CRC for
Water Sensitive Cities

Biofilters and wetlands for stormwater treatment and harvesting

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EXECUTIVE SUMMARY

Stormwater has emerged as a viable, alternative water resource. As a result, general urban runoff (*e.g.* stormwater that runs off streets and c¬ar parks) has been increasingly harvested in the past few years. The CRC for Water Sensitive Cities (CRCWSC) aimed to refine existing, and develop novel, stormwater harvesting technologies, building upon the proven concepts of Water Sensitive Urban Design (WSUD). Hence, a series of laboratory- and field-scale research projects were designed to investigate two of the most common WSUD technologies used in Australian stormwater harvesting schemes - biofilters and constructed wetlands - for their capacity to remove different pollutants (nutrients, faecal microorganisms and micro-pollutants).

It was confirmed that biofilter design and operational conditions greatly affect the overall pollutant removal capacity. For instance, nutrient removal capacity was shown to improve using a filter media with a low nutrient content and with the inclusion of a submerged zone (SZ) containing a low nutrient content carbon source. A mixed planting including both effective and ineffective plant species for nutrient removal (minimum 50% effective species) was suggested over a single plant species. Current, best-practice biofilters were found to sustain effective long-term phosphorous (P) removal without breakthrough, yet augmenting traditional biofilter media (loamy sand) with naturally occurring iron- and aluminium-oxide rich sand (mixing ratio 1:3 - 1:1 or 25 - 50% Skye sand) were found to further improve long-term P retention.

Research findings also demonstrated that well designed current stormwater biofilters are capable of achieving a 1 log reduction for bacterial indicators. The current biofilters were also capable of removing reference pathogens, particularly protozoa. It was revealed that the selection of plant species with an extensive root system and maintaining a steady SZ volume are important for faecal microorganism removal in current stormwater biofilters. However, faecal microbial removal performance in current stormwater biofilters was shown to be reduced following both extremely short and extended dry weather periods. A novel antimicrobial biofilter media was then developed using Cu^{2+}-immobilised zeolite (ZCu) coated with $Cu(OH)_2$, followed by heat treatment at 180°C (ZCuCuO180) and ZCu calcined at 400°C (ZCu400). The new layered biofilter media design using the novel antimicrobial media demonstrated capacity to achieve greater than a 2 log reduction of common indicator microorganisms without compromising the removal of other pollutants (*e.g.* total suspended solids, nitrogen (N), P and Cu). This new media design was also capable of reducing reference pathogen concentrations, with particularly high removal of protozoa (> 3 log) and greater than a 1 log reduction of reference bacterial and viral pathogens.

Stormwater biofilter systems built in accordance with current best practice met irrigation water quality standards for all tested metals: Al, Cr, Fe, Pb and Zn. When designed appropriately, biofilters can also meet drinking water standards for the same metals. However, biofilters were shown to have a shorter lifespan with respect to heavy metal accumulation in catchments that contain current or past industrial activity. Stormwater biofilter systems built in accordance with current best practice were also very effective for the removal of hydrocarbons and phthalates, but less effective for herbicides.

The laboratory- and field-scale research on biofilter pollutant removal performance also assisted in the development of assessment tools. As a result, two process-based models were developed to predict micro-pollutant and indicator bacteria removal in stormwater biofilters.

The research also revealed that there is no single biofilter design which can achieve optimal removal performance for a wide range of pollutants. Therefore, it is important to prioritize site-specific water quality objectives when designing biofilters for stormwater harvesting.

Constructed wetlands were generally shown to be promising in reducing indicator microorganism concentrations, yet a large variance in observed removal performance needs to be further investigated. A constructed wetland receiving pre-treated runoff from an industrial catchment was effective in reducing concentrations of nutrients, metals and the bacterial indicator, *E. coli*, but was less effective in reducing Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (COD was found to accumulate within the wetland during extended dry weather periods).

The recent research conducted by the CRCWSC has greatly assisted in developing and refining the treatment component of stormwater harvesting systems. Yet, some knowledge gaps still remain, and further research is required to understand the behaviour of some stormwater pollutant groups within these systems.

1 INTRODUCTION

Stormwater has emerged as a viable, alternative water resource. As a result, general urban runoff (*e.g.* stormwater that runs off streets and car parks) has been increasingly harvested in the past few years. It is widely recognised that **technological solutions that could deliver safe harvesting of stormwater on a large scale need further development** (Hatt et al., 2006, Mitchell et al., 2007). Hence, the Cooperative Research Centre for Water Sensitive Cities (CRCWSC) aimed to refine existing and develop novel stormwater harvesting technologies, building upon the proven concepts of water sensitive urban design (WSUD).

This report summarises a series of research projects designed to investigate two of the most common WSUD technologies used in Australian stormwater harvesting schemes, **biofilters and constructed wetlands**, for their capacity to remove different pollutants (nutrients, faecal microorganisms and micro-pollutants). Some insights into practical implementation arising from a range of field- and laboratory-scale work, and the direction of future research to further improve these WSUD technologies, are also presented.

2 BIO FILTERS

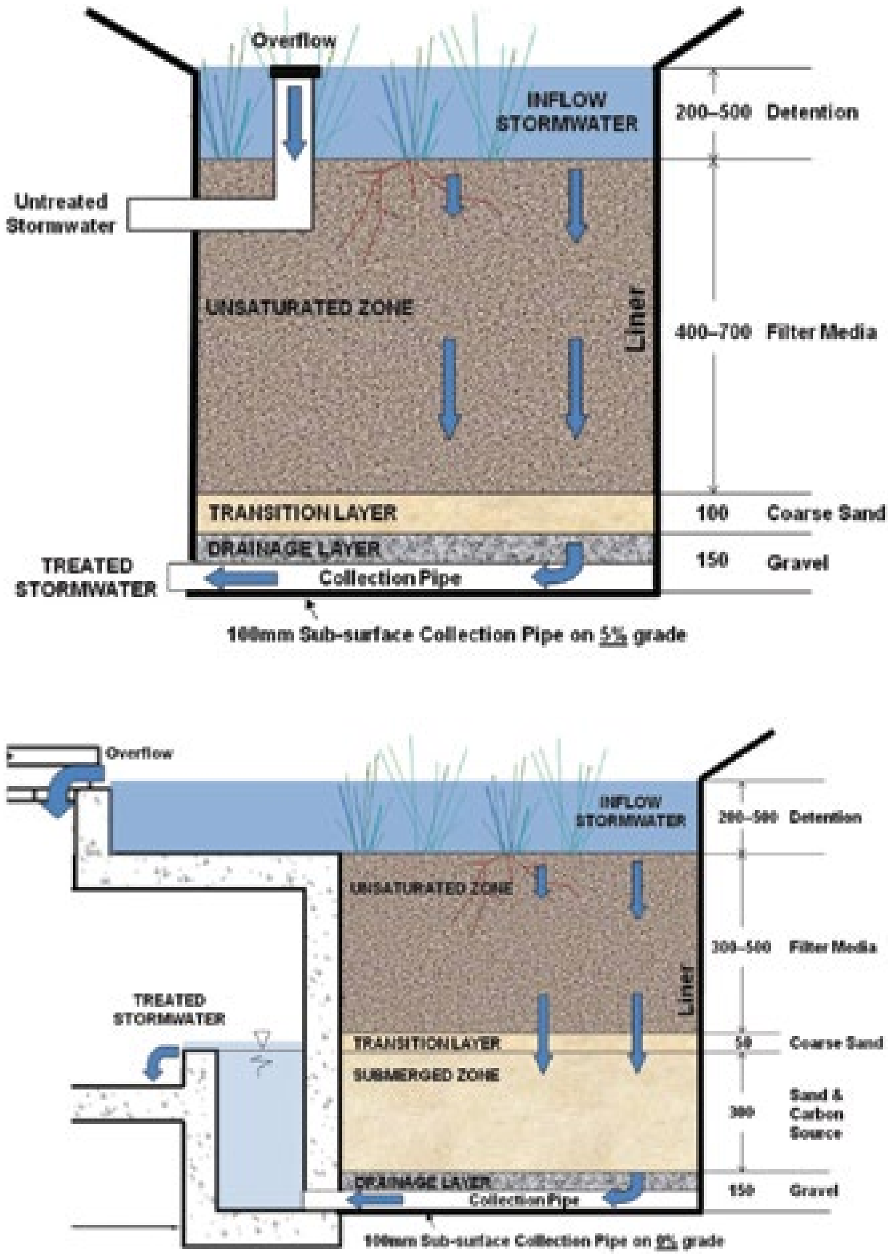


Figure 1: Schematic diagram of a lined biofilter without a SZ (top) and with a SZ (bottom) (Source: FAWB (2009)).

Biofiltration systems or biofilters, are soil-plant based filtration systems which can be easily scaled and incorporated into urban landscapes. A typical biofiltration system consists of a filter medium which generally has a high sand content and is underlain by a sand transition layer and a gravel drainage layer (Figure 1) (FAWB, 2009, Rusciano and Obropta, 2007). The systems can be lined, to collect as much water as possible (for harvesting or protection of nearby infrastructure), or un-lined, to promote infiltration. Depending on site conditions and system characteristics, a biofilter may or may not have a perforated collection pipe. Some biofilters comprise an elevated outflow pipe creating a submerged zone (SZ) at the bottom of the biofilter (Figure 1-bottom). This feature enhances water quality treatment and promotes healthier biofilter vegetation during extended dry weather periods (FAWB, 2009, Zinger et al., 2013).

Stormwater biofilters provide treatment through a combination of physical (sedimentation, mechanical straining), chemical (sorption, precipitation, redox reactions) and biological (plant and microbial uptake, microbial respiration) processes as the stormwater flows through dense vegetation and temporarily ponds on the surface before filtering through media (FAWB, 2009). The media, plants and the microbial community all play key roles in pollutant removal within biofilters. In particular, biofilter vegetation serves multiple roles by supporting microbial processes, promoting evapotranspiration, maintaining soil hydraulic conductivity, and taking up nutrients and heavy metals (Davis and McCuen, 2005, Bratières et al., 2008). However, the major mechanisms which govern the removal of a wide range of stormwater pollutants in biofilters are not fully understood, nor are the interactions between different biofilter designs and operational conditions. As such, recent research conducted by the CRCWSC has focused on the following three groups of stormwater pollutants of interest to ecosystem and public health:

Nutrients

Recent work focussing on nutrient removal in stormwater biofilters builds on the research conducted by the Facility for Advancing Water Biofiltration (FAWB) (Bratieres et al., 2008, Read et al., 2010, Read et al., 2008, Zinger et al., 2007a, Zinger et al., 2013). It also includes results from a recently completed Australian Research Council (ARC) Linkage Project on *Understanding the role of vegetation in nitrogen removal by biofiltration*, which was conducted by Monash University in partnership with the Western Australian Department of Water and Melbourne Water Corporation. Parallel studies were also recently completed investigating phosphorus (P) removal in biofilters, and the influence of mixed species plantings on nutrient removal. These research projects aimed to advance biofilter design for nutrient removal (nitrogen (N) and (P)) and focused on filter media composition, vegetation selection and submerged zone configuration. The work also aimed to investigate major mechanisms governing nutrient removal and long-term removal performance in stormwater biofilters.

Faecal microorganisms

The work conducted on faecal microorganism removal in stormwater biofilters had two focus areas. One of the studies was conducted to understand the mechanisms that govern faecal microorganism removal in current, best practice stormwater biofilters and how these processes are affected by various biofilter design and operational conditions. A parallel study was conducted to advance the current stormwater biofiltration technology for faecal microorganism removal by incorporating plant species that have good antimicrobial properties and by developing a novel, cost-effective, antimicrobial filter media.

Micro-pollutants

The third focus research area, micro-pollutant removal in stormwater biofilters, mainly considered the removal of metals and organic micro-pollutants (herbicides, oil and petrol derivatives, disinfectants, plastic production products, etc.). The capacity of current, best practice stormwater biofilter designs to reduce metal concentrations to meet standards for various end-uses (potable and non-potable) was investigated, as well as their performance across both short and long-term operation. A parallel study investigated the removal efficiencies of a range of organic micro-pollutants in two different, field-scale stormwater biofilters under challenging operational conditions.

The methods used to investigate each stormwater pollutant group and the key findings from each research project are only briefly presented in the following sections. **Further details of the experimental methods and results can be found in 30 journal and conference papers that were produced by CRCWSC researchers from the above projects** (see Appendix C for the full list of the publications).

2.1 Methods

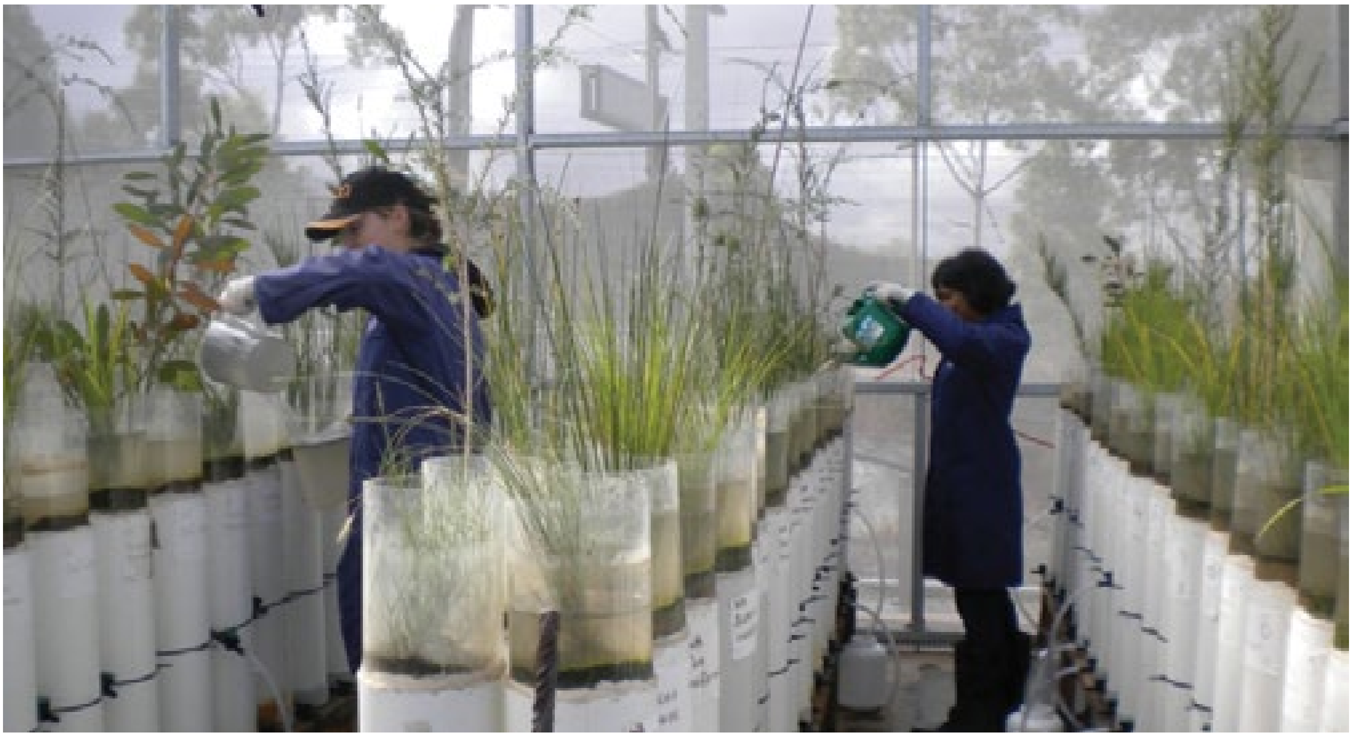


Figure 2: Dosing laboratory scale biofilter columns using semi-synthetic stormwater

Experimental approaches developed to study the three pollutant groups described above generally made use of laboratory-scale biofilter column studies. In all cases, biofilter column design characteristics were specified according to FAWB's Biofilter Adoption Guidelines (FAWB, 2009). The basic experimental procedure involved dosing the biofilter columns with semi-synthetic stormwater that was prepared to meet target pollutant concentrations (depending on the stormwater pollutant of interest, the exact composition of the stormwater varied from one study to another). The dosing volume and frequency were designed to reflect either typical or challenging rainfall conditions for major Australian cities of interest (as described in further detail below). Water quality samples were collected at the inlet and outlet of the biofilter columns to evaluate pollutant removal performance. In some studies, biofilter columns were opened at the end of the experimental dosing period and filter media and vegetation samples were analysed.

2.1.1 Nutrients

Four sets of laboratory-scale biofilter column experiments were carried out to study nutrient removal performances of stormwater biofilters.

The influence of plant species on N removal performance

A laboratory column study of 22 plant species and designs with and without a SZ was conducted across a 1.5-year period to investigate the mechanisms and influences driving N processing in biofilters (Payne et al., 2014a, Payne et al., 2014b, Payne et al., 2013, Pham et al., 2012). The laboratory experiment involved 220 single-plant columns and 20 non-vegetated control columns. The selected plant species included native grasses, sedges/rushes, shrubs/trees from Victoria and Western Australia as well as two lawn grasses (Table 1). These columns were subjected to 'wet' conditions, where the columns were dosed twice weekly with a volume that reflected typical rainfall conditions for either Melbourne or Perth, and 'dry' conditions, where the columns were dosed at approximately fortnightly intervals. Water quality samples were collected at the inlet and outlets and analysed for total nitrogen (TN), total phosphorus (TP), ammonium (NH_4^+), filterable reactive phosphorus (FRP) and nitrate/nitrite (NO_x) by a NATA (National Association of Testing Authorities)- accredited laboratory using standard methods and quality control procedures. In addition, ^{15}-NO_3^- (nitrate) isotope tracer was added to a sub-set of columns in order to assess internal nitrogen processes. At the conclusion of the study, all columns were opened and plants were harvested and washed to determine the dry weight of their above- and below-ground components. Roots and leaves were scanned and analysed to characterise root and leaf morphology.

Broad plant type	Western Australia	Victoria
Grasses	*Sporobolus virginicus* *Austrodanthonia caespitose* *Poa poiformis*	*Poa labillardieri* *Poa sieberiana*
Sedges/reeds	*Cyperus gymnocaulis* *Juncus kraussii* *Gahnia trifida* *Carex tereticaulis*	*Carex appressa* *Gahnia sieberiana* *Juncus pallidus* *Dianella revoluta* *Dianella tasmanica*
Shrubs/trees	*Melaleuca incana* *Astartea scoparia* *Hypocalymma angustifolium*	*Allocasurina littoralis* *Leptospermum continentale* *Hakea laurina*
Lawn grass	Velvetene™	Palmetto® Soft Leaf Buffalo

Table 1: Selected set of plant species tested for nitrogen removal performances (source Payne et al. (2014b)).

The influence of inter-species competition on N and P removal

A parallel laboratory-scale study of biofilters planted with either a monoculture or various combinations of a mixture of two species was conducted to assess the effect of mixed species plantings on nutrient removal (Ellerton et al., 2012). The two plant species selected for use were *Carex appressa* (shown to be effective for nutrient removal, Bratieres et al., 2008, Read et al., 2008) and *Lomandra longifolia* (ineffective for nutrient removal, Bratieres et al., 2008, Read et al., 2008). All design characteristics were specified according to FAWB's Biofilter Adoption Guidelines (FAWB, 2009) and the biofilter boxes were dosed with semi-synthetic stormwater for four months using a volume and pattern that reflected typical rainfall conditions for Melbourne. Water quality samples were collected at the inlet and outlets after 6, 12 and 18 weeks of operation and analysed for Total Suspended Solids (TSS), TN, NO_x, NH_4^+, TP and FRP by a NATA-accredited laboratory using standard methods and quality control procedures.

The influence of a retrofitted SZ on improving N removal performance

Following the completion of a large-scale biofilter column study (see Bratieres et al, (2008) for details), SZs were retrofitted to a subset of biofilter columns to investigate potential benefits from such interventions (Zinger et al., 2013). Of particular interest was whether stormwater biofilters with poor N removal could be enhanced if a SZ was retrofitted to create anaerobic conditions for effective denitrification. This was evaluated by measuring removal of N, P and metals in the retrofitted biofilter columns. Various, typical biofiltration configurations, that included freely draining 690 mm deep loamy sand media above a 140 mm deep transition layer and a 70 mm gravel layer planted with popular plant species (Dianella revoluta, Microlaena stipoides and Carex appressa), were tested for typical operational conditions over a period of 12 months. Following this, a SZ was created at the base of each column by fitting a riser pipe to elevate the outlet and treatment performance was monitored for a further five months.

The role of filter media in long-term P retention

Another laboratory-scale column study was conducted to investigate the role of filter media, vegetation and a SZ on P removal (Glaister et al., 2012, Glaister et al., 2013b, Glaister et al., 2014). Twenty biofilter columns, representing four different design configurations (Table 2), were monitored over a 12-month period of dosing with semi-synthetic stormwater using a volume and frequency typical of Melbourne rainfall. A key aim of this study was to investigate whether interactions between iron (Fe) and P could enhance the binding of P to the filter medium. The alternate design variable of particular interest in this study was the use of Skye sand, a naturally occurring iron- and aluminium-oxide rich sand, as the filter medium. In addition, the dosing frequency simulated wet and dry seasonal conditions. During wet periods, the columns were dosed twice weekly while the number of dry days between dosings was gradually increased from 6 to 18 days during the dry period.

Configuration	Filter Medium	Vegetation	SZ
Loamy sand, vegetated, submerged zone (LS-V-S)	Loamy Sand	*Carex appressa*	SZ
Skye sand, non-vegetated, submerged zone (SS-NV-S)	Skye Sand	Non-vegetated	SZ
Skye sand, vegetated, submerged zone (SS-V-S)	Skye Sand	*Carex appressa*	SZ
Skye sand, vegetated, no submerged zone (SS-V-NS)	Skye Sand	*Carex appressa*	No SZ

Table 2: Biofilter column design configurations tested to investigate phosphorus removal in stormwater biofilters (Source: Glaister et al. (2014)).

