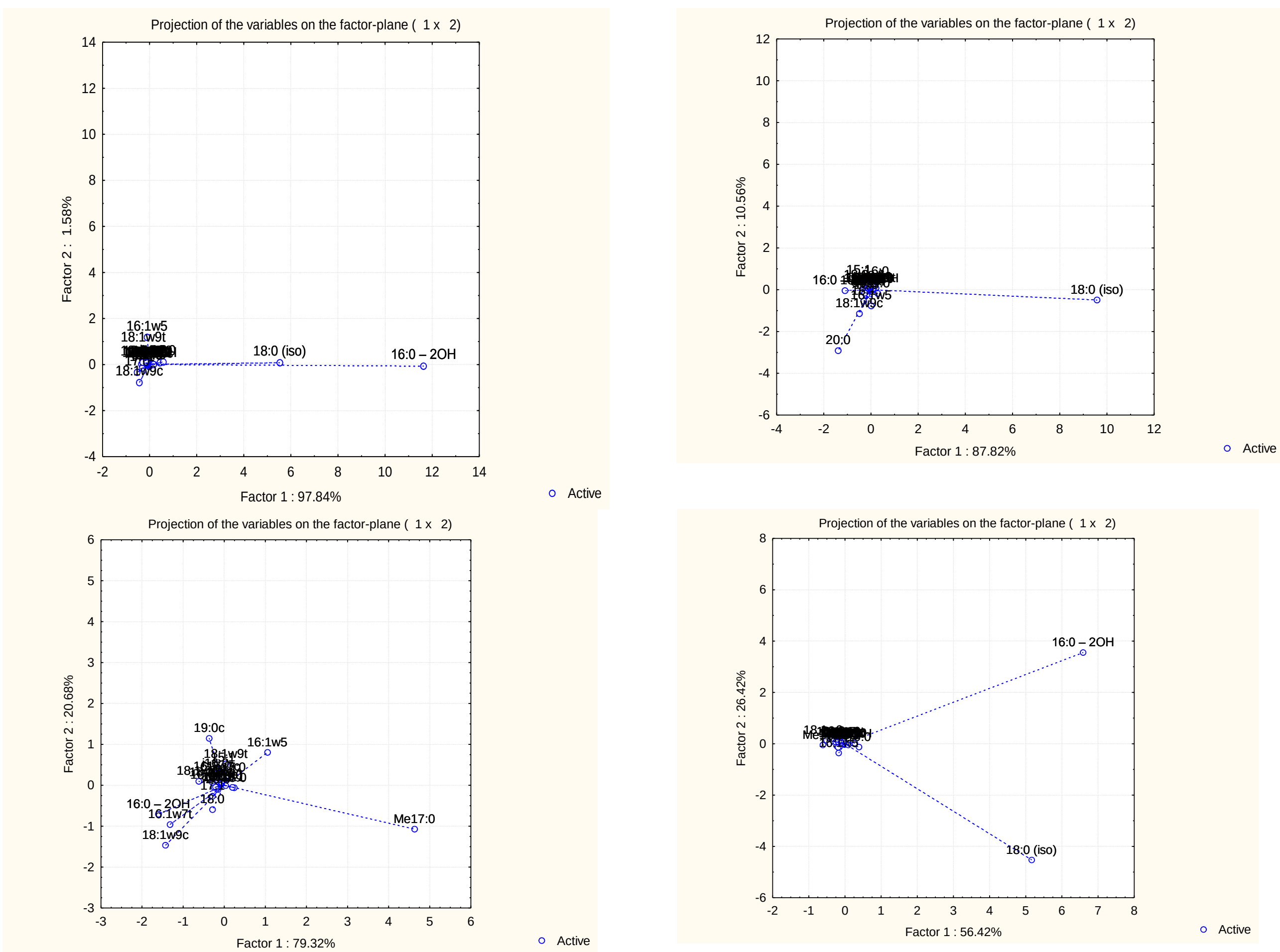

Figure 7.2: Scatter diagrams of PCA showing variation in PLFA pattern in top 0- 5 cm layers from the wetland media samples in all the beds (top left), top bed (top right), middle bed (bottom left) and bottom bed (bottom right)

The changes in PLFA patterns in both wetland technologies were dependent only on the type of species ($P = 0.000$). Presence or absence of vegetation and type of bed (whether a cascade wetland bed or the VFPCW bed) did not significantly affect the species' concentration in the top layers (0 – 5 cm) throughout the study period. Further analysis of the species' effects showed the significant differences were between the bacterial and fungi PLFA concentrations, bacterial PLFA concentrations being much higher than fungal concentrations.

PLFA patterns down the depth of the cascade wetland beds

PLFA analysis was carried out for samples from different layers of the cascade wetland beds (the unplanted [open-bed], the unplanted [covered-bed] and the planted beds). The general trend for all the beds shows a slight decrease in total bacterial PLFAs (from approximately 12 – 15 mol % to 8 – 10 mol %) up to a depth of 5 cm, followed by an increase with depth (to ≈ 25 mol % microbial abundance) (Figures 7.3 and 7.4). Gram-negative bacteria PLFAs increased (with depth) more than the gram-positive PLFAs. For the unplanted cascade wetland, analysis showed significant differences in the species' concentrations for different types of species ($P = 0.001$) (overall) and between beds ($P < 0.05$). However, analysis for individual beds (within bed analysis) showed no significant differences ($0.05 < P < 0.24$) for different layers within the same bed. The planted cascade wetland also showed no significant differences in specific species' concentrations within beds ($0.05 < P < 0.38$). The fungi biomarker abundance was very low except for the one case, middle unplanted cascade wetland bed (Figure 7.4). However, the concentrations were different between types of species *i.e.* fungi and bacteria ($P < 0.001$).

