Microbial hazards in urban stormwater and their removal through Water Sensitive Urban Design

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(*note*: Cover illustration by Simon Lloyd, imagery adapted from Springer Nature Limited_2015, '*Nature Reviews Microbiology*')

Contents

Executive summary

A review of current scientific literature was undertaken with the following key aims:

- To critically review performance data regarding the removal of faecal indicators and pathogens from urban stormwater by stormwater biofilters, constructed wetlands, green roofs and green walls
- To identify current knowledge regarding what factors, influence the removal of these microbial contaminants

Stormwater biofilters, constructed wetlands (CWs), green walls (GWs), and green roofs (GRs) are some of the vegetated treatment technologies used within water sensitive urban design (WSUD) schemes. These systems capture and treat stormwater for a range of pollutants prior to reuse or discharge into surface waters. The capacity of these technologies to remove microbial contaminants is a key performance aspect, needed, to inform what contribution WSUD can make toward the goal of bringing swimming back to the Parramatta River.

Microbial contamination in urban stormwater

The ingress of domestic sewage into the stormwater system is the most well-known source of waterborne pathogens (bacteria, viruses and protozoa). In addition, certain microorganisms have adapted to pipe infrastructure and may reside and reproduce in this environment (McLellan et al. 2018). Further, WSUD technologies provide habitat for urban wildlife which can be a source of faecal contamination. Therefore, pre characterisation of stormwater influent is a critical step when WSUD planning. Bichai and Ashbolt's (2017) stormwater use management plan (SUMP) may provide a useful guide for local councils in this respect.

Overall treatment performance

On average, a 90% (1 log reduction) in faecal indicator bacteria (FIB) can be expected via treatment with stormwater biofilters. Removal rates in constructed wetlands range between -119% (i.e. net export of indicator bacteria) through to 99% (2log reduction), with a mean removal of 56% (0.43 log reduction). Green walls and green roofs had the smallest number of studies with removal rates for greywater (in lieu of rain / stormwater) ranging from 0% removal to 99.9% removal (3.7 log reduction). WSUD technologies have also shown preliminary capacity to remove pathogens between 11 – 98% (0.05 - 1.7 log reduction)

Treatment type matters – stormwater biofilter, wetland, green roof or green wall?

The most reliable removal rates come from stormwater biofilters. This category of WSUD technology has also received the most attention in terms of design optimisation. The lower removal rates in CWs may be due to faecal contamination from urban wildlife (Meng et al. 2018), resulting in a baseline level of FIB in these systems (Hathaway et al. 2011). Given each technology included in this review share the common principles of biofiltration, it is likely similar removal rates could be achieved by all. However, more attention toward CW, GW and GR design is needed to achieve removal rates like those reported in stormwater biofilter (bioretention) literature.

Microorganism matters – virus, bacteria or protozoa?

Differing removal rates for bacteria, protozoa and viruses have been observed. Physical straining in filter media may remove larger protozoan pathogens. Whilst, adsorption and predation are the most likely factors influencing removal of smaller organisms (bacteria and viruses). This points to the need to monitor treatment performance for a wider range of pathogens. Importantly optimal design specifications and construction quality are pre-requisites to allow these physical and biological removal processes to take place.

Design specifications influence removal

In stormwater biofilters, the strongest evidence for design criteria that promotes FIB and pathogen removal includes deeper profiles (≥0.9m), low nutrient filter media, and the inclusion of a saturated zone (SZ). The combination of these increase moisture retention and total contact time between contaminated water and biologically enhanced filter media. Similar evidence shows extended retention time improves removal in CWs and GRs. Intensive GRs (i.e. systems with greater depth) will provide better scope for incorporation of this criteria. Relevant to all treatment types, some evidence suggests copper coated filter media may further enhance removal (Li et al. 2014b). Emerging research also suggests plant species from the Myrtaceae family may provide enhanced removal compared to current 'best practice' species (Galbraith et al. 2019).

The catchment scale – will WSUD reduce microbial contamination in the river?

The final concentration in effluent from individual WSUD technologies typically exceeds guideline values for primary contact recreation. These effluent concentrations are generally reported independent of any reduction in water volume. As such, slightly higher removal rates can be expected when hydrological performance (i.e. volume reduction) is taken into account (Hathaway and Hunt 2012). Regardless, the WSUD approach needs to combine vegetative technologies with other storage treatment devices and buffers to peak flow, to reduce FIB loads entering the Parramatta River toward recreational guideline values.

Further, empirical research is needed to understand the accumulated benefit of WSUD technologies to watershed health. Sydney waters' modelling study (Cetin et al. 2018) explored this via accounting for the volume reduction achieved by WSUD, however the treatment performance aspect identified in this review was not included in the model. Emerging empirical investigations at the catchment scale include the application of real-time control to WSUD, see Kerkez et al (2016) and Mullapudi et al (2018). This type of design optimisation may provide scope to further reduce microbial contamination in targeted locations.

1.0 Introduction

Traditional models of urban water management are under increasing pressure to adapt to a changing climate whilst meeting the demands of growing populations in urban areas. Alternative water sources are needed to augment the current reliance on potable supplies for both potable and non-potable uses. In addition, there is a growing movement to transform degraded urban waterways into places fit for water-based recreation (PRCG 2019). The existing network of drains and pipes that underlie our cities have historically been credited for safeguarding public health (Sedlak 2014). Today they are known to routinely transport microbial and chemical pollutants into surface waters, creating public health hazards for a range of water re-use applications.

To counter this, over the past two decades, many cities have been edging towards a sustainable model of urban water management. Water sensitive urban design (WSUD) is the approach intended to guide Australia's transition in this process (Fletcher et al. 2015; Lloyd et al. 2012). The vegetated drainage systems that typify the end product of WSUD can be found on urban streetscapes, building wall facades and rooftops, although the mainstream uptake of WSUD remains to be seen (Brown 2007). Despite this, urban stormwater recycling schemes are increasingly being investigated (Hatt et al. 2006) and innovative 'nature based' proposals are being put forward to buffer contamination in urban rivers and create niche environments where people can swim (Yarra Pools 2018). One of the key uncertainties in these scenarios is the risk to public health due to microbial contamination present in urban stormwater (Ahmed et al. 2019).

Stormwater biofilters, constructed wetlands, green walls and green roofs are some of the vegetated technologies used in WSUD schemes (here after referred to as 'WSUD technologies'). These systems capture and treat urban stormwater for a range of pollutants prior to reuse or discharge into surface waters. Laboratory and field scale experiments investigating the reduction of microbial contamination via WSUD technologies have increased over the past decade. It should be noted that uncertainty remains regarding the capacity of WSUD technologies to remove pathogenic microorganisms, and optimisation of the processes that govern this (Deletic et al. 2014).The core aim of this review paper is to summarise performance data concerning faecal indicator and pathogen removal by WSUD technologies. Data reported in the academic literature, industry reports and guidelines have been included together with a discussion of the factors that influence removal. To contextualise this performance aspect section 2.0 of the review highlights key waterborne pathogens in urban stormwater and their relationship to public health.

2.0 Microbial hazards in urban stormwater

Microbes reside on all of the surfaces in our cities including building facades, roads, plants, soil and subsurface soil, pipe infrastructure, animals and human bodies (King 2014). Rain washes these organisms into the urban drainage network where they are transported directly into surface waters (McLellan et al. 2015). To date, profiling of the microbial community in storm and receiving waters has largely been constrained to inference, based primarily on the monitoring of faecal indicator organisms (Carney et al. 2020).

Faecal indicator bacteria (FIB) are useful to detect the presence of sewage contamination (Petterson et al. 2016) but are unable to discern the origin of this contamination (Soller et al. 2015; McLellan et al. 2015). In addition a large number of recent studies have reported a poor correlation between FIB, and the viral and protozoan microorganisms that are more directly related to human health (Carney et al. 2020; McLellan et al. 2018; Page et al. 2016; Jiang and Chu 2004). This is unsurprising as the morphology, environmental stressors and survival mechanisms of bacterial, viral and protozoan organisms differ substantially (Lin and Ganesh 2013; Byappanahalli et al. 2012). It is important to monitor the prevalence of these three microbial domains within stormwater to avoid over or underestimating health risk when considering subsequent reuse (Zimmerman et al. 2016; Petterson et al. 2016).

The most recent Australian stormwater recycling guidelines (NRMMC-EPHC-NHMRC 2009)cited a lack of local or international data to have quantified bacterial, protozoan and viral pathogens in stormwater. Since 2009, investigations of microorganisms in stormwater have increasingly adopted molecular methods (as opposed to culture-based methods). This has led to the reporting of a more nuanced viral, protozoan and bacterial profile in urban storm and surface waters (Carney et al. 2020). Nevertheless knowledge of microbial communities and their virulence in stormwater remains an emerging area (Arnone and Walling 2007; Ahmed et al. 2019). Pre characterisation of stormwater influent is a critical step to inform WSUD planning (Chong et al. 2013).