In a complementary study, a survey of six field-scale biofilters in Brisbane and Melbourne was conducted to assess long-term accumulation of P in biofilters (Glaister et al., 2013a). These biofilters varied in age, size, filter media configuration, vegetation and catchment characteristics (Table 3). All biofilters were located in residential catchments, except for Cremorne Street, which was located within an industrial/commercial area. Filter media samples were subjected to a four-step sequential extraction scheme designed to measure P associated with four different phases (bioavailable P, P -adsorbed to iron oxyhydroxides, P associated with amorphous iron oxyhydroxides, and organic P).

Biofilter	Location	Year Constructed	Area	Ratio	Filter Media Depth	Sedimen Pre-treatment
Hoyland St.	Brisbane	2001	720 m^2	4%	700	Nil
Cremorne St.	Melbourne	2003/4	11 m^2	3.4%	400	Nil
Saturn Cres.	Brisbane	2006	20 m^2	2%	400	Nil
Wakerley	Brisbane	2006/7	2865 m^2	0.3%	800-1000	Pnd
Clifton Hill	Melbourne	2007	200 m^2	0.3%	500	Trap
Banyan	Melbourne	2008	3750 m^2	1.6%	400	Pond/Marshes

Table 3: Summary characteristics of the field biofilters tested for long-term P retention (Ratio refers to biofilter surface area as a proportion of the total catchment area) (Source: Glaister et al. (2013a)).

2.1.2 Faecal microorganisms

Understanding performance of current biofiltration designs

A range of of laboratory-scale biofilter column experiments were carried out to investigate how faecal microbial removal was affected by current biofilter design parameters (vegetation selection, inclusion of a submerged zone, filter media type, etc.) and operational conditions (antecedent dry weather period, inflow concentration, etc.) (Chandrasena et al., 2012a, Chandrasena et al., in press, Chandrasena et al., in preparation-a, Li et al., 2012).

These included a laboratory-scale study conducted to investigate the removal of three indicator organisms (Clostridium perfringens, Escherichia coli, and F-RNA coliphages) by current, best practice biofilter design (Li et al., 2012). Twenty-eight large biofilter columns (375 mm diameter) were dosed with semi-synthetic stormwater to mimic typical Melbourne rainfall over 3.5 months. The influence of a range of factors, including the presence of vegetation, depth and types of filter media, presence of a SZ, and intermittent wetting and drying, was investigated.

The influence of biofilter design and operational factors on faecal microbial removal

Another laboratory-scale study was conducted to investigate how the removal of *E. coli* in current, best practice biofilters was affected by varying operational conditions (inflow concentrations, antecedent dry weather period and *E. coli* particle association characteristics in inflow) and type/mix of vegetation (Chandrasena et al., 2012a). Twenty-five established biofilter columns (> 1.5 years of operation), built following current FAWB (2009) guidelines and without a SZ, were dosed with semi-synthetic stormwater to mimic typical Melbourne rainfall and samples were collected over a six-week period.

The *E. coli* removal performance of a subset of 7 plant species (from a broader set of 22 plant species tested for N removal performances (Section 2.1.1)) was also tested over an eight month period (Chandrasena et al., 2014b) (Table 4). Two types of designs, with and without a SZ, along with two non-vegetated controls (90 columns, 150 mm in diameter) were dosed with semi-synthetic stormwater to mimic typical Melbourne and Perth rainfall frequency. These columns were subjected to 'wet' conditions, where the columns were dosed twice weekly, and 'dry' conditions, where the columns were dosed at approximately fortnightly intervals.

Broad plant type	Western Australia	Victoria
Grasses	*Sporobolus virginicus*	*Poa labillardieri*
Sedges/reeds		*Carex appressa* *Dianella tasmanica*
Shrubs/trees	*Melaleuca incana*	*Leptospermum continentale*
Lawn grass		Palmetto® Soft Leaf Buffalo

Table 4: Selected set of plant species tested for *E. coli* removal performances (source Chandrasena et al. (in press)).

A further laboratory-scale biofilter column experiment was established to understand how faecal microbial removal processes were influenced by biofilter design (vegetation presence and type, filter media type) and operational conditions (extended drying periods, size and duration of wet weather events, and clogging of the filter media) (Chandrasena et al., in preparation-a). Importantly, these systems were evaluated using three faecal indicators (*E. coli*, F-RNA coliphagess and *C. perfringens*) and three reference pathogens (*Campylobacter* spp., *Cryptosporidium* oocysts and adenoviruses). A total of 30 biofilter columns (240 mm in diameter) with SZs were tested for a period of eight months. The biofilter columns were dosed twice weekly with semi-synthetic stormwater to mimic typical wet weather conditions in Melbourne while columns were left to dry up to 2, 4 and 6 weeks on selected occasions to test the interactive effects that biofilter design and extended dry weather periods have on faecal microorganism removal performance.

Major mechanisms governing the removal of faecal microorganisms in current biofilters

In addition to the above, controlled laboratory studies were conducted to investigate the mechanisms governing faecal microorganism removal (*i.e.*, retention and survival processes). Filter media and top sediment samples were taken from two different, field-scale biofilters (Monash carpark and Banyan Reserve) to investigate *E. coli* survival under controlled laboratory conditions (Chandrasena et al., 2014a). The study investigated how *E. coli* survival is influenced by temperature, moisture content, sunlight exposure, and presence of other microorganisms in filter media and top surface sediment over a period of five weeks. Another miniature column study (30 mm in diameter) comprised of eight non-vegetated columns dosed with semi-synthetic stormwater to investigate the relative contribution of microbial retention processes (straining, adsorption and desorption) on *E. coli* removal in stormwater biofilters (Chandrasena, 2014). Miniature columns filled with either washed sand (typical biofilter media) or glass beads (control media) were dosed with 4 pore volumes (PV) of semi-synthetic stormwater followed by 4 – 8 PV of nutrient solution free of any faecal microorganisms. Multiple samples were collected at the column inlet and outlet during dosing and at different media depths once the dosing period was complete and were analysed for *E. coli* concentrations.

Faecal microorganism removal achieved by current, best practice biofilters in the field-scale

Field monitoring was conducted at two biofilters used for stormwater harvesting to validate the laboratory-scale findings (Chandrasena et al., in preparation-b), as discussed below.

The Royal Melbourne Golf Club (RMGC) biofilter (Figure 3), which is a part of the stormwater harvesting system implemented at RMGC, was monitored for *E. coli* and *Campylobacter* spp. removal during normal operation for over 1.5 years (starting in May 2012) (Chandrasena et al., in preparation-b).

Six *in situ* challenge tests were conducted at a field-scale biofilter located at Monash University, Clayton (known as the Monash carpark biofilter) to investigate the removal performance of a set of pathogen indicators (*E. coli*, Enterococci, *C. perfringens*, F-RNA coliphages) and the reference pathogen, *Campylobacter* spp. under challenging operational conditions (large event sizes, extended drying and extremely wet conditions) (Chandrasena et al., 2012b, Chandrasena et al., in preparation-b). The biofilter was dosed with semi-synthetic stormwater spiked with raw sewage to mimic large storm events (2.4 -4 PV) and extreme dry/ wet conditions (antecedent dry period varying from ten hours to two weeks, Figure 4).

Figure 3: Royal Melbourne Golf Club biofilter monitored for faecal microbial removal performance (left) and autosamplers used for inflow sampling (right).

Figure 4: *In situ* challenge tests at Monash University biofilter.
Preparation of semi-synthetic stormwater in the inflow mixing tank (left) and application of semi-synthetic stormwater (right).

Next generation biofilters: novel antimicrobial filter media designs

Selection of antimicrobial coatings for optimal stormwater treatment

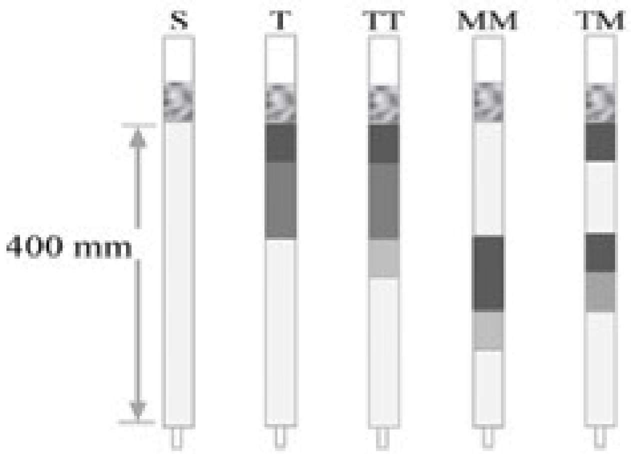
A miniature non-vegetated column study was conducted to identify potential antimicrobial filter media candidates which are resilient to different biofilter operational conditions (drying/wetting, infiltration rate, inflow salinity etc.) (Li et al., 2014a) (Figure 5). Zeolite and granular activated carbon (GAC) were each modified with ten types of antibacterial materials (metal ions, metal hydroxides, metal oxides and quaternary ammonium salts), however only 15 different media combinations were tested (Table 5). Their antibacterial properties and stability in natural stormwater were studied in gravity-fed columns for a period of 24 weeks covering ten simulated rain events and nine dry weather periods of varied duration (1-4 weeks). Water quality samples were collected at the inlet and outlets and analysed for *E. coli* concentrations while outlet samples were also analysed for heavy metal concentrations to investigate the stability of the media. Filter media samples were analysed at the end of the experiment to investigate the distribution and inactivation of *E. coli* by the modified media.

↑ Figure 5: Miniature experimental column set-up used for initial antimicrobial filter media screening test. Zeolite based media (10 types + control); GAC based media (5 types + control)

↓ Table 5: Combination of base media with antimicrobial agents tested in the initial screening test. (Source: Li et al. (2014a))

Antibacterial Agent	Modifier Filter Media	
	Zeloite-Based	GAC-Based
Nil	Z0	G0
Metal ions	Cu-Z,Zn-Z,Fe-Z,Zn/Cu/Fe-Zb	Cu-G[b]
Metal hydroxide	$Fe(OH)_3$-Z[b], Zn(OH)2-Z[b]	$Zn(OH)_2$-G, $Cu(OH)_2$-G[b]
Metal oxide	CuO-Z[b], TiO_2-Z[b]	TiO_2-G[b]
Quats[a]	SiQAC-Z[b], QAC-Z	SiQAC-G[b]

[a] Quats – Quaternary ammonium salts;
[b] Media have never been tested for water treatment, while all the listed media have never been tested for stormwater applications.



Layout of filter media in each design (bottom to top)

- **S –** 400 mm sand, 50 mm coarse gravel (control)
- **T –** 250 mm sand, 100 mm sand/ZCu400 mix (1:1), 50 mmZCu400, 50 mm coarse gravel
- **TT –** 200 mm sand, 50 mm Z0, 100 mm sand/ZCu400/ ZCuCuO180mix (2:1:1), 50 mm ZCu400/ZCuCuO180 mix (1:1), 50 mm coarse gravel
- **MM –** 100 mm sand, 50 mm Z0, 100 mm ZCu400/ZCuCuO180 mix(1:1), 150 mm sand, 50 mm coarse gravel
- **TM –** 150 mm sand, 50 mm Z0, 50 mm ZCuCuO180, 100 mm sand,50 mm ZCu400, 50 mm coarse gravel

Optimising Cu coating – making it effective and stable

A subsequent study was then conducted to prepare a stable, effective copper-zeolite media (either by calcination of Cu^{2+}-exchanged zeolite (ZCu) or *in situ* $Cu(OH)_2$ coating on ZCu), and to investigate the impact of salinity on the stability and *E. coli* removal performance of the novel antibacterial media (Li et al., 2014b). In total, seven types of antibacterial media were tested in the first stage of this study (Table 6). The aim of the second stage of this study was to design and investigate new sand filters with the two selected stable Cu-zeolite media types for improved bacterial retention and inactivation (Figure 6).Inlet and outlet water quality samples were tested for *E. coli* and total Cu concentrations. Treated water quality parameters (*E. coli* and total Cu concentrations) were compared against Australian drinking water standards, irrigation guidelines and recreational water quality guidelines (ANZECC, 2000, NHMRC, 2004, NRMMC et al., 2009).

← Figure 6: Different novel filter media designs tested for stable antibacterial media (Source Li et al. (2014b)).

Base Zeolite Media Size Fraction (mm)	Novel Antimicrobial Media
0.1-0.3 (graded)	$ZCu800_{0.1}{}^{a}$
0.3-0.6 (graded)	$ZCu_{0.3}{}^{a}$, $ZCu800_{0.3}{}^{a}$, $ZCu600_{0.3}{}^{a}$, $ZCu400_{0.3}{}^{a}$, $ZCuCuO400_{0.3}{}^{a}$, $ZCuCuO180_{0.3}{}^{a}$
0.1-0.6 (non-graded)	$ZCuCuO180^{b}$, $ZCu400^{b}$

Table 6: Different antibacterial media prepared by modifying Cu2+ exchanged zeolite through calcination and *in situ* Cu(OH)2 coating (Source Li et al. (2014b))

[a] Media tested in columns to investigate their stability and bacterial removal efficiency in test water of varied salinity;
[b] Media combined with washed sand in a variety of arrangements to investigate the promising layout for bacterial removal

Incorporating stable antimicrobial filter media into stormwater biofilters

A large-scale column study was subsequently conducted over 1.5 years (starting in February 2013) to examine the treatment performance of vegetated and non-vegetated biofilters that contained the copper-zeolite media (Li et al., 2014b). A total of 35 biofilter columns consisting of seven different novel antibacterial filter media designs were tested. The 400 mm deep filter media layer consisted of washed sand, natural zeolite and copper-zeolite in two types of configurations (Li et al., submitted, Li et al., in preparation);

- *Layered* – (listed from the top to the bottom of the filter media) 50 mm of ZCu400; 100 mm of washed sand (the top 50 mm of which was ameliorated with organic matter, fertiliser and trace elements as per Australian biofiltration design guidelines); 50 mm of ZCuCuO180 for pathogen inactivation during filtration; 50 mm of natural zeolite to adsorb any leached copper; and 150 mm of sand; and,

- *Mixed* - (listed from the top to the bottom of the filter media) 100 mm of ZCu400/ZCuCuO180 mixture (1:1 ratio in volume); 50 mm natural zeolite; and 250 mm of washed sand.

The *'layered'* design was investigated without vegetation (SCu), with Palmetto® Soft Leaf Buffalo (PBCu, the entire filter top surface was covered with grass), and with *Leptospermum continentale* (LCCu, one plant each filter). The same designs, but replacing copper-zeolite with natural zeolite, served as controls (named S, PB, and LC). In addition, one 'mixed' design planted with *L. continentale* was constructed to assess the potential of retrofitting existing biolfilters with antimicrobial media.

These systems were assessed for their pollutant removal efficiency for reference pathogens (*Cryptosporidium* oocysts, *Campylobacter* spp., and adenoviruses), faecal indicators (*C. perfringens*, *E. coli* and F-RNA coliphages), TSS and nutrients (TN and TP). Most importantly, the columns were subjected to a range of challenging operational conditions, including high volume events (1 in 3 month average recurrence interval (ARI)) and extreme dry weather periods (2 - 6 weeks).

2.1.2 Micro-pollutants

Retention of metals that pose a risk for water uses

A large-scale stormwater biofilter column study was conducted to evaluate the impact of design configurations and operating conditions on metal removal (Feng et al., 2012). The study focused on six metals that have been shown to pose a risk for stormwater harvesting and protection of aquatic ecosystems: aluminium (Al), chromium (Cr), lead (Pb) and zinc (Zn), copper (Cu) and iron (Fe). A range of factors were tested over eight months of operation in 120 large biofilter columns of 375 mm diameter, including vegetation selection (plant species), filter media type, filter media depth, inflow volume (loading rate), and inflow pollutant concentrations. Operational time was also considered to evaluate treatment performance over time. Outflow water quality was evaluated against irrigation and drinking water standards.

Long-term metal retention capacity of biofilters

To assess the likelihood that the retention capacity of filter media would be exhausted within the expected operational life, accelerated-dosing laboratory tests were conducted by Hatt et al. (2011). In that study, three different non-vegetated, soil-based filter media that are commonly used in stormwater biofilters were tested in two separate experiments: loamy sand, loamy sand mixed with 10% vermiculite and 10% perlite (by volume), and loamy sand mixed with 10% leaf compost and 10% mulch (by volume). Semi-synthetic stormwater was continuously pumped through small columns for 11 – 13 weeks; this equated to an equivalent of 12 – 15 years of operation for a system sized at 2% of its impervious catchment area under a typical Melbourne rainfall pattern.

In addition, a field survey was conducted to assess heavy metal (Cd, Cu, Pb and Zn) accumulation in biofilters (Hatt et al., in preparation). Twenty-nine biofilters were sampled over 14 sites in Melbourne, Sydney and Brisbane (Table 7). The sites represented a broad range of catchment and biofilter design characteristics (development type, urban density, current land-use, past land-use). Concentrations of heavy metals in the 29 surveyed biofilters were then compared to Australian and European (Dutch) soil quality guidelines (NEPC, 1999, NMHSPE, 2000)

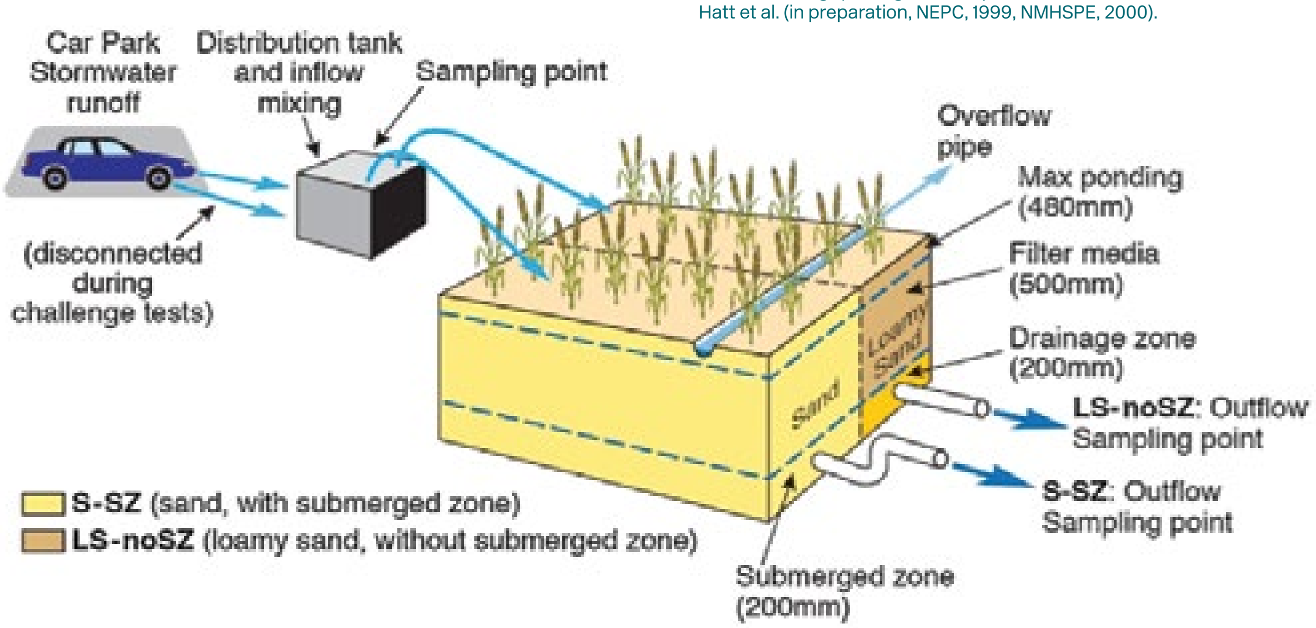
Removal of organic micro-pollutants under challenging operational conditions

Two series of *in situ* experiments were conducted, each consisting of three separate challenge tests (*i.e.*, six challenge tests in total) to investigate the organic micro-pollutant removal performance of field-scale biofilters (Zhang et al., 2012, Zhang et al., 2014) (Figure 7). Six challenge tests, three conducted in 2011 (inflow volume = 2.4 PV) and three in 2012 (inflow volume = 1.8-4 PV), were designed to mimic different operational conditions, ranging from challenging wet or dry conditions (antecedent dry periods from ten hours to two weeks) to typical operational conditions. Two biofilter configurations were studied: a configuration with loamy sand and no SZ (LS-no SZ) and another configuration that uses sand and a SZ (S-SZ). The organic micro-pollutants studied were: total petroleum hydrocarbons (TPHs), polycyclic aromatic hydrocarbons (PAHs), glyphosate, triazines (simazine, atrazine and prometryn), phthalates (dibutyl phthalate (DBP), di-(2-ethylhexyl) phthalate) (DEHP), Trihalomethanes (THMs) and phenols (phenol, pentachlorophenol).

Site	Catchment characteristics				Biofilter characteristics			
	Development	Urban density	Current land-use	Past land-use	Age (years)	Surface area (m^2)	Ratio (%)	Design annual inflow (m^3/m^2)
Streisand Dr, Brisbane	greenfield	low	res	bushland	0.5	20	1.8	53
Saturn Cr, Brisbane	greenfield	low	res	bushland	0.5	20	3.0	32
Donnelly Pl, Brisbane	greenfield	low	res	agr/peri	0.2	32.2	2.8	34
Hoyland St, Brisbane	retrofit	low	res	bushland	5	860	4.9	19
Cremorne St, Melbourne	retrofit	high	ind/comm	mixed	3	14.5	4.0	13
	retrofit	high	ind/comm	mixed	3	11	18.3	2.8
	retrofit	high	ind/comm	mixed	3	4.5	0.8	65
	retrofit	high	ind/comm	mixed	3	18	2.9	18
	retrofit	high	ind/comm	mixed	3	10	2.5	21
	retrofit	high	ind/comm	mixed	3	11	3.4	15
	retrofit	high	ind/comm	mixed	3	6	7.1	7.3
	retrofit	high	ind/comm	mixed	3	10	11.8	4.4
Alleyne Ave, Melbourne	retrofit	low	res	agr/peri	2	12	17.6	2.9
	retrofit	low	res	agr/peri	2	24.5	21.9	2.4
	retrofit	low	res	agr/peri	2	17	8.0	6.5
	retrofit	low	res	agr/peri	2	22	13.5	3.9
Point Park, Melbourne	renewal	high	comm	mixed	0.5	7	1.7	30
	renewal	high	comm	mixed	0.5	7	1.9	27
Hamilton St, Melbourne	retrofit	medium	res	mixed	3	4	0.1	416
Avoca Cr, Melbourne	retrofit	medium	res	agr/peri	3	5	4.2	12
	retrofit	medium	res	agr/peri	3	4	2.0	26
	retrofit	medium	res	agr/peri	3	4	1.3	40
Parker St, Melbourne	retrofit	medium	res	agr/peri	3	12	3.8	14
	retrofit	medium	res	agr/peri	3	14	8.9	5.9
	retrofit	medium	res	agr/peri	3	7	1.3	39
CERES, Melbourne	retrofit	medium	res	mixed	2	21.75	1.7	30
Bourke St, Melbourne	renewal	high	comm	mixed	1	1.44	1.4	36
Wolseley Gr, Sydney	renewal	high	res	mixed	7	330	21.9	4.3
Leyland Gr, Sydney	renewal	high	res	mixed	7	180	10.0	9.4

↑ Table 7: Summary characteristics of the field biofilters tested for long-term heavy metal accumulation. Age - age at time of sample collection; Ratio - biofilter surface area as a proportion of the impervious catchment area; res, residential; ind/comm, industrial/commercial; comm, commercial; agr/peri, agriculture/peri-urban; mixed, mixed urban. (Source: Hatt et al. (in preparation, NEPC, 1999, NMHSPE, 2000).

