Managed Aquifer Recharge and Stormwater Use Options: Investigation of stormwater impact on water quality and distribution infrastructure

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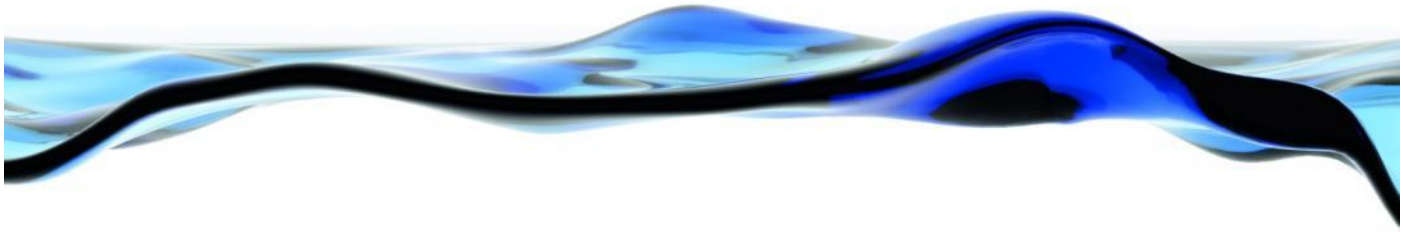

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Executive Summary

Objectives

This report focuses on the assessment of potential impacts of stormwater in trunk mains and reticulation pipes. Stormwater recovered from managed aquifer recharge (MAR) schemes in Australia is typically supplied for non-potable end-uses, such as outdoor irrigation, either as is or blended with recycled water. Future end use may potentially include potable water supply.

Whilst there has been extensive research on the distribution of mains and recycled water, the knowledge of stormwater and its impact on water distribution infrastructure is limited in the scientific literature, particularly for stormwater that has been treated through MAR. Therefore an experimental investigation was designed to evaluate the impact of stormwater on materials adopted in the water distribution network. The investigation aimed to determine:

(a) The risk that the water source may cause water quality issues, impact aesthetics or pathogen survival; and
(b) Interactions between the water source and the distribution network or household infrastructure that could impact the water quality received by the customer at the tap.

To answer such questions materials used in water distribution, copper, poly(vinyl chloride) and cement lining; were aged in buried pipe loops supplied with two distinct water sources, stormwater and baseline water. The assessment evaluated the impact of untreated stormwater as a worst case scenario, and explored parameters that apply to both non-potable and potable infrastructure. This report outlines the investigation and the findings from the research.

Experimental conditions

The stormwater was sourced from the Parafield stormwater harvesting and managed aquifer recharge scheme operated by the City of Salisbury, South Australia. It had been treated through a horizontal-flow constructed wetland and stored for variable periods in a confined carbonate aquifer and supplied to the pipe loop rig. The baseline water was sourced from a nearby drinking water distribution main which at this location had a negligible disinfectant residual <0.05 mg/L (i.e. below the Australian Drinking Water Guideline (ADWG) of 0.2mg/L for residual chlorine).

Each pipe loop was 48m in length, consisting of 36m PVC DN100 pipe, 6m DICL DN 100 pipe and a material swatch exposure section made of DN 150 PVC pipe. Water entered a holding tank for aeration and was recirculated at a constant pressure of 2 bar (200 kPa) and a constant flow rate of 140-145 L/min for a period of 16h per day and a quiescent period of 8h. Water was bled from the return line at a rate of approximately 1 L/min over the 16h run triggering inflow of new water at the same rate. No disinfection was applied to the water sources.

Environmental conditions in the rigs ranged between 14 to 36 °C (median 22.35 °C), pH 7.6 to 8.7 and 2 to 9.6mg/L dissolved oxygen during the test period. The temperature range exceeded 20°C, the typical maximum temperature in buried water distribution mains, but was still within the range reported at customer taps (maximum 37°C).

Material coupons were exposed to stormwater and 'baseline water' in parallel rigs for up to 10 months in 2012-2013. Coupons and water were sampled regularly and analysed for biofouling, chemical precipitation, changes in water quality and pathogens. In addition, sections of HDPE pipe (field pipe) that had been in service in an actual stormwater distribution network were also analysed for comparison.

Biofilms

Biofilms can impact water quality, aesthetics and harbour pathogens. Biofilms were observed in all three materials. The total microbial cell counts (a measure of living and inactive microrganisms) on

coupons were in the order of 10^5 to 10^7 cells/cm^2 and varied over the duration of the study when measured by flow cytometry. For copper and PVC, the cell counts were higher for coupons exposed to the stormwater than to baseline water, for all except two sampling times. But cement had no clear difference in the cell numbers between the baseline water- or stormwater-exposed coupons. The total cell counts in the field stormwater pipes were in the range of 10^6 cells/cm^2. However, when quantifying bacterial genes using quantitative polymerase chain reaction, cement lined iron and PVC coupons in the stormwater had higher 16S rRNA gene copy numbers as compared to copper coupons exposed to stormwater or to baseline water.

The culturable cell counts (i.e. measure of living microorganisms) at 22°C were in the order of 10^1 to 10^6 cells/cm^2, culturable cell counts at 37 °C were 10^1 to 10^5 cells/cm^2 and thermotolerant coliform cell counts at 37/45°C were 10^1 to 10^3 cells/cm^2 . None of the coupon materials stood out as having consistently higher or lower culturable cell numbers than the other materials. Neither was there a consistent difference in culturable counts at 22°C or 37°C between coupons exposed to baseline water and stormwater. However, the number of thermotolerant coliforms was larger in the stormwater system than in baseline water system.

Differences were observed in the bacterial and eukaryotic communities formed on the rig coupons made of various materials and exposed to baseline water and stormwater. Moreover, seasonal variations were observed in both bacterial and eukaryotic community composition and diversity.

A number of bacterial families and genera harbouring potential human pathogens were detected in both baseline water and stormwater systems, with larger numbers of genera observed in the latter indicating a potentially increased risk of exposure to pathogens with stormwater. The stormwater system also harboured sulfate and sulfur reducers which may cause pipe deterioration and odour problems, and a greater diversity of iron oxidisers which may contribute to iron deposits and discoloured water events. A number of nitrifying bacteria were observed in both baseline water and stormwater systems. A number of eukaryotic taxa containing bacterial grazers (amoebae and nematodes) were detected in both baseline water and stormwater, indicating that the biofilm communities are dynamic and the abundance of bacteria is able to support higher level eukaryotes. Thus, as expected, disinfection and operation control would be required for stormwater to control microbial development and manage associated potential health and water quality impacts.

Pathogens

The data indicates that the system supports amoebae genera which are pathogenic or contribute to bacterial pathogenisis. *Salmonella enterica*, *Campylobacter* spp. *Cryptosporidium parvum*, *Clostridium perfringens* and *Clostridium difficile* were not detected in the stormwater or baseline water pipe rig biofilm samples. Human adenovirus was detected in one copper coupon biofilm sample from the baseline water pipe rig in low numbers (on the threshold of detection limit).

Presence of *Legionella*, nontuberculous mycobacteria (NTM), *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in both baseline and stormwater exposed biofilms indicate a potential for regrowth and contamination of the water distribution systems. This further supports maintenance of a residual biocide in the piped water to reduce the risk of contamination of water supply from pathogens in dislodged biofilms.

Nutrients

Both water sources can support bacterial activity with biodegradable dissolved organic content (BDOC) and phosphorus concentrations. BDOC in baseline water rig had a median of 0.75mg/L and ranged from 0.3 mg/L to 1.3 mg/L, contributing 9-30% of the total organic carbon (TOC). In the stormwater rig, the BDOC median was 0.65mg/L, but the range was wider, <0.2 mg/L to 3.6 mg/L, contributing 9-48% of TOC. The respective maxima were equivalent to only 25% and 69% of typical maxima for the Little Para mains water supply. Passage of water through the rig caused increase in

Total Organic Carbon (TOC), but not of BDOC. The concentration of total nitrogen was 45% lower in stormwater than in baseline water.

Importantly, the potential risk for biofilm development and water aesthetics incidents appears to be closely linked to the source water quality. Residence time in the aquifer is one of the factors that influences water quality, and short residence time in the aquifer could restrict the attenuation of nutrients (DOC and TP) and produce a higher particulate content than baseline water, which could contribute to biofilm formation, water discoloration and lead to sub-saturation of carbonate minerals which increases the water reactivity towards cement.

Metals

The baseline water quality in the rig was compared to that of the Little Para mains water and was verified to have higher total iron, copper and turbidity readings, with mean values of 0.16±0.21 mg /L Fe, 0.006±0.003mg/L Mn and 2.80±4.11 NTU. The stormwater had mean and 95th percentile concentrations of 1.05±1.66 mg/L Fe and 3.65 mg/L Fe, which exceeded the 0.3mg/L Fe aesthetic Australian Drinking Water Guideline aesthetic (ADWG) value. The 95th percentile for baseline water, 0.49 mg/L Fe, also exceeded the ADWG. The mean concentrations of manganese for baseline and stormwater were below the aesthetic ADWG value (0.1mg/L Mn), being respectively 0.004±0.003 mg/L Mn and 0.049±0.053 mg/L Mn; whilst the respective 95th percentiles were 0.010 mg/L Mn and 0.151 mg/L Mn.

Thus removal of excess iron from the aquifer treated stormwater is recommended to prevent water discolouration. Aeration pre-treatment had been previously recommended (Page *et al* 2013). Under oxic conditions, Fe(2) was converted to Fe(3) which is generally insoluble. This reduced the soluble iron concentration but also caused precipitation of insoluble iron (oxy)hydroxide or oxides within the reticulation system, which was verified. Chlorination would also increase the redox state of the solution. Water within the baseline rig maintained super-saturation with respect to iron minerals. This was generally greater than the average Little Para treated drinking water quality, as the water entering the rig was elevated in iron concentrations and hence the likelihood for iron(3) precipitates to form is increased due to the reticulation network itself.

Sediments

Deposition of iron oxides on coupons was verified over time and was more severe in the stormwater rig, particularly on cement and PVC coupons. In contrast, aluminium and manganese precipitates were not detected during the trial.

All three materials (PVC, cement and copper) were subject to surface changes. Cement showed the largest variability for total cell counts with exposure; and despite PVC's lower surface roughness compared to cement, it was susceptible to sediment deposition and attachment with prolonged exposure. Copper coupons, which have biocidal properties, exhibited the least sediment attachment, but showed signs of oxidation resulting in surface discoloration in both baseline and stormwater. Therefore, it was concluded that sediment attachment was closely associated with biofilm development and the microbial community characteristics. However, a coupon aged for 29 weeks in stormwater when introduced to the baseline water experienced dissolution of the sediment layer, indicating its ease of removal with a change in water source.

An increase in the concentration of dissolved copper occurred in the baseline water rig and was attributed to the water supply line from the Greenfield site to the experimental rig. The stormwater line adopted the same material, but no copper increase was observed, which indicated less copper corrosion in stormwater compared to baseline water.