2.1 Stormwater ‘end-uses’

Current uses for recycled stormwater are predominantly non-potable. A survey by Hatt et al (2006) listed the most common end uses in Australia at the time, in descending order: ‘irrigation’ (gardens, sporting fields and public open space), ‘toilet flushing’, for ‘environmental flows’,’ industrial recycling’, and lastly ‘firefighting’. WSUD technologies have been applied individually or as part of a treatment train to capture and treat rainwater and stormwater for such uses where public exposure is relatively low (Hatt et al. 2006). Higher exposure scenarios include the use of surface waters for recreation or aquaculture, crop irrigation, indirect potable re-use and potable re-use (Hathaway and Hunt 2012; Chong et al. 2013). WSUD technologies can be combined with more advanced treatment processes to deliver water fit for these purposes (Page et al. 2016; Hatt et al. 2006).

Relevant to each type of end use is the survival of pathogens in the environment. Given favourable conditions, studies have reported the ability of bacteria (Byappanahalli et al. 2012), viruses (Aw 2019) and protozoan cysts (Carey et al. 2004) to survive extended periods of time (3 - 35 days+) in the environment. To manage these risks in low exposure schemes where WSUD technologies are used without advanced treatment, non-infrastructural measures such as temporarily limiting public access to irrigated spaces can be introduced (Page et al. 2016).

2.2 End-uses and health risk

Lim (2015) used a Quantitative Microbial Risk Assessment (QMRA) to rank viral infection risk for different stormwater end uses, finding irrigation of crops to be the highest risk followed by showering and then toilet flushing. In all exposure cases viruses need only be present in small numbers to cause illness (NHMRC 2008). Waterborne viruses can cause mild through to more severe illness or they may cause asymptomatic infection, making their impact difficult to trace (Rusiñol and Girones 2019). The predominant risks from pathogenic microorganisms in stormwater are acute (Khan and Byrnes 2016). Importantly, these same contaminants may result in chronic disease in vulnerable members of the community, such as those with compromised immunity (NAP 1998). For instance, enteroviruses are an important source of water-borne acute gastroenteritis in healthy populations, but may cause life-threatening chronic diarrhoea in children born with antibody deficiency (Jones et al. 2019).

Most epidemiological research is associated with recreational use of surface waters receiving stormwater discharge. Arnone and Walling's (2007)review reported higher levels of gastrointestinal infections and also risk of respiratory illness for swimmers in Los Angeles who swam near stormwater outlet drains, and in waters where viruses of enteric origin were detected. Both protozoa and enteric viruses are implicated as causing most of the world's waterborne disease outbreaks, notably with pathogens such as hepatitis E virus (HEV) which has caused many large epidemics with significant mortality (Efstratiou et al. 2017; Zimmerman et al. 2016; Kamar et al. 2014). Water-transmitted viral pathogens that are classified as having a moderate to high health significance by the World Health Organisation (WHO) include adenovirus, astrovirus, hepatitis A and E viruses, rotavirus, norovirus and other caliciviruses, and enteroviruses, including coxsackieviruses and polioviruses (Gall et al. 2015)

Microbial hazards in stormwater may be highly site-specific, meaning risk assessments and management plans tailored to local conditions is the favoured approach (Petterson et al. 2016; Bichai and Ashbolt 2017). Where sources of microbial contamination are identified as present or absent, treatment performance targets can be tailored accordingly (Bichai and Ashbolt 2017).

2.3 Sources of microbial contamination

The ingress of domestic sewage into stormwater is the key source of microbial contamination contributing to the risk profile in stormwater reuse schemes (Lampard J et al. 2017; Ahmed et al. 2019; Khan and Byrnes 2016). Untreated wastewater enters storm and surface waters via leaking sewer pipes, designed sewer overflows or septic tank seepage (Sidhu et al. 2012). Rainfall is known to trigger this cross-contamination, evidenced by increases in bacterial indicators and reference pathogens (by one-two orders of magnitude) entering stormwater treatment systems following rainfall (Petterson et al. 2016). Unsurprisingly rainfall has been linked to an elevated health risk for a range of end uses (Schreiber et al. 2019). Importantly bacterial markers for human faecal contamination have also been observed in stormwater during dry weather (Chong et al. 2013). As such site-specific knowledge of the condition of sewer infrastructure is important to identify the likelihood of faecal contamination occurring during dry and wet weather.

Faecal matter in stormwater is not always of human origin. Microbial source tracking (MST) techniques have revealed distinct bacterial genetic 'fingerprints' that link microbial communities detected in urban waters with their likely sources in the catchment (McLellan et al. 2015). Livestock and agricultural fingerprints have been found in upstream water reaches, and the 'imprint' of human, domestic animals and urban wildlife is marked in the waters fed by the city (McLellan et al. 2015). It is generally accepted that health risk is lower when sources of faecal contamination are linked to animals (Soller et al. 2015). Although some species, such as cattle are considered as carriers of high risk pathogens, and in general remediation strategies are more effective if sources are known (Ahmed et al. 2019). Further, WSUD technologies provide habitat for urban wildlife which can be a source of faecal contamination.

The re-introduction of nature / wildlife habitat into our cities in the form of wetlands, rain gardens and green roofs can themselves attract host organisms which may carry viral pathogens not of waterborne origin (Malan et al. 2009). In Australia, mosquitoes have been shown to breed on still edges of wetlands and carry diseases such as Ross River virus (Lõhmus and Balbus 2015). Much less has been reported about this potential transmission route of disease with reference to WSUD. The introduction of 'constructed wet environments' (Lõhmus and Balbus 2015) into our cities will require planning and design optimisation for more than waterborne pathogens. Importantly city design should not become risk-adverse but can better balance health risks and health benefits by integrating urban design and public health fields.

Another emerging source of microbial contamination is that borne inside pipe infrastructure itself. Referred to as 'infrastructure associated organisms', McLellan (2015) identified several microorganisms that seem to be residents of stormwater pipes in US cities with separated storm sewer infrastructure. This was deduced due to surprisingly little overlap of bacterial communities found between faecal samples, wastewater pipes, stormwater pipes and natural aquatic environments (McLellan et al. 2010). Most importantly organisms identified within these pipe environments have shown pathogenic potential (McLellan et al. 2015). This fingerprint of anthropogenic inputs is slowly becoming clearer in natural aquatic environments, which should allow more targeted source control strategies like WSUD to be put in place.

2.4 Key pathogens of concern

Table 1 presents a summary of the bacterial, protozoan and viral pathogens that have been detected in urban stormwater. Notably most were published after the stormwater recycling guidelines (2009) guidelines were published. Similar genera of bacteria, protozoa and viruses have been detected across distinct geographical regions. However, the prevalence of each genus varies considerably between these regions. Within regions, some studies have found temporal variability was more pronounced then spatial variability (Carney et al. 2020; McLellan et al. 2010). These differences are likely due to variations in local climate, health of the contributing population, land use and sampling methods used between studies (NAP 1998; NHMRC 2008; Ahmed et al. 2019; Hathaway and Hunt 2012)

Table 1 – Potential pathogens in urban stormwater

Genus	**Pathogenic strains**	**Mean/median Concentration or % positive detects***	**Associated illness**	**Host organism**	**Transmission into stormwater *** **
Bacteria					
Aeromonas	*A. caviae* *A. dhakensis* *A. veronii* *A. hydrophila*	3.4 x 10^4 (Rose and Jiménez-Cisneros 2019)	Gastroenteritis Septicaemia, wound infections	human	Sewage ingress;
Arcobacter	*A. cryaerophilus* *A. butzleri* *A. skirrowi*	2.2 x 10^6 (baseline) 1.8 x 10^7 (event) (Carney et al. 2020)	Gastroenteritis, bacteraemia	human, mollusc	Sewage ingress
Campylobacter	*C. jejuni* *C. coli* *C. lari*	72.7% all *spp* (24/33) 6.3% (*C. jejuni)* (Lampard J et al. 2017)	Gastroenteritis	human, poultry, wild birds, cattle	Sewage ingress, wildlife direct deposition