↓ Figure 7: Monash Carpark stormwater harvesting system and configuration of biofilter cells selected for *in situ* challenge tests (Source: Zhang et al. (2014))



2.2 Key Findings

The following sections present key findings from the recent research conducted by the CRCWSC focusing on the removal performance of stormwater biofilters across different types of pollutant.

2.2.1 Nutrients

N removal

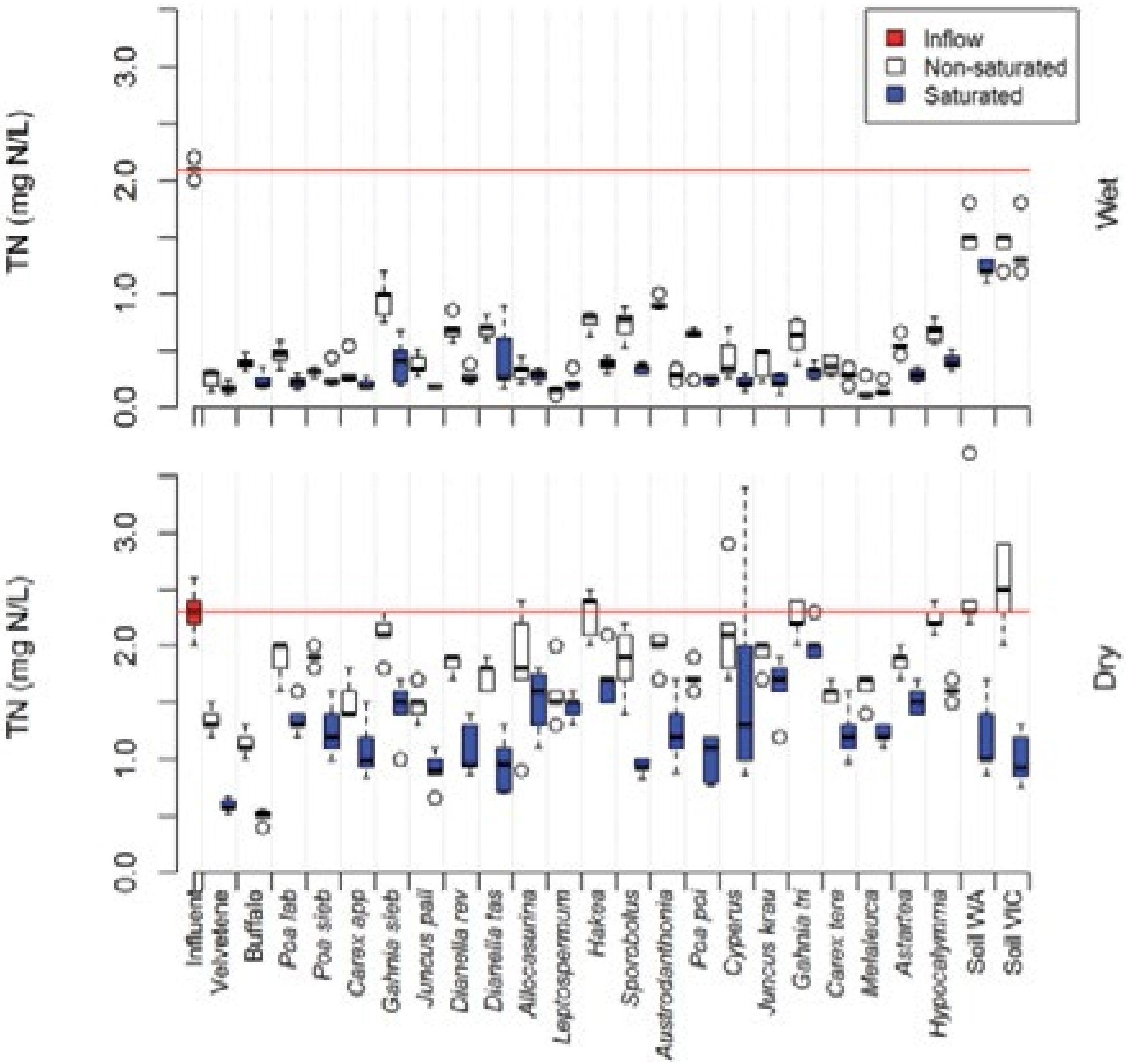
All plant species effectively removed N during wet periods (Figure 8), suggesting that plant selection is less important under wet conditions provided that the filter medium is carefully specified to minimise the risk of nutrient leaching. However, extended drying resulted in an overall decline in N removal performance and also increased variation between the species and design configurations tested. It is not surprising that presence of a SZ mitigated the performance decline after drying and acted to diminish performance differences between plant species (Payne et al., 2014c). It was also noted that the relative performance of some species changed from wet to dry conditions. For example, *L. continentale* and *C. gymnocaulis* were relatively high performers under wet conditions but performed comparatively poorly in the dry conditions (Figure 8). Inclusion of a SZ and species diversity in biofilters should be considered to ensure resilience against varying climatic conditions.

The TN removal performance of biofilters planted with less effective vegetations types (such as *D. revoluta*, *M. stipoides*) was shown to improve after retrofitting a SZ, mainly due to increased NO_x removal (data not shown, see Zinger et al. (2013) for details). However, TP removal was less efficient after retrofitting the SZ, possibly due to the presence of organic matter in the filter media within the SZ.

When looking into the key processes, it was also found that plant assimilation was the primary fate for nitrate (NO_3^-) removal, contributing an average 89-99 % of $^{15}NO_3^-$ processing in biofilter columns containing the most effective plant species, while only 0-3 % was denitrified and 0-8 % remained in the pore water (Payne et al., 2014a). Denitrification played a greater role for biofilter columns containing less effective species, processing up to 8 % of $^{15}NO_3^-$. Inclusion of a SZ was previously found to improve the removal of NO_3^- but this also led to decreased retention of organic N and P (Zinger et al., 2007b, Zinger et al., 2007c). However, subsequent research indicates that careful selection of the carbon source that is added to the SZ minimises the reduction in organic N removal and maintains effective P removal (Payne et al., 2013, Glaister et al., 2014).

In conclusion, **plant species selection is critical where extended dry periods are likely to be experienced. Table 8 shows a list of plants that are effective and less effective for N removal** (this adds to the list of effective plants identified earlier by FAWB, (2009)). **To enhance denitrification and ensure resilience against varying climatic conditions, a SZ should be included wherever possible with a low nutrient carbon source; appropriate materials include sugar cane mulch, pine chips (without bark) and pine flour ('sawdust').**

A mixed planting that included *C. appressa* (previously shown to be very effective in removing nutrients; improved removal of NO_x (and therefore TN) compared to a monoculture of *L. longifolia* - previously shown to be ineffective in removing nutrient (Bratières et al., 2008, Read et al., 2008)). It was demonstrated that the ratio of effective and ineffective plant species was not as important as the presence or absence of effective species (Ellerton et al., 2012). **To be on the safe side, it is therefore recommended that biofilters be planted with at least 50% effective species while the remaining species can be specified to accommodate other considerations, such as biodiversity and aesthetics.**



Effective species		Less effective species	
Carex appressa	Melaleuca incana	Microlaena stipoides	Hakea laurina
Melaleuca ericifolia	Juncus pallidus	Dianella revoluta	Allocasurina littoralis
Goodenia ovata	Leptospermum continentale	Leucophyta brownii	Hypocalymma angustifolium
Ficinia nodosa	VelveteneTM	Lomandra longifolia	Gahnia sieberiana
Juncus amabilis	Palmetto® Soft Leaf Buffalo	Banksia marginata	Gahnia trifida
Juncus flavidus		Pomaderris paniculosa	Austrodanthonia caespitosa
Carex tereticaulis		Astartea scoparia	

↑Table 8: Plant species found to be either effective or less effective for N removal in stormwater biofilters (Source: FAWB (2009) and Payne (2013))

↑↑Figure 8. Outflow TN concentrations (mg/L) across the plant species in October 2012 (wet period; top) and December 2012 (dry period; bottom) (Source: Payne et al. (2014c))

P removal

P removal was also shown to be consistently high, provided the filter medium contained a low nutrient content to prevent leaching (Figure 9), confirming the results of previous studies (Bratières et al., 2008, Read et al., 2008). Nevertheless, inclusion of vegetation and a SZ with low nutrient carbon source further improved P removal, particularly the dissolved component (Figure 9) (Glaister et al., 2014).

The results of a compressed-time small column experiment showed that augmenting the standard loamy sand filter material with Skye sand (a naturally occurring iron- and aluminium-oxide rich sand) increased the life expectancy, with respect to exhaustion of the P retention capacity of filter media, from less than 6 months to more than 2.5 years, (Glaister et al., 2014). However, when tested in laboratory-scale biofilter columns, significant differences in P removal performance were not evident in different filter media types, especially in the early stages of operation, where the phosphorus sorption capacity of the media was unlikely to be exhausted (Glaister et al., 2011). Some washout of fine, iron-rich particles was observed from the columns without a SZ, but not those with a SZ, suggesting that the water dynamics in the systems with a SZ (lower head loss as well as less drying and creation of cracks and macropores in the filter media) prevented washout of fines. In addition, it is recommended that Skye sand be mixed with the standard loamy sand filter medium at a ratio of 1:1 – 1:3, primarily to optimise increased sorption capacity versus capital costs, but also because this creates a smoother particle size distribution and will thus better retain fine particles.

A survey of six field-scale biofilters revealed that P accumulated in the top 10 cm of the filter media, irrespective of the design and age of the biofilters (Glaister et al., 2014), consistent with the findings of a previous laboratory-scale study (Glaister et al., 2013a), and in areas closer to the inlet. It was also found that the accumulated P was largely in the mineral and organic (*i.e.*, least mobile) phases, which suggests that P will be retained in the long-term. Further, there were no signs of P breakthrough, even after 12 years of operation, indicating that biofilters can be expected to sustain effective P removal in the long-term.

Highlights

It was confirmed that filter media with a low nutrient content and inclusion of a SZ containing a low nutrient content carbon source were shown to improve nutrient removal.

Plant species selection remains critical, particularly for N removal, and a mixed planting including both effective and ineffective plants species for nutrient removal is suggested over a single plant species (minimum 50% effective species).

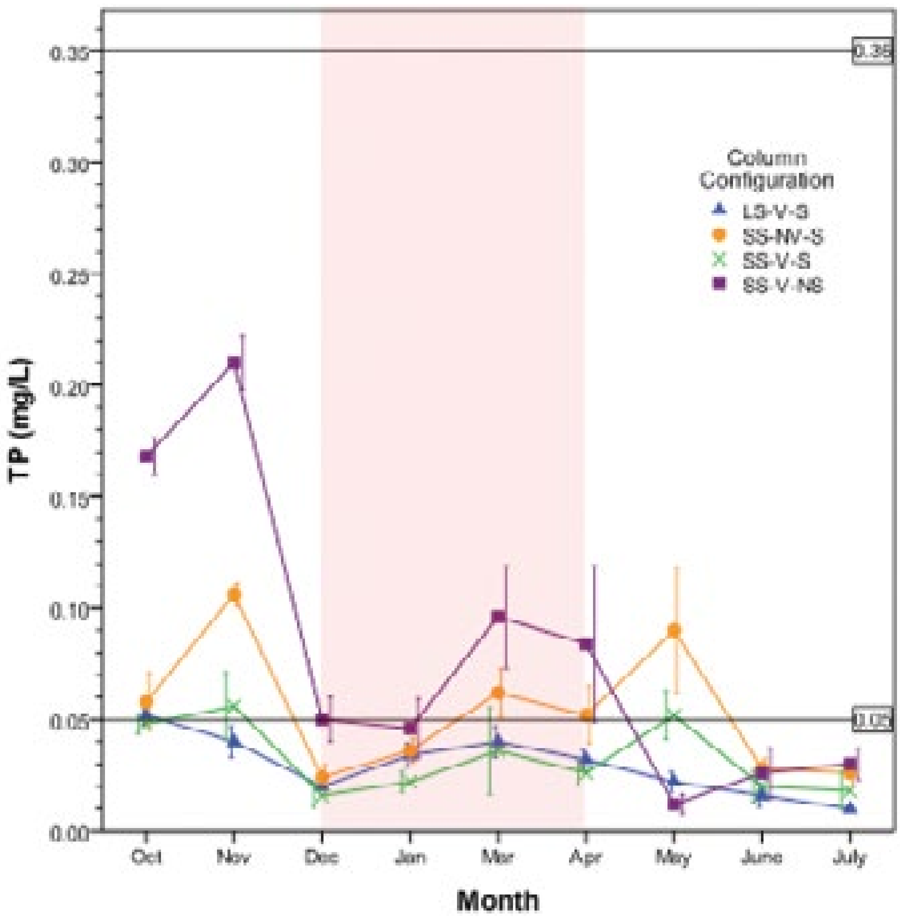


Figure 9. Mean (n=5) outflow TP concentrations (mg/L) from October 2011 to July 2012. Error bars represent ±1 standard deviation from the mean. LS-V-S: loamy sand media, vegetated with C. appressa, SZ; SS-NV-S: Skye sand, non-vegetated, SZe; SS-V-S: Skye sand, vegetated with C. appressa, SZ; SS-V-NS: Skye sand, vegetated with C. appressa, no SZ. The shaded area indicates the dry dosing period. The upper horizontal line denotes the inflow concentration while the lower horizontal line represents the water quality guideline concentrations for slightly disturbed lowland rivers in South-Eastern Australia (ANZECC, 2000) (Source: Glaister et al. (2014)).

Augmenting traditional biofilter media (loamy sand) with naturally occurring iron- and aluminium-oxide rich sand (mixing ratio 1:3 - 1:1 or 25 - 50% Skye sand) helps to improve long-term P retention.

Current, best-practice biofilters can be expected to sustain effective long-term P removal without breakthrough.

2.2.2 Faecal microorganisms

Understanding faecal microbe removal in current biofiltration designs

Level of treatment achieved by current best practice biofilters

The two field scale **stormwater biofilters (designed as per current FAWB guidelines (2009)) achieved around 1 log reduction in bacterial indicator (E. coli) concentrations** (Figure 10), consistent with the findings of a laboratory-scale column study (Figure 11). Removal rates of around 2 log reductions were observed for *C. perfringens* in both the field and laboratory studies (Figure 10, Figure 11, Figure 12). On the other hand, the viral indicator, F-RNA coliphages showed the highest and the most variable removal rates with average log reductions varying from 3 to 1 (Figure 10, Figure 11, Figure 12).

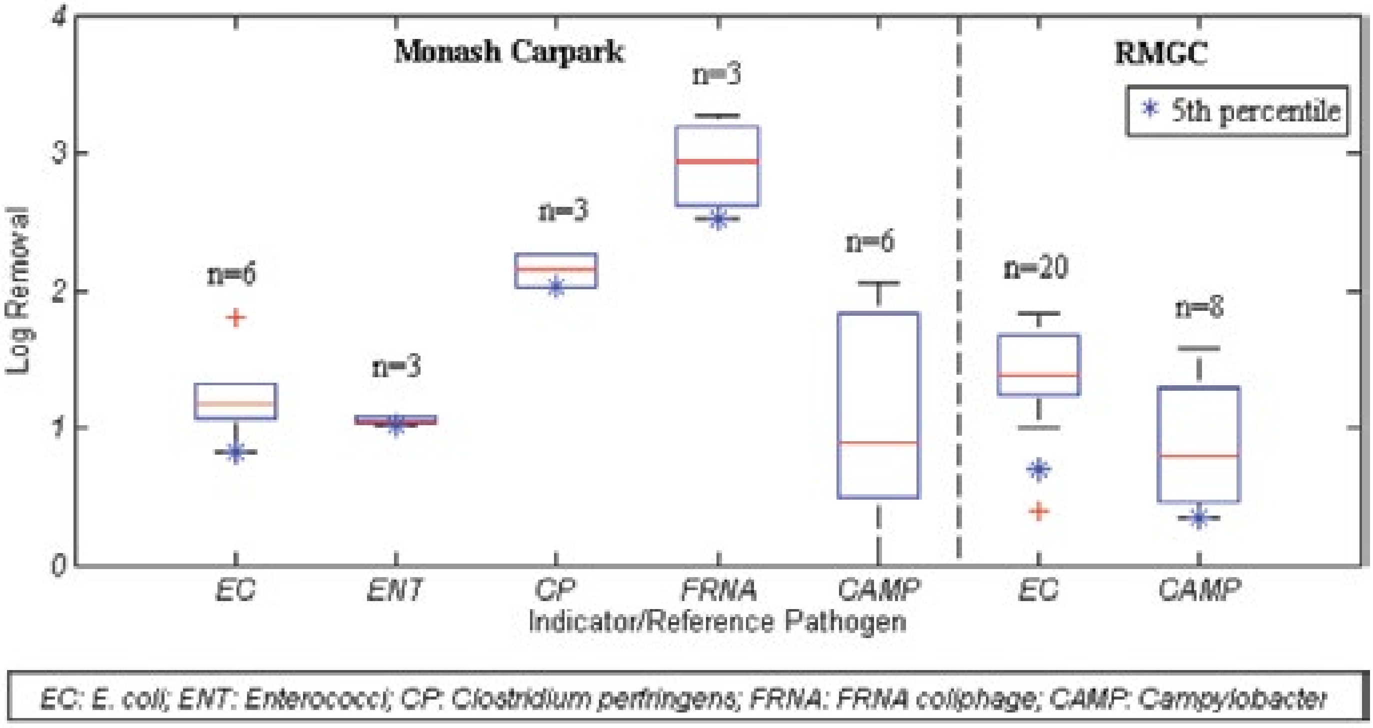
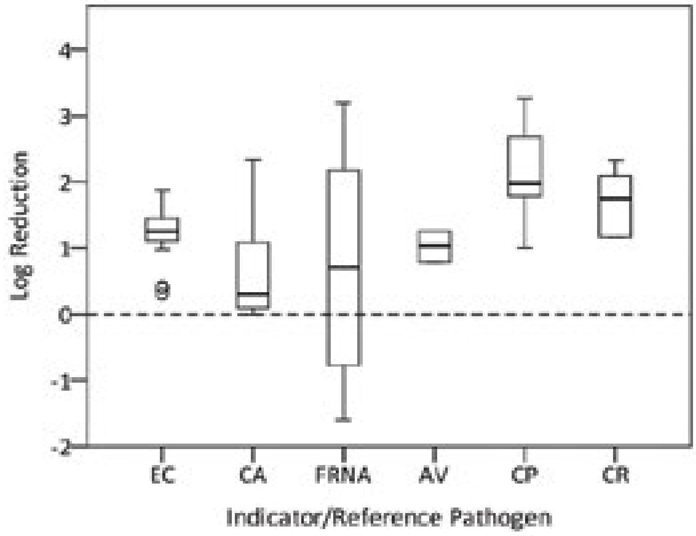


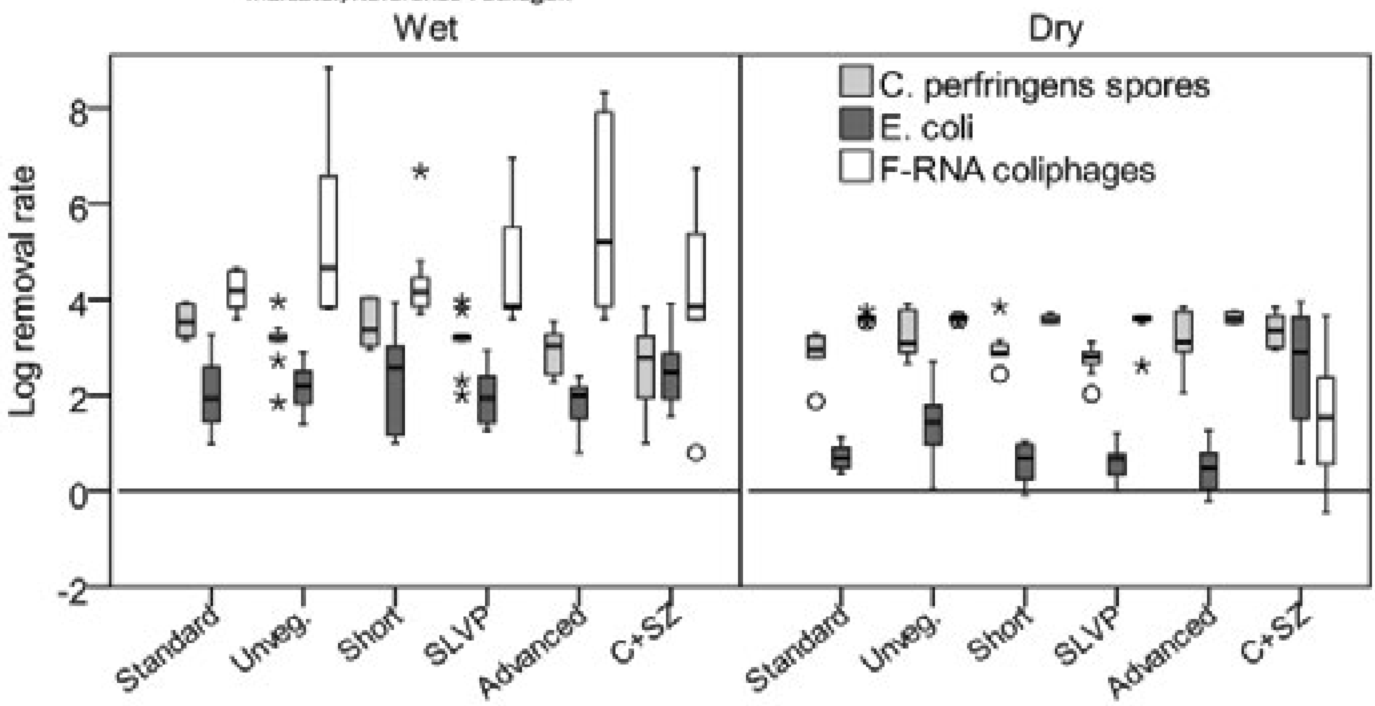
Figure 10: Indicator and reference pathogen removal at field-scale stormwater biofilters designed as per current FAWB (2009) guidelines. n - number of events sampled for each faecal microorganism

The data collected at the two field systems (during both challenge tests and stormwater monitoring, Figure 10) suggest that biofilters can also act as a barrier for reference pathogens (Chandrasena et al., in preparation-b). Biofilters were found to have the capacity to reduce Campylobacter spp. concentrations under field scale operation, with average log reductions of around 0.9. Laboratory-scale biofilters were found to achieve around 1 log reduction for adenoviruses (Figure 11). The highest average log reduction was observed for the protozoan reference pathogen, *Cryptosporidium* oocyts, which was around 1.7 (Figure 11).