Operating conditions in the rig were aerobic during the trial period, however severe sediment accumulation or long residence times (stagnant water), could lead in principle to the development of localised anoxic pockets that could favour anoxic or anaerobic bacteria development (e.g. sulphate

reducing bacteria) and could impact water aesthetics and cement corrosion. In addition, sediment could also shield microorganisms from disinfectant residuals in bulk water and allow biofilm development.

Colour

The stormwater rig displayed more variability in aesthetic properties than the baseline water rig, particularly for colour (range 2 - 94 HU, median 12 HU), compared to baseline water (range $\leq$1 – 3 HU, median 1HU). There were indications of slough off of biofilm from the surfaces of both rigs as verified in the increase in metal (Al, Cu, Fe,Mn, Ni, Zn) and turbidity on 1/10/2013, but these did not increase water colour. Iron was the main metal to pose a risk to aesthetics guidelines, however, despite the formation of insoluble iron and its deposition in the stormwater rig internal surfaces, the analysis indicated a greater influence of dissolved organic carbon in the source water on colour, with exccedance of the ADWG 15HU colour guideline associated with dates when the stormwater supply was highly coloured.

Implications for water distribution operation

The experimental conditions in the rigs differed in certain aspects from those in water supply distribution. In particular, buried water mains would be subject to less temperature fluctuation due to seasonal climate variability. Velocities in water distribution would also reach higher values, but are more transient depending on households' demand, which would lead to variable velocity profiles and impact resuspension of sediment and biofilm growth and slough-off. Disinfection of stormwater would also reduce microbial diversity and delay biofilm formation.

To summarise, the research examined a worst case scenario of undisinfected water sources within a buried distribution network subject to variability in seasonal water supply and network temperature. The iron concentration and the microbial diversity in stormwater were the major parameters that could cause water quality and public health risks in the distribution system. Iron oxidation products contributed to water discoloration and to sediment deposition on pipe surfaces, including PVC and cement. Copper was less prone to sedimentation. Biofilm formation and slough-off was also verified in both distribution rigs, however it was observed that colour was influenced not only by iron sediment, but by the BDOC in the source water. Pathogens identified in the stormwater rig were similar to those detected in the baseline rig. Biofilms counts in the two water sources were similar, however T.coliforms numbers were higher in stormwater. There were differences in microbial population in the two water sources and a wider microbial diversity was verified in stormwater (including sulfate and sulfur reducers, iron and nitrifying bacteria) which can evolve dynamically if conditions within the water distribution system permit.

In this experiment the stormwater source was a blend of stormwater recovered from ASTR and ASR wells. The aquifer treatment has been shown to produce lower median nutrient levels compared to baseline water in this, and also in other studies (Page et al 2013), provided residence time in the aquifer is managed. The experimental observations further indicate that the aquifer operation can directly influence the water quality, including colour and nutrients, in distribution infrastructure.

The type of end-uses for stormwater, either potable or non-potable, will be the main determinant of the level of treatment adopted, with more rigour required for potable end uses. In addition, the mode of supply for potable supplies will also influence the treatment steps. For example if stormwater was injected directly into a distribution network this would require stricter monitoring and control of water quality variability to ensure consistency in water quality in the network than if stormwater were pumped into a reservoir and blended with other supplies.

Overall, to prevent health risks and aesthetics impacts on the distribution network the recommendations are:

- Monitoring for verification and control of the MAR treated stormwater supply nutrient concentrations prior to supply. Proxy parameters can also be used for verification such as pH and EC;
- Oxidation and filtration of the stormwater for iron removal prior to injection into water distribution systems, to remove excess iron, which could react with residual chlorine, lead to sediment formation and contribute to water discoloration. This can be achieved via aeration or alternatively via chlorination or similar methods;
- Followed by disinfection and the maintenance of a disinfection residual in the network for biofilm and pathogen management.

Such maintenance requirements are in principle similar to those required for current mains water systems, which was expected.

Recommendations for further research

Suggestions for further improvement and additional verification would include:

- Experimental rig: Improved insulation of the pit lid to achieve a narrower temperature range in the rig to better represent buried pipe and limit microbial development ;
- Examination of pre-treatment techniques for stormwater iron removal and disinfection with pre-selected methods, followed by verification of the treated stormwater effects in the experimental network to examine biofilm and microorganism survival (and comparison to the results from this report); and
- Challenge testing a stormwater rig with an established biofilm and with disinfectant residual to examine the potential risk for pathogen survival and re-contamination.
- In addition, if the stormwater was intended for drinking purposes, the monitoring of disinfection by-products following disinfection, e.g. THMs, would also be recommended.
- The rig is sited so that it could be used to evaluate the effects of recycled water and recycled water blended with stormwater in water distribution systems to better identify disinfection requirements for pipes conveying waters with higher nutrient compositions for non-potable supplies.

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Acknowledgements

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1. Introduction

The Managed Aquifer Recharge and Urban Stormwater Use Options (MARSUO) project is a follow-up from the Aquifer Storage Transfer and Recovery (ASTR) project, to determine whether urban stormwater could be stored in aquifers and recovered to produce high quality water suitable for drinking water supply (October 2005-April 2009) (Page *et al*., 2009). The ASTR project showed significant promise for a wider range of uses of urban stormwater, but indicated that further research was required to address some aspects. In summary, the ASTR project found no immediate fatal flaws in water quality when comparing treated stormwater quality with the Australian Drinking Water Guidelines (NHMRC-NRMMC 2011). Results indicated that only simple low-cost treatments would be needed for iron removal, UV disinfection and chlorination in addition to the passive treatment provided during both wetland and aquifer storage. However, the ASTR project focused only on product stormwater quality from a wetland harvesting system over a three year period and did not explore the potential hazards in the stormwater catchment, appropriate for identifying the required preventive measures, including procedures by which stormwater is harvested, stored and treated before entering public water supplies, and the costs and operational requirements of these to make risks acceptable and consistent with current drinking water supplies. Among the research needs identified, knowledge on the potential impacts of stormwater harvesting on existing water and sanitation infrastructure is needed before the government can assess the merits of introducing stormwater into the drinking water system.

Activity 4, 'Infrastructure and water quality' of the MARSUO project, focuses on the assessment of potential impacts of stormwater in trunk mains and reticulation pipes. Hypothetically, the adoption of multiple sources and the distribution of such sources through a single distribution network could occur. Whilst there has been extensive research on the distribution of traditional and recycled water, the knowledge of stormwater and their impact on the materials found in distribution systems, including the potential for biofouling and chemical precipitation has received limited attention. Data on potential changes in aesthetics and odour of alternative supplies in relation to conventional supplies is also scarce.

1.1 Aims

The infrastructure and water quality assessment aims to answer the following research questions:

(c) What is the risk that the water source (stormwater treated by wetland and aquifer storage) may cause water quality issues – i.e. impact colour, pathogen survival; the associated operation and maintenance requirements for the distribution system or the life of assets; and

(d) What interactions between the water source and the distribution network or household infrastructure may impact the water quality received by the customer at the tap?

In order to answer those questions, an experimental investigation was conducted. The Parafield stormwater harvesting, managed aquifer recharge and reuse scheme operated by the City of Salisbury was used to examine the effect of stormwater on various pipe materials found in water distribution systems and to evaluate the potential for biofouling and chemical precipitation for treated stormwater using material swatches. The Parafield scheme supplies stormwater for non-potable end uses, however the assessment outlined in this report will examine parameters that are relevant to both potable and non-potable end uses. The analysis adopts a worst case scenario in evaluating the impacts in the absence of disinfection. This report outlines the experimental investigation and the findings from the research.

1.2 Assessment framework

The framework adopted for the investigation is summarised in Figure 1. It consisted of 3 stages:

A. Pre-assessment

   (a) Review of the literature on water, stormwater and ASR/ASTR water quality and biofilm formation in water distribution infrastructure.

   (b) Review of water supply pipe materials adopted in South Australia and the operation conditions of the distribution system (Section 2).

   (c) Consultation with key project stakeholders (SA Water, United Water/Allwater and Salisbury Council) and the project technical reference committee. This task aimed to document experiences with sediment precipitation and any other water quality issues which may occur in pipes which distribute groundwater supplies from limestone aquifers (e.g. in Eyre Peninsula and South East) and in mains water supply in metropolitan Adelaide (Section 2). In addition, the reference panel was consulted to define the scope of the project: water parameters, materials, water sources of interest and operating conditions.

B. Risk analysis

   (a) Review of the historical water quality data from the Parafield stormwater system for determination of potential risks to water aesthetics and biofilm growth and comparison with Adelaide mains water (Section 2).

   (b) Modelling of geochemical equilibrium for evaluation of the risk of precipitation and water aesthetic changes (e.g. iron, colour, hardness) of selected water sources (Section 2).

C. Experimental assessment

   (a) Design of an experimental rig for exposure of pipe materials to selected water sources; and

   (b) Monitoring program for periodic sampling of water sources and pipe materials for biofilm development, the evaluation of infrastructure interactions and impacts on water aesthetics and health risks (Sections 3 and 4).

   The assessment objectives were:

   (a) To examine predictions on precipitation, corrosion and biofilm growth;
   (b) To assess baseline and selected alternative supply water on quality, including health and selected aesthetic parameters. This also included the sampling of a pipeline used for non-potable stormwater from Parafield ASR/ASTR supplies, and in-situ evaluation of potential stormwater impacts on mains infrastructure materials using an experimental rig;
   (c) To determine the character of biofilm and microbial communities within drinking water mains and pipes conveying stormwater supplies ;
   (d) To determine the presence or absence of pathogens within the biofilm; and
   (e) To determine any additional water quality constraints imposed by water infrastructure protection over those required for human health protection.