Salmonella	*S. enterica (typhoidal salmonellae)*	26.2% (16/61) (Lampard J et al. 2017)	Fever, seizures, diarrhoea	human	Sewage ingress
Vibrio spp	*V. cholerae 01* *V. cholerae 0139* *V. Vulnificus*	2.6 × 10^5 (all *spp*) 71% (*V. cholerae, 0% toxi gene)* *(Siboni et al. 2016)*	Cholera, skin infections, gastroenteritis	human, fish, shellfish	Sewage ingress
Legionella spp	*L. pneumophila*	0% (0/39) (Lampard J et al. 2017)**	Legionnaires' disease; severe pneumonia	Amoeba	Potential growth in pipe infrastructure
Staphylococcus aureus	*S. aureus*	18.8% (6/32) (Lampard J et al. 2017)	Skin infections, bacteraemia	human	Sewage ingress
Escherichia coli (indicator)	*E.coli* O157:H7	10^3-10^6 (indicator only) (McCarthy 2009; Schreiber et al. 2019)	acute haemorrhagic diarrhea	Farm animals	Agricultural run-off
Enterococci (indicator)	N/A	4.03 x 10^2 (baseline) 2.805 x 10^3 (event) (Sidhu et al. 2012)	N/A	N/A	Sewage ingress
Clostridium perfringens (indicator)	N/A	2 × 10^1 - 2.3 × 10^4 (Schreiber et al. 2019)	N/A	N/A	Sewage ingress
Protozoa					
Giardia spp	*G. lamblia*	31.6% (12/38) 2.3-3666 cysts/100L (Schreiber et al. 2019)	Giardiasis; diarrhoea	Animal & human	Sewage ingress; pet faeces
Cryptosporidium spp	*C. parvum (GI, GII)* *C. hominis*	10.5% (4/38) 2.3-3666 cysts/100L (Schreiber et al. 2019)	Respiratory, gastroenteritis	Animal & human	Agricultural run-off. Wildlife faeces; run-off and direct deposition
Viruses					
Adenovirus	*HAdV*	50.7% (35 / 69) (Lampard J et al. 2017)	Respiratory & febrile illness, gastroenteritis	human	Sewage ingress
Norovirus	GI GII	33.9 GC/ 100 ml (Steele et al. 2018)	Gastroenteritis	human	Sewage ingress
Enterovirus	*Enterovirus*	22% (13/59) (NRMMC-EPHC-NHMRC 2009)	Gastroenteritis respiratory illness	human	Sewage ingress
Rotavirus	*Rotavirus A*	3.0x10^1 to 2.5x10^7 GC/L	Gastroenteritis	human	Sewage ingress

		(da Silva et al. 2019)			
Polyomavirus	JCPyV BKPyV	58.6% (17 /29) (Lampard J et al. 2017)	Kidney failure, brain disorders ****	human	Sewage ingress
Hepatitis A	GI, GII, GIII GIV, GV, GVI	76% (16/21) (Jiang and Chu 2004)	Hepatitis	human	Sewage ingress
Hepatitis E	GI GII	11.7% (2 /17) (Rutjes et al. 2009)	Hepatitis	human, pigs	Sewage ingress; agricultural input

*% positive = total no of positive detections of target organism; ***Legionella spp have been observed in stormwater in previous studies (Ahmed et al. 2019) hence its inclusion despite 0% detection in Lampard et al (2017) Australian study ***sewage ingress includes illegal connections, seepage and designed overflows ****effects immunosuppressed*

2.4.1 Bacteria

In McLellan's (2015) study of microbial communities in raw stormwater, within separated sewers in US cities, three bacterial species were consistently present: Acinetobacter, Aeromonas and Pseudomonas. These were designated as 'resident' organisms. Arcobacter and Trichococcus were noted to only be significant after known sewage ingress had occurred. Similarly, a study of the Botany Bay foreshore in Sydney found that significant increases in Arcobacter, correlated with rainfall triggered sewer overflows (Carney et al. 2020). *A. cryaerophilus* and *A. butzleri* have been linked to cases of bacteraemia and gastroenteritis primarily via shellfish consumption. The study in Botany Bay, grouped genetically similar strains into several 'ecotypes'. Ecotypes which were co-existing and genetically similar to pathogenic *A. cryaerophilus*, were found to be most abundant in Summer, and after rain. Importantly, the presence of Arcobacter during dry periods was dominated by non-pathogenic environmental strains such as *A.nitrofigilis.*

Campylobacter was also commonly detected in urban stormwater. *C. jeuini*, *C. coli* and C. *lari* are the main pathogenic species of concern within this genus (Sidhu et al. 2012; Koenraad et al. 1997). Previous health risk assessments have derived an increased risk of illness associated with different levels of exposure to Campylobacter in stormwater (Murphy et al. 2017) and surface waters (Koenraad et al. 1997). However a recent study by Siddiqee (2019) in Melbourne's Yarra River revealed only a small percentage of the total Campylobacter genetic identified was similar to that of *C. jeuini.* Similarly, a widespread survey of microorganisms in stormwater across ten different catchments in Australia revealed the consistent presence of Campylobacter, although less than 6 % of all samples contained species akin to *C. jeuini (Lampard J et al. 2017)*. As this genus is commonly detected in stormwater, further investigations are needed to understand the relative virulence potential (Ahmed et al. 2019) of the species members and how this might change between seasons and regions within Australia.

Most studies reviewed in this section of the report use molecular methods to understand species level information, thus building a rich microbial profile. In addition, most include enumeration of Enterococci and *E.coli* to provide a benchmark given their global role as faecal indicator bacteria (FIB). Studies in Australia (Sidhu et al. 2014; McCarthy 2009; Lampard J et al. 2017), Europe (Schreiber et al. 2019; Hörman et al. 2004), USA (Choi and Jiang 2005; McLellan et al. 2018) and China (He et al. 2012) show Enterococci and E.coli are prevalent between 10^3 - 10^6 CFU/100ml, with *E.coli* numbers generally higher then Enterococci. These include samples taken from stormwater drains, canals, creeks, lakes and rivers receiving stormwater discharge. More studies characterising 'raw' stormwater prior to discharge into surface waters are needed. Regardless, nearly all studies describe differences in abundance and survival of FIB in comparison to viral and protozoan reference pathogens.

2.4.2 Protozoa

Efstratiou et al (2017)collated 381 cases of r protozoa associated waterborne disease outbreaks reported globally between 2011 and 2016. Cryptosporidium *spp* and Giardia *spp* were identified as the causative agents for all 381 cases with no other protozoan microorganisms linked to reported cases of illness in this period. Giardia commonly infects domestic pets and is a zoonotic pathogen, meaning it is transmissible from an animal reservoir into humans to cause disease. Given this reservoir, it is found in domestic sewage and has been detected in stormwater impacted by sewage ingress (Schreiber et al. 2019). A range of 2.3-3666 cysts/100L was reported in raw stormwater samples taken from a separated sewer system in Germany (Schreiber et al. 2019). Schreiber suggested Illegal connections between the storm and wastewater pipes and runoff containing pet or rodent waste were the possible sources of this contamination. Microbial source tracking was not included in this study and would be needed to calculate the contribution of pet waste versus that of human origin.

Relative to Giardia spp, Schreiber's study reported lower average concentrations of Cryptosporidium *spp* in stormwater in the range of 10.3-22.5 oocysts / 100L. Further 85% of samples were free of this pathogen. This makes sense as Cryptosporidium are less prevalent in domestic sewage and more closely associated with waters impacted by agricultural run-off carrying waste from livestock such as cattle (Betancourt 2019). Importantly, low concentrations, less than 10 oocysts per litre, may be enough to cause cryptosporidiosis (Betancourt 2019). The strain *C.Parvum* is known to be found in the small intestine of 152 different mammals, including birds and mice (Carey et al. 2004). These animals may inhabit WSUD technologies though minimal information exists linking reservoir species other than farm animals to cases of cryptosporidiosis in humans.

2.4.3 Viruses

Viruses may not be as environmentally robust as bacteria, given they require a host cell to reproduce. Whereas bacteria can multiple in the environment. However viruses can survive in aquatic environments, without a host, for long enough to cause infection (Rusiñol and Girones 2019). They are also the smallest group of microorganisms requiring treatment by WSUD technologies. Given these characteristics virus survival in WSUD technologies is likely to differ to FIB

Adenoviruses, Enteroviruses, Rotaviruses, Noroviruses, Polyomaviruses and Hepatovirus A, were the most commonly detected viral species in the studies reviewed within Table 1. Adenovirus was the most widely distributed, found in the stormwater of most major Australian cities (Lampard J et al. 2017; Sidhu et al. 2012) and stormwater impacted surface waters in California (Jiang and Chu 2004; Choi and Jiang 2005) , Singapore (Vergara et al. 2016) the Netherlands (Lodder and Roda Husman 2005), and in recreational surface waters throughout most of Europe (Wyn-Jones et al. 2011). The low infectious inoculum (i.e. the number of virions required to cause disease) of many viral microorganisms, together with their small size, makes them the group requiring the most attention in future WSUD research.

These viruses are known to be present in domestic wastewater (Boehm et al. 2018) meaning cross contamination between the stormwater and wastewater networks is probable in all of these locations (Ahmed et al. 2016). Enterovirus and rotavirus have shown seasonal fluctuation, whereas Adenoviruses appear less affected by season. Adenoviruses have shown longer survival times in the environment with less seasonal variations then the other RNA viruses commonly detected (Rusiñol and Girones 2019). This is one of the reasons several researchers suggest using them as a viral indicator for recreational waters (Wyn-Jones et al. 2011; Lin and Ganesh 2013). The prevalence of these viruses in 'separated' systems and in surface waters highlights the public health challenge facing most cities transitioning toward a sustainable model of urban water management.

2.5 Regulatory context

These varied microbial hazards emphasise the need to use reference pathogens for each microbial domain (bacteria, protozoa and viruses) to pre-characterise stormwater in WSUD planning stages. Table 2 shows the indicators and reference pathogens used in the Australian guidelines for water recycling (NSW OW 2015). Adopting reference pathogens is further challenged by differing abundance and survival of organisms within the same reference domains. For example, while Adenoviruses have been advocated as a potential viral indicator, studies in tropical regions have found Noroviruses to be the most prevalent enteric virus group

(Aw and Gin 2011). This reinforces that type and number of pathogens in stormwater are dependent on regional and site-specific factors.