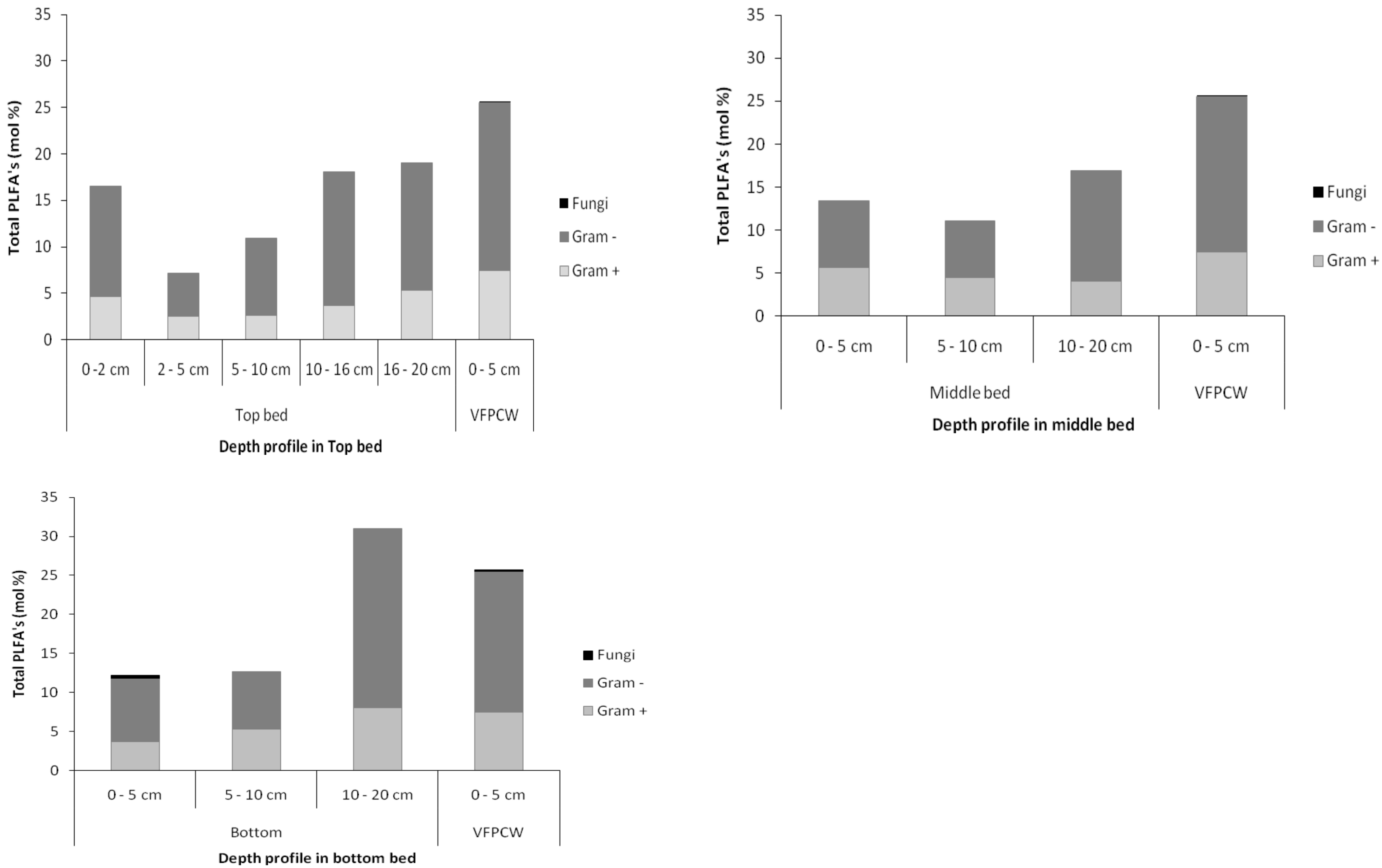

Figure 7.3: Microbial concentration profile by depth in the open bed cascade wetland and standard vertical flow constructed technologies

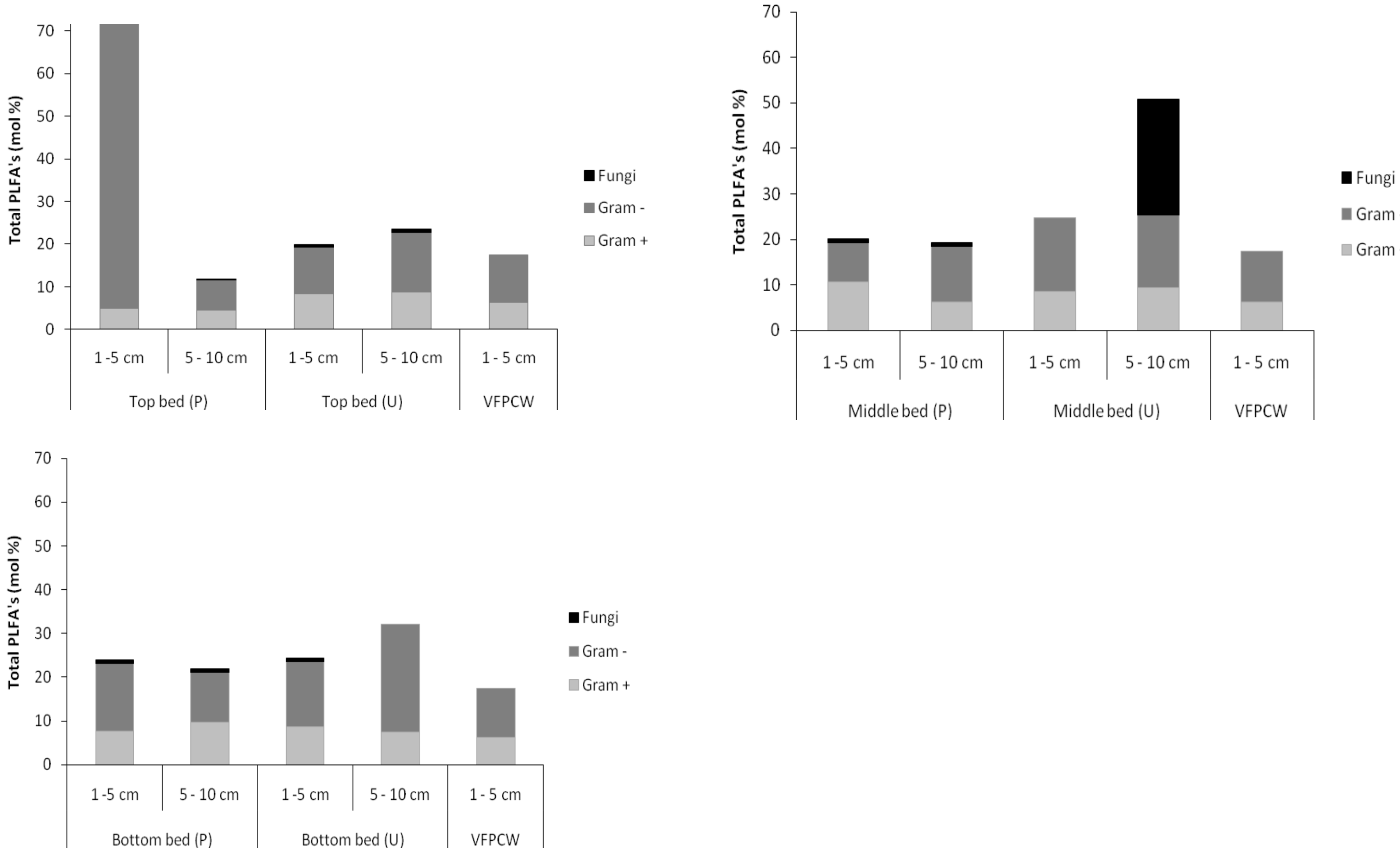

Figure 7.4: Microbial concentration profile by depth in the planted and covered cascade wetland and standard vertical flow constructed technologies