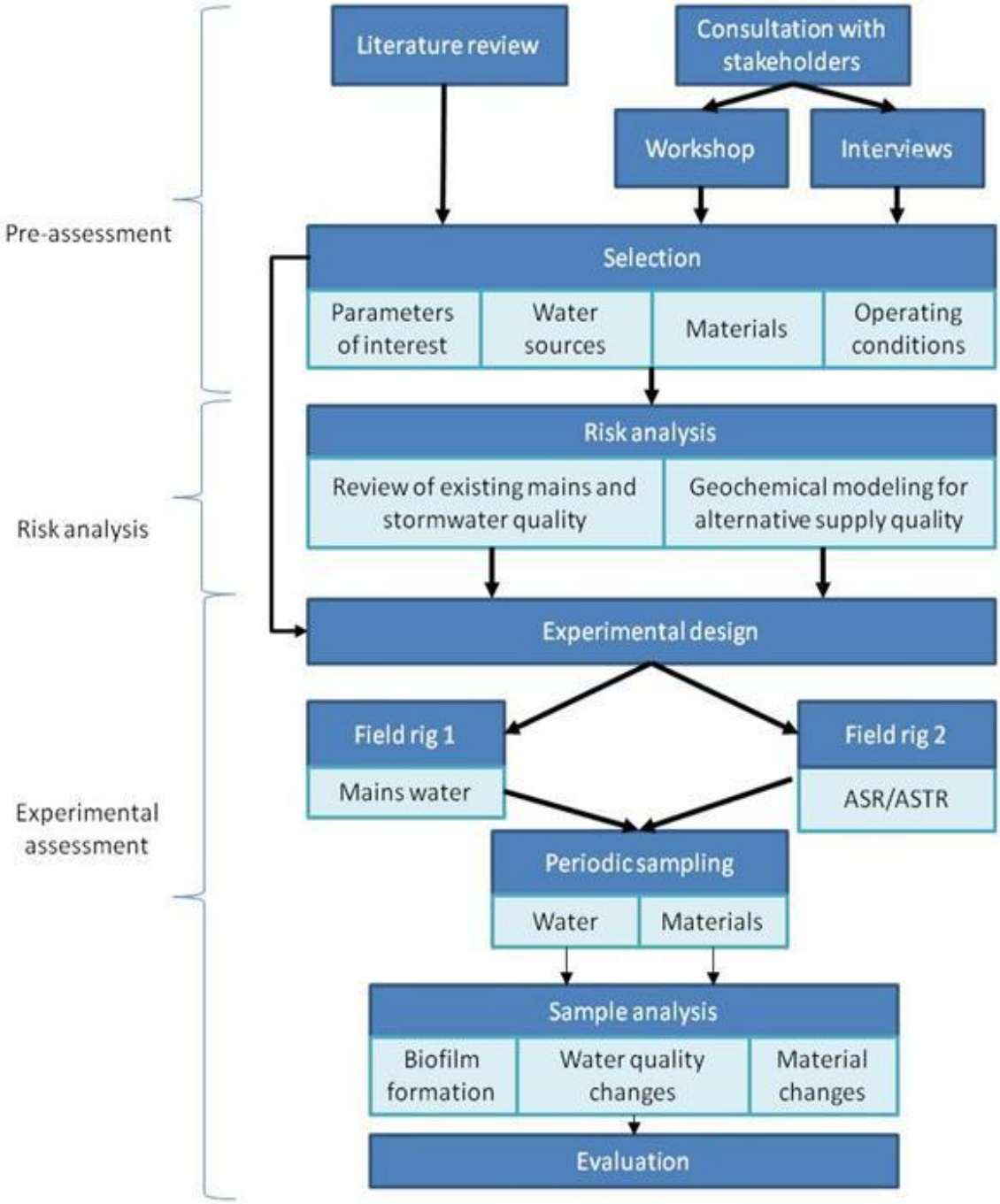


Figure 1. Framework for evaluation of stormwater impact on water distribution infrastructure.

2 Literature review

The water supply in the greater Adelaide region has steadily been diversified to a range of water sources: reservoir supplies, River Murray water, desalinated water, stormwater reuse, managed aquifers, rainwater and recycled water, to increase the future security of water supply.

The alternative water sources currently adopted are distributed though third pipe networks dedicated to supply of water from a single source or introduced into reservoirs and blended before distribution via the water supply network. Examples of the distribution models currently in use include:

- Localised dedicated distribution system for supply of treated stormwater for non-potable uses, e.g. Salisbury City Council distribution network for treated stormwater from Parafield and a network of other harvesting sites;
- Localised third pipe distribution for supply of blended recycled water and managed aquifer recharge (MAR) water, such as in Mawson Lakes;
- On-site distribution systems with sections of pipe infrastructure that alternate supply of rainwater or mains water to non-potable end uses. These are typically built with a backflow prevention valve to deter contamination of the mains water distribution network; and
- Blended desalinated water and reservoir water at Happy Valley treatment plant, distributed using the centralised water supply network.

However, in the future the adoption of a common distribution network to transport treated water from various origins or blended water from various sources could become more widespread.

In order to understand the impacts of operating distribution systems under such scenarios, it is important to understand the potential risks and impacts that alternative water supplies or changes in water conditions could have on infrastructure materials and the stability of their operation.

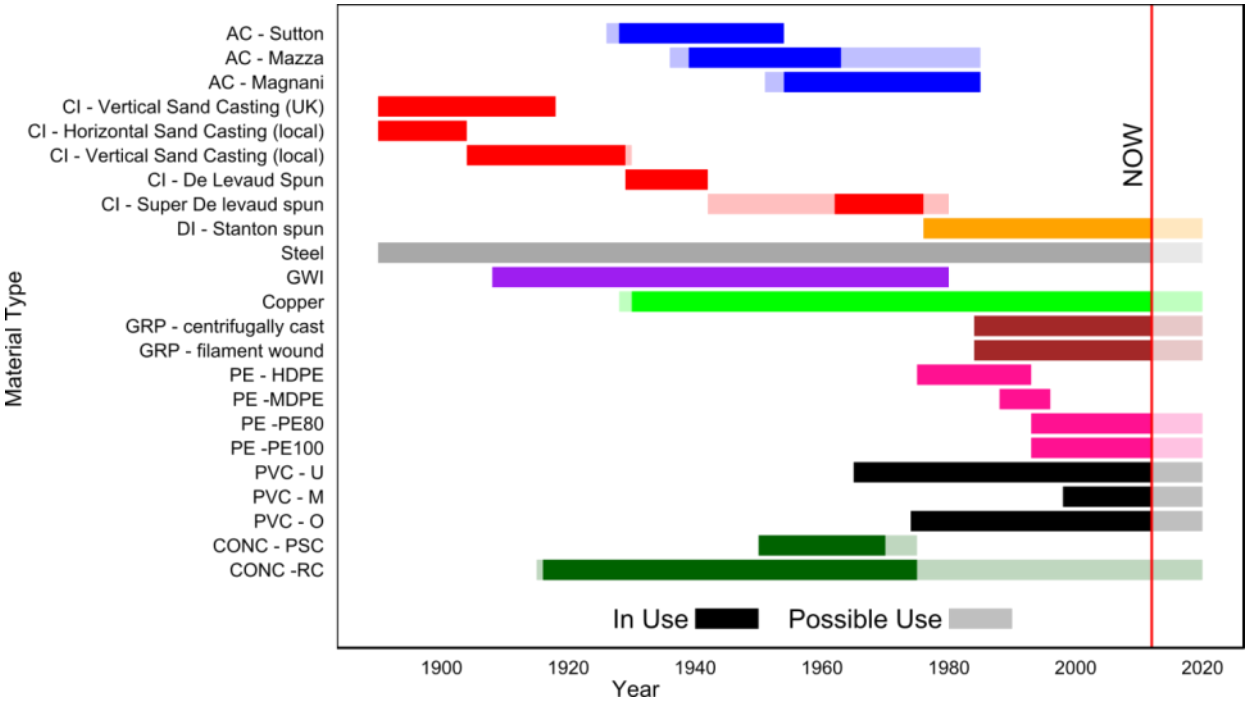
A preliminary search of scientific literature on stormwater and water quality in distribution interactions revealed an absence of publications on the topic. Whilst an extensive number of publications are available on stormwater, these refer mostly to the quality and quantity of run-off. Therefore the literature review has focused mainly on the interactions between water supplies and infrastructure materials in mains water distribution reported in the international literature.

Therefore this section explores:

(a) The global status of knowledge on water supply infrastructure and interactions associated with water quality;
(b) The characteristics of the distribution infrastructure in the greater Adelaide area, including the materials adopted and their potential reactivity to water quality; and
(c) The typical operating conditions adopted for water distribution in greater Adelaide.

2.1 Water quality interactions with infrastructure

A wide variety of pipe materials are adopted in the potable water distribution infrastructure. The pipes also differ in formulation and manufacture methods and hence individual characteristics depend on material type, manufacturer and the period of manufacture. For example, Figure 3 shows the typology of pipe assets adopted across Australia, with the increasing prevalence of new assets manufactured from ductile iron (DI) with internal linings, steel and polymeric materials in more recent years.



Legend: asbestos cement (AC), cast iron (CI), ductile iron (DI), glass reinforced pipe (GRP), glavanised wrought iron (GWI), high density (HD)/low density (LD) polyethylene (PE), poly (vinyl chloride) unplasticised (U)/ modified (M)/ oriented (O), concrete (CONC), reinforced concrete (RC).

Figure 2. Chronology of materials used in pressure pipes in Australia (Gould *et al.* 2012).

A summary of the major interactions between pipe materials and water quality in potable water is shown in Table 1.

Table 1. Pipe materials and interaction with water quality.

Material	Parameters	Reaction	Secondary impact
Copper	Bicarbonate, pH, redox, chloride, sulphate, DO, NOM and ammonia.	Microbial corrosion and pitting corrosion. Sediment release increases with pH.	Release of corrosion by-products.
Lead	DO, ammonia, sulphate, pH	Oxidation of Pb from solder or brass fittings.	Release of corrosion by-products.

Copper-zinc-brass alloys	Chloride, hardness, alkalinity and pH	Dezincification	Release of corrosion by-products.
Cement mortar lining and asbestos cement	Hardness, alkalinity and pH	Leaching of $Ca(OH)_2$ and Al.	Release of corrosion by-products. Changes in pH.
Iron	pH, alkalinity, DO, chlorine, inorganic ions	Corrosion	Release of corrosion by-products.

2.1.1 Metallic pipes

Corrosion of metallic pipes is caused by a range of mechanisms characterised by oxidation of metallic surfaces and the release of corrosion products or generation of tubercles. This is influenced by pH, alkalinity, water hardness, oxidant levels, dissolved organic carbon and sulphate, which act as electron donors and acceptors for sulphate reducing microorganisms (Yang *et al* 2014), and the concentration of inorganic ions, such as phosphate and orthophosphates, which promote the development of a protective layer halting the corrosion process (Benjamin *et al.* 1996). Consequences from corrosion may include the increase in particulate release, water discoloration, changes in organoleptic properties and other water quality characteristics.

2.1.1.1 Copper

Copper pipes are adopted in most households for the supply of water to the taps in the kitchen, bathroom, toilet, or laundry. Their popularity is largely due to corrosion resistance, strength, reliability, ease of use and durability. The corrosion resistance is attributed to the formation of a layer of CuO at the interface of the pipe, however environmental conditions can result in the breakdown of this layer leading to corrosion. Typical corrosion mechanisms include biocorrosion, which occurs in waters with low chlorination residual, and chemical corrosion, instigated by particular water quality characteristics, e.g. low pH and high carbonate content (O'Halloran *et al.* 2001). For copper pipes, the presence of contaminants or particulate material due to installation, the distribution system or corrosion coupled with stagnant or low velocity flows can create conditions for the formation of galvanic cells and lead to corrosion of the pipes, however perforation of the pipe due to corrosion generally takes 15 years or more to occur (Fernandes 1998).

Low pH has been shown to increase copper corrosion and by-product release (Boulay and Edwards 2001).Broo *et al.* (1998) has shown that high pH and low alkalinity decrease uniform corrosion of copper, as do high concentrations of inorganic species, such as chloride and calcium. The rate of copper corrosion and by-product release can also be reduced by decreasing natural organic matter concentrations in water (Broo *et al.* 1998, Boulay and Edwards 2001).