Table 2 - Indicators and reference pathogens in AGWR

Microbial Hazard	**Reference pathogen**	**Surrogate/Indicator**
Protozoa	Cryptosporidium parvum	Clostridium perfringens (spores are used)
Viruses	Amalgam of rotavirus and adenovirus	Somatic coliphages F–RNA coliphages
Bacteria	Campylobacter	Escherichia coli Enterococci

***Source**: AGWR Section 3.2.1, Table 3.6, and Table 5.3 in (NSW OW 2015)*

In lieu of site-specific microbial water quality data, the NRMMC-EPHC-NHMRC (2009) guidelines provide Log Reduction Values (LRVs) that stormwater effluent must meet for a range of end uses with reference to the above pathogen groups. They are conservatively based on limited data and likely overestimate risk, which can deter the uptake of WSUD initiatives (Bichai and Ashbolt 2017).

More accurate health risk assessments can be made via the collection of site-specific data and the use of QMRA (Petterson et al. 2016; McBride et al. 2013). These steps are key features of the Water Safety Plan approach that has improved public health outcomes related to drinking water supplies, and from which Bichai and Ashbolt (2017) have transferred the principles to stormwater harvesting schemes. Their site-specific validation approach is a promising direction, suitable to be trialled for WSUD planning in Australia.

3.0 The WSUD approach

WSUD is an approach that integrates the management of the urban water cycle (potable supplies, stormwater, groundwater and wastewater) into the planning, design and construction of urban environments (Wong 2006; Lloyd et al. 2012). It is a framework utilised by disciplines spanning architectural design and planning, engineering, water quality and social sciences. The core principle of restoring pre-development flow regimes in urban environments guides strategic scale decisions through to site-specific technologies that prioritise storm, waste or grey water as valued resources (Lloyd et al. 2012). Conceiving stormwater as a resource for reuse and re-imagining urban waterways as recreational assets are natural steps in the progression of cities towards this sustainable model of urban water management.

3.1 WSUD technologies

A variety of types of physical infrastructure fall under the umbrella of WSUD technologies. Examples can be living systems, such as vegetated swales, stormwater biofilters, green roofs, green walls, constructed wetlands and riparian buffers (Hatt et al. 2006; Sharma et al. 2018). And non-living, such as rainwater tanks, permeable pavements, downpipe diversions (Sharma et al. 2018). These technologies share the common aim of intercepting stormwater at or near the source of runoff, allowing it to be retained for re-use, infiltrated into groundwater, or released back into the storm sewer network (Roy et al. 2008). The capture of stormwater by WSUD allows microbial loads to be reduced via passive treatment, but also via reduction of peak flows which cause sewers to overflow into stormwater (Talebi and Pitt 2019).

Performance data has been collated for the following WSUD technologies: stormwater biofilters (aka rain gardens and bioretention systems), constructed and floating wetlands, green walls and green roofs. The literature search included peer-reviewed journals, conference proceedings, technical reports and guidelines published between 2000-2020. The databases used for the search were: Web of Science, Scopus, Pubmed and Google Scholar. The following Boolean string was entered into the databases, *("green wall*" OR "green roof" OR "blue roof"), (wetland*), (biofilter* OR bioretention) AND (pathogen* OR "indicator bacteria" OR protoz* OR virus*) AND ("storm water" OR stormwater).* Figure 1 shows the results of the search categorised by type of WSUD technology.

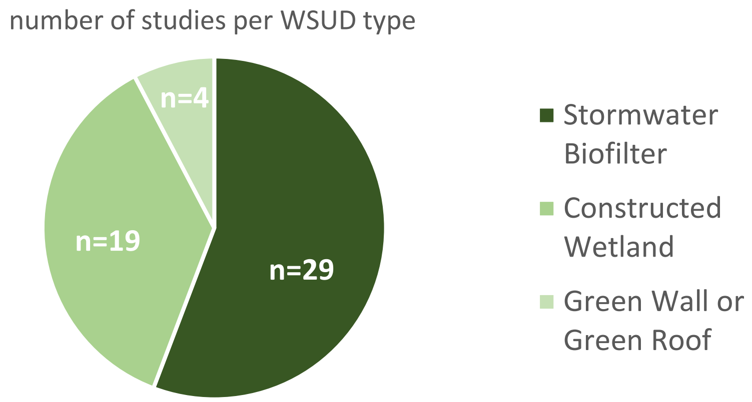


Figure 1 – Literature search results (52 studies in total, LRV data extracted from 33)

Publications concerning the design optimisation and performance of 'Stormwater biofilters' have been the most common over the past decade, and n=*29* studies have been included in this review. Followed by constructed wetlands n=*19.* Green roofs and Green walls n=4 have received very little research attention with regards to removal of microbial contaminants. These technologies share the common principle of biofiltration, that is, the process of filtering water through biologically enhanced filter media (Payne et al. 2019). The following descriptions of each WSUD technology have been adapted from those described in the experimental work within the reviewed literature:

Stormwater biofilter; rain garden; bio-retention system; A vertical infiltration system consisting of a vegetated basin overlaying a specified mix of porous filter media, including loam, sand, soil and mulch. Stormwater is collected in the surface basin and percolates through the filter media. The bottom of the system can be lined with an underdrain allowing subsequent conveyance or re-use. It may also be unlined for infiltration into underlying soils (Rippy 2015; Peng et al. 2016)

Constructed wetland (CW); An engineered system designed to mimic natural aquatic ecosystems. The most common sub-type of CW utilised for stormwater treatment is known as a Free Water Surface (FWS) wetland and is designed to retain its water level at or near ground surface level throughout the year (Headley and Tanner 2012; Kadlec and Wallace 2009). They consist of one or more basins generally divided into: an inlet zone for sediment settling, a densely planted macrophyte zone, and open water areas in-between. There are many variants of (CW's) in terms of how the media, flow and planting is arranged. For a full description of types and applications refer to Kadlec and Wallace 2009.

Floating wetland (FW); A variant of FWS wetland where the defining difference is that emergent macrophytes are not rooted in the wetland's sediment. Instead they are planted into a floating mat on the surface of the water, resulting in a dense network of plant roots suspended beneath the mat. The mat is typically an engineered substrate housed in a buoyant frame (Headley, T. R. & Tanner, C. C. 2006; Li et al. 2010)

Green roof; A layer of vegetation grown on building roof surfaces. Vegetation is planted into a designed mix of light-weight media which typically sits above a layer of geotextile filter material, drainage cells and a water poof membrane (IWC 2020). For systems designed with stormwater control or treatment in mind, a layer of cells for water storage are included beneath the filter media (Winward et al. 2008). Two types exist: intensive or extensive, best delineated by the total depth of growing medium on the rooftop. Extensive ≤ 150mm and Intensive ≥ 150mm.

Green wall; Vegetation grown in light weight filter media that is housed within a structure designed to be attached to a vertical building face (Prodanovic et al. 2017). The structure can be mounted internally or externally, and the type of structure varies, including: planter boxes, pre-vegetated panels, or geotextile blankets with pockets for filter media and plants (IWC 2020).

4.0 Indicator and Pathogen Log Reduction Values (LRV)

Microbial treatment performance is commonly reported as a reduction in the concentration of FIB or pathogens between influent and effluent samples. A log reduction value (LRV) is a measure of this difference. Of the 52 reviewed, LRVs were extracted from 33 studies. All 33 used *E.coli* and or Enterococci to describe removal performance. Of these 33, 8 also included viral surrogates, namely Somatic or F+ coliphages. The same 8 also included the protozoan surrogate *C.perfringens.* Only 6 monitored pathogens in combination with the above-mentioned indicators. A collection of these studies is provided in table 3.