The fungal:bacterial biomass ratios ($F{:}_{tot}B$) in the upper layers of all the treatment beds (0.0 – 0.05) in this study (Table 7.1) were generally lower than for soil (0.06 - 0.17) as reported by Bardgett *et al.* (1996). However slightly higher $F{:}_{tot}B$ ratios were obtained for the middle and bottom beds of the cascade wetland than in the top beds, which shows that the top beds were under stress probably due to the high and varying levels of xenobiotics in the influent raw grey water. Increase in $F{:}_{tot}B$ ratio was observed in the lower (latter) beds (direction of flow through the treatment system). As the wastewater passess through serial beds nitrogen mineralization increases This may be associated with the increase in the $F{:}_{tot}B$ ratio.. During the period when temperatures were generally higher (20^{o}C), fungal PLFAs were also marginally higher in the planted wetland systems than before (Figure 7.4) probably due to occurrence of some plant decay in the wetlands as litter turnover was taking place. The unusually high fungal PLFA level in the unplanted middle is difficult to explain as the unplanted cascade wetland beds were covered by this time. However, two weeks prior sampling, the covers were removed from the beds for routine maintenance (when the piping system and valves were cleaned and replaced) but were accidentally not put back until after a week. A possible explanation may be that there was a build-up of plant litter (falling from surrounding trees at the pilot study site) resulting in high C/N ratio (Bardgett *et al.*, 1996) and due to insufficient grey water treatment, which probably provided poor substrate for microbial growth (Swift *et al.*, 1979).

Table 7.1: Ratios of fungi to bacteria and gram negative to gram positive bacteria in the wetland beds

Relationship	Type of bed	Bed depth	Top bed	Middle bed	Bottom bed	VFPCW
F:$_{tot}$B	Planted	Upper layer (1 - 5 cm)	0.004	0.049	0.034	0.000
		Middle layer (5 – 10 cm)	0.026	0.044	0.040	
	Unplanted	Upper layer (1 - 5 cm)	0.027	0.000	0.032	
		Middle layer (5 – 10 cm)	0.030	1.000	0.000	
G^-:G^+	Planted	Upper layer (1 - 5 cm)	13.431	0.793	2.006	1.798
		Middle layer (5 – 10 cm)	1.602	1.943	1.186	
	Unplanted	Upper layer (1 - 5 cm)	1.343	1.853	1.706	
		Middle layer (5 – 10 cm)	1.601	1.669	3.316	

Principal component analysis showed that the all variations in the VFPCW were explained by the first principal component, while in the bottom beds more than 90% of the variation was explained by two factors and in the top and middle beds, variations were explained by more than two factors. After a baseline monitoring period of 16 months (64 weeks), the microbial concentration variations in all the cascade wetland beds were decreasing and approaching steady concentrations, as shown by the general diminishing microbial levels in Figure 7.1. The VFPCW, which was in its 5th year of operation, exhibited fewer variations in the microbial levels throughout the monitoring period. This is in line with observations by Langergraber *et al.* (2003) that biomass concentration stays fairly constant in constructed wetlands for wastewater treatment, once steady state condition is reached. Hence the (major) factor explaining the variations in the VFPCW may have been the age of the media, with respect to period after reaching steady-state condition. This is also confirmed by the increasing microbial basal activity and species richness in the cascading wetlands, with time, in the deeper layers (Chapters 5 and 6). The hydraulic loading rate was not changed for the VFPCW, but stochastic fluctuations in organic strength of the influent grey water and changes in environmental conditions did not affect the microbial levels in the VFPCW.

7.5 DISCUSSION

PLFAs of lower chain length (< C_{20}) observed in this study suggest that the majority of the PLFAs in the wetland media were from the contribution of micro organisms (within the media) as opposed to plant organic matter (Jandl *et al.*, 2002). Bacteria dominance in the treatment wetlands over fungal species concentrations in all the wetlands was obvious. The pattern of bacteria PLFAs in all the surface layers (0 – 5 cm) cascade wetland beds showed peaks and troughs at same points in the time-axis, which clearly confirms the influence of abiotic factors, most likely the prevailing environmental conditions. These trends were not seen in the VFPCW. This suggests either acclimation of the microorganisms or that the presence of non-marginal plants may have contributed significantly to the consistent microbial PLFA levels in the VFPCW by buffering the microorganism from constant changing weather conditions.

Microbial growth and activity were dependant on the influent grey water quality (Schimel, 1995) and changed correspondingly with pH (*i.e.* resource quality decreased while pH increased from 6.9 – 7.8) as treatment occurred. This correlated with the changes in the microbial levels especially for bacteria as shown by the differences in PLFA levels between beds. This is confirmed by the different levels across the system from top bed to bottom bed, whereby the bottom bed, especially, has very low levels of microorganisms. With pH of the wetland system being circum-neutral, fungi PLFAs were expected to be very low in all the treatment beds, because presence of fungi becomes significant in conditions of low pH (and low resource quality) (Bardgett *et al.*, 1996). These characteristics and their relation to microbial communities showed the evolution of media characteristics and the importance of organic (loading) strength in relation to performance and microbial dynamics of the wetland treatment systems. This provides evidence that increased organic strength (resource quality) of the influent grey water increases the dominance of the gram-positive bacteria (and indeed the bacteria community) in the receiving beds. Bacteria respond to changing conditions and stress in ecosystems through slow growth as they (especially gram-positive) adapt to metabolising complex carbon substrates (Waldrop *et al.*, 2000). Gram-negative bacteria on the other hand are affected by pH increase, which leads to disruption of their cytoplasmic membranes and eventual death (Avery *et al.*, 2009). Presence/absence of plants determines physicochemical characteristics, such as texture, pH, and nutrient content, and may possibly have had an indirect effect on treatment performance. Climate is also one of the main factors influencing microbial properties and nutrient dynamics as shown in soil microorganism studies (Wardle, 1992). However, the direct effect of climate (temperature) on bacterial organisms is low (Kadlec and Reddy, 2001), and also varies depending on other aspects of the wetland system. To illustrate, the VFPCW was uniquely different from the cascade wetlands in that it had non-marginal plants (*Phragmites australis*) which grow 2 - 6 metres tall and has leaves 20 – 50 cm broad, which provided insulation to the media. This was not the case in the cascade wetlands, where there was little or no insulation. Microbial mediated reactions are affected by temperature, with much greater changes (reduced impact) typically at lower temperatures (<15 ^{o}C) compared to higher temperatures (20 – 35 ^{o}C) (Kadlec and Reddy, 2001). Bacteria are also known to alter their membrane lipids in response to the temperature stress (Tunlid and White, 1992), which may indirectly affect their metabolism and hence the overall treatment performance of a wetland system.