Chlorine has been shown to both increase and reduce the corrosion and release of copper in water supply systems depending on pH. Boulay and Edwards (2001) verified that 0.7 mg Cl_2/L had no significant impact on corrosion at pH 7, but increased copper release at pH 9.5. Other researchers verified that basic pH 9.3 and 2 mg Cl_2/L resulted in a lower corrosion rate compared to non-chlorinated (Edwards and Ferguson 1993, Edwards *et al.* 1999). Whilst for acidic pH (<7) and concentrations of less than 10 mg Cl_2/L, corrosion rates were reportedly higher (Atlas *et al.* 1982, Hong and MacCauley 1998, Stone *et al.* 1987, Singh and Mavinic 1991 in Boulay and Edwards 2001).

Copper is present in uncontaminated surface waters at very low concentrations, usually less than 0.01 mg/L. In drinking-water copper concentrations are on average 0.05 mg/L, but they can range

from ≤0.005 to 0.8 mg/litre at the customer tap, with the primary source most often being the corrosion of interior copper plumbing (NHMRC and NRMMC 2011).

2.1.1.2 Iron and steel

Steel and iron in pipelines can undergo localised or general corrosion. General corrosion is caused by the proliferation of short-lived cathodic and anodic sites. Whilst localised corrosion is caused by localised non-uniformities in the pipe or by water quality adjacent to such defects, resulting in processes such as tuberculation (formation of mounds of corrosion products) and pit corrosion (Benjamin *et al.* 1996).

The corrosion of iron pipelines increases the roughness of the pipe resulting in head loss and the formation of by-products that, if suspended in water, can increase the turbidity and colour in the water. The increase in roughness facilitates the attachment of biofilm to the pipe wall, in addition the biofilm can shield bacteria from the chlorine dissolved in the water, reducing its effectiveness as a disinfection agent (Frias, Ribas et al. 2001). Likewise, a higher concentration of particulate matter, onto which bacteria can attach itself, has a similar shielding effect, reducing the effectiveness of the chlorine and increasing micro-organism survival (Gauthier 1999).

Frateur *et al.* (1999) verified no significant changes in the rate of corrosion for chlorine levels between 0 and 2mg/L, despite faster chlorine consumption. Corrosion occurs at a faster rate in water with high dissolved oxygen levels and at higher temperatures (e.g. in Summer). However, the release of iron (Fe^{2+}) particles to water is favoured by anaerobic conditions (Sarin 2001) and can also be mediated by bacterial communities (Herrara and Videla 2009, Yang *et al* 2014).

2.1.1.3 Lead

Lead pipes are found in early water supply distribution systems in Europe (Davidson *et al* 2004), but are uncommon in Australia (Gould *et al* 2012). Lead corrosion results in the formation of scales of lead carbonate ($PbCO_3$) or basic lead carbonate ($Pb_3(CO_3)_2(OH)_2$), lead oxide and lead phosphate ($Pb_9(PO_4)_6$) (the latest in orthophosphate treated water) (Davidson *et al* 2004). The ADWG sets a maximum health guideline concentration of 0.01mg Pb/L for mains water (NHMRC and NRMMC 2011).

2.1.2 Cement materials

Asbestos and cement lined pipes spun in factory or *in situ* can be subject to corrosion of the cement layer due to calcium hydroxide leaching, carbonation (CO_2 attack) and sulphate attack, i.e. swelling caused by reaction of sulphate ions and tricalcium aluminate (Muster and Davis 2011). Calcium hydroxide leaching occurs if the [Ca^{2+}] in water is lower than the equilibrium concentration at surface. Thus soft waters, characterised by low carbonate and bicarbonate content, and low alkalinity are aggressive to cementitious materials, whilst hard waters, characterised by high alkalinity and calcium concentration, have a protective effect. A range of factors impact the rate of corrosion including water quality, retention time, cement type, surface to volume ratio, buffer capacity of water, method of lining and age of lining.

Internal pipe corrosion occurs in soft and acidic water when calcium hydroxide (free lime) is leached out into the water from the cement matrix. Initially calcium hydroxide is removed as free lime, however when free lime is exhausted hydrated calcium silicates decompose to calcium hydroxide which is then leached out of the pipe. Schock *et al*. (1981) also indicated that calcium, iron, manganese and silica provide protection against corrosion by precipitating onto the inner surface of the pipe and inhibiting the dissolution of both calcium hydroxide and release of asbestos fibres.

Fujita *et al* (2014) conducted elemental analysis of suspended solids in water distribution networks (>20years old) in Hitachi city, Japan, and verified the prevalence of calcium in the suspended solids for water distribution networks with mortar lining, i.e the breakdown of mortar lining contributed to the suspended solids in aged water distribution systems (>40 years old). However, the original water supply characteristics were not described. The potential for microbially mediated corrosion has also been verified, where microbial adhesion and biofilm can result in localised anaerobic micro-environment that that favour the leaching of free lime and calcium silciates (Wang *et al* 2011).

Research into the effects of inhaled and ingested asbestos fibres on animals was carried for many years and showed no links to cases of gastrointestinal cancer (Olson, 1974, Milette *et al.* 1980); nor has the ingestion of asbestos fibres been shown to be harmful to the digestive tract (Buzio *et al.,* 2000, Milette *et al.* 1981, Toft *et al.* 1984).

2.1.3 Polymeric materials

Migration of additives from PVC and PE pipes into water occur during service as excess additives and by-products are removed from the pipe surface. The type and concentration of additives leached from a pipe depend on the individual pipe matrix, the additives present and the water exposure conditions. For instance, uPVC pipe formulations in Australia do not adopt organotin or lead stabilisers used in overseas formulations (Davis *et al.* 2007, Whelton and Nguyen 2013).

Studies on migration of additives are conducted using mostly stagnant water tests, followed by the identification of selected chemicals or indirect parameters, such as total organic carbon (TOC) or total chlorine reduction, as a proxy measure for all combined organic compounds in water (Zhang *et al* 2014). Majority of studies to date examined unplasticised PVC (uPVC), high density polyethylene (HDPE), low-density polyethylene (LDPE) and crosslinked polyethylene (PEX) varieties (Whelton and Nguyen 2013). Among polymeric pipes, uPVC pipes are the most widely characterised regarding formulation and water interactions in laboratory and actual networks (Burn *et al* 2005). Less is known about the formulations and additives from newer polymer formulations, such as mPVC, oPVC, fPVC, bimodal PE pipes, or FRP-epoxy (Whelton and Nguyen 2013).

Studies on migration of inorganic metal stabilisers from unplasticized polyvinylchloride (U-PVC) and organics additives from PE pipes indicate that the concentrations of known compounds leached are not considered health hazards (Burn *et al*. 2005, Davis *et al.* 2007, Whelton and Nguyen 2013).

Leaching is the most intense in the initial 24h-48h of operation and decreases with time to trace levels (Burn *et al.* 2005, Whelton and Nguyen 2013, Zhang *et al* 2014). The leaching of inorganic stabilisers from uPVC occurs at higher rates under conditions of low pH, high total dissolved solids (TDS), high temperature and in presence of UV (Al-Malack 2001), turbulent flow and has been well characterised (Burn *et al.* 2005).

Leachates from PE pipe include a diverse range of organic compounds (lubricants, antioxidants, stabilisers and degradation by-products). A limited range of those compounds have been studied, with concentrations detected in contact water and biofilm often in μg/L, however not all studies have focused on the impact of those compounds on water quality (Whelton and Nguyen 2013).

Table 2 provides a summary of recent references on the water quality. However, comparison between studies and the application of the data to the selection of materials for actual networks is difficult due to the limited number of studies, the different pipe formulations and manufacturers, and often the variable and incomplete reporting of tests conditions (temperature, pH, water quality and the surface-to-volume ratio) in the literature. Furthermore, water quality and exposure characteristics adopted in migration studies typically differ from those found in actual distribution networks.

Table 2. Studies on total organic carbon (TOC) changes in migration tests for PVC and PE pipes.

Study	Pipe (origin)	TOC (mg/L)	TOC migration (m^2. d)	Test conditions reported
Forslund (1991)	PVC (Denmark)	$\leq 0.1 * 10^{-3}$	n.d[#]	Distilled water at 23°C for 3x 24h.
	LDPE, HDPE and PEX (Denmark)	<0.1	n.d	Distilled water at 23°C (LDPE, HDPE) or 60°C (PEX only).
Khiari *et al* (2002) (in Whelton and Nguyen 2013)	PVC (USA)	no change	n.d	n.d
Skjevrak *et al* (2003) (in Whelton and Nguyen 2013)	PVC (Norway)	no change	n.d	n.d.
Durand (2005) Durand and Dietrich (2007) (in Whelton and Nguyen 2013)	HDPE and PEX (USA)	0.5-2.5	n.d	Water with and without residual chlorine at 23•C, exposure duration not disclosed.
Koch (2007)	PVC (Germany)		<0.1	Demineralised water 23°C and 60°C after 9 days with or without 1mg/L free chlorine
Zhang et al (2014)	PVC (China) PE (China)	0.01-0.02 0.03-0.08	0.1-0.3 0.03-0.08	Synthetic water (pH 8, 100mg/L hardness and 2mg/L total chlorine) for 7 X 24h per batch) at 25±5°C

[#] Not determined (n.d).

2.1.3.1 Effect of organic stabilisers on TOC

Migration tests of uPVC pipe reported no change in TOC concentrations (Khiari *et al* 2002, Skjevrak *et al* 2003); or a small increase in TOC (Forslund 1991, Koch 2007, Zhang *et al* 2014). TOC migration rates reported were less than 0.1mg/m^2.d (at 23°C and 60°C after 9 days in demineralised water) for two German pipes (Koch 2007) and 0.1-0.3mg/m^2.d (after seven batches of synthetic water at 25±5°C) for Chinese pipes (Zhang *et al* 2014). Final TOC concentrations in water were less than 0.1 µg/L TOC (after three deionised water batches for 3 days each at 23°C) in Danish tests (Forslund 1991) and 0.01-0.02mg/L TOC (after 9 days in synthetic water) in Chinese tests (Zhang *et al* 2014).

Some migration studies of PE pipes reported increase in TOC concentrations (Whelton and Nguyen 2013, Durand 2005, Durand and Dietrich 2007, Zhang *et al* 2013). In overseas studies, PEX often had a larger increase in TOC than LDPE and HDPE (Durand 2005, Durand and Dietrich 2007, Zhang *et al* 2014). TOC migration rates ranged within 0.001- 0.032 mg/m^2.d for German PE and PEX (in demineralised water at 23°C for 72h) (Koch 2007) and 0.1-0.4 mg/m^2. d for chinese pipe (synthetic water) (Zhang *et al* 2014). TOC concentrations in water were less than 0.1 mg/L TOC for 15 out of

19 brands of Danish LDPE, HDPE and PEX (after 9 days in distilled water at 23°C or 60°C) (Forslund 1991); 0.03-0.08mg/L TOC for Chinese PE (in synthetic water) (Zhang *et al* 2014) and 0.5-2.5mg/L TOC for American HDPE and PEX pipes (in water with and without residual chlorine at 23°C), with the larger TOC concentrations in chlorinated water (Durand 2005, Durand and Dietrich 2007 in Whelton and Nguyen 2013). Zhang *et al* (2014) also compared new PVC, PE and polypropylene (PP) pipes manufactured in China and reported that PVC had the lowest migration rate and the least impact on TOC, whilst PE displayed the highest impact.