→ Figure 11: Indicator and reference pathogen removal at laboratory-scale stormwater biofilters designed as per current FAWB (2009) guidelines. Indicator/Reference pathogen abbreviations: EC – *E. coli*; CA – Campylobacter spp.; FRNA – F-RNA coliphage; AV - adenovirus; CP – *C. perfringens*; CR – *Cryptosporidium* oocysts. (Source: Chandrasena et al. (in preparation-a))

↓ Figure 12: Indicator microorganism removal affected by antecedent dry weather period in different biofilter designs. 'Wet' sampling events had an antecedent dry weather period < 2 weeks and 'Dry' events had antecedent dry weather period ≥ 2 weeks. (Source: Li et al. (2012))



Comparing reference pathogens and indicator removal performances

An important question is whether the faecal indicator microorganisms can be used to monitor the removal performance for pathogenic organisms. Campylobacter spp. removal was observed to be consistently lower than the bacterial indicator *E. coli* at both the laboratory- and field-scale (Figure 10 and Figure 11). However, on average, the removal performance of *C. perfringens* (indicator for protozoa) was comparable to that for the corresponding reference pathogen, *Cryptosporidium* oocysts, (Figure 10 and Figure 11). Log reduction of the indicator virus, F-RNA coliphage, was highly variable, yet the average log reduction was comparable to that of the adenovirus (Figure 10 and Figure 11).

Major removal processes and how they are impacted by design and operational conditions

The removal performance of faecal microorganisms is governed by their retention and subsequent survival within biofilters (Chandrasena et al., 2012a, Li et al., 2012). Experimental evidence suggested that the bacterial indicator *E. coli* was retained in the top-most layers of the biofilter media, due to physical straining/filtration, while the remaining microorganisms were reversibly adsorbed throughout the biofilter media, plant roots, etc. during wet weather periods (Chandrasena, 2014). These microorganisms then experienced die-off during subsequent dry weather periods due to the hostile prevailing environment in the biofilters (temperature, presence of other microorganisms, solar irradiance, etc.) (Chandrasena et al., 2014a). Nevertheless, at least some of these retained microorganisms can desorb during subsequent wet weather periods and eventually be washed out in the biofilter outflow (Chandrasena et al., 2012a, Chandrasena et al., 2013).

Faecal microorganism removal in stormwater biofilters is affected by a range of biofilter design characteristics, which were often found to have interactive effects on the major microbial removal processes described above. Consequently, the overall removal performance can increase or decrease depending on the dominant effects prevailing at any given time. **A biofilter which consists of vegetation species with an extensive root structure (such as *L. continentale*, M. incana and C. appressa) was shown to improve microbial removal performance due to enhanced retention** (Chandrasena et al., 2012a, Chandrasena et al., in press, Chandrasena et al., in preparation-a) (Figure 13).

Faecal microbial removal performance improved in the presence of a SZ (Figure 13). It was found that the additional treatment provided during antecedent dry weather periods (via natural die-off, competition and predation, etc.) reduced faecal microorganism levels within the SZ. The 'old' SZ water (*i.e.*, that retained from the previous inflow event) therefore had a lower faecal microorganism concentration than the freshly treated stormwater (*i.e.* water passing immediately through the biofilter; 'new' water).

Both extremely short and extended antecedent dry weather periods were found to reduce faecal microbial removal in stormwater biofilters. Desorption of previously attached microorganisms decreased removal performance in wet weather events which followed short antecedent dry periods, due to inadequate time for die-off to occur (Chandrasena et al., 2012a). Increased infiltration rates, due to the formation of cracks and macropores during extended antecedent dry weather periods, also decreased removal performance in subsequent wet weather events (Chandrasena et al., in preparation-a). Inclusion of a SZ can mitigate some adverse effects of drying especially on bacterial removal in stormwater biofilters (Li et al., 2012). However, it is also important to maintain a steady SZ volume over extended dry weather periods in order to take advantage of the buffering capacity of a SZ (Figure 14).

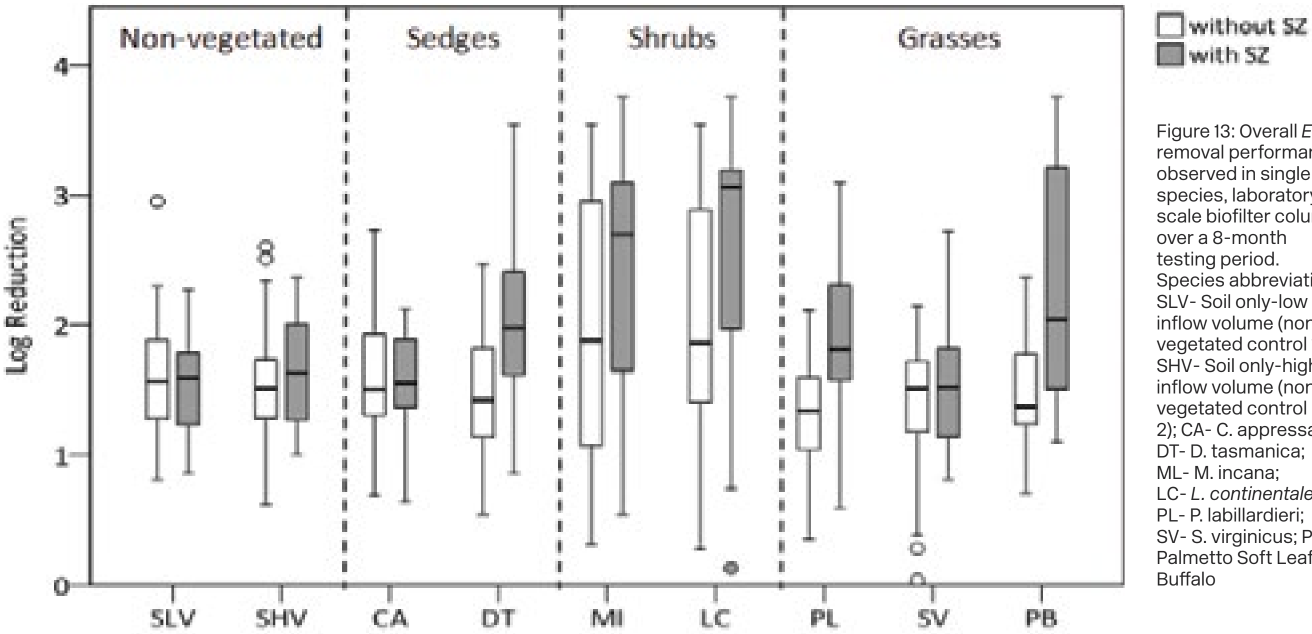


Figure 13: Overall *E. coli* removal performance observed in single species, laboratory-scale biofilter columns over a 8-month testing period. Species abbreviations: SLV- Soil only-low inflow volume (non-vegetated control 1); SHV- Soil only-high inflow volume (non-vegetated control 2); CA- C. appressa; DT- D. tasmanica; ML- M. incana; LC- *L. continentale*; PL- P. labillardieri; SV- S. virginicus; PB- Palmetto Soft Leaf Buffalo

Highlights

If well designed, current stormwater biofilters are capable of achieving 1 log reduction for bacterial indicators.

Current biofilters are also capable of removing reference pathogens, particularly protozoa. However, the removal performance of reference pathogens for bacteria behave differently to their indicators.

Selection of vegetation species with an extensive root system (such as *L. continentale*, M. incana and C. appressa) and maintaining a steady SZ volume are important for faecal microorganism removal in current stormwater biofilters.

Faecal microbial removal performance in current stormwater biofilters is reduced following both extremely short and extended dry weather periods.

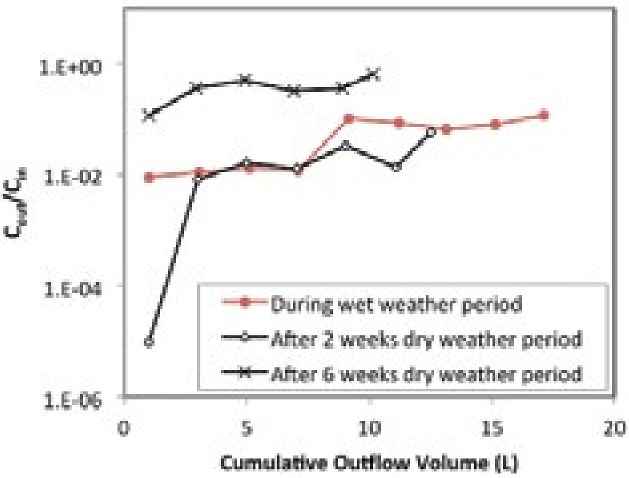


Figure 14: Change of normalised outflow *E. coli* concentration (Outflow *E. coli* concentrations are presented as a fraction of the inflow concentration) with cumulative outflow volume as observed in a laboratory-scale biofilter columns planted with *L. continentale* during three sampling events with different antecedent dry periods. All three sampling events had the same inflow volume (20L) and yet different outflow volumes were observed due to a volumetric reduction of water in the SZ during the antecedent dry weather period (Source: Chandrasena et al. (in preparation-a)).

Next generation biofilters: novel antimicrobial filter media designs

Selection of antimicrobial coatings work best for the stormwater treatment

Of the 15 antimicrobial media tested (all modified granular activated carbon (GAC)- and zeolite-based materials), Cu^{2+}-immobilised zeolite (ZCu) and $Cu(OH)_2$-coated GAC yielded the best *E. coli* removal. Over 2 –log reductions were achieved using a 40 minute contact time. However, the stability of these media required improvement as Cu concentrations in the effluent were well above current Australian stormwater harvesting guidelines (NHMRC, 2009).

Optimising Cu coating – making it effective and stable

The stability of the ZCu antibacterial media was improved, where it was coated with $Cu(OH)_2$ and heat treated at 180°C, resulting in a novel media (ZCuCuO180). This media showed minimal Cu leaching and consistently high *E. coli* removal (2 log reductions) with a contact time of just 20 minutes (Li et al., 2014a) (Figure 16-left). Contact-based inactivation and enhanced adsorption were hypothesized to be the main processes contributing to the improved removal by ZCuCuO180. The media was also effective at inactivating bacteria captured by the columns; indeed, during events, those microbes which were captured through adsorption processes were effectively inactivated by the antimicrobial media during the drying periods between events (Figure 16-right). Furthermore, ZCu400 media (ZCu heated at 400°C) was also able to inactivate captured microbes during drying periods (Figure 16-right) even though it was not effective in providing high *E. coli* log reductions during shorter wet weather periods.

Incorporating stable antimicrobial filter media into stormwater biofilters

The stable antimicrobial filter media, ZCu400 and ZCuCuO180, were incorporated into current stormwater biofilter design using a layered filter media arrangement. The optimal arrangement was found to comprise a ZCu400 layer at the top of the filter media (to inactivate captured bacteria during drying periods) and ZCuCuO180 layer halfway through the filter media (to capture and inactivate microbes during wet events) (Li et al., 2014b). This design yielded an average *E. coli* log reduction of 2.4 and the concentration of *E. coli* in the effluent (101 MPN/100 mL) met the USEPA target concentration for *E. coli* in fresh water (USEPA, 2001) .

The design with novel, layered filter media and plant species *L. continentale* (LCCu) was found to achieve greater than 1 log reduation for all tested microorganisms. However, the LCCu design was only found have relatively high log reductions for *E. coli*, Campylobacter spp. and F-RNA coligphages compared to the control layered design without any antimicrobial filter media (LC) (Figure 18). Conversely, the LCCu design was either counterproductive or less effective at removing the *C. perfringens*, *Cryptosporidium* oocysts and adenovirus compared to the LC design without any antimicrobial filter media (Figure 18). The pathogen indicator removal rates for this LCCu design met the guideline for unrestricted irrigation (NHMRC, 2009). The removal of the protozoan reference pathogen, *Cryptosporidium*, was comparable to that of the indicator, *C. perfringens* (3.1 and 2.1 log, respectively). However, the removal of Campylobacter spp. (1.1 log) and adenoviruses (1.2 log) was found to be much lower than that of their indicators (*E. coli* – 3.0 log; F-RNA coliphages - 4.1 log, respectively) (Li et al., in preparation).

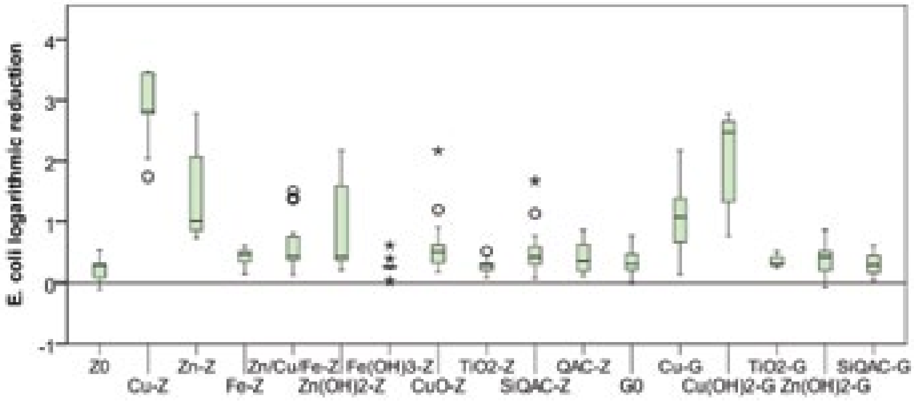


Figure 15: *E. coli* log reduction rate by 15 types of antimicrobial media. Two modified base media types: Z – zeolite, G- granular activated carbon with each of the antimicrobial compound coated into base media. QAC: hexadecyltrimethyl ammonium chloride; Z0 and G0: control media without any modification (Source: Li et al. (2014a)).

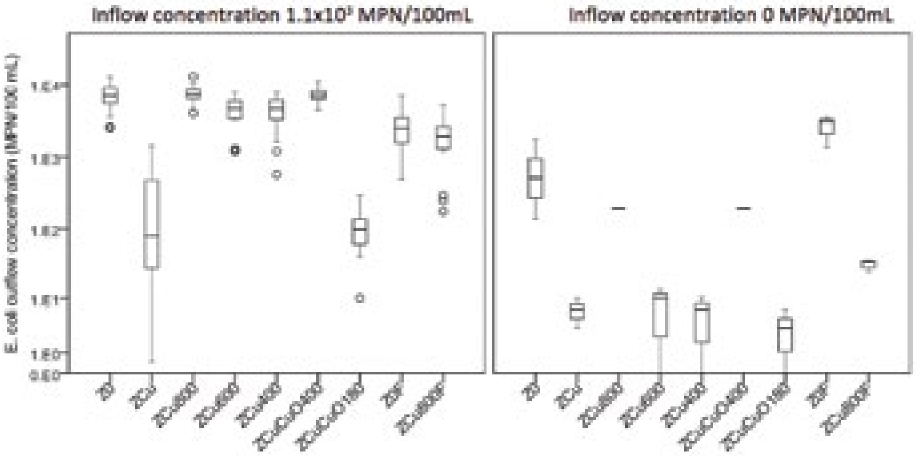


Figure 16: *E. coli* inflow and outflow concentrations from ZCu media and Cu(OH)2-coated media: Z0 –uncoated graded zeolite (0.3-0.6mm), ZOF –uncoated graded zeolite (0.1-0.3mm), ZCu180, 400, 800 – *in situ* Cu(OH)2 coated and acclimated at 180, 400, 800 oC.

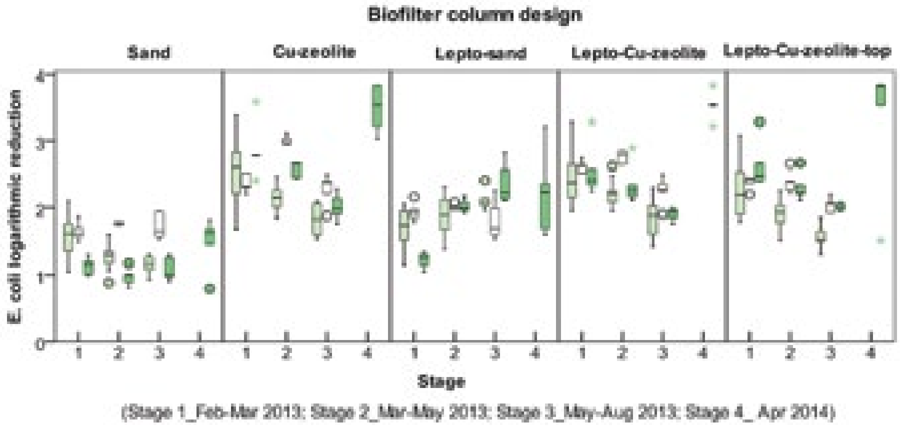


Figure 17: *E. coli* log removal rate by five biofilter designs over three types of weather during four stages of monitoring. Biofilter column designs: Sand – non-vegetated control, Cu-zeolite – non-vegetated novel layered filter media design, Lepto-sand – vegetated control with *L. continentale*, Lepto-Cu-zeolite - vegetated novel layered filter media design with *L. continentale*, Lepto-Cu-zeolite-top – alternative vegetated novel mixed filter media design with *L. continentale*; Sampling events: typical – antecedent dry weather < 2 weeks; Dry weather - antecedent dry weather ≥ 2 weeks, challenging – 1-in-3 month ARI event (Source: Li et al. (submitted)).