The low ratios of the fungal PLFA to the sum of the abundance of bacterial PLFA ($F:_{tot}B$) obtained in this study (Table 7.1) coincided with periods of low TOC removal (as indicated by the higher residue TOC in the treated water) (Chapter 5 and 6), suggesting a relationship between high effluent TOC (*i.e.* low removal), low fungal activity and level of stress. The observed changes in $F:_{tot}B$ ratios provide evidence on how efficient microorganisms are as indicators of rapidly changing environmental conditions in the wetland ecosystem and the importance of resource quality (organic strength or loading rates) in ensuring a sustainable wetland system (Drenovsky *et al.*, 2004; Nielsen *et al.*, 2002). The $F:_{tot}B$ is used to estimate the fungal:bacterial ($F:_{tot}B$) biomass ratio. The ratio also describes the spatial variation of the type of microbial community and monitor recovery of media from intensive management (Zeller *et al.*, 2001). Studies by Zeller *et al.* (2001) also showed that less stressed soil systems (less intensively or unmanaged treatments) have higher mineral N concentration.

Acclimation and history (long operation time) of a ‘planted wetland ecosystem’ is a factor that controls the extent of microbial shifts as observed in the VFPCW and this also explains the decreasing levels of microbial organisms in the cascade wetland which had been in operation for less than a year. The shift in PLFA patterns from gram-negative to gram-positive indicate a changing physico-chemical environment in the cascade wetland beds probably as a result of release chemical species, presumably sodium and ammonium ions, from the long surfactant chains in the grey water. The increase of pH, also evident in the cascade wetland, as the grey water progressed through the system, also affects the ratio of gram negative-to-gram positive PLFAs. The slightly lower pH of the planted cascade wetland effluent (compared to the unplanted) is influenced by the presence of vegetation (Aciego Pietri and Brookes, 2009; Cotter and Hill, 2003; Grayston *et al.*, 2004).

7.6 CONCLUSIONS

This study has shown that the biological degradation (microbial levels) within a bed depends on the type of organisms in relation to resource quality, pH and abiotic factors as well as presence of vegetation and number of treatment beds in the wetland system. The acclimatisation status and condition of the treatment beds for removal of pollutants was shown to be related to the levels and ratio of bacterial and fungal PLFAs. The study also confirmed that decomposition channels are bacteria-based in ecosystems that are ‘stressed’ as a result of high levels of xenobiotics in the influent wastewater (higher organic strength). These observations provide evidence on how efficient microorganisms are as an indicator of rapidly changing environmental conditions in the wetland beds and the importance of influent grey water organic strength and loading rates in ensuring a sustainable and efficient wetland system.

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7.8 SUPPORTING INFORMATION

Appendix 7.1: Univariate results for 3 factorial analysis: Species, bed type and vegetation

Test hypotheses:

1. H_o: Mean abundance levels (concentration) are the same in all the beds (*i.e.* $\mu_{top} = \mu_{middle} = \mu_{bottom} = \mu_{VFPCW}$)
2. H_o: Mean abundance levels are the same all types of beds (*i.e.* $\mu_{planted} = \mu_{unplanted}$).
3. H_o: Mean abundance levels (concentration) are the same for all species (*i.e.* $\mu_{GP} = \mu_{GN} = \mu_{FUN}$)
4. H_o: Differences in species levels among the beds are independent of the type of bed (planted or unplanted), A x B interaction.
5. H_o: Differences in mean species concentrations among the beds are independent of the type of species, A x C interaction.
6. H_o: Differences in mean species concentrations in the different bed types (planted or unplanted) are independent of the type of species, B x C interaction.
7. H_o: Difference in mean species concentrations in the different beds are independent of the other two factors, A x B x C interaction.

	Effect	*Degrees of freedom*	*Concentration SS*	*Concentration MS*	*Fcrit* $_{0.05}$ *(1)*	*Calculated F*	*P*	*Conclusion*
1.	Bed type (factor A)	3	13.898	4.633	2.79	0.1299	0.942	Accept H_o
2.	Vegetation (factor B)	1	18.555	18.555	4.03	0.5204	0.474	Accept H_o
3.	Species type (factor C)	2	1822.766	911.383	3.18	25.5608	0.000	Reject H_o
4.	A x B	2	42.198	21.099	3.18	0.5917	0.557	Accept H_o
5.	A x C	6	129.517	21.586	2.29	0.6054	0.725	Accept H_o
6.	B x C	2	43.592	21.796	3.18	0.6113	0.546	Accept H_o
7.	A x B x C	4	350.766	87.691	2.56	2.4594	0.056	Accept H_o
	Error	54	1925.397	35.656				
	Total	74	4346.686					

Note: There are no critical values for $\text{U}_2 = 54$, so the values for the next lower DF, $\text{U}_2 = 50$, were utilized.

We conclude that species levels are not the same for the different types of species present (*i.e.* abundance level depend of type of species and not bed type of whether bed is planted or not). Hence the role that each species plays in organic carbon degradation is what determines the levels.

Appendix 7.2: Univariate results for 2 factorial analysis: Species, bed depth in the unplanted prototype (No distinction was between the different beds)

Test hypotheses:

4. H_o: Mean abundance levels are the same all types of bed depths.
5. H_o: Mean abundance levels (concentration) are the same for all species (*i.e.* $\mu_{GP} = \mu_{GN} = \mu_{FUN}$)
6. H_o: Differences in mean species concentrations at different bed depths are independent of the type of (*i.e.* are the same for different) species, A x B interaction.

	Effect	*Degrees of freedom*	*Concentration SS*	*Concentration MS*	*Fcrit $_{0.05}$ (1)*	*Calculated F*	*P*	*Conclusion*
4.	Bed depth (factor A)	2	54.21	27.10	3.55	3.71	0.045	Reject H_o
5.	Species type (factor B)	2	611.11	305.56	3.55	41.85	0.000	Reject H_o
6.	A x B	4	81.59	20.40	2.93	2.79	0.057	Accept H_o
	Error		131.4329	7.3018				
	Total		878.3429					

We conclude that the species concentrations are different at different bed depths in all the beds and that they are also different depending on the type of species. However, in the species' concentration among the different depths do not depend on the type of species (*i.e.* GP, GN or FUN).

Appendix 7.3: Single factor ANOVA for total bacterial PLFA in the top bed unplanted prototype at different depths

Anova: Single Factor

*H*o: The mean concentration of total bacterial PLFAs is the same at all the layers (depths down the bed profile) *i.e.* $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Layer 1: 0 – 2 cm	3	43.49	14.497	32.532
Layer 2: 2 - 5 cm	3	24.1	8.0333	0.8170
Layer 3: 5 – 10 cm	3	44.04	14.68	54.571
Layer 4: 10 – 16 cm	3	49.27	16.423	19.891
Layer 5: 16 – 20 cm	3	49.75	16.583	3.2244

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Conclusion*
Between Groups	146.5409	4	36.6352	1.6497	0.2371	3.4781	Accept H_o
Within Groups	222.0697	10	22.2070				
Total	368.6105	14					

We conclude that the mean total bacterial PLFAs are the same at all layers, from top to bottom, in the top bed.