Table 3 – Indicator and pathogen Log Reduction Values (LRVs) extracted from the literature

WSUD technology	**Location**	**Indicators and pathogens**	**Mean/median Influent**	**FIB LRV or % ***	**Pathogen LRV or %**	**Source**
stormwater biofilter	Melbourne, Australia	E.coli E. faecalis C. perfringens FRNA coliphages C.jejuni	1.70*10^4 (FIB)	3.0	n/a	(Jung et al. 2019)
stormwater biofilter	Melbourne, Australia	E.coli Campylobacter spp	6.27*10^4 - 2.02*10^5 (FIB) 10 - 30 MPN/L (C)	1.39, 1.23	0.98, 0.88	(Chandrasena et al. 2016)
stormwater biofilter	Melbourne, Australia	E.coli	2.0*10^4	1.7	n/a	(Chandrasena et al. 2014b)
stormwater biofilter	Melbourne, Australia	E.coli	4.97*10^3 *	1.15*	n/a	(Chandrasena et al. 2012)
stormwater biofilter	California, USA	E.coli enterococci FRNA coliphage	1.38*10^5 (EC) 2.90*10^4 (ENT) 1.80*10^2 (F+)	0.47 (EC) 0.41 (ENT 1.03 (F+) *	n/a	(Kranner et al. 2019)
stormwater biofilter	North Carolina, USA	E.coli Enterococci	1.30*10^2 (EC) 3.75*10^2 (ENT)	70&89% EC & ENT -119 & -102% EC & ENT	n/a	(Hathaway et al. 2011)
stormwater biofilter	Melbourne, Australia	E.coli C.perfringens FRNA coliphages	2.3*10^5 (EC) 5.2*10^3 (CP) 2.1*10^4 (F+)	1.6 (EC) 3.1 (CP) 4.32 (F+)	n/a	(Li et al. 2012)

stormwater biofilter	Melbourne, Australia	E.coli (Copper coated media subset)	1.37*10^4	3.44	n/a	(Li et al. 2014a)
stormwater biofilter	Melbourne, Australia	E.coli C.Perfringens Campylobacter Cryptosporidium Adenoviruses	2.8*10^4 (EC)	1.2 (EC) 2.1 (CP)	0.7 (C) 1.7 (CR) 1.0 (A)	(Chandrasena et al. 2017)
stormwater biofilter	Melbourne, Australia	E.coli	4.77*10^4 *	1.81, 0, 4.94, 0.88	n/a	(Shen et al. 2019)
Green wall	Melbourne, Australia	E.coli	1.5*10^3	60 - 99%	n/a	(Prodanovic et al. 2017)
Green wall	Melbourne, Australia	E.coli	2.17*10^4	82%*	n/a	(Prodanovic et al. 2020)
Green roof	London, United Kingdom	Total Coliforms E.coli Enterococci Clostridia Pseudomonas aeruginosa	2.51*10^5 (TC) 6.3*10^2 (EC) 5.01*10^2 (ENT) 1.0 *10^3 (C) 2.51*10^4 (PA)	3.7 & 1.5	3.9 (PA)	(Winward et al. 2008)
Natural' floating wetland	Nakivubo wetland, Uganda	total coliforms	n/a	2.0 & 1.0	n/a	(Kansiime and van Bruggen 2001)
Constructed wetland	North Carolina, USA	E.coli Enterococci	8.29*10^2 (EC) 9.42*10^2 (ENT)*	-105%EC 56% ENT*	n/a	(Hathaway and Hunt 2012)
Constructed wetland	Sydney, Australia	Enterococci C. Perfringens	6.1*10^3	85%	n/a	(Davies and Bavor 2000)
Constructed wetland	Melbourne, Australia	E.coli Campylobacter	2.6*10^3 (Base EC) 1.0*10^4 (event EC) 9.8*10^2 (C)	0.96 (EC)	0.05 (C)	(Meng et al. 2018)
Constructed wetland	Melbourne, Australia	E.coli Somatic coliphages Clostridium perfringens	10 - 8.7*10^4 (EC) 3 -6.3*10^3 (SC)	0.83 (EC) 0.45 (SC) 1.2 (CP)	n/a	(Petterson et al. 2016)

Abbreviations: EC = E.coli, ENT = Enterococci, C = Camplyobacter, CP = Clostridium perfringens, F+ = FRNA coliphage, SC= Somatic coliphage, PA = Pseudomonas aeruginosa, CR= Cryptosporidium spp, G= Giardia spp, A = adenoviruses
* mean or median calculated by author from available data

4.1 Stormwater biofilters

FIB removal in the literature is highly variable, with average removals reported from 2 log reduction (LRV = 2) through to net leaching (LRV < 0) (Rippy 2015). Removal rates were more pronounced within studies. For example, in North Carolina, Hathaway (2011) sampled field scale stormwater biofilters and observed mean

LRVs of -1.9 to 1. In a laboratory scale study in Melbourne Australia, Shen (2019) trialled the real time control of stormwater biofilters achieving median LRV's of 0 – 5.85. These living technologies will inherently deliver variable results (Payne et al. 2019). In addition, differences in design, operational characteristics, local climate (Bonilla et al. 2015)and construction installation quality makes comparing the 'same' technology difficult. An LRV of 1 (90% reduction) for FIB can generally be expected via treatment with stormwater biofilters (Deletic et al. 2014).

A subset of studies assessed removal of reference pathogens and or surrogates for viral and protozoan pathogens, namely: Campylobacter *n=3* studies (Chandrasena et al. 2017; Chandrasena et al. 2016; Jung et al. 2019) FRNA or somatic coliphage *n=4* (Kranner et al. 2019; Jung et al. 2019; Li et al. 2012; Petterson et al. 2016) Adenovirus *n=1* (Chandrasena et al. 2017)Cryptosporidium *n=1* (Chandrasena et al. 2017) and the protozoan surrogate indicator *C.perfringens* spores *n=3* (Petterson et al. 2016; Jung et al. 2019; Li et al. 2012) Campylobacter (bacteria) removal varied between LRV 0.05 – 0.7 and was consistently lower than the indicators for bacteria, *E.coli* and Enterococci. LRVs for the viral indicators (FRNA or somatic coliphages) ranged from 0 to 4.32. The average LRV for Adenovirus in the same study was 1. LRVs for the protozoan indicator (*C.perfringens)* ranged from 1 – 3.1 compared to the LRV for protozoan pathogen Cryptosporidium oocysts of 1.7. More studies monitoring reference pathogens are needed to make meaningful comparisons concerning organism removal in stormwater biofilters.

As illustrated above stormwater biofilters have shown capacity to remove reference pathogens at low levels. However, an insufficient volume of data exists to suggest an average range of removal. The study by Chandrasena et al (2017) is most notable due to it being one of the few to assess removal performance for both reference pathogens and indicators across all three microbial domains: Virus, bacteria and protozoa. The laboratory scale biofilters used in this study achieved removal rates per organism between LRV 0.7 and 2.1, refer table 2. Most notably, removal rates for indicators and pathogens were quite different, with opposite trends being observed between microorganisms in response to a variety of drying and wetting regimens (Chandrasena et al. 2017). This finding demonstrates in greater detail the observation made by many (Boehm et al. 2018; Carney et al. 2020; Ferguson et al. 2003; Petterson et al. 2016; Bichai and Ashbolt 2017)that FIB alone do not provide the nuance needed to accurately assess health risk.

It should be noted that most of the performance data concerning stormwater biofilters is generated from laboratory scale studies. This has been the appropriate scale for much of the pioneering work optimising design parameters. One of the most difficult field conditions to replicate in the laboratory is stormwater influent with 'natural' strains of target species. Most studies use a 'synthetic' or hybrid stormwater mix, with cultured bacterial strains. More field scale studies are needed to assess long term performance under a variety of challenging conditions (Bichai and Ashbolt 2017; Peng et al. 2016) A valuable growing resource for field based microbial data is the 'International Stormwater BMP database' of which the results are summarised in (Clary et al. 2008). The data extracted from Clary's study included influent and effluent concentrations from 14 field scale 'bioretention' systems and 4 wetlands. The International database also includes several operational green roof sites. Some effluent concentrations for E.coli have been monitored however no influent concentrations were recorded to derive LRVs. As more pilot studies and full-scale systems are implemented in the field, a global dataset of this nature becomes a valuable resource for future modelling and validation guidelines.

4.2 Constructed Wetlands

Much less data exists for the removal of FIB in constructed wetlands receiving stormwater. 13 studies were found in this literature search reporting FIB removal. Only two studies (Meng et al. 2018; Sidhu et al. 2010) monitored the removal of viral and protozoan reference pathogens in constructed wetlands. Sidhu et al (2010) piloted the operation of a constructed reedbed as a pre-treatment step within a managed aquifer recharge scheme designed to recycle stormwater for potable reuse in Adelaide. Stormwater was captured in the reedbed where it resided for an average of 10 days before discharge to groundwater. The residence time (in days) to achieve a one log reduction for the following microorganisms was reported as follows: E.coli(4) Salmonella typhimurium(5), E. faecalis(6), Cryptosporidium oocysts(33) and Adenovirus(87). The reedbed was one component of this system which in isolation achieved an LRV of 2 (99%) for FIB and a LRV of 0.1-0.3 for Cryptosporidium oocysts.

One of the challenges to interpreting influent and effluent bacterial loads in constructed wetlands is the potential input of faecal matter directly into the water body from local wildlife. Meng et al (2018) observed between 55 – 208 waterfowl on each of their visits to sample a constructed wetland receiving stormwater from a peri-urban area in Melbourne. The wetland achieved an average LRV for the indicator *E.coli* of 0.96. The corresponding reference pathogen Campylobacter achieved a much lower removal, with a median LRV of 0.05. Further, several of the sampled events showed a net increase in Campylobacter between the influent and effluent. Based on previously reported concentrations of *E.coli* and Campylobacter *spp* in waterfowl faeces, Meng et al (2018) calculated sufficient numbers of waterfowl were present to contribute to the Campylobacter *spp* and E.coli loads in the outflow samples. This is important as previous health risk assessments have shown knowledge of contamination origin, i.e. human or animal, has allowed health risks to be adjusted accordingly and innovative schemes to go ahead (Roser et al. 2006)

Another challenge is understanding the spatial distribution of pathogenic microorganisms within these systems. Davies (2003) studied concentrations of indicator bacteria Enterococci and *C.perfringens* in both the water column and sediment of two stormwater constructed wetlands and two retention ponds in Sydney. Outflow concentrations for these indicators were consistently lower than inflow in the wetlands, with a mean LRV of slightly below 1 (85% reduction). Notably they found the bacterial loads in the sediment to be much higher than in the water column. For example, concentration of *C.perfringens* spores in the wetland sediment were consistently 3-4 orders of magnitude above those in the water column. This shows that spatial patterns of indicator bacteria likely vary throughout wetlands given the different conditions for microorganisms to thrive or die.