typical sampling event
Dry weather sampling ever
challenging event

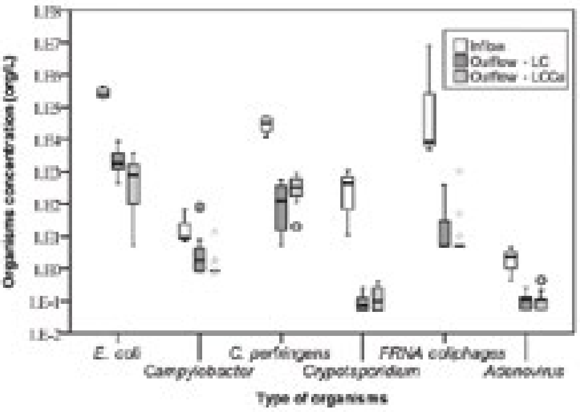
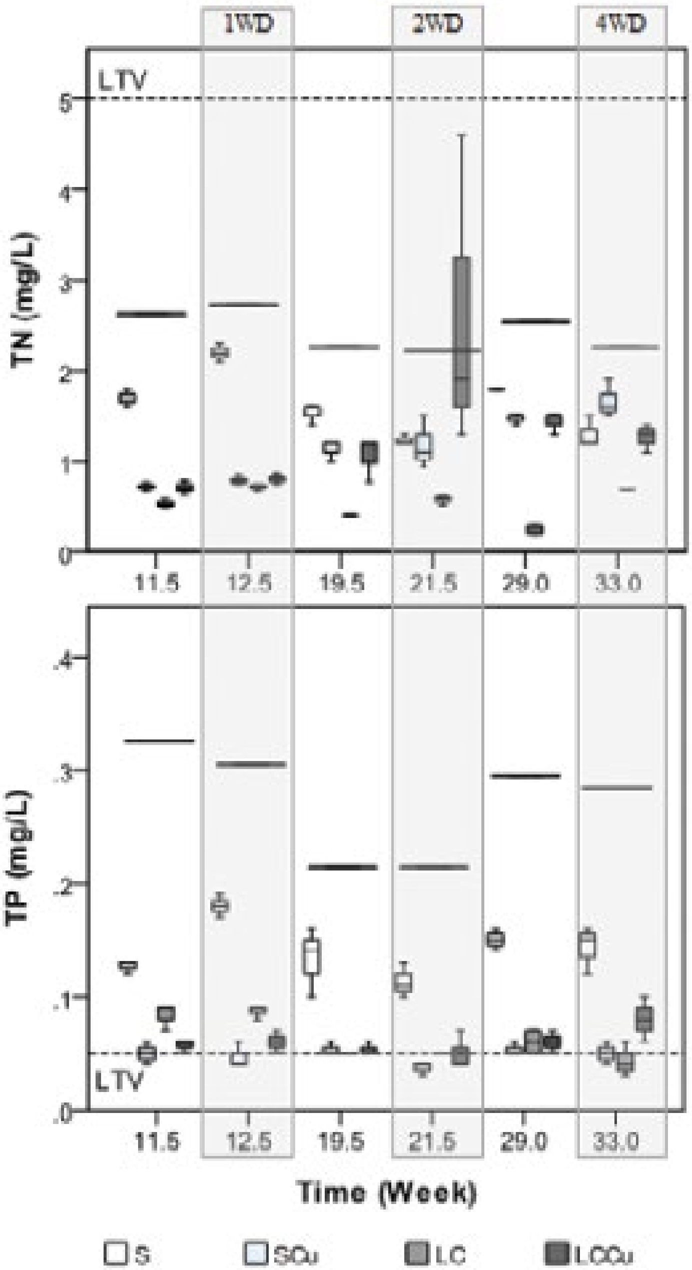
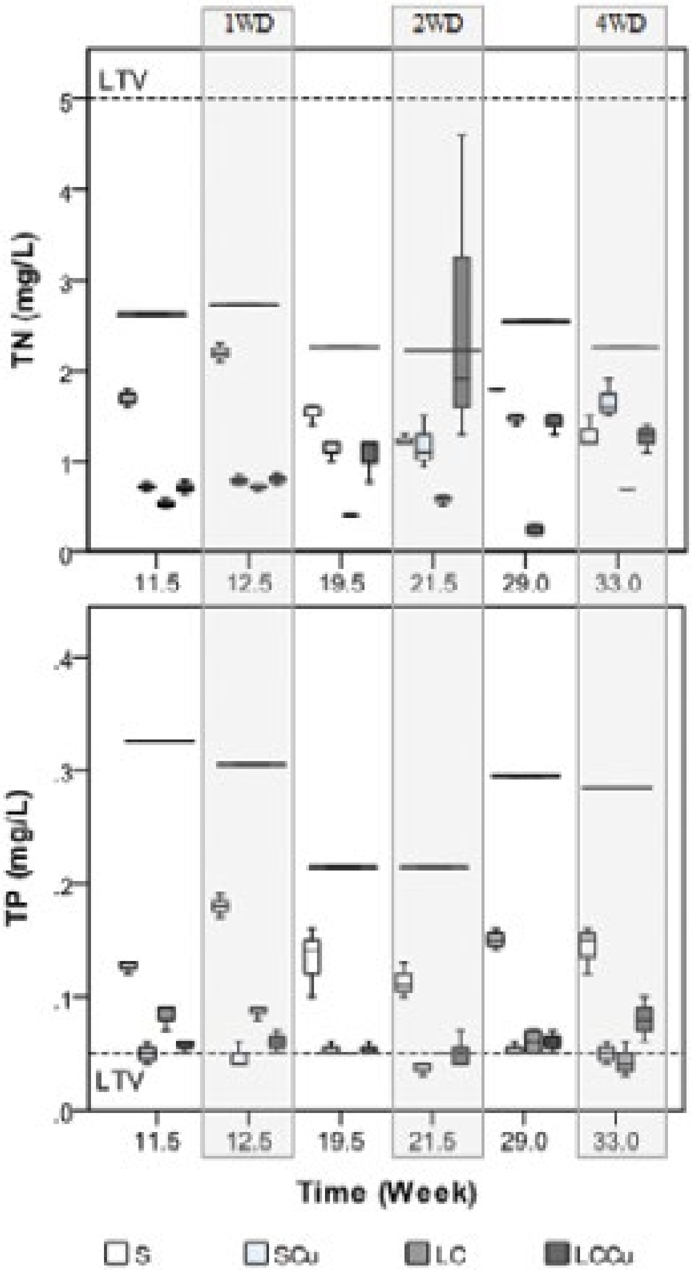


Figure 18: Reference pathogen and indicator removal performances over 1.5 years during four challenging events. LC – vegetated control with *L. continentale*, LCCu - vegetated novel layered filter media design with *L. continentale*.

The new design consisting of the novel antimicrobial media was found to perform consistently well under variable operating conditions (intermittent wetting-drying regimes and storm event volumes), unlike the current best practice design with traditional filter media. However, the novel design was found to be sensitive to reduced temperatures and surface clogging. Importantly, incorporation of the antimicrobial media into biofilter design did not compromise removal of other pollutants (*e.g.* TSS, TN, TP and total Cu). Indeed, the removal rates were almost the same as those found in biofilters of the same design but without ZCu layers (Li et al., in preparation)(Figure 19). However, the layered designs where the antimicrobial media was placed in the middle of the filter media profile did impact vegetation development. As a result, future research will trial placing the novel media at the very top of the biofilter, well above the plant roots. In this way, the roots will be protected from the negative impact of the Cu media and the full benefits of plants to biofilter functioning may not be compromised.





Highlights

ZCuCuO180 (Cu^{2+}-immobilised zeolite (ZCu) coated with $Cu(OH)_2$ followed by heat treatment at 180°C) and ZCu400 (ZCu calcined at 400°C) are two newly developed antimicrobial filter media that improve pathogen removal when incorporated into stormwater biofilters.

The new layered biofilter media design consisting of the novel antimicrobial media is capable of achieving greater than 2 log reduction of common indicator microorganisms without compromising the removal of other pollutants (*e.g.* TSS, TN, TP and Cu).

This new layered biofilter media design is also capable of reducing reference pathogen concentrations, with particularly high removal of protozoa (> 3 log) and greater than 1 log reduction of reference bacterial and viral pathogens.

Performance of the newly developed layered biofilter media design is less affected by intermittent drying/ wetting conditions and the size of storm events, but may be reduced during cold temperatures and in clogged biofilters.

Figure 19: TN and TP inflow (horizontal line across each week) and outflow (sub-boxplots) concentrations and removal rates for four biofilter designs during 3 typical sampling events (open area) and 3 drying weather sampling events (shaded area). LTV- Australian long term irrigation trigger value; ARQ – Australian runoff quality load reduction. S – non-vegetated control, SCu – non-vegetated novel layered filter media design, LC – vegetated control with *L. continentale*, LCCu - vegetated novel layered filter media design with *L. continentale*, (Source: Li et al. (in preparation))

2.2.3 Micro-pollutants

Retention of metals that pose a risk for water use

The current stormwater biofilter designs met irrigation standards for all tested metals (Al, Cr, Cu, Fe, Pb and Zn), but drinking water standards were only met for Pb, Cr, Cu, and Zn (Feng et al., 2012). The outflow concentrations of Al were substantially higher than Australian drinking water guidelines and would require additional treatment, while Fe was close to, and in some cases even met, the targets.

Vegetation and filter type were found to be significant factors for the treatment of metals (Feng et al., 2012). In particular, deep filter media resulted in increased outflow concentrations of Fe, Al, Cr, Zn and Pb, likely due to leaching and mobilisation of metals within the media. Biofilters could be optimised to increase Fe removal by sizing them to at least 4% of their catchment area, planting with an effective plant species (such as C. appressa), specifying a depth of 300 – 500 mm, and increasing the organic content of the filter media (Feng et al., 2012).

Long term metal retention capacity of stormwater biofilters

A field survey conducted to assess heavy metal accumulation in biofilters in Melbourne, Sydney and Brisbane revealed that Zn was the only metal (of the four tested) with concentrations in exceedance of Australian and European (Dutch) soil quality guidelines (NEPC, 1999, NMHSPE, 2000)(Table 9). It was also revealed that catchment characteristics have a strong influence on heavy metal concentrations in the surface layer of biofilters (Hatt et al., in preparation). **Biofilters in catchments that contain current or past industrial activity are therefore expected to have a shorter lifespan with respect to heavy metal accumulation in the filter media and therefore require more intensive maintenance to avoid exceeding thresholds in the soil quality guidelines.**

	Cd	Cu	Pb	Zn
Measured mean concentration (mg/kg)				
	0.85	32	40	302
Mean concentration grouped by site characteristics (mg/kg)				
Urban Density				
low	0.5	18	13	85
medium	0.9	23	38	212
high	1.0	45	57	49
Current Land Use				
residential	0.7	20	25	143
commercial	0.4	16	8.7	105
industrial/commercial	1.5	64	84	734
Trigger values (mg/kg)				
Australia: human health[i]	40	2000	600	14000
Australia: ecological[ii]	3	100	600	200
Dutch: human/ecological[iii]	12	190	530	720

Table 9. Mean surface (0 - 2 cm) heavy metal concentrations in field-scale biofilters and soil guidelines (Source: Hatt et al. (in preparation)).

[i] Health Investigation Levels: human exposure settings, based on land use for parks, recreational open space and playing fields (NEPC, 1999)

[ii] Interim urban Ecological Investigation Levels (NEPC, 1999)

[iii] Intervention values, indicating when the functional properties of the soil for humans, plant and animal life is seriously impaired (NMHSPE, 2000)

Accelerated-dosing laboratory tests conducted by Hatt et al. (2011) revealed that the retention capacity of the biofilter for Zn was exhausted, but not for Cd, Cu and Pb following 12 – 15 years of simulated operation for a filtration system sized at 2% of its impervious catchment area under a typical Melbourne rainfall pattern. These experimental results were used to provide a framework for the design of biofilters. The predicted time to exhaustion of the Zn retention capacity for two climatic regions in Australia (Melbourne and Brisbane) are presented in Table 10. These calculations are based on the assumption that dissolved metals are first sorbed in the top layers of the filter until all the exchange sites are fully occupied, at which point metals will migrate through to underlying layers where they will be trapped, and so on. It can be seen that, if the filter is shallow or small relative to its catchment, its retention capacity will be rapidly exhausted (Table 10). This would be problematic for biofilters as revegetation would be required each time the media is replaced. Therefore, to ensure that systems last for at least 10 years they should be at least 0.5 m deep and sized to at least 2% of their impervious catchment area in Melbourne, while for the same depth they should be sized to 4% of their impervious catchment area in Brisbane.

Media	**Depth** *(m)*	**Years to breakthrough** *(% of impervious catchment)*							
		Melbourne				**Brisbane**			
		1	**2**	**3**	**5**	**1**	**2**	**3**	**5**
LS	0.3	4	9	14	23	2	4	6	10
	0.5	7	15	23	39	3	7	10	18
	0.7	10	21	32	54	5	10	15	25
LSVP	0.3	3	7	7	17	1	3	4	8
	0.5	5	11	11	29	2	5	8	13
	0.7	8	16	24	41	3	7	11	19
LSC	0.3	5	11	17	29	2	5	8	13
	0.5	9	19	29	48	4	9	13	22
	0.7	13	27	41	68	6	12	19	31

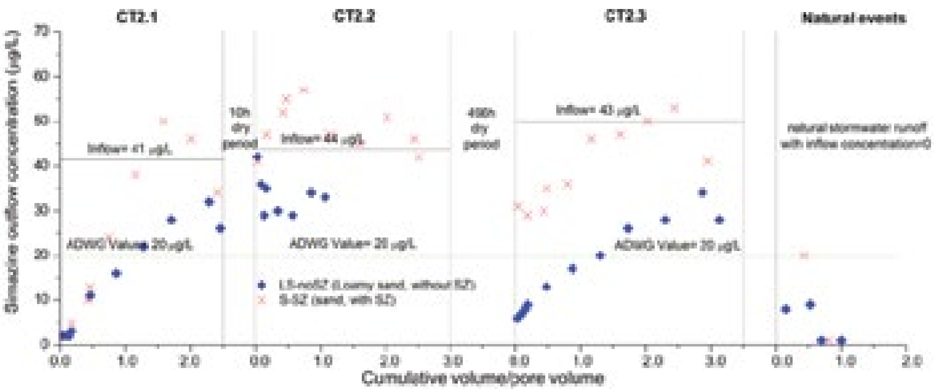
Table 10. Predicted time (years) to breakthrough of dissolved Zn for filters in two different climates and with varying filter media properties, depth, and size relative to the catchment. LS, loamy sand; LSVP, loamy sand with vermiculite and perlite; LSC, loamy sand with compost and mulch. Melbourne: temperate climate, annual rainfall = 650 mm. Brisbane: sub-tropical climate, annual rainfall = 1200 mm (Source: Hatt et al. (2011)).

Removal of organic micro-pollutants under challenging operational conditions

Biofilters were initially shown to have a good capacity for the removal of herbicides under challenging test conditions (Table 11), but these herbicides may accumulate in the filter media leading to a high possibility of break-through (Figure 20, Zhang et al,). However, the challenge tests showed that concentrations of glyphosate, the most commonly used pesticide in Australian cities, in the outflow were always below Australian Drinking Water values (Table 11). In all cases media with higher organic content performed better as a result of a higher adsorption capacity.

Miscellaneous Organic Chemicals (MOCs) such as Polycyclic aromatic hydrocarbon (PAHs) and Total Petroleum Hydrocarbons (TPHs), and phthalates (DBP and DEHP) were never detected in biofilter outflows, indicating excellent removal by biofilters (Table 11, Zhang et al, (2014)). Phenols were detected in the outflow only once in the six challenge tests conducted, and this occurred after a highly challenging dry weather event. While PCPs were detectible, this was only the case in the system with a SZ and after an extremely challenging dry or wet event. The removal of chloroform exhibited load removal rates of between 20% and 50% (Table 11).

		Measured concentrations						
		Inflow ± STD	**Outflow EMC** *(µg/L)*					
		(µg/L)	**LS-no SZ**			**S-SZ**		
	1st series tests	12700±707	**CT1.1**	**CT1.2**	**CT1.3**	**CT1.1**	**CT1.2**	**CT1.3**
	TPHs	1950±353	<100	<100	<100	<100	<100	<100
Herbicides	Glyphosate	55±13	NA	54	100	NA	41	105
	Atrazine	47±6	14	**34**[1]	17	**32**	**65**	23
	Simazine	53±4	3	11	6	7	**25**	7
	Prometryn	33±5	4	9	2	13	**26**	5
Phthalates	DBP	24±10	<1	<1	<1	<1	<1	<1
	DEHP	43±15	<1	<1	<1	<1	<1	<1
Trihalomethanes (THMs)	Chloroform		9	24	19	15	49	28
	2nd series tests	4300±220	**CT2.1**	**CT2.2**	**CT2.3**	**CT2.1**	**CT2.2**	**CT2.3**
	TPHs	10±2.6	<100	<100	<100	<100	<100	<100
Polycyclic Aromatic	Pyrene	17±6.6	<1	<1	<1	<1	<1	<1
Hydrocarbons (PAHs)	Naphthalene	1600±100	2	2	2	3	1	3
Herbicides	Glyphosate	48±6	99	116	187	29	106	70
	Atrazine	42±3	**25**	**28**	**27**	**35**	**42**	**49**
	Simazine	50±4	**22**	**32**	**24**	**33**	**49**	**43**
	Prometryn	42±4	11	14	15	20	**29**	**32**
Phthalate	DBP	17±8	<1	<1	<1	<1	<1	<1
	DEHP	59±7	<1	<1	<1	<1	<1	<1
Trihalomethanes (THMs)	Chloroform	27±6	32	38	40	40	47	49
Phenols	PCP	203±15	1	6	4	2	**19**	**11**
	Phenol		2	1	18	1	3	106



↑Table 11: Measured inflow concentrations and outflow even mean concentrations (EMC) during six *in situ* challenge tests. Two biofilter designs were tested: LS-no SZ – biofilter with a loamy sand filter media and without a SZ, S- SZ – biofilter with a sand filter media and inclusion of a SZ. (Source: Zhang et al. (2014))

[1] Bold indicates mean value exceeded ADWG value.

←Figure 20: Change of Simazine concentrations in the 2nd series of challenge tests (Source: Zhang et al. (2014))

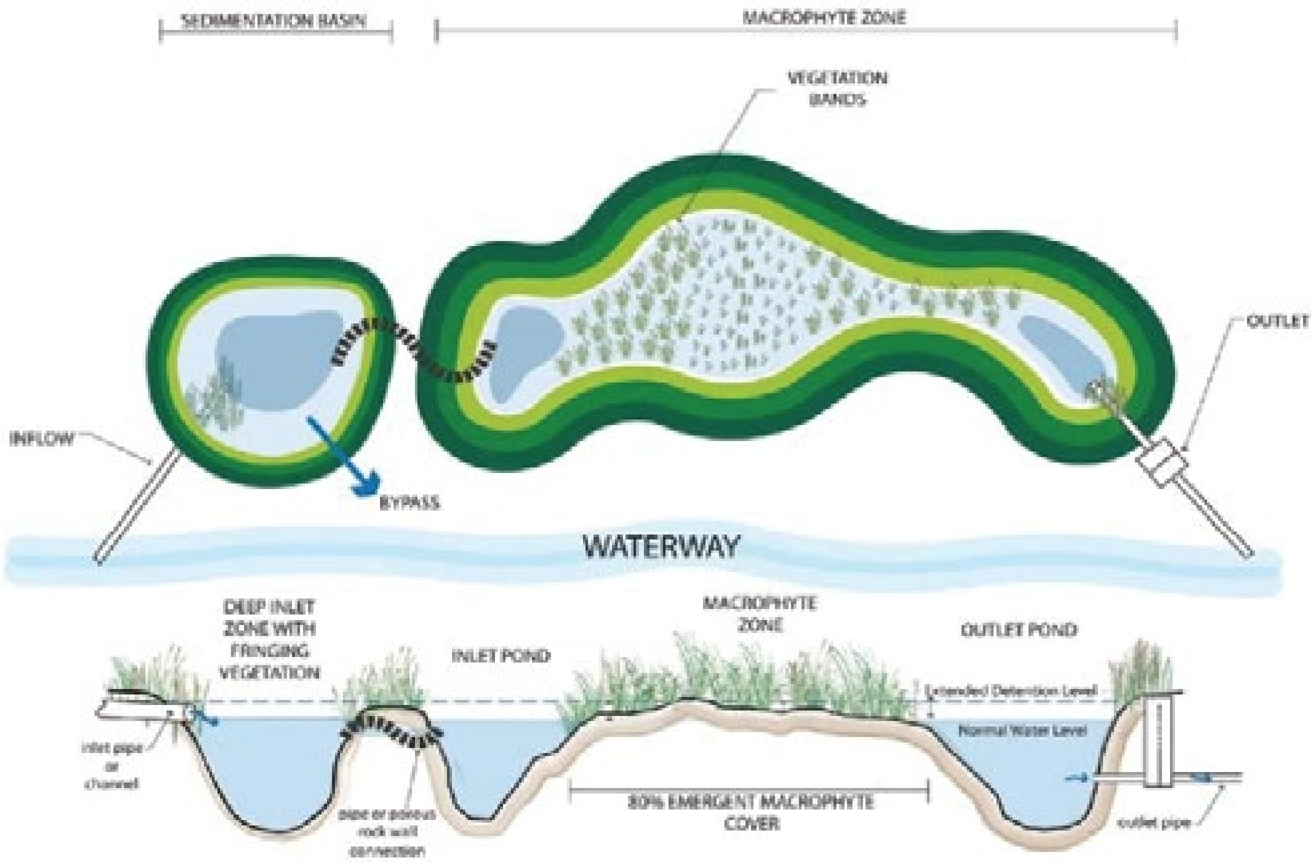
Highlights

Stormwater biofilter systems built in accordance with current best practice can meet irrigation standards for all tested metals: Al, Cr, Fe, Pb and Zn. If they are designed appropriately they can also meet drinking water standards for the same metals.

Biofilters are expected to have a shorter lifespan with respect to heavy metal accumulation in catchments that contain current or past industrial activity.

Stormwater biofilter systems built in accordance with current best practice are very effective in removing hydrocarbons and phthalates, but less effective for herbicides.

3 WETLANDS



↑Figure 21: Schematic diagram of a constructed wetland. (Source: Melbourne Water (2010))

Constructed wetlands are artificially created marsh systems, which regularly fill and drain and also provide an aesthetic value and wildlife habitat (Melbourne Water, 2010). Generally, surface flow constructed wetlands are commonly used in stormwater harvesting schemes across Australia (CWSC, 2010). Surface flow constructed wetlands designed for stormwater treatment typically have an open water inlet zone, a macrophyte zone (which consists of the majority of the system), and an open water outlet zone (Figure 21).

Water treatment is achieved through a combination of sedimentation, filtration, sorption and biological uptake as the water flows through different sections of the constructed wetland (CWSC, 2010). Vegetation plays a major role in treatment, including providing (1) surfaces to support biofilms which promote pollutant adsorption and uptake (2) oxygen transfer into the water column and sediment and (3) uptake of nutrients (4) supporting microbial processing in the substrate (5) alteration of the flow dynamics, which can enhance sediment retention and promote effective hydraulics if planted in consistent bands (Metcalf and Eddy, 1991, Brix, 1994, Kadlec, 2008, Greenway, 2010).

Research conducted by the CRCWSC included an in-depth literature review and a preliminary investigation into the constructed wetlands capacity in removing a range of stormwater pollutants of interest to ecosystem and public health.

3.1 Methods

Parameter	Methods Used	Reporting Limit
TSS	Filtration and dry weight	<0.5mg/L
TN	Digestion and FIA	<0.02mg/L
TP	Digestion and FIA	<0.01mg/L
Metals (Full Scan)*	ICP-MS (VIC-CM050 C)	<0.0001/<0.001mg/L
Total Petroleum/Recoverable Hydrocarbons (TRH/TPH)	Purge and Trap GCMS (VIC-CM047 & VIC-CM030)	<0.04/<0.1mg/L
Oil and Grease	Soxhlet extraction/Gravimetric	<1mg/L
pH	Probe (VIC-CM060 B)	-
Biological Oxygen Demand (BOD)	Dissolved Oxygen 5 Day (VIC-CM028)	<2mg/L
Chemical Oxygen Demand (COD)	Reactor Digestion (VIC-CM052)	
Methylene Blue Active Substances (MBAS)	Colorimetric analysis UV/Vis (APHA 5540 C)	<0.1mg/L
Monocyclic Aromatic Hydrocarbons (MAH)	Purge and Trap GCMS (VIC-CM051 & CM047)	<0.001/<0.003mg/L

Table 12. Water quality parameter analytical methods and reporting limits

* A full metals scan includes Al, antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), Cu, Fe, Pb, manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and Zn.

A detailed literature review was conducted by Monash University on the design and performance of current stormwater harvesting technologies (CWSC, 2010). This literature survey provided a summary of reported removal performances in different constructed wetlands worldwide and also information and lessons learned from 22 case studies across Australia for stormwater harvesting. A research project has also recently commenced to investigate the pathogen removal capacity of constructed wetland.