Appendix 7.4: Univariate results for 3 factorial analysis: Species, bed depth and wetland regime (*i.e.* planted or unplanted) (No distinction was between the different beds)

Test hypotheses:

1. H_o: Mean abundance levels (concentration) are the same in all the bed depths (*i.e.* $\mu_{1\text{-}5cm} = \mu_{5\text{-}10cm}$)
2. H_o: Mean abundance levels (concentration) are the same for all species (*i.e.* $\mu_{GP} = \mu_{GN} = \mu_{FUN}$)
3. H_o: Mean abundance levels (concentration) are the same all types of beds (*i.e.* $\mu_{planted} = \mu_{unplanted}$).
4. H_o: Differences in species concentrations among the different layers (depths) are independent of the type of species, A x B interaction.
5. H_o: Differences in mean species concentrations among the different layers (depths) are independent of the bed type (planted or unplanted), A x C interaction.
6. H_o: Differences in mean species concentrations in the different bed types (planted or unplanted) are independent of the type of species, B x C interaction.
7. H_o: Difference in mean species concentrations in the different layers (depths) are independent of the other two factors, A x B x C interaction.

	Effect	Degrees of freedom	Concentration SS	Concentration MS	*F*crit $_{0.05}$ (1)	Calculated *F*	P	Conclusion
1.	Bed depth (factor A)	1	70.17	70.17	4.26	0.78	0.385	Accept H_o
2.	Species type (factor B)	2	1880.0	940.35	3.40	10.50	0.001	Reject H_o
3.	Vegetation (factor C)	1	9.84	9.84	4.26	0.11	0.743	Accept H_o
4.	A x B	2	117.15	58.57	3.40	0.65	0.529	Accept H_o
5.	A x C	1	159.43	159.43	4.26	1.78	0.195	Accept H_o
6.	B x C	2	47.04	23.52	3.40	0.26	0.771	Accept H_o
7.	A x B x C	2	279.90	139.95	3.40	1.56	0.230	Accept H_o
	Error	24	2149.62	89.57				
	Total	35	4713.85					

We conclude that the species concentrations in the top two layer (1-5 cm and 5-10 cm) of the planted and unplanted prototype beds only depend on the type of species that are being considered (*i.e.* GP or GN or FUN).

CHAPTER 8: GENERAL DISCUSSION AND SYNTHESIS

GENERAL DISCUSSION

8.1 DISCUSSION AND SYNTHESIS

This thesis has addressed a number of challenges i) characterise the link between cleaning and personal care products, and grey water characteristics, and ii) describe the performance of a novel multiple shallow cascading bed constructed wetland for treatment of grey water. The preceding literature review and technical papers have all contributed towards these aims and include discussions on the implications for grey water recycling. Here, the findings are brought together for evaluation and further analysis of the implications of cleaning and personal care products on grey water and performance of the novel multiple shallow bed constructed wetland design.

The overall aim of this research was to assess the performance of the novel shallow (0.2 m deep) constructed wetland design and compare the performance to a more typical vertical flow SSF wetland (with a depth that falls within the standard range of 0.6 – 1.2 m) (Taniguchi *et al.*, 2009). The consideration for having shallow beds in the novel design and the chosen depth of 0.20 m was from observations that the main degradation of substances in standard wetlands takes place in the upper 0.2 m of the reactor-beds regardless of the bed depth (Langergraber *et al.*, 2003; Platzer, 1999; Felde and Kunst, 1997). The cascading bed design was a way of increasing water turbulence in order to increase oxygen transfer into the treatment beds. To understand the implications of this project, the work is discussed in form of answers to the research questions presented in Section 2.5 in Chapter 2, which are outlined below:

1. What is the impact of general chemical properties of the household cleaning and personal care products on grey water quality?
2. Can toxicity of grey water be predicted from the household cleaning and personal care products?
3. How does the performance of the prototype for grey water treatment compare with the standard constructed wetland?
4. What is the influence of microbial community structure in the wetland beds on treatment performance?

The initial literature investigated the physico-chemical properties of grey water and different designs and configurations of constructed wetlands and their performance in the treatment of grey water. The review indicated that factors impacting on constructed wetland designs are related to knowledge of grey water characteristics (or lack of it) in terms of the type of chemicals contained in it and inadequate understanding of the internal biochemical dynamics of the treatment wetlands. The review also suggested that the ability to understand the internal processes occurring in the treatment beds would help in design considerations and enable controlled operation within limits that ensure consent for reuse standards. Presently, the aspect of raw grey water that is readily controlled in wetland treatment is loading rates. It was therefore hypothesised that the ability to predetermine and even control overall chemical quality of grey water from type of products and their usage (user behaviour) could possibly lead to generation of grey water with pre-determined characteristics ("controlled pollution") thus making it 'easily treatable'. This could in turn allow system designers to tailor the constructed wetland designs and operations to the lifestyles of the users and their choice/types of cleaning and personal care products. However, this study did not address the contribution of user behaviour on the premise that type of product would be just as informative. The discussion of the tested research question follows below: -

8.1.1 Impact of product type on grey water characteristics

8.1.1.1 What is the impact of general chemical properties of the household cleaning and personal care products on grey water quality and treatability?

In urban water reuse, light grey water (bathroom/shower grey water only) (Jefferson *et al.*, 1999) is of particular interest due to its low pollution load and high availability. The exact character of such grey water is dependent on the amount of water used and the choice of the products used by the individual(s) generating the grey water. However, the influence that product choice has on the character, biodegradability and treatability of grey water is not well understood. The combination of product type and usage of the products may affect characteristics of grey water such as treatability (COD:N:P ratio) and toxicity (EC_{50} values) and negatively impact the receiving ecosystem. These findings are presented in Chapter 3 where significant differences were generally observed between laundry/cleaning product categories, such as Wash Up liquids, All Purpose Cleaners and Fabric Conditioners, and personal care product categories, such as Shower Gels, Bath Crèmes and Shampoos. The study has shown that 'product use' (category) can be used to predict characteristics of grey water but the same is difficult to predict using product brand types. To illustrate, personal care products (Shampoos, Hair Conditioners, Shower Gels, and Bath Crèmes), were found to be less toxic compared to laundry and cleaning products (All Purpose Cleaners, Washing Up liquids and Fabric Conditioners) which is in agreement with observations in literature that light grey water is less toxic and easier to treat than laundry or kitchen grey water. However, grey water characterisation based on branding or type of product is not straight-forward. Indeed, product branding and/or labelling (1 = branded, 2= own brand and 3 = eco-brand) only showed a significant correlation with pH ($r_s = 0.25$, $p < 0.05$), whose positive correlation to total Phosphorus was significant ($r_s = 0.39$, $p < 0.05$) and TOC ($r_s = 0.24$, $p < 0.05$). This suggests that the pH can be associated with product branding, and concentrations of total Phosphorus and TOC as discussed in Chapter 3 (Section 3.4). This is interesting because levels of microorganisms in the different ecosystems have been shown to be related to pH and resource quality (Wardle, 1992; Frostegård *et al.*, 1993).