The wetlands outperformed the ponds and were suggested to be the better approach when attempting to intercept stormwater and reduce microbial loads discharging into recreational water environments (Davies and Bavor 2000) Conversely, Hathaway and Hunt (2012)found treatment wetlands in California to have a lower removal performance during the warmer swimming season months compared to the non-swimming season in USA and that 'wet ponds' (retention ponds) achieved higher removal rates than wetlands (2012) study.

No performance data was found regarding the use of floating treatment wetlands (FTW) for the removal of indicator bacteria or reference pathogens from urban stormwater. However their potential to reduce pathogen loads in degraded urban waters is promoted by those who apply these systems commercially (SPEL 2020; Harris Environmental 2019). It is feasible to assume they will offer a similar level of removal as other vegetative systems. Tanner and Headley (2012) briefly discuss FTW's potential to buffer microbial contamination based on performance data from naturally occurring floating wetlands. Kansiimme and van Bruggen (2001) reported LRVs of 1 and 2 by naturally occurring FTWs within a larger wetland system in Uganda that receives mixed storm and wastewater discharge. FTWs are appealing due to their modular design, flexible arrangement and ability, however more research is needed concerning their capacity to buffer microbial contamination and the optimal sizing and arrangements for this performance aspect.

4.3 Green walls and green roofs

No performance data was found for the removal of indicator organisms or pathogens from urban stormwater or 'raw' rainwater by green roofs or green walls. However, a small number of green wall and green roof studies (Winward et al. 2008; Chowdhury and Abaya 2018; Prodanovic et al. 2019) have investigated the treatment of greywater. These have been included in Table 2 as benchmarks for future studies focused on stormwater harvesting. The study by Winward et al profiles a commercial system called GROW. Greywater is pumped into a series of planted troughs lined on a rooftop. The troughs are filled with expanded clay media and 'gravel chippings'. The system was tested with a 'low' pollutant load and a 'higher' pollutant load greywater influent. The system achieved up to a LRV of 3.7 (total coliforms) under the low strength testing and a LRV of 1.5 for the high strength. Grey water typically has a lower viral load then wastewater but is generally higher then stormwater (Arden and Ma 2018). The key difference that will challenge green roofs treating stormwater is the intermittent flow. A blend of grey and stormwater water streams can be explored in future investigations to overcome this.

Part of the interest in optimising green walls and green roofs for stormwater treatment is they do not require the same amount of public space as other WSUD technologies. Although the challenge with appropriating building facades and roofs are limitations on the total weight and depth of the green wall or green roof. Deeper profiles have been linked to better FIB removal in stormwater biofilters (Hathaway et al. 2011; Zhang et al. 2010). Regardless lightweight filter media is key. Prodanovic's (2017)study trialled a range of light weight filter media to find which performed best for a wide range of pollutants. E.coli was the only microorganism included in the study. Removal of between 60 – 100% was observed. Reliable performance for pollutant removal was linked to a steady flow of greywater influent. Notably expanded clay media, the same utilised by the GROW system was trialled and considered not suitable due to high nutrient leaching.

4.4 LRV Summary

The LRVs show that WSUD technologies can effectively remove FIB with a small amount of evidence to suggest pathogen removal also occurs. Despite the reported variability, an LRV of 1 (90% removal) for FIB and reference pathogens should be expected. Table 3 compares removal rates and ranges for FIB between the WSUD technologies included in this report. The mean *E.coli*, Enterococci or Total Coliform removal rates, for individual treatment systems, were extracted from the literature to calculate the total means shown in table 3.

Table 3 – Treatment performance (LRVs) by WSUD type

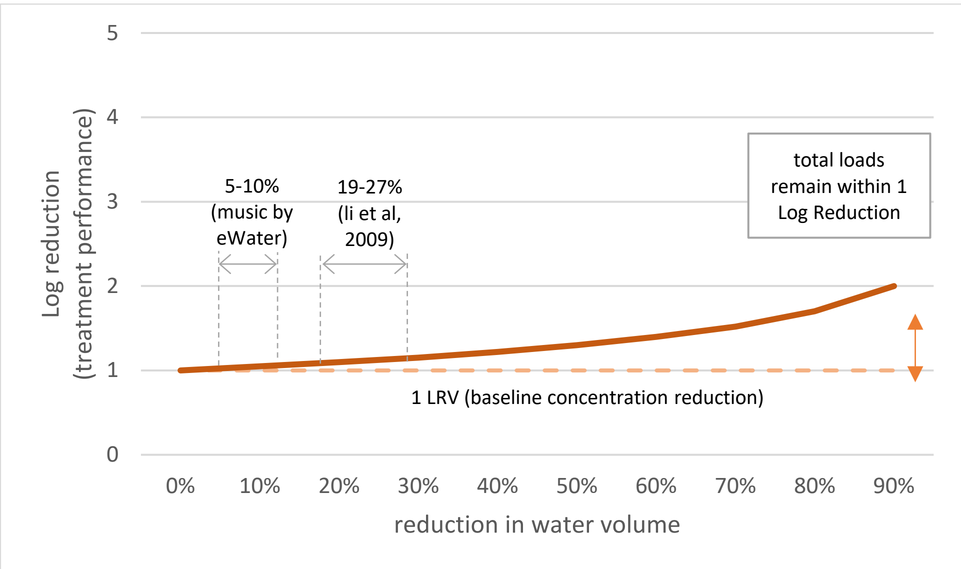
WSUD Technology	# Studies	Removal range		Mean LRV	Mean % removal
		low	High		
Stormwater biofilters	18	-0.4	4.9	0.9	87%
Constructed wetlands	13	-0.7	2.0	0.4	63%
Green roofs and green walls	4	0.0	3.7	*	*

*insufficient no of studies to derive significant mean

Virus and protozoa removal will be the limiting factor for any of these treatment processes (NRMMC-EPHC-NHMRC 2009). This means, more research is needed monitoring removal of viral and protozoan organisms by WSUD technologies in both the field and the lab. To understand how often 'treated' water meets current guidelines, Shen et al (2019) and Meng et al (Meng et al. 2018) compared individual sampling events to guideline requirements for stormwater end use scenarios (NRMMC-EPHC-NHMRC 2009) and recreational water quality requirements (NHMRC 2008). Shen et al found 28% of samples met guideline values (NHMRC 2008) for secondary contact recreation. A further 5.4% met dual reticulation requirements (NRMMC-EPHC-NHMRC 2009) for indoor and outdoor use and irrigation of commercial food crops. Meng et al (2018) observed fewer instances where effluent samples met guideline requirements. For example LRVs for *E.coli* were below guideline requirements for municipal and irrigation of non-food crops <25%, while campylobacter concentrations exceeded threshold concentration values for all 'end-use' scenarios.

These effluent concentrations are generally reported independent of any reduction in water volume. As such, slightly higher removal rates can be expected when hydrological performance (i.e. volume reduction) is taken into account (Hathaway and Hunt 2012). Figure 2 is a conceptual chart showing additional reductions in bacterial loads that may be achieved when reductions in outflow volume, such as evapotranspiration and infiltration, are considered. A mass water balance model is needed to calculate real values. Regardless, the WSUD approach needs to combine vegetative technologies with other storage treatment devices and buffers to peak flow, to reduce FIB loads entering the Parramatta River, toward recreational guideline values.

Figure 2 - baseline concentration (LRV of 1) combined with volumetric reduction



* this same relationship can be observed with different baseline concentrations

5.0 Processes governing microbial removal

The WSUD technologies discussed in this review are likely to share similar processes governing the removal and survival of microbial hazards from stormwater. The following section, therefore, groups WSUD technologies together under four process headings: operational characteristics, design specifications, biological processes and physio-chemical processes.

5.1 Operational / environmental conditions

Stormwater influent characteristics, rainfall, antecedent dry periods, temperature (air, water & soil), local wildlife, age of WSUD technology and maintenance have all previously been described as operating or environmental conditions that will influence the transport and fate of pathogens in biofiltration systems (Peng et al. 2016; Meng et al. 2018; Chandrasena et al. 2014a). Storm events are the most well-known factor to influence removal performance. Jung et al (2019) showed that as event size increased removal rates decreased. Larger events are associated with increased pollutant inflows, which have also been used as a predictor of removal performance, particularly in CW's (Arden and Ma 2018). Lastly, storm events may resuspend sediment from the bed of CW's which has been shown to contain higher concentrations of FIB in comparison to the water column (Davies and Bavor 2000).