To summarize, tests had different experimental conditions and pipes had different origins, thus studies indicate that some PE pipe formulations can increase TOC, but the effect differs markedly with pipe origin and manufacturer.

2.1.3.2 Chlorine consumption

Zhang *et al* (2014) reported a positive correlation between TOC and total chlorine consumption in the evaluation of chinese PVC, PE and PP pipes in lab studies. Less than 0.2 mg/L of chlorine was consumed after 48h exposure to consecutive batches of synthetic water; and after 168h (or 7days), the residual chlorine concentrations in water remained at higher than 1mg/L for all pipes (original concentration was 2mg Cl/L). PE had the highest chlorine consumption rate, consuming 1 to 3 times more chlorine than uPVC in synthetic water.

2.2 Biofilm development

Biofilm is a complex structure of microbial cells that attaches to pipe surfaces and is typically comprised of microorganisms, exopolysaccharides and water (Blanco *et al.* 2011). Sediment and biofilm provide a habitat for growth of microorganisms, given their potential for shielding microorganisms from disinfectants in water (Makris *et al* 2011).

Biofilm characteristics depend on the number and diversity of species in water supply, the concentration and type of biodegradable organic matter, the hydraulic regime, the supporting material and the disinfectant residual in water. However, biofilms in potable water systems have little qualitative and quantitative uniformity and large variation in population composition (Percival and Walker 1999).

Biofilm growth is dependent on temperature, pH, growth rate, lag time, cell yield, microorganism species, nutrients, sediments and disinfectant residuals (Makris *et al* 2011, Adhikari *et al.* 2012). Temperatures higher than 15°C and neutral pH (7.3-7.9) favour bacterial growth (Martin *et al.* 1982).

Servais *et al.* (1993) evaluated the correlation of assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC) levels to heterotrophic plate count (HPC) growth and recommended a maximum BDOC < 0.15mg/L to prevent biofilm growth, whilst LeChevallier *et al*. (1987,1991) recommended a maximum AOC of 0.05 mg/L.

In addition, inorganic nutrients also have a role in microbial growth and phosphorus can become a rate limiting parameter in water distribution, particularly in waters rich in organic matter (Sathasivan *et al.* 1997, Miettinen *et al*. 1997). Chu *et al.* (2005) evaluated the impact of inorganic nutrients (ammonium, nitrate, phosphate) on biofilm growth in laboratory studies. The biofilm growth rate increased at concentrations of 0.5 mg N/L and 0.01 mg P/L but below these concentrations no effect was observed.

Sathasivan *et al.* (1997) verified a linear correlation for heterotrophic plate count (HPC) in laboratory tests with distribution water for concentrations from 0 to 5 μg P/L, but a much slower growth rate at higher P concentrations, whilst Miettienen *et al.* (1997) verified that for waters with high AOC, limiting phosphorus concentrations to 10 μg PO_4-P/L hindered bacterial growth in water distribution.

2.2.1 Biofilm attachment on various pipe materials

Biofilm attachment on pipe materials is influenced by the roughness of the pipe surface (Figure 3), which allows biofilm attachment, and the water quality characteristics which allow biofilm growth. Higher heterotrophic plate counts (HPC) and higher biofilm density are observed typically in decreasing order in metallic ferrous pipes, epoxy, cement lined surfaces, steel, plastics (Clement *et al.* 2003, Niquette *et al.* 2000, Le Chevallier *et al.* 1997, Tsvetanova 2006, Pedersen 1990) and copper (Schwartz *et al.* 1998).

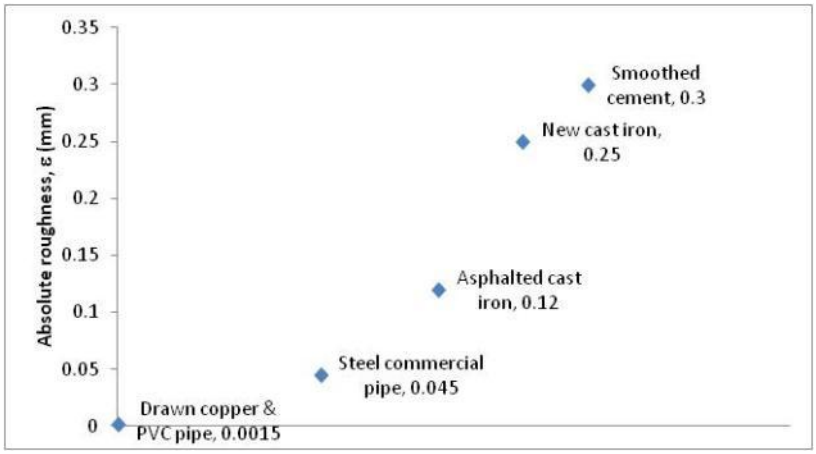


Figure 3. Surface roughness of common pipe materials.

Niquette *et al.* (2000) reported the increase in biofilm density with materials in the following order: PE < PVC < Cemented steel < Asbestos cement < Cemented cast iron < tarred steel < grey iron<corroded iron. Clement *et al.* (2003) verified a similar effect on the density of biofilm for PVC, cement, epoxy and cast iron. Niquette *et al.* (2000) also verified that the biofilm density developed on a range of cement based materials (cement-coated steel, cement-coated cast iron and asbestos cement) was higher than that supported by polymeric materials, but lower than in iron and steel pipes. Pedersen (1990) compared biofilm development on stainless steel and PVC surfaces in drinking water. No difference was observed in the amount of cells on hydrophilic glossy steel surfaces and hydrophobic PVC surface (Pedersen 1990). He also verified that rougher matt steel surface had more cells than the electro-polished steel. On rougher surfaces, cells are better shielded from the bulk flow and more substratum surface area may be available. In contrast, Geldreich and Le Chevallier (1999) reported that UK studies verified four times higher coliform isolations for samples collected from plastic taps compared to metal taps.

Schwartz *et al.* (1998) compared the formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems in Germany using total cell counts (DAPI), respiration activity and heterotrophic plate counts (HPC) for copper, steel, PVC and HDPE. Copper had the least total cell counts, HPC and respiration activity, whilst the last three materials displayed no significant differences in readings among themselves. Roger *et al* (1994) also reported less biofilm organisms on copper compared to PVC and polybutylene pipe. This has been attributed to the biocidal effect of copper on many microorganisms found in drinking water systems (Kirmeyer and LeChevallier 2001, Jungfer *et al* 2013) or alternatively to the lower surface energy of copper pipe restricting initial microorganism attachment (Assanta *et al* 1988). Whilst the biocidal effect delayed

biofilm formation, the effect tended to be overcome after prolonged exposure time (e.g. > 3 months in Jungfer *et al* 2013). Jungfer *et al* (2013) postulated that the death of microbe cells and release of cellular compounds provided nutrients for other bacteria which eventually formed a biofilm layer over the surface and reduced the contact with copper.

Studies on PE and PVC pipe materials have shown no major differences in biofilm development between the two polymers, as previously mentioned in section 2.1.1 and in comparisons including uPVC, PVC and MDPE (Momba and Makala 2004, Niquette *et al.* 2000). However, Tsvetanova (2006) reported a higher bacterial density in PE compared to PVC during the first 70 days of biofilm formation, but this tended to become less prevalent with biofilm maturity. Whilst Zacheus *et al.* (2000) reported a higher cell volume for PE than PVC, but a similar HPC.

The migration of organic compounds from polymeric pipes was previously described in section 2.1.3. Whilst it has been postulated that leaching of organic compounds may provide additional carbon sources for microorganisms, Whelton and Nguyen (2014) reported that not enough is known about the interaction between contaminants from polymer pipes and biofilm. Skjevrak *et al* (2005) detected 2,4-di-tert-butylphenol (2,4 DTBP), a degradation by-product from a HDPE antioxidant in biofilm in a pipe in service at 22 weeks in Denmark, but no traces were detected for longer service (up to 52 weeks), which was attributed to either the reduction of the diffusion rate from the pipe and/or breakdown of the compounds by microorganisms in the biofilm. Some European studies (Anselme *et al* 1986, Cathorne *et al* 1990, Brocca *et al* 2002, Nielsen *et al* 2005, 2007 in Whelton and Nguyen 2013) reported the presence of degradation by-products in sections of in-service polyethylene pipe networks in Europe, but the impact on biofilm and on water quality was not examined.

Overall, besides copper which can have anti-microbial properties, biofilm development and initial adhesion is mainly controlled by the material roughness and by the flow regimen within a pipe. However, Percival *et al* (1999) examined biofilm growth and sloughing on stainless steel at velocities of 0.32 m/s to 0.96m/s and concluded that surface roughness impacted only initial biofilm development, as once the biofilm was established similar biomass weight was verified in steel samples despite of roughness.

2.2.2 Bacterial populations in biofilm

Storey and Kaucner (2009) examined the development of opportunistic pathogens in potable water and recycled water distribution across Australia. Their study verified that the incidence of pathogens, fecal indicators and heterothrophic bacteria varied for distribution systems across the country. In Adelaide, coliforms and aeromonads were detected in recycled water biofilm, but not in the biofilm in the potable mains water distribution (Storey and Kaucner 2009).

In potable water distribution networks of copper, PVC, PE-HD and steel in Germany, Beta and Gamma Proteobacteria were dominant on PVC and PE-HD, whereas steel was dominated by Beta Proteobacteria (detected by fluorescence in situ hybridization, FISH). Legionella was detected on PE, PVC and steel at all sites, but not always on copper (Schwartz *et al.* 1998). Fecal Streptococci was detected only on PE (Schwartz *et al.* 1998). Chlorine dioxide was added in a concentration of 0.12 ±0.16 mg/L according to the German drinking water regulations. The distribution water contained a chlorine dioxide residual of 0.05 ± 0.11 mg /L (Schwartz *et al.* 1998).

On the other hand, Camper (1996) reported the same heterotroph and coliform numbers in PVC as in corroded iron. Whilst, Kirmeyer *et al.* (2001) reported the same HPC on oxidised iron deposition on cement lined pipe as on CI.

Ferrous pipes are subject to corrosion. The corrosion of metallic surfaces reduces scouring and can allow growth of iron bacteria, sulphate reducing bacteria and nitrate reducing heterotrophs

(Tuovinen and Hsu 1982). The formation of tubercles and corrosion products can also shield bacteria from disinfectants (LeChevallier *et al.* 1987). Coliforms have been found in tubercles in cast iron pipe after cement lining has broken away in water distribution system (LeChevallier 1987) and lab studies (Clement *et al.* 2003), on lead pipe, cement lined cast iron and cast iron pipe (Kirmeyer *et al* 2001 in Clement *et al.* 2003). On the other hand, Dubiel *et al* (2002) reported that for biofilm could also result in protection of the metal substrate as a result of specific microbial populations combining aerobic and anaerobic microorganisms.

2.2.3 Disinfectant residuals

Biofilm is more resistant to disinfectant than suspended organisms (Costerton *et al.* 1987). Hence both free chlorine and monochloramine undergo reduction in efficacy against biofilms.