The findings also suggest that the different ingredients used in household products, including surfactants, builders, bleaches, enzymes, fragrances and other xenobiotics compounds, influence the chemical characteristics of grey water such as pH, biological oxygen demand and conductivity differently. This implies that the products' chemical ingredients broadly fall into two categories, those for general cleaning and those personal hygiene/washing which leads to the distinctions observed in these product types. Studies by Simon (2008) found that BOD_5 and COD levels were higher in eco-friendly products (430% and 50% respectively) than in own brands, but their biodegradation in porous pot studies produced similar effluent quality. From this, it can be claimed that eco-friendly products have higher biodegradation rates, but then this was against initial high concentrations compared to the other brands. Hence the ability to predict grey water chemistry is complex and cannot be achieved by only considering the product brands because it also depends on being able to control or factor in consumer usage of both the products and water and biodegradability of the products. The eco-friendly products also showed higher biodegradation but the initial nutrient concentrations in these products were also higher than in other brands. High biodegradation enhances cometabolism. But according to Burgess (1999) the mere availability of nutrients determines the species diversity and community structure of biological systems and hence the ability of that community to undertake cometabolism. In case of xenobiotics, cometabolism is the main mechanism for biodegradation. The findings in this study (Chapter 3) however are in line with observations in literature (Jefferson *et al.*, 1999; Friedler and Butler, 1996) that characteristics of grey water vary with source (*i.e.* bath, shower, bath tub, kitchen, laundry). This study has produced evidence that differences between light grey water and high strength grey water result from the actual product which result in this demarcation *i.e.* personal washing and cleaning products. In addition use of Microtox and MicroResp toxicity tests has revealed that the high sensitivity of Microtox results is useful for ranking products or indeed grey water according to concentrations of surfactants and oxidants contained in it.

8.1.1.2 Can the toxicity of grey water be predicted from knowledge of cleaning and personal care products used in the household?

The Microtox toxicity analysis which uses marine pure bacteria culture was found to be more sensitive than the MicroResp method which was done on soil microorganisms. Although Microtox toxicity analysis is a relatively easier and quicker method, it produced different results from the MicroResp and therefore cannot be used a direct predictor of grey water toxicity to soil (and wetland) microorganisms. On the other hand, MicroResp is useful for predicting impact of grey water of soil health, which is very important for grey water irrigation reuse (Sharvelle *et al.*, 2008). As seen in Chapter 4, chemicals in grey water when applied to soil can produce both beneficial (stimulation) and detrimental (inhibiting) effects. Microtox analysis only predicted detrimental effects due to the high sensitivity of the *Vibrio fischeri* to strong oxidants and surfactants (Cloete, 2003). This observation was also made by Knops *et al.* (2009) who obtained high toxicity responses for hydrogen peroxide (oxidant) and LAS and CTMAB (surfactants). Nevertheless, analysis of the toxicity test results showed that most of the household products are unlikely to be used in high enough quantities to cause significant negative effects (toxicity) in the short-term if the resultant untreated grey water was used for irrigation (Chapter 3, Table 3.3). This study has shown that there is potential to predict physico-chemical and toxicity of grey water based on product type. Detailed information of the ingredients and biodegradability of the cleaning and personal care products is necessary to be able to say more about the grey water characteristics. Parameters such as COD:TOC ratio, TOC and to some extent nutrient concentrations can also be used to predict potential toxicity of grey water.

8.1.2 Performance of the novel shallow bed constructed wetland design

8.1.2.1 How does the performance of the cascade wetland for grey water treatment compare with a standard constructed wetland?

The treatment performance by the different variants of the cascade wetland are described and compared to the standard VFPCW in Chapters 5 and 6. The percentage removals for key parameters (Table 8.1) show that the different variants of the cascade wetland (open bed, covered bed and planted) performed very well with regards to removal of conventional water quality parameters (BOD, COD, turbidity, pH, conductivity and indicator organisms) and exhibited robustness for varying loading and organic strengths. To illustrate, removal for BOD_5 generally met the reuse standard of 10 mg. L^{-1}. However comparison of the tested CW versions showed the following order: open beds cascade wetland > standard VFPCW > planted cascade wetland = covered beds cascade wetland, with the open bed cascade wetland meeting the reuse standard virtually throughout the monitoring period. However, reduced performance was noted for the highest hydraulic and organic loading rates tested (Section 5.3.1, Chapter 5). Comparable and in some periods, better indicator pathogen removal concentration against the standard VFPCW and other types of constructed wetlands (Winward *et al.*, 2008; Dallas and Ho, 2005) were also observed for the cascade wetlands. All the cascade wetland versions only met the Australian Total coliform reuse standard of 4 $\log_{10}$ concentration but not the more stringent USEPA reuse standards of 2 and 1 $\log_{10}$ concentration for Total coliform and *E* coli respectively (USEPA, 2004) which were only met at low influent organic strength. Organics are known to reduce bacterial adsorption in porous media by competing for adsorption sites (Stevik *et al.*, 2004) which reduces the affinity of bacterial surfaces for adsorption. This explains the reduced removal performance at high organic strength in the cascade wetlands. Despite suitability of the constructed wetland technology having been limited to wastewaters with turbidities below 50 NTU (Kadlec and Wallace, 2009), the cascade wetland design performed consistently well even for turbidity levels that were well over 50 NTU and met the reuse standard of < 2 NTU. The role of plants in removal of conventional parameters was perceivably low (Chapter 6). Although plants significantly affect the removal of pollutants in horizontal subsurface systems with long hydraulic retention times, their role in intermittently loaded vertical flow SSF systems indeed appears to be minor (Stottmeister *et al.*, 2003).

Table 8.1: Comparison of treatment performances of the cascade wetlands and the standard VFPCW design

	DO (% saturation of effluent)	**BOD_5**	**Turbidity**	**COD**	**TOC**	**Surfactant**			**Indicator organisms***	
						Anionic	Cationic	Non-ionic	Total coliform	E coli
Open bed Prototype	92.7	99.1	91.0	92.0	59.4	85.5	-	-	3.1	2.3
Covered bed Prototype	89.1	98.5	87.3	95.7	63.3	60.5	35.6	50.8	2.9	2.7
Planted Prototype	86.3	99.0	93.4	95.2	65.8	83.4	33.3	49.4	3.2	2.2
Standard VFPCW	65.7	99.7	82.3	95.5	64.6	80.2	74.2	43.6	2.8	2.1

*Note:

1) log_{10} concentration reduction
2) Average percentage removals reported for standard VFPCW are for the whole monitoring period of 17 months.