Prolonged periods of dry weather between rain events have proven one of the most challenging conditions for stormwater biofilters (Deletic et al. 2014). Fissures can develop right through the profile of the biofilter due to excessive drying, which has been shown to increase infiltration rates and reduce FIB removal due to inflow water having reduced contact time with filter media (Li et al. 2012; Rippy 2015) More recent research has found the impacts of dry weather to be organism specific. For example, in an Australian laboratory study simulating dry weather, removal rates for Adenovirus remained steady whereas the indicator for viruses (FRNA coliphages) increased in numbers, (Chandrasena et al. 2017). Actually, Jung found a certain amount of 'drying' out may be beneficial (Jung et al. 2019). Deliberate, intermittent drying has shown benefit in other studies of slow sand filtration devices used as point source filters of rainwater (Verma et al. 2017). The use of smart valves, gates and sensors, may allow better control of this drying/wetting aspect that troubles passive biofiltration systems. Future studies exploring the real time control of these operational conditions may see more reliable removal rates.

Temperature is known to be a key stressor to pathogen survival in the environment (Aw 2019), therefore microclimate and time of year will likely influence removal performance. Hathaway (2012) grouped effluent samples from several field scale wetlands and stormwater biofilters into swimming and non-swimming seasons. Treatment efficiency was reduced during the warmer swimming season months. Notably influent concentrations were also higher in the warmer months. These assumptions are based on the behaviour of FIB alone. Monitoring studies of 'raw' stormwater have shown some species to be seasonal only in certain climatic zones (McLellan et al. 2015) and others to be unaffected by season (Siddiqee et al. 2019; Wyn-Jones et al. 2011). Process monitoring is needed for a range of species in distinct climatic zones at different times of the year to be able to correlate removal performance with ambient seasonal conditions. In addition simple decisions can be made re situating devices in the public domain where microclimate may increase solar exposure.

5.2 Design specifications

One of the key design considerations intended to combat the adverse effects of dry weather on stormwater biofilter performance, is the inclusion of a saturated zone (SZ). Saturated zones are created in biofilters, via raising the level of the outlet, on the pipe that underlies the system. This creates a saturated layer at the base of the biofilter that holds moisture for longer periods in between rain events (Rippy 2015). Rippy et al (2015) found that configurations with SZ's consistently achieve higher removal rates than those without SZ's in terms of FIB removal. This has been partly attributed to a 'dilution' or 'old water' effect, where water from previous storm events remaining in the system mixes with 'new' inflow water diluting the overall pollutant load (Kranner et al. 2019; Jung et al. 2019). A 300mm deep SZ has been shown to be sufficient to achieve the desired retention of moisture between rain events (Chandrasena et al. 2014b). Some US studies have reported no enhancement of FIB removal with the inclusion of a SZ, however they did observe improved removal of other pollutants (Nabiul Afrooz and Boehm 2017). This multi-pollutant performance potential seems to warrant the inclusion of a SZ as a key design feature.

To overcome some of the operational limitations to biofilter performance, investigations into the real time control of these systems has emerged. Kerkoz and Mullapudi et al (2016; Mullapudi et al. 2018) retrofitted field scale constructed wetlands and retention ponds with a series of 'smart' valves, gates and pumps to inlet and outlet pipes in order to more actively control flow. The valves and gates are triggered by water level sensors and meteorological forecasts and water level sensors. Shen et al (2019) trialled the first study of this kind in Australia, piloting the optimisation of stormwater biofilters fitted with similar equipment. The key aim was to negate the poor removal rates observed during short dry periods (<12hrs) and prolonged dry periods (≥14 days) by regulating flows to maximise hydraulic retention time and overall moisture retention between events. The results of this study were mixed however this was primarily attributed to experimentation with the 'operational flow settings' as opposed to the variability intrinsic to most 'living' WSUD technologies.

The role of vegetation is uncertain with several studies reporting minimal difference in mean LRVs between vegetated and unvegetated controls (Nabiul Afrooz and Boehm 2017; Chandrasena et al. 2017) The presence of vegetation has been more clearly linked to moisture retention in the filter media and this is thought to improve FIB and pathogen removal (Li et al. 2014b; Chandrasena et al. 2014b). Further the root structures of different plants are known to alter and maintain hydraulic conductivity and this directly influences FIB removal (Le Coustumer et al. 2012; Li et al. 2012; Chandrasena et al. 2019).

A recent investigation re-evaluated 'best practice' plant species with the aim of optimising pathogen removal. Leaf extracts from plant species within the Myrtaceae family were found to have the highest antimicrobial potential (Galbraith et al. 2019). *Melaleuca fulgens*, *Callistemon viminalis*, and *Leptospermum lanigerum* all demonstrated superior pathogen reduction potential compared to the current 'best practice' species *carex appressa.* Combinations of these higher performing plant species is recommended versus monoculture planting (Galbraith et al. 2019). In the only study of microbial removal by floating wetlands Kansiime and van Bruggen 2001 found that *Cyperus papyrus* growing on naturally occurring floating mats outperformed *Miscanthidium violaceum* species due to greater biofilm surface area provided by the Papryus roots.

Filter media type and depth impacts removal rates. The Cooperative Research Centre for Water Sensitive Cities (CRC WSC) adoption guidelines for stormwater biofiltration systems (Payne et al. 2015) recommend <5% organic matter to be used in the media profile of stormwater biofilters, to avoid nutrient leaching. Green walls and green roofs typically exceed this with higher nutrient loads and use of fertilisers. Prodanovic et al. 2017 tested several lightweight filter media types, assessing removal rates for a range of pollutants, including *E.coli.* 'Perlite' and 'coco coir' media achieved the best removal rates taken into account all pollutants, including *E.coli.* In stormwater biofilters, copper coated zeolite showed improved removal (LRV up to 3.44). However, it may not yet be commercially available, or it may be cost prohibitive to local councils. Lucas et al (2019) trialled alternative filter media combinations to the sand mixes specified in the CRC WSC guidelines. They found no extra leaching in mixes containing 65% recycled palm fronds though did not assess removal for microbial contaminants. Room for experimentation of other recycled materials seems to exist for this design aspect.

Deeper profiles have been linked to better FIB removal in stormwater biofilters (Hathaway et al. 2011; Zhang et al. 2010). Hathaway (2011) compared two field scale bioretention systems in North Carolina and concluded that the difference in profile depth between the two systems was a major reason for differences in removal rates. The deeper of the two (0.6m) versus (0.25m) was found to have superior hydrologic function and this is the main reason removal rates for FIB were higher than the shallow system. Studies investigating the reduction in peak flows by WSUD technologies have shown systems ≥ 0.9m in depth to have superior hydrological performance, as they promote higher evapotranspiration and infiltration (Li Houng et al. 2009). Therefore, it is recommended that this depth be used to optimise both microbial treatment and hydrologic function.

5.3 Biological processes

Optimal design specifications and construction quality are pre-requisites to allow the following physical and biological removal processes to take place. Rippy (2015) posits that the evolution of the microbiome inside biological filtration systems sets the foundation for subsequent removal of FIB. However, it is the area least quantified and understood in the literature (Peng et al. 2016). Research across a wider range of biological filtration systems used for greywater, wastewater and stormwater suggest predation, adsorption to biofilm, natural die-off and microbial competition to be key removal mechanisms (Arden and Ma 2018; Verma et al. 2017)

Maximizing surface area for biofilm growth via filter media and plant selection is a common aim for most natural treatment technologies. Though the actual role of the biofilm (the thin extracellular matrix that sticks to plant roots and filter media) is not well understood. Some suggest it may even inhibit removal performance (Nabiul Afrooz and Boehm 2017) or harbour pathogens. Others suggest it provides a surface for competition, predation and adsorption (Rippy 2015; Peng et al. 2016; Prodanovic et al. 2017) Proponents of floating wetlands particularly emphasise the role of the biofilm, as in lieu of filter media, plant roots coated in biofilm are suspended in the water column (Li et al. 2010) and thought to provide a key removal mechanism. Novel modifications of floating wetlands include ‘curtains’ (Li et al. 2010)that hang beneath the floating structure, to increase the surface area for biofilm to grow and increased biological activity to take place.

The inclusion of a saturated zone to maintain moisture can also facilitate the process of Predation. Predation by protozoan micro-fauna(2-50um), nematodes (30um – 1mm) and microzooplankton is thought to be one of the most significant processes for reducing numbers of pathogenic microorganisms in constructed wetlands and stormwater biofilters (Davies and Bavor 2000; Morató et al. 2014). An individual protozoan organisms can graze on between 5-73 bacteria per hour, though variation in species cell wall thickness will make some organisms more susceptible to grazing then others (Rippy 2015). Li et al (2012)suggests maintaining a higher soil moisture content through the inclusion of a saturated zone may prolong survival of some protozoan microorganisms that will graze on viral and bacterial pathogens. Further, operational conditions such as soil temperature have been linked to prosperity of protozoan grazers and subsequent removal of FIB (Chandrasena et al. 2014a).