A field monitoring program was conducted by Monash University from August 2012 to April 2014 in a constructed wetland which receives pre-treated stormwater runoff from an industrial site in Melbourne's west (the site cannot be specifically identified due to a confidentiality agreement). The wetland receives water from large, on-site bunded areas that enclose several large fuel storage tanks. Runoff is initially detained in a large holding pipe before being released into a cyclonic separation interceptor. From there it moves into a sedimentation pond, followed by the wetland system before being discharged to the stormwater system and finally Hobson's Bay. Wet weather water quality samples were collected at the inlet and outlet using automatic samplers. These collected discrete, flow-weighted samples which are then combined to form one composite sample for each sampling point (ten at the inlet and eight samples at the outlet). Dry weather samples were manually collected from the water column near the outlet on a weekly basis, provided the antecedent dry weather period was at least three days (47 dry weather samples). All water quality samples were analysed by a NATA-accredited laboratory for the parameters shown in Table 12 using standard methods and quality control procedures.

3.2 Key Findings

3.2.1 Review of faecal microorganism removal capacity

Eight out of twenty two Australian stormwater harvesting schemes surveyed were found to have wetlands incorporated (CWSC, 2010). Treated stormwater was mostly used for irrigation and/or aquifer storage and recharge. Constructed wetlands were incorporated as an element of secondary treatment, following litter traps and sedimentation tanks to improve the efficiency of treatment provided by the wetlands. However, almost all of the systems had a disinfection step prior to stormwater reuse for irrigation with restricted access, despite the potential for water sensitive urban design technologies such as wetlands to meet the water quality thresholds required for irrigation with a restricted access end-use (CWSC, 2010).

A review of the literature on pathogen removal capacity revealed that there is a paucity of pathogen removal performance data in constructed stormwater wetlands and only indicator microorganism removal performance data are available (Meng, 2014). **Constructed wetlands were generally shown to be promising in reducing indicator microorganism concentration, yet a large variance in removal performance remains** (Table 13). The differences in removal performances were suggested to be caused by (1) different inflow characteristics, (2) various system designs (which caused some processes to be more or less influential) and (3) the sampling method employed (*i.e.* many reports use grab samples to evaluate performance, which is insufficient for such dynamic systems) (Meng, 2014).

Reference	**Location**	**Removal Efficiency**				
		Thermotolerant coliforms	**Enterococci**	***E. coli***	**Heterotrophic bacteria**	**FRNA coliphages**
Davies and Bavor (2000)	Plumpton Estate, NSW, Australia	79%	85%		87%	
Davies et al. (2003)	Sydney, Australia	26% to 99%				100%
Birch et al. (2004)	Sydney, Australia	-260.6% to 36.9%				
Mendez et al. (2009)	North Sydney, Australia	56% to 98%				
Hathaway et al. (2009)	Charlotte, USA			33% to 96%		
Díaz et al. (2010)	California, USA			-554% to 95%		
Hathaway and Hunt (2010)	Coastal North Carolina, USA	-676% to 77%	52% to 60%	-9% to -13%		
Beutel et al. (2013)	Washington State, USA	88%				
Stenstrom and Carlander (2001)	Flemingsberg, Sweden			99%		

Table 13: Summary of indicator microorganism removal in constructed stormwater wetlands. (Source: Meng (2014))

3.2.2 Treatment performance at an industrial Melbourne site

Preliminary water quality results are shown in Table 14. Of the 24 metals analysed, only five were detected (Al, Cu, Fe, Pb and Zn); results for the remaining metals are not shown. Inlet and outlet water samples were also tested for a range of organic micro-pollutants but almost all the measured levels were below the lowest reporting level (Please refer to Appendix A for the list of tested organic micro-pollutants and their measured concentrations).

Pollutant	**Wet weather**			**Dry weather**
	Inlet (n = 10)	**Outlet (n = 8)**	**Average reduction (%, n = 4)**	**Outlet (n = 47)**
TP	0.09 (0.04 – 0.14)^{9}	0.07 (0.04 – 0.08)^{8}	25.8	0.06 (0.03 – 0.19)^{47}
TN	1.1 (0.75 – 1.66)^{10}	0.83 (0.63 – 1.19)^{8}	24.6	0.72 (0.39 – 1.40)^{47}
TSS	15.50 (3.91 – 33.5)^{10}	5.70 (4.28 – 9.76)^{8}	28.5	4.45 (1.73 – 17.25)^{47}
TOC	8.10 (4.79 – 28.2)^{8}	10.00 (6.56 – 14.7)^{7}	-13.3	15 (12 – 24)^{33}
DOC	7.30 (4.34 – 18.45)^{8}	9.00 (6.13 – 14.7)^{7}	-24.3	14 (11 – 21)^{33}
BOD	1 (1 – 2)^{4}	1.50 (1 – 2.65)^{4}	-12.5	2 (1 – 6)^{33}
COD	17 (14.45 – 26.85)^{10}	23.5 (17.7 – 27.3)^{8}	-35.9	28 (20 – 59)^{47}
E. coli	1151 (297.1 – 4632.1)^{8}	559 (201.5 – 1144.3)^{6}	26	257 (5 – 2142)^{34}
Al	0.64 (0.27 – 2.425)^{10}	0.305 (0.091 – 0.955)^{8}	37.2	0.13 (0.023 – 2.39)^{46}
Cu	0.007 (0.003 – 0.012)^{10}	0.003 (0.001 – 0.005)^{8}	50.0	0.002 (0.001 – 0.006)^{40}
Fe	0.95 (0.334 – 3.195)^{10}	0.43 (0.301 – 1.19)^{8}	33.2	0.76 (0.272 – 2.9)^{47}
Pb	0.015 (0.005 – 0.027)^{10}	0.003 (0.002 – 0.011)^{8}	68.6	0.002 (0.001 – 0.009)^{38}
Zn	0.3 (0.162 – 0.363)^{10}	0.078 (0.059 – 0.162)^{8}	56.7	0.078 (0.059 – 0.162)^{47}
TRH	0.1 (0.05 – 0.165)^{6}	0.1 (0.05 – 0.1)^{6}	0.0	0.1 (0.1 – 0.2)^{26}
Oil & Grease*	0.05 (0.05 – 7.725)^{1}	0.05 (0.05 – 1.643)^{0}	9.9	<1^{0}
MAH	0.001^{0}	0.001^{0}	-	0.001^{3}
MBAS	0.05 (0.05 – 0.155)^{2}	0.075 (0.05 – 0.165)^{3}	-25.0	0.05 (0.05 – 0.2)^{23}

Table 14. Summary of pollutant concentrations at both in the inlet and outlet during wet weather events and the outlet during dry weather periods. Median values are presented together with the 5th and 95th percentiles shown in parentheses. The percentage reduction was calculated for each wet weather event where there was a paired inlet and outlet concentrations. Superscript values indicate the number of samples which were above detection. Units are mg/L for all except *E. coli* (org/100mL).

* Only one sample from entire wet weather samples was determined above detection for oil and grease, therefore the percentage reduction cannot be accurately calculated.

Wet Weather

A constructed wetland with an industrial catchment was effective at reducing TN (24.6% concentration reduction), TP (25.8% reduction) and TSS (28.5% reduction) from stormwater runoff. In addition, heavy metals (Al, Cu, Fe, Pb and Zn) were well removed (> 33% concentration reduction) (Table 14, Figure 22). The calculated concentration reductions were lower than the values reported in CWSC (2010) for TP (46-60% reduction) and TSS (58-85% reduction). These differences in removal performance may be due to inherent differences in system characteristics and inflow water quality. It should be emphasised that most of the inflow pollutant concentrations observed in this constructed wetland were very low and almost within the range of background concentrations (Table 14).

The reductions in nutrients and metals may have been assisted by the extremely dense biomass of wetland plants, which primarily comprised of the semi-submerged aquatic plant Cumbungi. However, the presence of such a large mass of organic material may also be the cause of the increases in TOC and DOC at the outlet. Wetland carbon cycles are very complex and involve numerous processes, however the decay of organic matter does lead to significant increases in carbon within the ecosystem (Kadlec and Wallace, 2009).

BOD, and more so, COD increased from the wetland inlet to the outlet. Elevated COD levels relative to BOD levels can be attributed to the presence of humic materials in the wetland environment (Kadlec and Wallace, 2009). It should be noted that the majority of samples during wet weather had BOD levels below or at just detection and therefore no significant performance evaluations could be made under these conditions. In contrast, during dry weather the majority of samples (70%) had BOD levels at or above the detection limit. This increase in BOD concentration may result from the volume reduction driven by evapotranspiration (Karathanasis et al., 2003). COD was also higher during dry weather, relative to wet weather events, which may be due to the decomposition of wetland plant material (Kadlec and Wallace, 2009). Wetlands possess non-zero

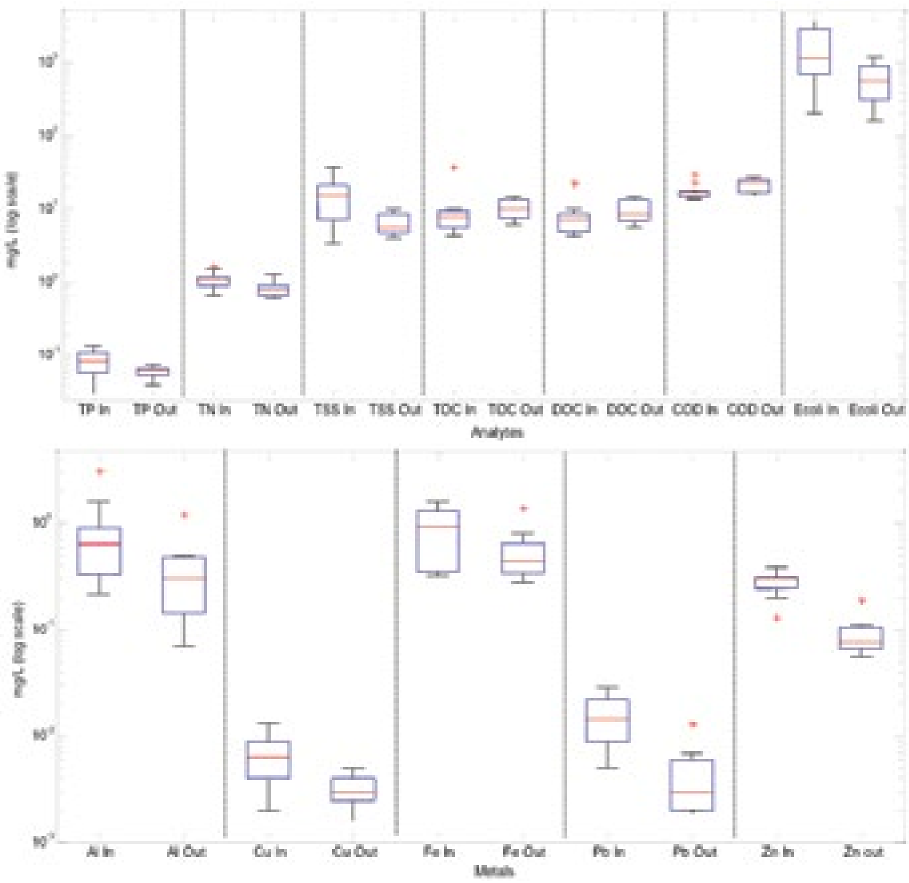


Figure 22. Range of wet weather inflow and outflow pollutant concentrations that were regularly detected within the wetland. TP- Total phosphorus; TN – Total Nitrogen; TSS – Total Suspended Solids; TOC – Total Organic Carbon; DOC – Dissolved Organic Carbon; COD – Chemical Oxygen Demand. Units are mg/L for all except *E. coli* (org/100mL).

background levels of BOD and COD, usually within the range of 1-6mg/L and 10-100mg/L respectively, depending upon the type of wetland (Kadlec and Wallace, 2009). Most values measured at the study site thus far were below or at the lower end of this non-zero background range and may therefore simply be naturally occurring background concentrations of BOD and COD found within the wetland and inflow waters. Alternatively, the COD found in these waters may be attributed to other organic compounds which were not analysed in the current project. Regardless, these values are in the lower bands of the range typically found in industrial stormwater (Duncan, 1999).

Very few samples contained detectable levels of hydrocarbons; indeed, the only detections that occurred were in two of these eight bands analysed (TPH C15-C28 in one inflow sample and TRH C16-C34 in just two inflow samples and one outflow sample). Of the samples that did have detectable results, values did not exceed 0.2 mg/L, *i.e.*, only slightly above the reporting limit. Given the industrial nature of catchment, the lower levels of hydrocarbon detected in the wetland indicates a good level of pre-treatment provided by the cyclonic separation interceptor located upstream of the wetland.

Dry Weather

Figure 23 presents a time-series of dry weather pollutant concentrations. Most pollutants show consistent trends throughout the year and are unaffected by rainfall. BOD, TP and several metals remain at low concentrations regardless of the flow through the wetland. However, it is evident that without flushing of the system, some analytes become more concentrated between rainfall events, particularly in the case of COD. Long dry periods during the warmer months between October 2012 and January 2013 as well as November 2013 to February 2014 show steady increases in COD until the occurrence of rainfall increases steadily and flushes the system consistently. There may also be leaching of nutrients and residual organic compounds into the system during these periods from the resuspension of soil particles, with pollutants attached, by water bird activity during low water levels, decomposition of plant matter or from the breakdown of organic chemicals in the water (Greenway, 2010).

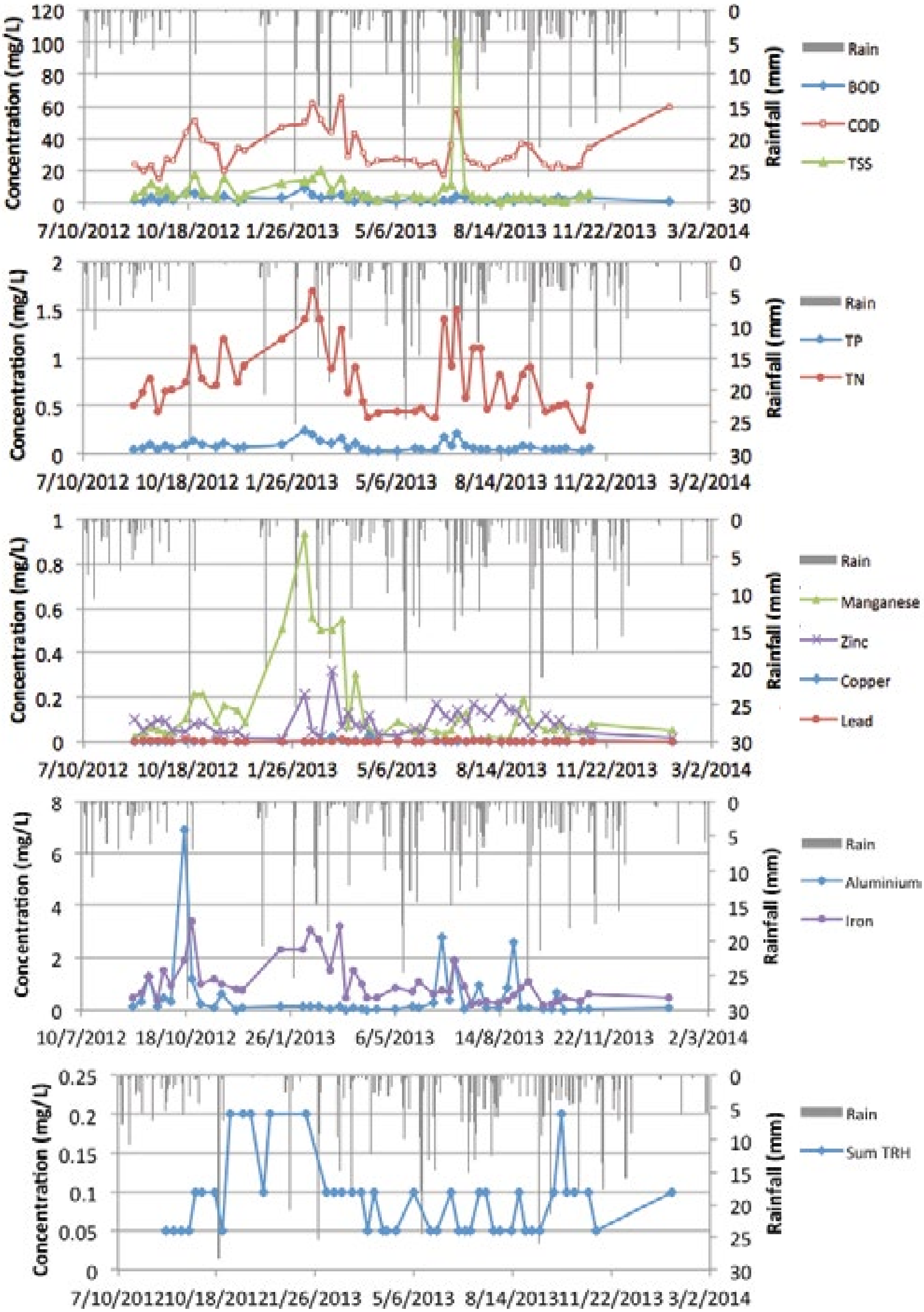
Highlights

Constructed wetlands were generally shown to be promising in reducing indicator microorganism concentrations, yet a large variance in observed removal performances needs to be further investigated.

A constructed wetland receiving pre-treated runoff from an industrial catchment was effective in reducing concentrations of nutrients, metals and the bacterial indicator, *E. coli*, but was less effective in reducing BOD and COD (COD was found to accumulate within the wetland during extended dry weather periods).

Wetland outflow concentrations of BOD, TP, Cu and Pb remained steady throughout the wetland operation regardless of wet/dry weather conditions.

→Figure 23. Time-series of dry weather pollutant concentrations and rainfall at the outlet of the wetland.


Concentration (mg/L)
Rainfall (mm)
Rain
BOD
COD
TSS
TP
TN
Manganese
Zinc
Copper
Lead
Aluminium
Iron
Sum TRH
7/10/2012
10/18/2012
1/26/2013
5/6/2013
8/14/2013
11/22/2013
3/2/2014
10/7/2012
18/10/2012
26/1/2013
6/5/2013
14/8/2013
22/11/2013
2/3/2014

4 PRACTICAL IMPLEMENTATION

Transferring the knowledge gained from the wide range of laboratory- and field-scale research conducted by the CRCWSC is of high importance for utilising the full potential of stormwater harvesting in Australia. However, it is acknowledged that most of the current in-depth research work has been carried out in relation to biofilters, with less focus on constructed wetlands. **Therefore, the practical implications presented below are limited to biofilters.**

4.1 Implications for the use of biofilters for stormwater treatment

It should be noted that this report is not intended to provide design guidelines for stormwater biofilters and these implications are bound with the uncertainties in each individual research project. Therefore, these implications should not be considered as design guidelines. Furthermore, it is important to note that there is no single biofilter design which can achieve optimal removal performance for a wide range of pollutants. Therefore, it is important to prioritize site-specific water quality objectives when designing biofilters for stormwater harvesting. The implications for the use of biofilters for stormwater treatment are:

Nutrient removal

- Select media with a low nutrient content, to avoid nutrient leaching.
- Include a SZ where extended dry weather is frequent; add a low nutrient content carbon source to maintain organic N and P removal.
- Select efficient plant species (as per Table 8 above) to ensure N removal is maintained; this is especially the case in climates where extended dry periods are likely.
- Ensure species diversity in biofilters, with at least 50% effective species while the remaining species can be specified to accommodate other considerations (such as biodiversity, aesthetics and resilience against varying climatic conditions).
- Consider harvesting the plant biomass to minimise nutrient release upon plant senescence. This may also serve to maintain pollutant uptake capacity.

Faecal microorganism removal

- Utilise *L. continentale*/M. incana or a mix of *L. continentale*, M. incana and *C. appressa* to yield better faecal microorganism removal.
- Include a SZ to improve the removal of faecal microbes, but caution should be applied if the biofilter is operating in a region where extended dry weather periods are frequent. In such instances, either employ a methodology to replenish the SZ volume during extended dry weather periods, or divert the initial outflow from the first event after an extended dry weather period, to avoid lower water quality; and/or
- Incorporate the novel antimicrobial layered filter media design with ZCu400 and ZCuCuO180 layers to improve faecal microorganism removal.

Micro-pollutant removal

- For effective long term (>10yrs) removal of heavy metals, use a biofilter depth of least 0.5 m and sized to at least 2% of its impervious catchment area in Melbourne (4% in Brisbane).
- To optimise biofilter Fe removal (to reach drinking water standards) increase the biofilter area (*e.g.* to at least 4% of its impervious catchment area in Melbourne), plant with an effective plant species (*e.g.* C. appressa), specify a media depth of 300 – 500 mm, and increase the organic content of the filter media (However, it is important to note that increasing the organic matter content of the filter media can compromise removal of other pollutants such as nutrients and reduce the filtration rate).
- For optimal removal of organic micro-pollutants use a filter media with higher soil organic matter content. However, again this requires caution as too much organic matter in the filter media can compromise nutrient removal and decrease the filtration rate, especially during challenging wet periods.
- Further work is required to optimise biofilters for the removal of herbicides.

4.2 Assessment tools of stormwater biofilters

Two process-based models were developed to predict pathogen and organic micro-pollutant removal in stormwater biofilters. These were developed based on the laboratory and field experiments presented in Section 2.1.2 and 2.1.3. A brief summary of these two models is presented below.

Pathogen model

A preliminary model was developed to simulate *E. coli* removal performances in stormwater biofilters (Chandrasena et al., 2013). The model simulates three key faecal microorganism retention and survival processes. Adsorption and subsequent desorption were simulated as significant wet weather processes, while die-off was taken as a contributing process during dry weather periods (*i.e.* periods between wet weather events; Figure 24-left). Wet weather processes were modelled in a very simplified manner as a function of available *E. coli* in the filter media and pore water velocity. Die-off was modelled as a simple first order rate process. The model was then tested against laboratory scale data presented in Chandrasena et al. (2012a). The preliminary model showed good agreement with the measured datasets (Figure 24-right). It was found that of the three simulated processes, adsorption is the governing process in the model. However, since this is only a preliminary predictive model, it requires improvement to incorporate the effects of, for example, a submerged zone and vegetation selection on microbial removal, and further testing against field data in the future.