Monochloramine is more effective than chlorine, generating a more stable residual, less taste and odour (Neden *et al.* 1992) and better biofilm penetration than chlorine (Le Chevalier *et al.* 1998b). However, *P.aeruginosa* adapts to monochloramine at low doses (<0.5mg/L). Zhou *et al.* (2009) examined the impact of chloramine and free chlorine residuals to control of biofilm accumulation on copper and stainless steel, verifying less HPC formation on Cu coupons.

Hallam *et al*. (2001) compared the potential for biofilm growth on different materials in water distribution systems and found that the influence of chlorine disinfectant can be more pronounced than that of a material alone, with mean biofilm activity ranked for glass < cement < MDPE < PVC.

Momba and Makala (2004) compared biofilm on PVC, unplasticised PCV (UPVC), medium density polyethylene (MDPE) and cement-based materials (cement, asbestos cement) with ca 2.5 mg/L initial chlorine followed by ca 1.5 mg/L monochloramine. Under such conditions, the cement-based materials supported significantly less fixed bacteria than plastic-based materials. But no significant difference was observed between the same generic types of materials (Momba and Makala 2004).

On the other hand, ozonation increased the concentration of AOC in water and viable numbers and cell volume of heterotrophic bacteria on the surfaces (Schwartz *et al.* 1998).

2.2.4 Biofilm and water quality

The effect of biofilm to the quality of the distribution system has been investigated by a number of authors (Holden *et al.* 1995, Melosi 2000, LeChevallier 2003, Percival and Walker 1999, Percival *et al* 1999). Biofilm has been implicated in the pitting corrosion of copper plumbing and changes to taste and odour of water (Gauthier 1999).

Biofilm has been shown to facilitate the entrapment and accumulation of iron and manganese particles on surfaces, and upon slough-off released iron and manganese, increasing suspended solids, discoloration and turbidity in bulk water in laboratory studies (Ginige et al 2011, Percival et al 1999).

Percival and Walker (1999) reviewed the public health risks associated with biofilm in potable water distribution. Biofilm provides a protective environment where many microbes (including pathogenic species) can adhere and grow protected from biocide penetration (Percival and Walker 1999, LeChevallier 2003). Shearing of biofilm can reduce biofilm development but does not prevent the growth and maturation of heterogeneous biofilm, thus bacterial numbers still increased through passage in the water distribution network. Holden *et al* (1995) noted that the biofilm formation and corrosion on coupons differed with the orientation in a pipe, and concluded that corrosion and biofilm growth were influenced by similar conditions as corrosion, including temperature and disinfectant residual.

2.3 Water discoloration

Discoloration in water distribution systems can be attributed to a range of materials and is often associated with the changes in the system that lead to mobilisation of accumulated particles in a network (Vreeburg and Boxhall 2007, Seth *et al* 2004). Particles can be organic or inorganic material originating from source waters, biological growth and biofilm slough off (Ginige *et al* 2011), by-products from corrosion of liners and pipes, residues from water treatment and chemical reactions within the distribution network (Vreeburg and Boxhall 2007) .

The accumulation of iron and manganese particles from the water supply, infrastructure degradation and/or from sediments accumulated in the distribution can be a common contributor to the discoloration of water in the distribution network (Seth *et al* 2004, Ginige *et al* 2011).

A number of studies have evaluated water discoloration events associated with infrastructure materials.

Husband and Boxhall (2011) compared water discoloration by sampling pipe networks, at intervals 1-year apart, across 15 sites supplied with groundwater, groundwater/surface water blend and surface water after network flushing in the UK. Materials tested were unlined cast iron (CI), PE, uPVC, AC and CICL. They verified that the re-incidence in risk of discoloration for field samples decreased for materials in the sequence: CI(surface water)>CI(groundwater)>uPVC/PE.

The transfer of iron from pipe walls to water is responsible for the "rusty water" phenomena observed in old pipes and is caused by the combined effect from corrosion, dissolution of the scales and scouring due to hydraulic changes (Sarin 2001). The aesthetic ADWG guideline for iron is 0.3 mg Fe/L, after which discoloration and sediment are typically noticed (NHMRC and NRMMC 2011).

Cerrato *et al.* (2006) analysed manganese and iron sediment in PVC and iron distribution pipes. Manganese and iron soluble particulates were introduced into the distribution system from reservoir water at concentrations of 0.2-0.3 mg Mn/L and 0.1-1 mg Fe/L and became insoluble during distribution, resulting in black sediment in tap water. They reported that PVC networks developed more manganese colour problems than iron networks, especially under intermittent flow. This was because manganese particulates were easier to dislodge from polymeric pipe surfaces, whilst iron pipes tended to incorporate manganese into tubercles in the corroded surface.

Corrosion of interior copper plumbing under hard water conditions can lead to copper concentrations equal or greater than 1.5-2 mg/L and blue-green water (O'Halloran *et al.* 2002, Comber and Gunn 1996, Edwards *et al* 2000) and in one extreme case overseas, a concentration of 22 mg/L has been reported (WHO 2004). The Australian Drinking Water Guidelines recommends a maximum copper concentrations of 1 mg/L in mains water to prevent aesthetics issues, whilst 2mg/L and 3mg/L are considered respectively as the discoloration and taste thresholds of water (blue water) (NHMRC and NRMMC 2011).

2.4 Taste and odour

Le Chevallier *et al.* (1996) reported more odour and taste complaints for unlined ferrous pipes compared to other lined pipes.

Dietrich *et al* (2004) examined the literature on taste thresholds (i.e. detection by 50% of test subjects) attributed to copper salts: 2.6mg/L Cu for tap water in a study in Chile (Pizarro *et al* 2001) and 13mg/L Cu for spring water with 200mg/L alkalinity for a US study (Cohen et al 1960); whilst for distilled water in laboratory studies lower concentrations applied: 2.5 mg/L Cu in Chile (Pizarro *et al*

2001), 6.6mg/L Cu in the USA study (Cohen et al 1960) and 0.5mg Cu/L in laboratory studies (Dietrich *et al* 2004). Thus, highlighting the subjectivity of taste perception.

For polymeric pipes, no odour, flavour and taste have been associated with the migration of additives from PVC pipe (Forslund 1991, Burn *et al* 2005, Koch 2007, Khiari *et al* 2002, Marchesan and Morran 2004). However, organoleptic changes have been verified in some PE pipe (HDPE, MDPE, PEX, multilayered PEX, etc) studies conducted in Australia, the USA, the UK and Denmark (Whelton and Nguyen 2013). The Australian study, Marchesan and Morran (2004), found five out of twenty-five PE pipes from Australia/ New Zealand failed the additive migration test, whilst all the twenty-one PVC pipes passed the test and imparted no flavour.

In summary, organoleptic changes in water are complex to evaluate as they depend on personal sensitivity and the background characteristics of the water customers are used to.

Water discoloration, taste and odour problems can also be associated with microbial activity (Makris et al 2014). For example some algal species and actinomycetes produce odorous compounds geosim and 2-methyisoborneol and iron and sulphur bacteria are often asssociated with discoloured water and metallic taste events in iron pipes (Makris et al 2014).

2.5 Impact of residence time on water quality

Stagnant water causes loss of disinfectant residual and sediment accumulation. Donlan and Pipes (1988) observed an inverse relationship between water velocity with biofilm counts and water quality, particularly for dead-end lines. In addition, water hammer and intermittent flow caused release of more bacteria in water than constant flow (Opheim *et al.* 1998).

Vreeburg and Boxhall (2007) conducted laboratory analysis of sedimentation and discoloration formation in polymeric pipes, recommending a minimum velocity of 1.5m/s for network maintenance.

2.6 Infrastructure for water and stormwater distribution in SA

SA Water manages the water supply distribution network in South Australia, including in the greater Adelaide region. SA Water's water supply network comprises 26,674km of pipe and supplies 1,162,000 people (SA Water 2013a). The pipe assets in the water distribution and reticulation network range in diameter size from 20mm to 2100mm, in material type and ages, with the earliest assets installed in 1872 according to the assets database (Gould *et al*. 2012). Furthermore, the North-South interconnection system will add 32km of additional pipework to the existing system and allow desalinated water to be transferred to Happy Valley reservoir.

In addition to traditional drinking water supplies, a number of alternative water supply schemes have been developed. *Water Proofing Northern Adelaide*, constructed a system of wetlands, managed aquifer recharge (MAR) and a 120 km distribution network for the harvesting, treatment and supply of stormwater for irrigation in the cities of Playford, Salisbury and Tea Tree Gully (Waterproofing Northern Adelaide Regional Subsidiary 2010). Similarly, *Water Proofing the West* will also capture stormwater and adopt similar treatment for distribution to business and residential customers in the City of Charles Sturt and is expected to be completed in 2015 (City of Charles Sturt 2013). Both schemes adopt HDPE purple pipe for their water distribution network. The two schemes have the capacity to supply up to 16.1GL per year of water. Recycled wastewater from Bolivar Treatment Plant can provide 18 GL/yr water to irrigators and to residential developments such as Mawson Lakes (Government of SA 2009). The Glenelg and Adelaide Parklands (GAP) recycled water project has the

capacity to supply up to 3.8 GL per year recycled water to the Adelaide Parklands and the city of Adelaide, whilst Christies Beach treatment plants also supply recycled water for irrigators (SA Water 2013).

2.6.1 Materials adopted in water and stormwater distribution

The main typology of materials adopted for water distribution assets in greater Adelaide is shown in Figure 4. Asbestos cement and cement lined metal pipes (cast iron cement lined (CICL), ductile iron cement lined (DICL) and mild steel cement lined (MSCL)) constitute the majority of the network, covering respectively 46% and 35% of the total network length (M.Nicholas, United Water, pers.comm., 2011).

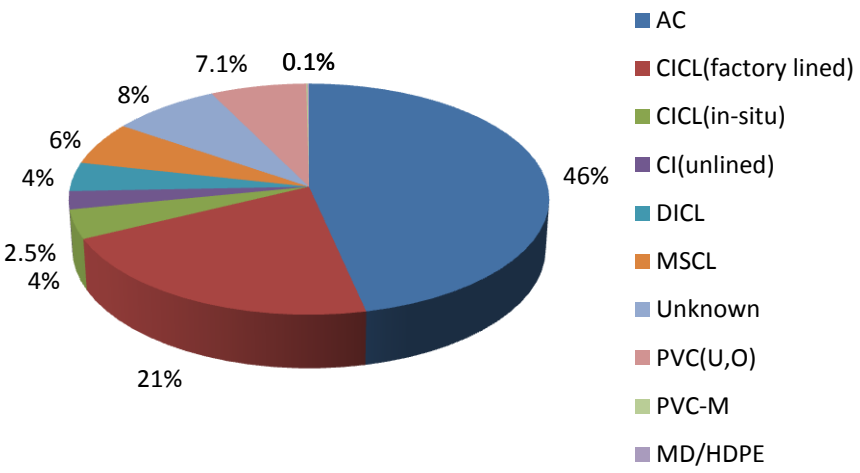
Plastic pipes constitute 7.2% of the network, but their contribution is increasing as they are the material of choice for expansion of the network and for replacement of pipe failures (M.Nicholas, United Water 2011).