Selecting favourable microclimatic conditions to locate WSUD technologies may also facilitate removal processes. Natural decay and inactivation of pathogens occurs in response to sunlight, pH, temperature, salinity, nutrient availability and moisture content (Byappanahalli et al. 2012; Aw 2019; Carey et al. 2004) Survival also depends on where in the biofilter pathogens are distributed, with the top layer believed to provide the most hostile environment to FIB due to sunlight and other trapped toxicants (Chandrasena et al. 2014a). Given the diverse array of microorganisms in stormwater it is possible that the some microclimatic conditions may be inhospitable for one species and promote growth for another (McLellan et al. 2015).

Specification of low nutrient filter media mixes also creates hostile conditions for FIB survival. Whilst several studies have shown FIB can survive in soils and sediment for up to 30 days (Hathaway et al. 2011) some strains of *E.coli* are unable to adapt to low nutrient soils (Zhang et al. 2010). Specification of low nutrient filter media mixes therefore seems appropriate despite the challenges this presents to plant establishment.

5.4 Physio – chemical processes

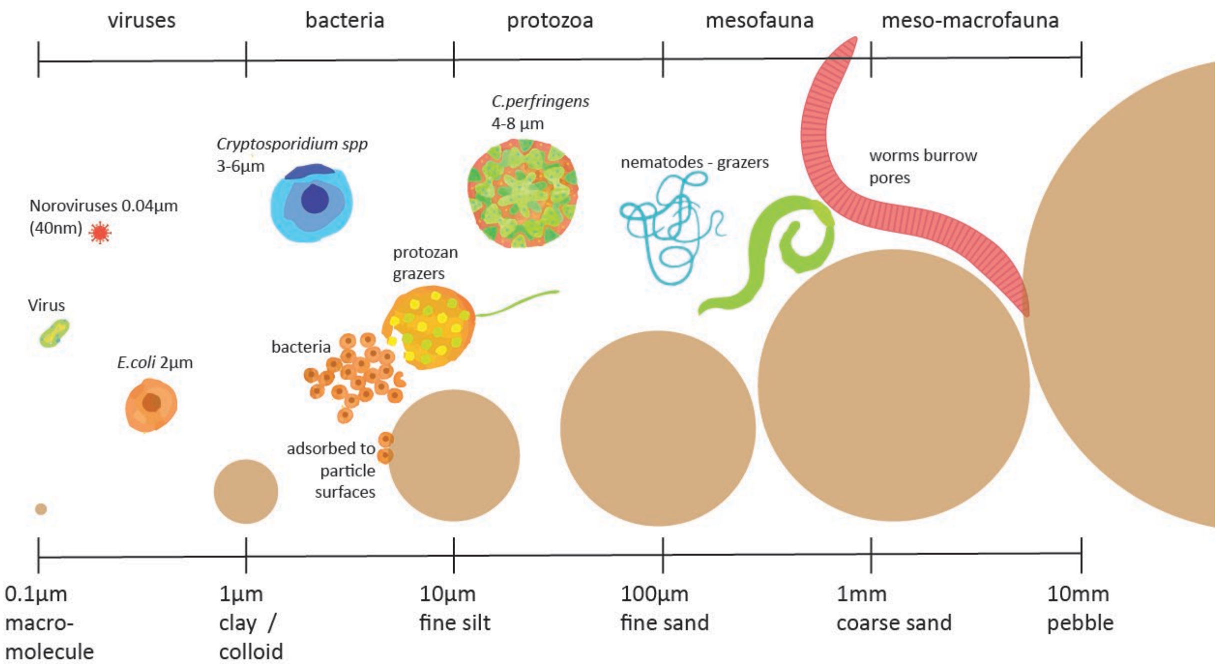
Due to variations in morphology of bacteria, protozoa and viruses (Fig 3) the dominant removal processes will vary between species (Peng et al. 2016). In general organisms with larger particle size are more likely to be removed by physical and chemical processes (Rippy 2015). For example protozoan spores, cysts or oocysts range in size from 1um to 60um (USEPA), meaning certain organisms will be trapped in surface pores or wedged in narrower pores as they transit through the filter media (Rippy 2015). This physical straining is a likely removal process in systems with porous media such as stormwater biofilters (Peng et al. 2016; Kranner et al. 2019), green walls (Fowdar et al. 2017; Prodanovic et al. 2017) and green roofs. Chandrasena reported higher removal rates for Cryptosporidium oocysts (3-6 micrometres (μm) and C.*perfringens* (4-8 μm) then E.coli (2 μm) and Adenovirus (0.90 μm) in stormwater biofilters and attributed this to physical capture of the larger organisms.

Bacteria (2 – 3µm) and viruses (< 1µm) are able to pass through filter media pores and may instead be removed via adsorption to media surfaces, plant roots, biofilm and sediment (Schillinger and Gannon 1985). Davies and Bavor (2000) observed bacterial reductions from the water column in vegetated wetlands, add attributed this to bacteria adsorption to fine, medium and coarse particles settling in the wetland. In contrast, viruses have shown poor adsorption to settling particles in wetlands designed for wastewater treatment (Arden and Ma 2018). Regardless designing wetlands to maximise retention time will best facilitate this removal process.

The fact viruses may not adsorb in the same way as bacteria may be preferable for eventual removal for two reasons. Firstly, particle bound microorganisms have shown a greater resistance to predation (Schillinger and Gannon 1985)and secondly, they may eventually reside and survive in the sediment bed and banks, posing a subsequent health risk (Siddiqee et al. 2019; Davies and Bavor 2000). There is a need then to understand the microbial profile in relation to the sediment profile when characterising raw stormwater for WSUD planning.

Importantly, remobilisation of these trapped or previously adsorbed pathogens is possible (Chandrasena et al. 2014a)which may account for some studies that have observed higher effluent loads then influent following storm events. Some of these remobilised organisms may not be infectious, however several of the molecular methods used in the published literature do not differentiate between living and dead genetic material. Therefore monitoring methods that differentiate between activated and inactivated microorganisms need to be used when assess health risk (Chandrasena et al. 2017).

Figure 3 Filter media particles and microorganisms. (Not to scale)



(note: information re particle sizes adapted from Peng at al (2016) Rippy et al (2015))

6.0 Conclusion

Treatment performance

The LRVs extracted from the literature reflect the capacity for a range of WSUD technologies to reduce microbial contamination in urban stormwater. A 90% reduction in FIB is achievable by these types of biofiltration technologies. Most of this performance data was taken from investigations of stormwater biofilters (aka bioretention; rain gardens) with substantially less available for constructed wetlands and minimal for green walls and green roofs. Concerning pathogens, only a handful of studies from the categories combined, have monitored pathogen removal. These few studies and the larger suite of stormwater monitoring studies demonstrate the variation in removal rates between pathogens and FIB.

Design optimisation

Stormwater biofilters have received the most attention in terms of design optimisation. Further, the most reliable removal rates have been achieved by stormwater biofilters. The strongest evidence for design criteria that promotes FIB and pathogen removal includes deeper media profiles (≥0.9m), low nutrient filter media, and the inclusion of a saturated zone (SZ). The combination of these increase moisture retention and total contact time between contaminated water and biologically enhanced filter media. Given each technology included in this review share the common principles of biofiltration, it is likely similar removal rates could be achieved by all. However, more attention toward CW, GW and GR design is needed to achieve removal rates like those reported in stormwater biofilter (bioretention) literature.

Validating biofiltration technologies

The narrative from sustained researchers in this field has transitioned, logically, from design optimisation to methods for validation (Zhang et al. 2015). Reliable microbial performance is a critical component from a public health perspective, which has not been included in previous stormwater biofilter validation frameworks (Payne et al. 2019). Bichai and Ashbolt's stormwater use management framework (SUMP) (2017) takes the principles from Water Safety Plans (WSP) used to manage drinking water catchments and applies these to stormwater harvesting schemes. Quantitative Microbial Risk Assessment (QMRA) features as a core tool in this process framework. This framework provides a useful direction for WSUD planning at local and catchment scales.

Catchment scale improvements and the future of swimming in the river

Microbial performance has mostly been assessed at the scale of individual units, or laboratory mesocosms. As individual treatment barriers, the effluent from WSUD technologies is usually above guideline thresholds for primary contact recreation. Some have investigated treatment performance at different times of the year, for example, suggesting treatment capacity may vary between swimming season and non-swimming months (Hathaway and Hunt 2012). Emerging empirical investigations, at the catchment scale, include the application of real time control to WSUD, see Kerkez et al (2016) and Mullapudi et al (2018). This type of design optimisation may provide scope to further reduce microbial contamination, in targeted locations, and at specific times of the year.

There is a need to investigate the combination and distribution of WSUD technologies at the catchment scale. This is difficult as Kuller et al (2017) identified the ad-hoc planning and realisation of WSUD that has occurred at the catchment scale. Because of this, modelling is one way forward. Sydney Waters' (2018) study simulated several WSUD scenarios, though the model did not account for the treatment performance potential by individual WSUD technologies, such as those discussed here. This review collates available data on this performance aspect. As councils increasingly pilot the types of WSUD technologies discussed in this report, additional LRV data can be acquired from these field scale systems to support future modelling studies.

7.0 References

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