Extensive formation of the biological active layer (*Schmutzdecke*) was experienced in the top bed of the open bed cascade wetland and it had to be removed every 4 months. This phenomenon was not observed in the other wetland technologies, which clearly shows the influence of the combination of direct sunlight energy and high resource quality (high influent strength) grey water on the reproduction of bacteria in the top unplanted bed. Although clogging phenomenon was not experienced, there were indications that continued operation at higher loading rates, might have resulted in clogging in the top open bed cascade wetland. The biological layer, though it reduced infiltration, was the only explanation for better removal performance of open bed cascade wetland compared to the other technologies (Ottova *et al.*, 1997). It is interesting to note also, that the covered beds showed no signs of either accelerated formation *Schmutzdecke* or signs of clogging. This behaviour has been associated with presence of plants in wetland systems (Brix and Arias, 2005; Brix, 1997). Clearly, clogging is linked to rate of growth of microorganisms (and biofilm formation) in the beds, and this study has shown that it can be controlled by any form of 'insulation or covering', *i.e.* plants or actual covers. In the case of the open (unplanted) beds, this can be controlled through the hydraulic and organic loading rates and the frequency of the 'fill and drain cycles'. The higher dissolved oxygen saturation in the effluent from the open bed cascade wetland (Table 8.1) suggests higher oxygen transfer capacity in this wetland.

Three factors showed to be determinants for removal of surfactants: 1) presence of plants or plant stumps, 2) age with respect to deposition of organic matter and 3) charge of the surfactant. Removal of anionic surfactants in the open bed cascade wetland was initially below 50 % but started to pick up by the third month of operation. This is in line with slow sand filtration mechanisms where the crystalline structure of clean quartz sand produces a negative charge in the filter bed, which only attracts positively charged colloidal matter. Colloidal matter of organic origin (*e.g.* bacteria) and anionic chemical species and other negatively charged species are not attracted (Scholz, 2006) until the break-in period, at which point negative charges start to be adsorbed. For anionic and cationic surfactant removal, adsorption was probably the main removal mechanism, which is enhanced by long residence time (as observed in the VFPCW). As observed in the VFPCW, presence of additional surface to support microorganisms in the media (the planted wetlands) (Huang *et al.*, 2004; Thomas *et al.*, 2003; International Association on Water Quality, 2000), opposite charges between media and the pollutants (*e.g.* cationic surfactants) (McAvoy *et al.*, 1994; Zoller, 1994) and increased deposition of organic matter in the media (Steinmann *et al.*, 2003) most probably resulting from long history of vegetation in the wetland system also contribute to the removal mechanism of surfactants. Degradation of surfactants has also been associated with an increase in bacterioplankton density. This explains the higher anionic surfactant removals in the open bed prototype where removal resulted in increase of microbial activity as the surfactants were used up as a growth substrate (García *et al.*, 2001) which also contributed to the frequent *Schmutzdecke* formation. Non-ionic surfactant removals were appreciable and almost the same in all the wetland technologies. Nonetheless the highest removals were observed for the charged surfactant species, which suggest the combination of chemisorption (involving interactions between the surfactant species and the media). However, more work is necessary to confirm the actual removal mechanisms for surfactant species. Overall, the cascade wetland, which had the same total effective media depth as the single reactor-bed standard VFPCW, achieved removal levels that were comparable and in some cases better than the VFPCW under all conditions. This confirms that aerobic unsaturated removal pathways or higher oxygen transfer systems (vertical flow sub-surface systems), enhanced by the shallow bed and cascading design, are more appropriate for grey water treatment.

Various studies have shown smaller versions of the constructed wetland technology, which can be used on site and in small communities and individual houses (Gross *et al.*, 2007; Li *et al.*, 2009; Shrestha *et al.*, 2001; Fittschen and Niemczynowicz, 1997). This study has gone further to show the aspects of the technology that determine sustainability and good performance for grey water treatment. This will contribute to design and operation considerations of the constructed wetlands for grey water treatment especially in areas where there are problems with conventional centralised treatment systems and where grey water recycling is amenable.

8.1.2.2 What is the influence of wetland microbial community structure on grey water treatment performance?

Another way of looking at the treatment performance of the wetlands is by taking into consideration the internal microbial structure. This study has shown that wetland treatment performance is influenced by the design as well as operational mode (flush frequency). As discussed in Chapter 5, during fill-and-drain air is drawn into the bed by convection as the bed drains, which then gets trapped and is distributed during filling-up (Forquet *et al.*, 2009). So there are essentially two ‘ways’ in which pollutant attenuation takes place, (i) during vertical motion (filling and draining), as the water flows down the bed, physical straining of suspended matter and adsorption of colloids occurs, and (ii) between filling and draining when water is stagnant in the bed, when microbial contact with the water results in increased biodegradation. Time is crucial for both because on the one hand it affects the velocity of the water as it moves vertically during filling and draining (this is a function of bed depth) and on the other hand, it affects the contact time with microorganisms in between filling and draining. The relationship between contact time and microbial (PLFA) concentration is obvious where higher contact time corresponds to higher microbial levels (Chapter 7, Figure 7.2). As hydraulic loading rate was increased due to increase in fill-and-drain frequency, microbial contact time with the water decreased which resulted in lower microbial levels as observed by the lower bacterial PLFA concentrations. The overall effect was reduced treatment performance at higher loading rates. These observations help to emphasise that removal is indeed a factor of interaction between the mobile (grey water) and the solid compartments (media and biomass) of the wetland (Kadlec and Wallace, 2009). This is crucial for controlling microbial structure in the wetland beds which is crucial for biological degradation processes and the interactions that take place. Interestingly though, no significant differences were observed in catabolic richness between individual layers in the cascade wetlands (Chapter 6) as the comparison of catabolic diversity between the beds of the cascade wetlands revealed no significant differences. This perhaps is an indication that the beds in the cascade wetland developed similar microbial communities, as also shown in studies by Sletyr *et al.* (2009), because they had similar physiochemical factors such as media grain size distribution and pH of the effluent water, oxygen-content and only differing in organic strength of the influent grey water.

Strength of the influent grey water (resource quality) was a crucial factor in determining the microbial levels (especially for bacteria) but not the microbial compositions (*i.e.* bacterial and fungal ratios). But as discussed in Chapters 3, resource quality (chemical characteristics), is function of product type, *inter alia*. Hence the following factors; the wetland design (number and size of beds), operational mode and source of the influent wastewater, all contribute to the microbial structure and overall performance of the wetland technology. Microbial PLFAs in the cascade wetlands were also influenced by nutrient dynamics and climate (Chapter 7). Interestingly, plants do not feature highly in these factors and so they did not significantly affect the levels of microorganisms. This is in agreement with observations by other researchers (*e.g.* Brisson *et al.*, 2006) who indicated that in SSF CW only small performance differences exist due to vegetation. Knowledge of these factors is very important for good performance and extended lifespan of any wetland design through controlled operation. For instance, during very low temperature periods, plants can be cut, and the beds covered to ensure continued appreciable treatment activity. Nonetheless, as discussed in Chapter 7, the role of plants may indeed be crucial in the long term through contributions to additional surfaces for microorganisms and background organic matter which contribute to treatment performance.