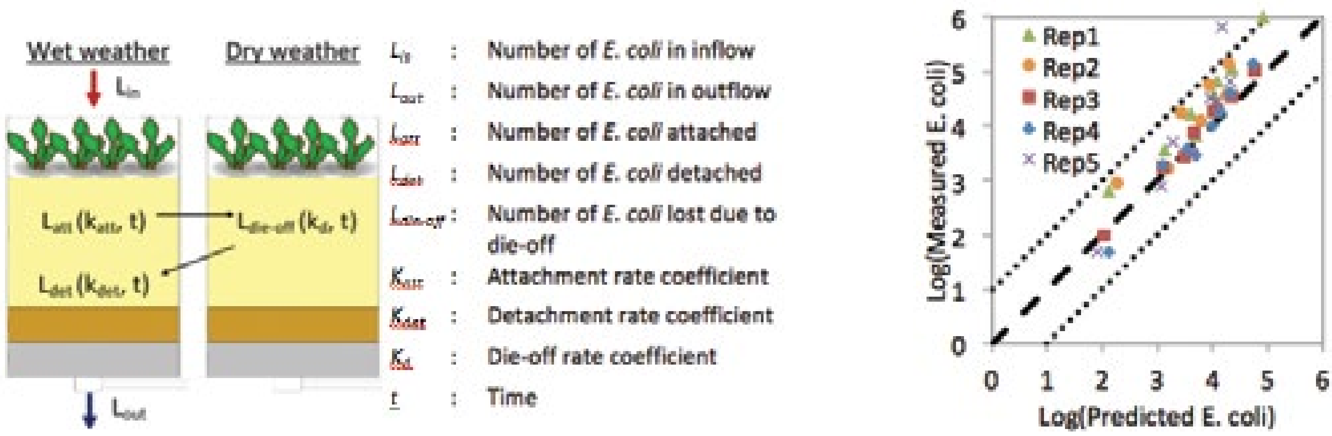


Figure 24: conceptual diagram of preliminary pathogen removal model (left). Model performance for a laboratory scale biofilter column planted with C. appressa, using the optimized parameter sets (right). Dashed lines indicate the 1:1 line between modelled and measured *E. coli* concentrations, while dotted lines indicate error bars (+/- one order of magnitude). Rep - replicate biofilter columns (Source: Chandrasena et al. (2013))

Micro-pollutant model

A process based model of water and micro-pollutant transport though stormwater biofilters was developed (Randelovica et al., in preparation). The model simulates water flow and micro-pollutant removal processes within three regions of a biofilter; (1) the ponding zone, (2) filter media, and (3) the submerged zone. Sorption, degradation and volatilisation were simulated in this model as the three key processes that govern the behaviour of micro-pollutants. Sorption processes were modelled by a chemical non-equilibrium two-site sorption model while degradation is presented with a first-order decay model. Therefore this model can be easily applied to any micro-pollutant if its key removal mechanisms are known (*e.g.* for the removal of pesticides, sorption and biodegradation are the predominate processes, while volatilisation can be neglected).

The model was used to simulate the fate of five organic micro-pollutants (herbicides: atrazine, prometryn, simazine, glyphosate and THMs: chloroform) in the Monash carpark biofilter. It was tested for variable and challenging operational conditions; *e.g.* different inflow volumes, dry and wet period dynamics, and inflow pollutant concentrations. The model was calibrated and independently validated on two separate datasets. The model has been most successful in predicting the fate of simazine, atrazine and prometryn. Further, the modelled concentrations of glyphosate reflect reasonably well measured data for challenging wet events, but model performance was reduce in events following an extended dry period. The calibrated model parameters were in agreement with the available literature values, which makes the use of this model promising for the tested groups of organic pollutants. However, a thorough sensitivity and uncertainty analysis will have to be conducted before the model is recommended for application into practice.

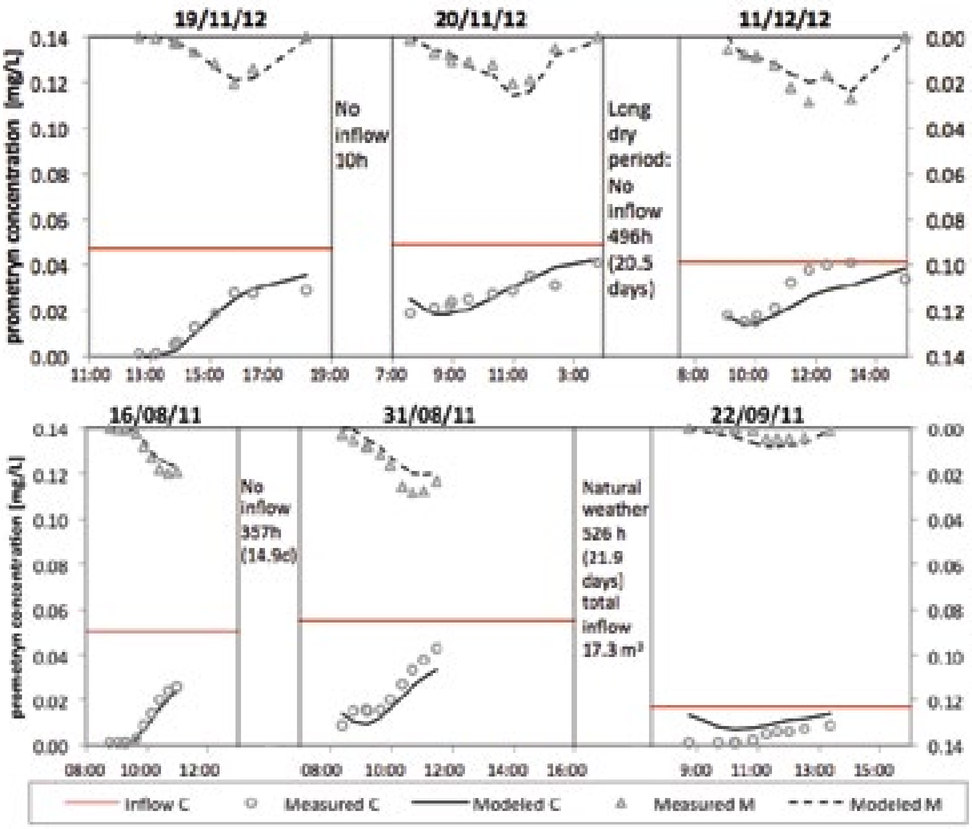


Figure 25: Inflow and outflow concentration and pollutant flux time series for Prometryn in S-SZ (sand with submerged zone) biofilter: first row – calibration dataset; second row – verification dataset. C – Concentration; M – flux. (Source: Randelovica et al. (Randelovica et al., in preparation)).

5 CONCLUSIONS AND FUTURE RESEARCH

This report presented a series of research projects designed to investigate the capacity of biofilters and constructed wetlands to remove different pollutant groups including nutrients, faecal microorganisms and micro-pollutants. Research projects conducted under the CRCWSC have contributed to broaden our knowledge on the behaviour of different pollutant groups within stormwater biofilters, including the influence of different biofilter design components and operational conditions (as previously discussed in Section 2.2, 3.2 and 4 of this report).

5.1 Key Findings

In summary, it is evident that WSUD technologies, particularly biofilters, once designed properly, are capable of reducing a range of stormwater pollutants to meet the water quality criteria required for different end uses (*e.g.* irrigation, recreational use with primary/ secondary contact). Importantly, a set of biofilter design parameters achieving optimal removal performances for one type of stormwater pollutant may not yield the optimal results for another type of stormwater pollutant. Therefore, is important to select appropriate design characteristics (*e.g.* system size, filter media composition and depth, vegetation and SZ, etc.), which are able to achieve specific water quality objectives for the stormwater harvesting/reuse system in question. It is also important to understand that biofilter performance can be adversely affected by challenging operational conditions (*e.g.* extreme wet/ dry conditions, variable inflow concentrations).

The recent research conducted by the CRCWSC has greatly assisted in developing and refining this treatment component of stormwater harvesting systems. However, a number of knowledge gaps remain in understanding the behaviour of some stormwater pollutant groups within these systems. Hence, the following section provides some directive for future research to further refine stormwater treatment technologies, such as biofilters and constructed wetlands.

5.2 Future Research

Biofilters

It was found that plant uptake significantly contributes to the retention of nutrients within biofilters, yet some nutrient release will invariably occur upon plant senescence. Seasonal and long term plant harvesting has been proposed as a solution, yet its effectiveness still needs to be tested, not only for nutrient removal performance but also for maintaining the hydraulic performance of biofilters. In addition, it is well known that soil microbial communities play a critical role in nutrient removal within biofilters, yet there is a very limited knowledge (almost non-existent) on how design and operating conditions affect the microbial communities involved in nitrogen processing. These important dynamics warrant further investigation.

Communities of soil fauna are also an integral part of biofiltration systems, and may play a critical role in the maintenance of infiltration capacity (*e.g.* preferential flow paths created via warm holes). The infiltration capacity of biofilters is directly linked to the removal of many stormwater pollutants and therefore further investigation into this aspect would be beneficial.

Currently there is no data on long-term faecal microorganism removal performances in either current best practice biofilters or next generation biofilters with novel antimicrobial filter media. Yet such data could be very important given the finding that the retentive capacity of biofilters can become exhausted for some pollutants (metals) over time. Therefore, investigating long-term faecal microorganism removal performance is recommended. There is also a very limited data set on reference pathogens and indicators other than bacteria (*E. coli*). Therefore more data should be collected for these microorganisms to evaluate the capacity of biofilters (both current best practice and next generation) to remove stormwater pathogens, especially at the field-scale.

The performance of the novel antimicrobial filter media decreased during cold weather and in clogged biofilters. Therefore, further investigation into improving the performance of the novel antimicrobial filter media under these conditions would be beneficial. It was observed that plant growth rate is relatively slow in the presence of this media and this may due to the presence of high levels of Cu in the filter media. Hence, it is recommended to investigate the Cu-tolerance of different plant species, which may identify species which can be incorporated into the next generation of stormwater biofilters fitted with novel antimicrobial filter media.

Only a preliminary predictive model was developed for pathogen removal in stormwater biofilters and this model is not capable of incorporating some biofilter design parameters, such as inclusion of a SZ and use of different plant species. Therefore, improvements are recommended for the preliminary predictive model for pathogen removal in stormwater biofilters.

Although biofilters are very good for the removal of heavy metals, limited knowledge is available on the accumulated heavy metals, and therefore it is difficult to assess the potential for bioremediation. Further investigation looking into the long-term fate of heavy metals in biofilters would be useful. Similarly, although petrol and oil derivatives are well removed by biofilters, currently there is no data on long-term organic micro-pollutant removal performances in biofilters. However, this could be very important given that biofilters were found to have exhausted retention capacity for some pollutants over time. This drives a necessity to investigate long-term organic micro-pollutant removal performances. Further, it was found that current best practice biofilters are less effective in removing herbicides. Therefore, further investigation to understand the key removal processes and seek ways to improve biofilter design to facilitate the removal of herbicides is warranted.

Constructed wetlands

Since the recent research conducted on constructed stormwater wetlands is limited to a detailed literature review and an on-going field monitoring program, an extensive investigation focusing on pathogen and micro-pollutant removal is required. Some of the key areas which warrant further investigation are:

- major mechanisms governing faecal microorganism removal in constructed wetlands;
- influence of wetland design and operational conditions on overall faecal microorganism removal;
- contribution of the direct deposition of faecal microorganisms into wetlands (*i.e.* waterfowl contributions);
- capacity of constructed wetlands to remove stormwater micro-pollutants;
- major mechanisms governing micro-pollutant removal in constructed wetlands; and
- influence of wetland design and operational conditions on overall micro-pollutant removal.

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7 APPENDIX A: ORGANIC MICRO-POLLUTANT WATER QUALITY RESULTS FOR A CONSTRUCTED WETLAND RECEIVING PRE-TREATED RUNOFF FROM AN INDUSTRIAL SITE IN MELBOURNE

			2012-04-23		2012-04-27		2012-05-03		2012-05-27		2012-06-07	
Micro-pollutant		**Unit**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**
	Methylene Blue Active Substances (MBAS)	mg/L	<0.1		0.2		<0.1	0.1	<0.1	<0.1	<0.1	<0.1
	Oil	mg/L	<5				12	<5	<1	<1	<1	<1
Benzene Toluene Ethyl benzene Xylenes and Naphthalene (BTEXN)	Benzene	mg/L	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.01*	<0.01*
	Toluene	mg/L	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.01*	<0.01*
	Ethyl Benzene	mg/L	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.01*	<0.01*
	Xylene - m & p	mg/L							<0.002	<0.002	<0.01*	<0.01*
	Xylene - o	mg/L							<0.001	<0.001	<0.01*	<0.01*
	Total Xylenes	mg/L	<0.003		<0.003		<0.003	<0.003	<0.002	<0.002	<0.01*	<0.01*
	BTEX (Sum) mg/	mg/L							<0.002	<0.002	<0.01*	<0.01*
Monocyclic Aromatic Hydrocarbons (MAH)	Styrene	mg/L	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.01*	<0.01*
	Cumene	mg/L	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.01*	<0.01*
	Trimethylbenzene	mg/L	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.01*	<0.01*
Total Petroleum/ Recoverable Hydrocarbons (TRH & TPH)	C6-C9	mg/L	<0.1		<0.1		<0.1	<0.1	<0.1	<0.1	<0.4	<0.4
	C6-C10	mg/L					<0.1	<0.1	<0.1	<0.1	<0.4	<0.4
	C6-C10 minus BTEX	mg/L					<0.1	<0.1	<0.1	<0.1	<0.4	<0.4
	C10-C14	mg/L	<0.1		<0.1		<0.1	<0.1	<0.04	<0.04	<0.04	<0.04
	C15-C28	mg/L	<0.1		<0.1		**0.1**	<0.1	<0.1	<0.1	<0.1	<0.1
	C29-C36	mg/L	<0.1		<0.1		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	TRH>C10-C16	mg/L					<0.1	<0.1	<0.04	<0.04	<0.04	<0.04
	TRH>C16-C34	mg/L					**0.2**	**0.1**	<0.1	<0.1	**0.1**	<0.1
	TRH>C34-C40	mg/L					<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Sum of TRH >C10-C40	mg/L					**0.2**	**0.1**	<0.1	<0.1	**0.1**	<0.1
Polycyclic Aromatic Hydrocarbons (PAHs)	Naphthalene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Acenaphthylene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Acenaphthene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Fluorene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Phenanthrene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Anthracene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Fluoranthene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Pyrene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Benz(a)Anthracene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Chrysene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Benzo(b)Fluoranthene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Benzo(k)Fluoranthene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Benzo(a)Pyrene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001

			2012-04-23		2012-04-27		2012-05-03		2012-05-27		2012-06-07	
Micro-pollutant		**Unit**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**	**Inlet**	**Outlet**
	Dibenz(a,h)Anthracene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Benzo(g,h,i)Perylene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Indeno(1,2,3-d)Pyrene	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
	Total PAH	mg/L							<0.0001	<0.0001	<0.0001	<0.0001
Organochlorine pesticides (OCPs)	Glyphosate	mg/L							<0.03	<0.03	<0.03	<0.03
	Chlordane	mg/L							<0.001	<0.001	<0.001	<0.001
	Diuron	µg/L							<0.02	<0.02	<0.02	<0.02
Polychlorinated biphenyls (PCBs)	Aroclor 1016	mg/L							<0.001	<0.001	<0.001	<0.001
	Aroclor 1221	mg/L							<0.001	<0.001	<0.001	<0.001
	Aroclor 1232	mg/L							<0.001	<0.001	<0.001	<0.001
	Aroclor 1242	mg/L							<0.001	<0.001	<0.001	<0.001
	Aroclor 1248	mg/L							<0.001	<0.001	<0.001	<0.001
	Aroclor 1254	mg/L							<0.001	<0.001	<0.001	<0.001
	Aroclor 1260	mg/L							<0.001	<0.001	<0.001	<0.001
Triazines	Ametryn	mg/L							<0.01	<0.01	<0.01	<0.01
	Atrazine	mg/L							<0.01	<0.01	<0.01	<0.01
	Prometon	mg/L							<0.01	<0.01	<0.01	<0.01
	Prometryn	mg/L							<0.01	<0.01	<0.01	<0.01
	Propazine	mg/L							<0.01	<0.01	<0.01	<0.01
	Simazine	mg/L							<0.01	<0.01	<0.01	<0.01
	Terbuthylazine	mg/L							<0.01	<0.01	<0.01	<0.01
	Simetryn	mg/L							<0.01	<0.01	<0.01	<0.01
	Terbutryn	mg/L							<0.01	<0.01	<0.01	<0.01
Phthalates	Butyl Benzyl phthalate	mg/L							<0.003	<0.003	<0.003	<0.003
	Dibutyl phthalate	mg/L							<0.003	<0.003	<0.003	<0.003
	Diethyl phthalate	mg/L							<0.005	<0.005	<0.005	<0.005
	Dimethyl phthalate	mg/L							<0.005	<0.005	<0.005	<0.005
	Di-n-octyl phthalate	mg/L							<0.005	<0.005	<0.005	<0.005
	Dioctyl phthalate	mg/L							<0.005	<0.005	<0.005	<0.005
Trihalomethanes (THMs)	Chloroform	mg/L							<0.001	<0.001	<0.001	<0.001
	Dichlorobromomethane	mg/L							<0.001	<0.001	<0.001	<0.001
	Dibromochloromethane	mg/L							<0.001	<0.001	<0.001	<0.001
	Bromoform	mg/L							<0.001	<0.001	<0.001	<0.001
	Trihalomethanes, Total	mg/L							<0.001	<0.001	<0.001	<0.001
	4-Chloro-3-Methylphenol	mg/L							<0.001	<0.001	<0.001	<0.001
	2-Chlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001
	2,4-Dichlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001
	2,6-Dichlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001
	Pentachlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001
Phenols(Halo)	2,3,4,5-Tetrachlorophenol	mg/L							<0.001	v0.001	<0.001	v0.001
	2,3,4,6-Tetrachlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001
	2,3,5,6-Tetrachlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001
	2,4,5-Trichlorophenol	mg/L							<0.001	<0.001	<0.001	<0.001

Table A. 1: Inlet and outlet water quality results for micro-pollutants

*LORR Limit of Reporting has been raised due to high moisture content, insufficient sample or matrix interference.

8 APPENDIX B: ENVIRONMENTAL AND PUBLIC HEALTH MICROBIOLOGY LABORATORY (EPHM LAB)

The EPHM laboratory utilises a range of molecular and culture based techniques for the monitoring of bacterial content in a range of environmental contexts. In the past year there has been extensive development and integration of molecular techniques into our existing skill portfolio. Cross-university collaborative projects have also extended our research capabilities in the field of environmental monitoring. Available techniques and projects currently utilising each are outlined below.

Escherichia coli

Projects:

Western Industrial Site, UCLA collaborative project, Monash Carpark Biofilter, Faecal Microorganisms Removal in Stormwater Biofilters, Novel Biofiltration Media for Pathogen Removal from Stormwater, Royal Melbourne Golf Club.

Techniques:

1.
AS 4276.21 (2005) – Method 21. "Examination for coliforms and Escherichia coli – Determination of Most Probable Number (MPN) using enzyme hydrolysable substrates".

Method utilises Colilert (Idexx Laboratories), Quanti-tray and Quanti-tray sealer to quantify, by MPN, *E. coli* and total coliforms, at concentrations from 1 to 2419 organisms, per 100mL sample. The Colilert method has been demonstrated to be a rapid, cheap and highly specific method for the detection of sub-types of *E. coli* from complex water samples (in preparation). Unlike other detection methods, the small cost (<$10 per sample) and ease of measurement allows comparative testing of multiple water samples within a short period of time.

Applications: Environmental water surveys (river, stormwater, sewage, biofiltration inflows and outflows etc.).

2.
Quantification of viable *E. coli* in soil environments.

Optimization of *E. coli* extraction method from Yarra river soil and biofilter filter media using AS 4276.21 (2005) – Method 21 (as described above) and AS 1289.2.1.1 (2005) for final quantification (Schang et al., 2012.)(Fricker et al., 1997). Bacteria are ubiquitous in the environment. The FIO *E. coli* has been demonstrated to survive within diverse soil matrices (Chandrasena et al., 2013). Consequently, disease potential (through FIO detection) should be considered during environmental monitoring regimes. The optimised method utilises the specificity and cost benefits of the AS 4276.21 (2005) – Method 21 for the testing of multiple soil samples.

Applications: Environmental soil samples, biofiltration columns media, faecal material.

3.
PCR detection of total viable *E. coli*.

The presence of viable microbes is required to cause disease. Within any environmental samples there is a complex mix of viable and non-viable organisms. Thus, to fully elucidate disease potential it is important to recognise the total number of viable microbes within a sample. Oligonucleotide probes can be used, in conjunction with quantitative PCR (qPCR) analysis, for the specific detection of *E. coli* within complex samples. To enable the specific detection of viable organisms samples, propidium monoazide (PMA) treatment, for the selective removal of dead cell DNA from water, soil and biofilter derived samples, has been incorporated into qPCR analysis (Chandrasena et al., 2013, Moynihan et al., 2013, Topp et al., 2003). Within EPHM, primers targeting the - -D-glucuronidase gene (uidA) (Fricker et al., 1997, Moynihan et al., 2013) and conserved regions of the 16S rRNA gene (Grey and Steck, 2001) have been used to detect the concentration of total *E. coli* (viable and non-viable). The uidA and 16S rRNA genes are essential for *E. coli* survival and are present in all sub-types of the species, which represents a distinct advantage over culture-based assays. Furthermore, enhanced assay sensitivity enables the detection of as little as 10 cells, irrespective of starting sample volume. The cost of performing the assay is dependent on the number of samples applied.

Applications: Environmentally derived soil and water samples, faecal material, biofiltration media, stormwater, raw sewage.