Unlined cast iron pipes comprise only 2.5% of the network and are diminishing in length as they are replaced with PVC pipe upon failure (M.Nicholas, United Water, pers.comm., 2011).

Plastic materials adopted in reticulation include modified and oriented polyvinylchloride (M-PVC and O-PVC), medium density polyethylene (MDPE) and high density polyethylene (HDPE) for pipe diameters of less than DN375.

At the dwelling level, the water infrastructure was traditionally made of copper pipe, but polyethylene pipe has in recent years become widely adopted in new developments due to its lower cost.

The infrastructure for alternative water supplies, including recycled water and stormwater, adopts mainly polymer pipes. The stormwater distribution infrastructure in Adelaide uses mostly HDPE pipe, given that stormwater is used mostly for non-potable applications, such as irrigation or industrial applications.



Legend: Asbestos cement (AC), cast iropn (CI), cast iron cement lined (CICL), ductile iron (DI), mild steel cement lined (MSCL), medium density (MD)/ High density (HD) polyethylene (PE), poly(vinyl chloride) (PVC) unplasticised (U), oriented (O), modified (M).

Figure 4. Representation of pipe materials in the potable water distribution for greater Adelaide (as percentage of total length of assets) (M.Nicholas, United Water, pers.comm., 2011)

2.6.2 Operating conditions

The potable water distribution network is designed to operate within the range of 0 to 1 m/s, with an average velocity of 0.5 m/s (C.Hewitson, United Water, pers. comm., 2011) and at a minimum pressure of 200 kPa (SA Water, pers. comm., 2014). Residence time in the network will vary, with the night time period of 12 am to 5am characterised by the lowest velocities.

Stormwater supply at the Parafield network is operated seasonally. Stormwater is supplied during the Summer period at pressures ranging from 150 to 600 kPa throughout the distribution network.

The operating conditions for the stormwater supply use average velocities in the range of 1 to 1.5 m/s, with a maximum velocity of 2.5m/s during peak season (B.Naumann, Salisbury City Council, pers.comm., 2011).

2.6.3 Performance of water distribution network

Consultation with the reference panel and key project stakeholders such as SA Water and United Water indicated that few water aesthetics incidents were observed in the water supply network, and that maintenance programs including mains flushing were in place and effectively managed potential risk areas (C.Hewitson, United Water, pers.comm., 2011). SA Water also manages the provision of blended desalinated and mains water to the water supply distribution network to ensure that water quality is stable and in compliance with aesthetics and health ADWG.

The stormwater supply from the City of Salisbury is designed for the supply of industrial and institutional customers for irrigation of open spaces or industrial use, and no water quality complaints had been recorded given the intended uses (Salisbury Council, 2011).

2.7 Risk analysis for stormwater

The stormwater supply in the Parafield scheme is a blend of aquifer storage and recovery (ASR) and aquifer storage transfer and recovery (ASTR) recovered water. In ASR water is injected and extracted from the same well, whilst in ASTR the injection and recovery are in different wells, which provides added water treatment by extending the residence time in the aquifer. The blend of ASR/ASTR recovered water is stored in two tanks (combined volume of 0.6ML) at the Parafield site. Information collected from the literature review, from a workshop on distribution water quality issues with stakeholders (conducted in 2011) and examination of records for the water quality of the Parafield ASR/ASTR schemes (Page *et al* 2013) was used to determine potential risk factors associated with water aesthetics and biofilm development for the individual wells. Table 2 summarises the water quality records of stormwater following wetland treatment (WE) and aquifer recovery (RW), compares it to selected ADWG parameters, and outlines the outputs from this preliminary risk analysis.

2.7.1 Water aesthetics

Page *et al* (2013) evaluated water quality parameters for stormwater, wetland-treated and aquifer recovered water (ASR and ASTR) quality for a decade of monitoring of the Parafield scheme. Aesthetics parameters were found to occasionally exceed ADWG values, such as high colour due to high iron concentrations, occasional turbidity and high salinity caused by entrainment of brackish groundwater in the recovered water.

The median concentrations of total manganese and iron in recovered stormwater (RW1, RW2) exceed the respective ADWG aesthetic limits, 0.3mg Fe/L and 0.1mg Mn/L, indicating a high risk of colour formation and sediment deposition in the stormwater blend. The wetland (WE2) had lower concentrations of iron, manganese and total dissolved solids than recovered stormwater, but comparable TN and TP concentrations.

The median concentration recovered from ASR was 0.38 mg/L and from ASTR was 0.36 mg/L. The measurement of soluble iron from the ASTR operation indicated that iron in the groundwater of the MAR storage zone is predominantly dissolved.

The 95th percentile manganese concentrations recovered from Parafield were above the aesthetic guideline of 0.1 mg/L, but the median values were lower with 0.04 mg/L from ASR and 0.06 mg/L from ASTR, which are below the health based Mn guideline of 0.5mg/L (Page et al 2013).

The median turbidity for the ASR and ASTR of 1.1NTU and 0.7 NTU, were below the ADWG aesthetic value of 5 NTU. However, the 95th percentiles were above the ADWG aesthetics guidelines at 16 NTU and 6 NTU for the ASR and ASTR, respectively.

In the ASR, the median colour, 1.95HU, was within the ADWG aesthetic guideline (15HU), but the 95th percentile just exceeded it at 16HU. However, the ASTR median, 21HU, markedly exceeded the aesthetic guideline. The median values in the stormwater were also higher than the average concentrations in the mains water supply in the Adelaide region.

Thus a noticeable colour is expected in the blended stormwater, due to high iron concentration in the MAR, however for other parameters (manganese and turbidity) the impacts on water colour and aesthetics will depend on the time of extraction and the blend ratios of the water sources.

2.7.2 Cement corrosion

Wetland water, with a Langelier index (LI) of -1.56 is more aggressive than RW. However, after aquifer storage, the LI for the 95th percentile of the ASR and ASTR stormwater is positive (LI > 0), indicating that the water would be less aggressive to cement than the mains water in Adelaide, which is characterised by a negative LI between -0.82 and -1.98.

Thus less cement corrosion would be expected from exposure to RW compared to mains water.

2.7.3 Biological growth and disinfection

Aquifer treatment can be designed to reduce viruses, protozoa, bacteria and inactivate viruses, provided a suitable residence time in the subsurface is guaranteed, and the aquifer performance is validated, being an effective treatment for pathogen reduction (Page *et al* 2013).

Water recovered from the ASR and ASTR operations generally remained below the ADWG aesthetic guideline value for nitrogen species, aside from the 95th percentile ammonia concentrations from Parafield ASTR (Page *et al* 2013). Median total phosphorus at 0.03 mg/L was above 0.01 mg P/L, below which phosphorus becomes rate limiting for biological growth. Median values of 1mg BDOC /L and 0.4mg BDOC/L for the respective ASR and ASTR exceed 0.15mg BDOC/L, the concentration that allows biofilm development (Servais 1993).

Thus, if microorganisms are introduced into a network, the nutrient concentrations in RW will be suitable for biofilm growth. Therefore, disinfection of the stormwater for treatment for pathogens prior to third pipe and drinking water use would be recommended (Page *et al* 2013). The rate of biofilm formation and the potential for its detachment and impact on stormwater aesthetics would

depend on the disinfection efficacy and operating conditions in a distribution network. Because high iron and turbidity concentrations can impact disinfection efficacy, removal of excess iron would be recommended as a precursor to disinfection (Page *et al* 2013).

Given the DOC and the potential for NOM in stormwater, verification of disinfection effectiveness, by-products and residuals would also be recommended if the stormwater was intended for drinking purposes. Page *et al* (2013) has previously evaluated the concentration of disinfection by-products in the form of bromoform, chloroform, dichloroform, dibromoform and total trihalomethanes (THM) formation potential (FP) in stormwater in the Parafield wetland and the ASR and ASTR schemes. The 95th percentiles reported for the wetland outlet, the ASR (RW1&2) and the ASTR (RW1&2) groundwater were respectively: 218 μg/L , 73μg/L and 128μg/L for chloroform FP, 36 μg/L, 21 μg/L and 22μg/L for di-bromoform FP,69 μg/L , 45μg/L and 55μg/L di-Chloroform FP, and 295μg/L , 141μg/L and 205μg/L for total trihalomethanes (Page *et al* 2013), hence the recovered water was below the ADWG total THM of 250μg/L, but wetland stormwater exceeded it. In our experimental investigation, disinfection by-products were not evaluated as no disinfection was applied to the source waters.

2.7.4 Summary

The Parafield stormwater system produced a blend of ASR and ASTR stormwater, however it was uncertain what the stormwater supply composition generated over the year would be, as it depended on the mix ratios between the two ASR and ASTR sources. The risk analysis indicated that:

- The recovered stormwater (RW) from the individual wells (ASR and ASTR) had the potential to impact water aesthetics and quality due to the high iron content in the groundwater. The high iron concentrations would increase the consumption of disinfectants such as chlorine in a system. The risk associated with other parameters, such manganese and turbidity from the water supply and their impact on discoloration is uncertain given the RW concentrations and variability reported.

- Cement dissolution due to RW is not expected, instead RW may be beneficial to the durability of cement and concrete in distribution based on LI.

- The RW quality would allow microorganism growth, due to the nutrient availability. The rate of formation of sediment and biofilm is associated with the operating conditions in a network, hence their impact on water quality are yet unknown.

- Iron removal is recommended to reduce colour episodes and sediment formation. Further disinfection and chlorine residual would be recommended to control microbial growth.

Table 3. Preliminary risk analysis for water quality and aesthetics.