8.1.3 Implications of the shallow and cascading bed wetland design for grey water treatment

This novel design has shown applicability for small-scale internal grey water treatment and reuse. Treatment performance was very good and comparable to biological-based and advanced treatment technologies (such as discussed by Pidou *et al.*, 2008) meeting stringent reuse standard for all conventional physico-chemical parameters. Safety of the treated water for irrigation reuse was shown through the high removal of surfactants. Ergonomically, the design is safe, easy to operate and service at household level, and can be adapted to suit the constraint and aesthetics of any garden. The novel design has also shown that, the required specific surface area for the constructed technology can be reduced and still achieve high treatment performance required for various reuse options.

Shallow beds enable near complete aerobic conditions to prevail throughout the treatment bed through increased oxygen transfer into the beds as evidenced by the higher (> 90 %) dissolved oxygen saturation (Chapters 5 and 6). Shallow beds also enable faster and balanced colonisation of microorganisms throughout the treatment bed than is the case in standard 0.6 – 1.2 m deep constructed wetlands. This was shown by the catabolic evenness (Chapter 6) and even distribution of bacterial PLFAs (Chapter 7) in individual beds of the cascade wetlands. Compared to other technologies, the cascade wetland design showed flexibility, because it was operated in three different variants i) with plants, ii) with covers and iii) with no covers and no plants. Therefore it can be adapted to different situations depending on the need for the treated water (type of reuse), availability of space, climate conditions and user preferences. In addition, if any of the upper beds bed become exhausted, for instance the top bed starts clogging, then only the top bed needs to be removed or rested and the lower two beds can handle the load or can be moved up to create space for a new one at the bottom. This provides already acclimated media beds in the system and hence the lifespan of the whole treatment wetland can be prolonged.

Perhaps the areas that would still have environmental implications are inadequate removal of indicator pathogens which necessitates disinfection or modification of the design (this is the case with the constructed wetland technology as a whole). The bench-scale study (Chapter 4) showed that using a combination of finer grains in the bottom beds and silver coated sands has the potential to improve removal of indicator microorganisms. This could reduce the need for the conventional chemical disinfection of the treated grey water which can lead to production of harmful by-products as shown by Winward *et al.* (2008). Chlorine disinfection can result in the formation of harmful disinfection by-products and plant essential oils frequently contain high concentrations of phenols and other chemicals which are toxic to the environment. Ultraviolet light on the other hand requires the use of significant amounts of energy, which may negatively impact the environment through the process of energy production. From the results obtained in this study (in the open bed cascade wetland) (Chapter 5), filtration only step can be included at the back-end of the system using finer grain sizes to remove residue microbial particles from the main wetland treatment. Another issue arising from this thesis is the amount of time spent flushing pipes and removing the *Schmutzdecke* in the open bed cascade wetland design, which translates to increased risk of exposure to pathogenic organisms. This makes the other variants of the cascade wetland, the covered and planted version, more appealing and user friendly.

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CHAPTER 9: CONCLUSIONS AND FUTURE WORK

9.1 FINAL CONCLUSIONS AND FUTURE WORK

9.1.1 Conclusions

The present thesis has improved the understanding of potential impacts of cleaning and personal care products on grey water characteristics. This is very important because it contributes to the treatment technology requirements of grey water for recycling purposes. A greater understanding of design and operation considerations for the constructed wetland technology to achieve optimum results for grey water reuse has also been established. The following conclusions can be drawn from this work:

1. Physico-chemical characteristics and toxicity of cleaning and personal care products were found to be related to the different grey water types. Differences were observed generally between cleaning products and human washing products, which was attributed to the differences in ingredients (especially oxidants and surfactants) and pH between these two broad groups but these are not directly related to water quality and its treatability (*Research question* 1).

2. It was found that for a particular type of grey water, the physico-chemical characteristics and toxicity cannot be easily predicted from the branding, (or price), or whether the product is labelled as being eco-friendly, but that other socio-economic factors need to be considered as well (*Research question* 2).

3. The novel shallow cascading bed wetland performed very well for all the hydraulic rates at which it was tested and showed robustness for occasional fluctuations in influent grey water strength. Performance was affected at higher loading rates (greater than 15 g BOD m^{-2} d^{-1} equivalent to 185 mg BOD. L^{-1}) (*Research question* 3).

4. On the design aspects, shallow bed depth (≈ 0.2 m) were found to provide higher removal rates of conventional water quality parameters than the standard constructed wetland. Good performance was exhibited by the cascade wetland meeting stringent world-wide reuse standards. Protection of the wetland bed

surface from direct impacts of changing weather conditions either by means of plants or covers directly impacted on the performance of the novel technology by slowing down formation of *Schmutzdecke* in the most active bed, the top bed, just enough without compromising of effluent quality (*Research question* 3).

5. For operational aspects, variable parameters such as hydraulic and organic loading rates, and frequency of ‘intermittent fill-and-drain’ cycles and the contact period between the grey water and the solid compartments of the wetland bed were found to be crucial in the overall performance of the novel wetland design (*Research question* 3).

6. Removal of surfactants is very important especially if the treated grey water is meant for irrigation. The study established the design aspects that enhanced removal of surfactants from grey water as i) presence of plants or plant stumps, ii) age with respect to deposition of organic matter iii) wetland depth with respect to retention time and perhaps iv) charge difference between the media and the chemical species requiring removal (*Research question* 3).

7. Faster and balanced colonisation of microorganisms was observed in the shallow beds than is the case in standard constructed wetlands as was shown by the catabolic evenness and similar bacterial PLFAs profiles. This also resulted in reduced redundancies in pockets of the wetland beds which contributed to high treatment performance. Comparison between cascade wetland beds also revealed similarities in catabolic diversity and composition of microbial communities (*Research question* 4).

9.1.2 Recommendations for further work

A number of areas where further research would be beneficial have been identified during the course of this research. These are outlined below:

1. There is a need to establish the actual ingredients in the cleaning and personal care products that give rise to differences in toxicity and other properties in grey water in order to understand the factors that are peculiar to specific brands and differently labelled products. This would help to predict impact of the choice

and usage of individual products on characteristics of grey water. Currently product labelling only carries trade names of some ingredients. This made it difficult to assess the impact of product types/choice of grey water characteristics.

2. Further studies are required to assess the sorptive capacity of the different media to model removal processes of surfactants, and the factors that affect their removal according to the different speciation. Where wetlands are vegetated the symbiotic bacteria introduced by plant root systems need to be studied in order to establish their role in surfactants removal.

3. In depth studies are required on microbial grouping and functions in wetlands in relation to design and operation. Use of community DNA analytical methods, such as Denaturing Gradient Gel Electrophoresis (DGGE), might be suited for this work in order to get a clearer picture.

4. The cascade wetland was tested for daily volumetric throughput of up to approximately 140 L d^{-1}, but there is need to model its performance for different volumes to cater for different household sizes or circumstances where amounts of grey water produced per day vsry widely.

5. Further studies are required at pilot level to assess the level of improvement of disinfection that silver coated sand can bring to wetland treated grey water. Of interest would be to establish the bed volumes, contact time, filtration run and mixture ratios with uncoated sands.