4.
Isolation of environmental isolates.

Evidence indicates significant differences (behavioural, growth, pathogenicity) can occur between bacterial strains isolated from environmental contexts and those passaged within laboratories (Huijsdens et al., 2002). Consequently, EPHM laboratory isolates and maintains a strain database of select bacteria, such as *E. coli* and Campylobacter sp. for use in comparative studies where the use of environmentally derived isolates are essential. The strains are isolated, by culture methods, based on biochemical confirmation of identity. The bacterial genus and species are then confirmed by Sanger Sequencing (Micomon Platform, Monash University) across the 16S rRNA gene.

Applications: Comparative survival studies, bacterial subtyping for identification of putative human-health related organisms.

Enterococci sp.

Projects:

Western Industrial Site.

Techniques:

1.
ASTM D6503-99 (2009). “Standard Test Method for Enterococci in water using Enterolert”.

Method utilises Enterolert (Idexx Laboratories), Quanti-tray and Quanti-tray sealer to quantify, by MPN, Enterococci concentrations from 1 to 2419 organisms per 100mL sample. The Enterolert method is a rapid, cost effective method for the evaluation of Enterococci concentrations within wastewater, stormwater and recreational waters (Budnick et al., 1996). Unlike other culture-based enumeration methods, ASTM D6503-99 (2009) does not require filtration of samples prior to culture, which in turn reduces on-costs (←$10 per sample) and increases the number of samples that can be processed within a timeframe.

Applications: Environmental water surveys (river, stormwater, sewage, biofiltration inflows and outflows etc.).

Campylobacter spp.

Projects:

Western Industrial Site, Monash Carpark Biofilter, Faecal Microorganisms Removal in Stormwater Biofilters, Royal Melbourne Golf Club.

Techniques:

1.
AS 4276.19 (2001). “Water microbiology – Examination for thermophilic Campylobacter spp. – Membrane filtration”.

The EPHM laboratory has evaluated and optimised a five tube MPN method of AS/NZ 4276.19 (2001) method (Appendix 1). Comparative studies with a NATA certified laboratory (ALS Environmental, Scoresby) have been undertaken (N = 84) and a positive correlation between results derived from the two laboratories ($\sigma = 0.58$, $p = <0.001$) has been observed. Comparative study data also demonstrated that EPHM had an overall higher sensitivity of detection ($P = <0.001$) to that of the commercial laboratory. The data reveals the inter-laboratory reproducibility of the optimised method and the possibility of under-estimation of disease risk if comparative studies of assay sensitivity are not undertaken.

Applications: Enumeration of Campylobacter sp. from a range of water sources (estuarine, freshwater, biofiltration inflows/outflows etc). The assays have also been extended for use in with soil, sediments and faecal material.

2.
Due to the procedural costs of undertaking AS 4276.19 (2001) EPHM laboratory has conducted a long term study on the use of an alternative Campylobacter MPN-PCR based assay (Appendix 1). The use of the PCR-based assay would reduce the current in-laboratory costs from $67.27 per water sample to $15.63 per assay (prices exclusive of staffing costs). Water samples (N=147) were collected under a variety of climatic and hydrological conditions and concurrently underwent the optimised AS 4276.19 (2001) method and MPN-PCR assay. Results show a significant correlation between data derived from both methods (- = 0.79, p = ←0.001). The data collected from this study demonstrate that the MPN-PCR assay can be used with no significant loss in assay sensitivity or reproducibility, and thus represents a significant cost saving. Furthermore, the diagnostic accuracy of the assay was found to significantly exceed the current inter-laboratory accuracy of AS 4276.19 (2001) method (Henry, in preparation).

Applications: Enumeration of Campylobacter sp. from estuaries, stormwater, wastewater, wetlands and biofiltration systems. The assays have also been extended for use in with soil, sediments and faecal material.

3.
Molecular typing of environmental isolates by Restriction Fragment Length Polymorphism (RFLP).

Typing of bacterial strains can reveal information pertaining to epidemiology, bacterial serovar/ subtype, source and specific environments in which bacteria can survive. Campylobacter can be isolated from a range of sources including agricultural material, faeces, food (chicken), stormwater, wastewater and recreational waters. Thus, the identification of disease-causing serotypes has direct human health implications. RFLP of the flaA gene is a commonly employed method for the molecular typing of virulent Campylobacter isolates (Budnick et al., 1996, Fricker et al., 1983, Yakub et al., 2002). The method enables the rapid and specific identification of pathogenic Campylobacter at a minimal of cost. The information can then be cross-correlated with hospital databases to identify strains known to cause human disease within the local population. To date, studies conducted within EPHM have demonstrated the existence of diverse pathogenic Campylobacter subtypes within the Melbourne region.

Applications: Data derived from molecular typing studies can be applied to aid disease mitigation efforts, epidemiological studies and quantitative risk assessment (QMRA) models.

4.
Species specific detection of Campylobacter in complex samples.

Four different Campylobacter species (C. jejuni, C. coli, C. lari and C. upsaliensis) have been identified by the WHO as the major causative agents of the diarrheal disease, campylobacteriosis (Nachamkin et al., 1993, Taboada et al., 2013). The disease is spread through ingestion of contaminated food and water sources. Thus knowledge of the presence and proportion of pathogenic species within environmental settings is important for epidemiology and disease mitigation. Within the EPHM laboratory, oligonucleotide probes are used in conjunction with qPCR analysis and Sanger sequencing for species specific detection of Campylobacter within water, sediment and faecal samples. Probes targeting the 16S rRNA, lpxA, mapA and ceuE genes have been successfully applied for the species specific identification of environmental Campylobacter isolates from water and faecal sources (Pond, 2005). The application of molecular tools, such as PCR, may help to circumvent some of the limitations and costs associated with current culture-based isolation methods, and expedite the time between sample collection and reporting.

Applications: Epidemiological research, QMRA and microbial source tracking studies

5.
Isolation of environmental Campylobacter sp. isolates.

EPHM laboratory maintains a strain database of Campylobacter isolates for use in comparative studies. To date, over 200 Campylobacter strains have been collected and biochemical identity confirmed using the AS 4276.19:2001 method. The identities of a subset of these strains have been established by Sanger Sequencing (Micomon Platform, Monash University) across the 16S rRNA gene region, multilocus sequence typing (MLST) and RFLP of the flaA gene. The strain database represents a one-of-a kind asset in the study of pathogenic Campylobacter in the Melbourne region offering a link between environmental source and human health outcomes.

Applications: Epidemiological surveys, microbial source tracking, QMRA and a tool to aid disease mitigation strategies.

Pseudomonas aeruginosa

Projects:

Microorganisms Removal in Stormwater Biofilters

Techniques:

1.
Pseudomonas aeruginosa isolations from environmental sample are performed using Pseudomonas Agar Base (PAB) medium (Oxoid) supplemented with cetrimide (200mg/L) and Nalidixic acid (15mg/L) (CN selective supplement, Oxoid). 1mL of sample is directly added into 9mL of LB Irgasan enrichment broth. Samples are analysed in triplicates using serial dilution from 10-1 to 10-4. Inoculated broths are incubated at 37oC for 18h on a shaking platform. After incubation 25µL of each broth is plated onto PAB-CN plates and incubated at 37oC for 24 to 48 hours in aerobic condition. In all cases, three plates are inoculated per dilution. Once, growth on plate is sufficient or after 48hours, 3 colonies are selected from each plate and transferred into 100µL of LB broth before being incubated for a further 24h at 37oC under agitation (180rpm). Colony identification and confirmation is then done using ecfx PCR screening to test the presence of Pseudomonas aeruginosa using the protocol describe in Lavenir et al. (2007). From the number of positive samples, the MPN per unit of sample (MPN/mL) is calculated using a standard statistical MPN table (Oblinger and Koburger, 1975).

Applications: Data derived from these studies can be applied to aid determine microbial efficiencies of stormwater harvesting systems, disease mitigation efforts, epidemiological studies and quantitative risk assessment (QMRA) models.

Salmonella typhimurium

Projects:

Faecal Microorganisms in Stormwater.

Techniques:

1.
AS 4276.14-1995. “Water Microbiology : Detection of Salmonellae”

The culture-dependent protocol utilises selective bacterial culture media, filtration and biochemical tests to isolate Salmonella sp. from environmental samples. Upon application to selective media Salmonella sp. are identified by the yellow appearance of colonies, indicative of xylose fermentation. Biochemical assays are required to confirm bacterium identity. Though specific to a genus level, the test is unable to differentiate between disease-causing (S.

typhi, S. typhimurium, S. enterica) and non-disease associated strains. Total costs to conduct AS 4276.14-1995 is sample number, type and biochemical test dependent, but usually <$10 per assay.

Applications: For the specific enumeration of Salmonella sp. from a range of water sources. The assay has also been optimised for use with estuarine soils and bed sediments.

2.
Species specific detection of Salmonella typhimurium

Salmonella typhimurium is one of the leading causes of non-typhoidal gastroenteritis worldwide (Best et al., 2003, Brown et al., 2004, Klena et al., 2004). A foodborne pathogen, the global incidence of disease has been estimated to be 2.8 million cases/year (Majowicz et al., 2010). However, the bacterium has been demonstrated to survive in recreational waters, which may offer an alternative, and under-recognised, route of infection (Majowicz et al., 2010). Within the EPHM laboratory, oligonucleotide probes are used in conjunction with qPCR analysis for specific detection of S. typhimurium within water and sediment samples. The probes target known virulence associated genes invA, hila and h-1i (Boehm et al., 2012, Catalao Dionisio et al., 2000) (Marathe et al., 2013, Chiu and Ou, 1996) to identify strains with human disease potential. The application of molecular tools, such as PCR, expedites the time between sample collection and reporting and also enables the specific detection of S. typhimurium with the potential to cause human disease.

Applications: Data derived from these studies can be applied to aid disease mitigation efforts, epidemiological studies and quantitative risk assessment (QMRA) models.

Bacterial Community Profiling

Projects:

Faecal Microorganisms Removal in Stormwater Biofilters, Novel Biofiltration Media for Pathogen Removal from Stormwater.

Techniques:

1.
16S amplicon sequencing of total bacterial communities.

Bacteria are ubiquitous in the environment and have many essential biological, biochemical and biogeochemical roles (Way et al., 1993). Understanding the diversity, ecology and environmental relationships between microorganisms and their exogenous environment can provide fundamental information on system dynamics and performance under a variety of conditions. The advent of high-throughput sequencing technologies have enabled researchers to mine deeper, than was previously possible with culture based techniques, to gain insight into the true complexity of microbial communities. To achieve this EPHM laboratory, in conjunction with the Micromon Next-Generation Sequencing Platform (Monash University, Melbourne), has optimised a procedure for the detection of total bacterial content within complex samples. A PCR assay is conducted to amplify a variable region within the ubiquitous 16S rRNA gene of all microbes present within a sample. The amplified products are then applied to an Illumina MiSeq V2 high-throughput sequencer for analysis of the genetic content. Once sequenced, bioinformatics analysis is undertaken to identify all microbes within the original sample to the genus level. The further application of specific bioinformatics pipelines enables the identification of some bacterial genus to the species level, increasing the specificity of the assay. Comparative analysis between samples allows the research to develop a picture of the dynamics within a system under a range of parameters. Cost of undertaking the Illumina MiSeq V2 sequencing is dependent on the number of samples applied, sample preparation, PCR assays and bioinformatic analysis applied. Further information on the cost of this service can be obtained from the Micromon Sequencing Platform (https://platforms.monash.edu/micromon/).

Applications: Development of new risk assessment and environmental monitoring system with focus on identification of bacterial pathogens, microbial source tracking, computational modelling of system dynamics, QMRA model development, understanding of microbial dynamics within biofiltration and stormwater systems. Modification of the probes to target specific genes can also further applications to include investigation of disease-potential and understanding of metabolic processes within complex microbial systems.

Molecular Tools

Projects:

UCLA collaborative project, Faecal Microorganisms Removal in Stormwater Biofilters, Novel Biofiltration Media for Pathogen Removal from Stormwater.

Techniques:

1.
Isolation on DNA

Accurate detection and identification of disease-related pathogens is essential to the improvement of human health outcomes. Molecular tools, such as PCR and 16S amplicon sequencing, represent rapid pathogen-detection methods. However, isolated high-quality DNA is required for highs levels of sensitivity, specificity and accuracy of molecular assays to be achieved. Environmental soil, water and faecal samples contain known inhibitors which often prevent their successful application to PCR based assays (Vanwonterghem et al., 2014). In the EPHM laboratory, we have optimised a procedure for the isolation of DNA from a range of environmental sources (water, soil, human and animal faeces) using the MoBio DNA extraction systems. MoBio are recognised as one of the best adapted systems for environmental DNA extraction due to their inclusion of inhibitor removal technologies (Schrader et al., 2012). Assay costs are source dependent but range from $6-$11 per sample.

Applications: The high quality DNA isolated using these procedures has, to date, been subsequently applied to community profiling, quantitative PCR for detection of environmental pathogens and microbial source tracking assays.

2.
Isolation of RNA.

Bacteria cause disease through the production and release of virulence factors. The levels to which these factors are produced are dependent on a variety of external parameters (temperature, oxygen, nutrients etc.). Researchers can monitor the conditions under which expression of specific factors is greatest (and therefore more likely to cause disease) through measurement of RNA. The isolation of total RNA from complex samples can be technically demanding. However, in the EPHM laboratory, we have optimised a procedure for the recovery of RNA from soil, sediments and faecal material using the MoBio RNA isolation systems. The total costs are source dependent but range from $11-$20 per sample for RNA purification. The high quality RNA is subsequently reversed transcribed for application in quantitative Real-Time PCR (qRT-PCR) based assays. The assays detect the absolute quantity of a virulence factor within a sample and can sensitively discriminate external parameter relationships associated with increases/decreases in bacterial disease potential.

Applications: These assays should be applied when decreased human health outcomes are observed and considered to be correlated with the presence/absence of a particular environmental factor.

9 APPENDIX C: LIST OF PUBLICATIONS

Nutrients

1. Ellerton, J. P., Fletcher, T. D.and Hatt, B. E. (2012). Mixed plantings of Carex appressa and Lomandra longifolia improve pollutant removal over a monoculture of *L. longifolia* in stormwater biofilters. 7th International Conference on Water Sensitive Urban Design. Melbourne, Australia.

2. Glaister, B. J., Fletcher, T. D., Cook, P. L. M. and Hatt, B. E. (2014). Co-optimisation of Phosphorus and Nitrogen Removal in Stormwater Biofilters: the Role of Filter Media, Vegetation and Saturated Zone. Water Science & Technology. 69(9), 1961-1969

3. Glaister, B. J., Cook, P. L. M., Fletcher, T. D.and Hatt, B. E. (2013). Long-term phosphorus accumulation in stormwater biofiltration systems at the field scale. 8th International Conference on Water Sensitive Urban Design. Gold Coast, Australia.

4. Glaister, B. J., Fletcher, T. D., Cook, P. L. M.and Hatt, B. E. (2013). Co-optimisation of Nitrogen and Phosphorus Removal in Stormwater Biofilters: the Role of Filter Media, Vegetation and Saturated Zone. Novatech 2013. 8th International Conference on Planning and Technologies for Sustainable Urban Water Management. Lyon, France.

5. Glaister, B., Fletcher, T. D., Cook, P. L. M. and Hatt, B. E. (2012). Advancing biofilter design for co-optimised nitrogen and phosphorus removal. 7th International Conference on Water Sensitive Urban Design. Melbourne, Australia.

6. Glaister, B., Fletcher, T. D., Cook, P. L. M.and Hatt, B. E. (2011). Can stormwater biofilters meet receiving water phosphorus targets? A pilot study investing metal-oxide enriched filter media. 15th International Conference of the IWA Diffuse Pollution Specialist Group on: Diffuse Pollution and Eutrophication. Rotorua, New Zealand, IWA.

7. Payne, E. G. I., Fletcher, T. D., Cook, P. L. M., Deletic, A. and Hatt, B. E. (2014). Processes and drivers of nitrogen removal in stormwater biofiltration. Critical Reviews in Environmental Science and Technology. 44(7), 796-846.

8. Payne, E., Fletcher, T. D., Russell, D. G., Grace, M. R., Cavagnaro, T. R., Evrard, V., Deletic, A., Hatt, B. E. and Cook, P. L. M. (2014). Temporary storage or permanent removal? The division of nitrogen between biotic assimilation and denitrification in stormwater biofiltration systems. PLOS ONE 9(3): e90890.

9. Payne, E. G., Pham, T., Cook, P. L. M., Fletcher, T. D., Hatt, B. E. and Deletic, A. (2014). Biofilter design for effective nitrogen removal from stormwater - influence of plant species, inflow hydrology and use of a saturated zone. Water Science & Technology 69(6): 1312-1319.

10. Payne, E. G. I., Pham, T., Cook, P. L. M., Fletcher, T. D., Hatt, B. E. and Deletic, A. (2013). Biofilter design for effective nitrogen removal from stormwater – influence of plant species, inflow hydrology and use of a saturated zone. Novatech 2013. 8th International Conference on Planning and Technologies for Sustainable Urban Water Management. Lyon, France.

11. Payne, E. G. I., T. Pham, B. E. Hatt, T. D. Fletcher, P. L. M. Cook and A. Deletic (2013). Stormwater biofiltration - the challenges of inorganic and organic nitrogen removal. 8th International Conference on Water Sensitive Urban Design. Gold Coast, Australia.

12. Pham, T., E. G. Payne, Fletcher, T. D., Cook, P. L. M., Deletic, A. and Hatt, B. E. (2012). The influence of vegetation in stormwater biofilters on infiltration and nitrogen removal: preliminary findings. 7th International Conference on Water Sensitive Urban Design. Melbourne, Australia.

Faecal microorganisms

1. Chandrasena, G. I., Pham, T., Payne, E. G., Deletic, A. and McCarthy, D. T. (2014). *E. coli* removal in laboratory scale stormwater biofilters: Influence of vegetation and submerged zone. Journal of Hydrology, 519, Part A, 814-822.

2. Chandrasena, G. I., Deletic, A. and McCarthy, D. T., (2014). Survival of Escherichia coli in stormwater biofilters. Environmental Science and Pollution Research. 21(8), 5391-5401.

3. Chandrasena, G. I., Deletic, A. and McCarthy, D. T., (2013). Evaluating Escherichia coli removal performance in stormwater biofilters: A preliminary modelling approach. Water Science and Technology 67(11): 2467-2475.

4. Chandrasena, G. I., Ellerton, J., Deletic, A. and McCarthy, D. T., (2012). Evaluating Escherichia coli removal performance in stormwater biofilters: A laboratory-scale study. Water Science and Technology 66(5): 1132-1138.

5. Chandrasena, G. I., Deletic, A. and McCarthy, D. T. (2012). A Preliminary Model on *E. coli* Removal in Stormwater Biofilters. 9th International Conference on Urban Drainage Modelling. Belgrade, Serbia.

6. Chandrasena, G. I., Filip, S., Zhang, K., Osborne, C. A., Deletic, A. and McCarthy, D. T. (2012). Pathogen and indicator microorganism removal in field scale stormwater biofilters. 7th International Conference on Water Sensitive Urban Design, Melbourne, Australia.

7. Chandrasena, K. K. G. I., Ellerton, J., Deletic, A. and McCarthy, D. T. (2011). Removal of Escherichia coli in Stormwater Biofilters. 12th International Conference on Urban Drainage. Porto Alegre, Brazil.

8. Li, Y. L., McCarthy, D. T. and Deletic, A. (2014). Stable copper-zeolite filter media for bacteria removal/inactivation in stormwater. Journal of Hazardous Materials. 273: 222-230.

9. Li, Y. L., Deletic, A. and McCarthy, D. T. (2014). Removal of *E. coli* from urban stormwater using antimicrobial-modified filter media. Journal of Hazardous Materials. 271: 73-81.

10. Li, Y. L., Deletic, A., Alcazar, L., Bratieres, K., Fletcher, T. D. and McCarthy, D. T. (2012). Removal of Clostridium perfringens, Escherichia coli and F-RNA coliphages by stormwater biofilters. Ecological Engineering. 49: 137-145.

11. Li, Y. L., McCarthy, D. T. and Deletic, A. (2012). Removal and inactivation of *E. coli* from water using copper modified natural zeolite. 7th International Conference on Water Sensitive Urban Design. Melbourne, VIC, WSUD 2012.

12. Li, Y. L., McCarthy, D. T. and Deletic, A. (2011). Treatment of pathogens in stormwater by antimicrobial-modified filter media. 12th International Conference on Urban Drainage, Porto Alegre, Brazil.

Micro-pollutants

1. Feng, W., Hatt, B. E., McCarthy, D. T., Fletcher, T. D. and Deletic, A. (2012). Biofilters for stormwater harvesting: Understanding the treatment performance of key metals that pose a risk for water use. Environmental Science and Technology. 46(9), 5100-5108.

2. Hatt, B. E., Steinel, A., Deletic, A. and Fletcher, T. D. (2011). Retention of heavy metals by stormwater filtration systems: Breakthrough analysis. Water Science and Technology. 64(9), 1913-1919.

3. Zhang, K., Randelovic, A., Page, D., McCarthy, D. T. and Deletic, A. (2014). The validation of stormwater biofilters for micropollutant removal using *in situ* challenge tests. Ecological Engineering. 67:1-10

4. Zhang, K.F., Tian, X., Page, D., Deletic, A. and McCarthy, D.T. (2014). Use of Water Sensitive Urban Design systems for biofiltration of urban stormwater: laboratory biodegradation batch studies. 5th International Slow sand and Alternative Biological Filtration Conference, Nagoya, Japan.

5. Zhang, K.F., Aguiar, L., Randelovic , A., McCarthy, D.T. and Deletic, A. (2013). Determination of operational and challenge conditions for validation of stormwater biofilters and wetlands. 8th International Water Sensitive Urban Design Conference, Gold Coast, Australia.

6. Zhang, K. F., Filip, S., Chandrasena, G. I., McCarthy, D. T., Daly, E., Pham, T., Kolotelo, P. and Deletic, A. (2012). Micro-pollutant removal in stormwater biofilters: a preliminary understanding from 3 challenge tests. 7th International Conference on Water Sensitive Urban Design, Melbourne, Australia.

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