		Metropolitan Adelaide			Parafield Wetland		Aquifer groundwater					
Parameter	Units	Mains water at tap [a]			Outlet[b]		ASR 1 &2 [b]		ASTR-RW1 &2 [b]		Guideline values	Risk
		Catchment averages	**Min**	**Max**	**Median**	**95th %**	**Median**	**95th %ile**	**Median**	**95th %ile**		
Iron(t)	mg/L	0.008-0.032	<LDL	1.3	0.45	1.3	0.38	3.7	0.36	5.5	0.1[d] , 0.3[c] colour	Colour
Iron(s)	mg/L				0.13	0.49	1.31	2.8	0.3	5.7	0.2-0.3 sediment	Sediment
Manganese (t)	mg/L	0.0016-0.0042	<LDL	0.1	0.04	0.21	0.041	0.15	0.06	0.31	0.03 colour [d] 0.1 sediment[c]	Colour sediment
Manganese (s)	mg/L				0.02	0.15	0.08	0.14	0.06	0.31		
Total dissolved solids (TDS)	mg/L	306 - 429	180	500	125	200	240	710	300	344	600 [c]	
Alkalinity	mg $CaCO_3$ /L	46-69	28	95	59	118	145	201	157	213	<60 corrosive >200 scaling risk	
Bicarbonate	mg/L	55.9-84.0	34	115	72	144	165	228	192	260		
Sulphate	mg/L	43-63.6	25.2	77.7	9.6	21.8	24	62	24	42	250[c]	
Chloride	mg/L	115-161	43	220	28	54	35	146	63	84	250[c]	
Calcium	mg/L	18.6-29.97	12.8	35.4	19	36	39	47	45	69		
Langelier index		-1.18 to -0.82			-1.56	-0.24	0.06	0.52	0.14	0.52	<0 Aggressive to CL >0 Deposition on CL (protective).	
pH		7.2-7.5	6.8	8.1	7.0	7.6	7.8	8.0	7.8	7.9	6.5-8.5[c]	
T (°C)		19.3-20.2	9.0	37.0	14.3	21.6	18	19	19	20		
Turbidity	NTU	0.16-0.25	0.1	11	3.6	13	1.1	16	0.7	5.9	5[c]	
True colour	HU	1-1.98	<1	10	26	82	1.1	15.9	21	36	15[c]	
Nitrate+Nitrite as N	mg/L	0.0547-0.115	<LDL	0.5	<LDL	0.03	< LDL	052	< LDL	0.023	0.5 [e] (biofilm) 0.5[c]	
Ammonia as N	mg/L	0.0057-0.010	<LDL	0.1	0.01	0.09	0.09	0.32	0.14	5.75		
Total Kjeldahl	mg/L	0.21-0.32	0.1	1.5	0.35	0.86	0.17	0.77	0.32	5.69		

		Metropolitan Adelaide			Parafield Wetland		Aquifer groundwater					
Parameter	Units	Mains water at tap [a]			Outlet[b]		ASR 1 &2 [b]		ASTR-RW1 &2 [b]		Guideline values	Risk
nitrogen (TKN)												
Total nitrogen (TN)	mg/L				0.36	0.86	0.18	0.77	0.32	5.69		Biofilm
Filterable reactive phosphorous (FRP)	mg/L mg/L	0.0050-0.0057			0.01	0.04	0.02	0.03	0.02	0.03		
Total phosphorous (TP)	mg/L	0.0070-0.0108	<LDL	0.1	0.04	0.10	0.029	0.11	0.03	0.23	0.01 [e] biological	Biofilm
Biological dissolved organic carbon (BDOC)	mg/L						1	1.3	0.40	1.77	0.15 [f] biological	Biofilm
Dissolved organic carbon (DOC)	mg/L	2.63-5.36	1.6	8.0	4.7	11	2.3	3.3	3.90	6.43		
UV254 (unfiltered)					0.18	0.26			0.21	0.59		
Disinfectant residual	mg/L	0.2-0.37	<0.1	2.1	ND	ND	ND		ND	ND		

[a] Average values from Five year statistics for customer taps by Water treatment systems from 1/7/2005 to 30/6/2010 for Anstley Hill, Barrossa, Happy Valley, Hope Valley, Little Para and Myponga systems (SA Water), United Water, (2011), [b] Parafield Wetland outlet (Page et al 2013), , [c] Aesthetic guidelines from the Australian Drinking Water Guidelines (ADWG) (NHMRC–NRMMC 2011), [d]J. Lucas. United Water,pers.comm. (2011), [e] Chu et al (2005), [f] Servais (1993); LDL: lower detection limit, ND: not determined.

3 Experimental Evaluation

The review of the literature (section 2) indicated that limited data was available on the impact of stormwater on water aesthetics or on its interaction with water distribution infrastructure. The preliminary risk analysis for the stormwater from the Parafield study site (section 2.7) indicated the potential for biofilm development, iron deposition and sedimentation, but less risk of corrosion of cement lining according to water quality characteristics compared to mains water. Therefore a dedicated experimental rig was built to evaluate the interaction between the water sources and selected distribution materials under controlled operating conditions.

Following discussion with the reference panel, the materials selected for evaluation were cement lining from ductile iron metal pipe, plastic pipe (O-PVC) and copper pipe. Cement lined pipes have the largest representation in the existing water distribution system in Adelaide, plastic pipes are the material of choice for new distribution networks with PVC having the lowest cost, and at the dwelling level, water pipes are comprised of either copper or polyethylene. However, aesthetic incidents are most commonly reported for copper pipes. These three pipe types (PVC, cement lined and copper) were machined into coupons to test exposure to stormwater subjected to treatment by wetlands and ASR/ASTR, with unchlorinated baseline water as the control in a field rig.

Coupons were extracted at regular intervals over the study period for evaluation of biofilm development and surface characterisation. Water quality was monitored through grab samples and continuous monitoring of selected parameters. Infrastructure material change was monitored through surface appearance and morphology evaluation of coupons. Biofilm formation and composition was monitored to identify the microorganism population and to examine the potential for pathogen development.

The experimental exposure and the characterisation methods are summarised here and detailed in the respective sections:

- Description of experimental site, rig set-up and exposure conditions are described in sections 3.1 to 3.4.
- Water quality evaluation: used grab samples and analysis of physical and chemical parameters including pH, dissolved oxygen, electrical conductivity, temperature, colour, turbidity, metals, nutrients, inorganic and selected organic compounds (section 3.5). This was followed by geochemical modelling of water quality changes (section 3.6).
- Material characterisation: assessment of surface changes and sediment deposition on coupons was determined using optical microscopy, colour measurements, dry mass and X-ray spectroscopy (for identification of inorganic compounds and elemental analysis) (section 3.7).
- Quantification of microorganisms numbers: sampling techniques for biological assessment were described (section 3.8.1), total cell counts (i.e. biological matter as non-culturable and culturable cell numbers) using flow cytometry in biofilm and in bulk water (section 3.8.2); and culturable cell counts (i.e. living microorganisms capable of growth) (section 3.8.3).
- Identification of microorganisms and pathogen species: conducted using DNA extraction for species identification (sections 3.8.4); detection of pathogens, non-turberculosis mycobacteria and Legionella in water samples (section 3.8.5); and characterisation of microbial community (bacteria and eukaryotes) (sections 3.8.6 and 3.8.7) .

3.1 Test site location

The test site was located on lot 102, Salisbury Highway, Mawson Lakes, SA (GPS coordinates 138.6024, -34.804723) (Figure 5). The site was located in close proximity to SA Water's Greenfields Mixing Tank that stores the blended treated effluent from the Bolivar Wastewater Treatment Plant and stormwater from Parafield used to supply the Mawson Lakes Recycled Water Scheme (Figure 6).

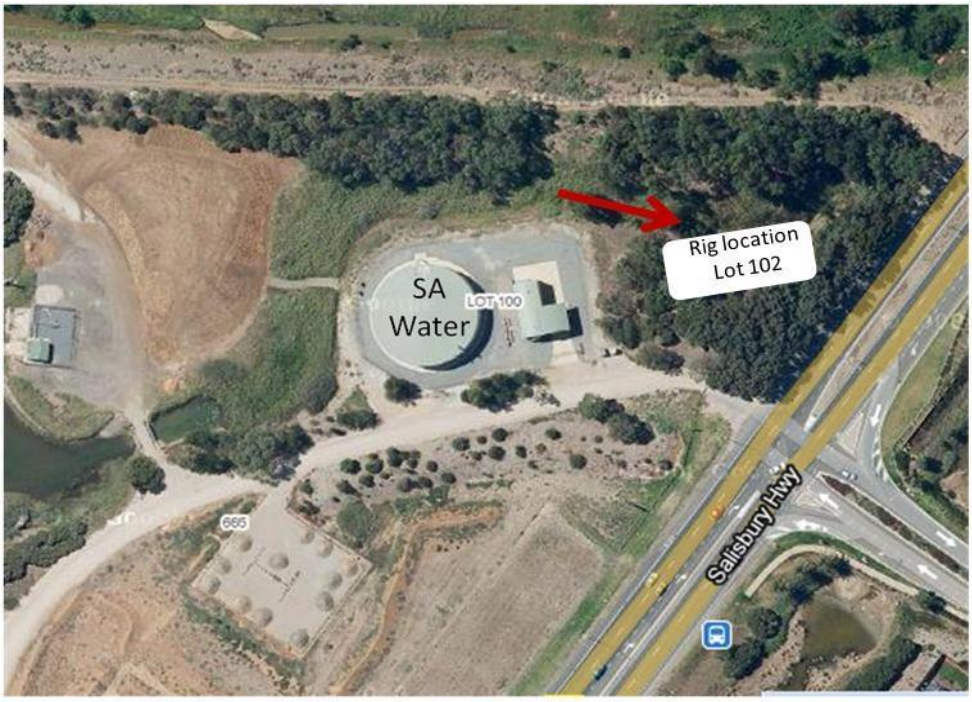


Image: http://www.maplandia.com/australia/airports/parafield-airport/

Figure 5. Location of experimental rig for water quality and infrastructure interaction.

Figure 6. SA Water's Greenfields Mixing Tank facility that supplied recycled stormwater and baseline water to the biorig experimental site. This facility supplies a blend of DAFF treated wastewater and recycled stormwater from the mixing tank to the Mawson Lakes Recycled Water Scheme.

3.2 Experimental rigs

Two pipe rigs were constructed for testing stormwater and baseline water. Each pipe rig was comprised of a pipe loop circuit and both were identical in design and operational conditions. Continuous supply of recycled stormwater and baseline water was obtained from the SA Water's Greenfields Mixing Tank site as shown by the respective purple and blue lines in Figure 6.

The two water sources supplied to the rigs were:

- Stormwater treated in the Parafield wetland, injected, stored and recovered from the lower Tertiary marine sediments of the Port Willunga Formation, a well-cemented sandy limestone (T2) aquifer via the aquifer storage and recovery (ASR) and aquifer storage transfer and recovery (ASTR) systems, and stored in 0.6 ML storage tanks at the Parafield stormwater harvesting site, prior to supply to the Greenfields Mixing Tank site via a direct pipeline; and
- Baseline water obtained from a mains water supply pipe at the Greenfields Mixing Tank site via a pipeline connection.

The pipe loop circuit is shown in Figure 7 and consisted of:

- Biofilm rig: A 3.2m length section of PVC DN150 pipe which holds thirty-eight coupons of various materials for monitoring biofilm development based on modified robbins device. The biofilm rig was sunk 0.6 m into the ground, and was located in a metal covered hutch to allow access for sample removal (Figure 8 a, b).
- Water quality line: A 36m pipe loop of DN100 M-PVC and 6m DN100 DICL pipe allowed interaction between the inner pipe surface and water. Water sampling points were located at the inlet to the rigs and at return point from the lines, shown as points S1 and S2 (inlet sampling) and S3 and S4 (return sampling) in Figure 7). The line was buried to a depth of 0.6m, and
- A control room which housed the circulation pumps, 200 L storage header tanks and the flow control and monitoring instrumentation for each loop (Figure 8 c, d and e).

Water was introduced into a 200 L header tank and circulated through the pipe loop using a pump (Lowara ITT CEA 370/3/A-V 240/380-415 50) controlled by a Vasco variable speed drive coupled to an Omni P1600-100 pressure sensor. Entry into the header tank was designed to promote the aeration of the water supply by gravity. No disinfection treatment was applied to either of the water sources.

The pump operation was controlled by a timer. During pump operation the flow rate was maintained at a constant speed by setting the variable speed drive to run at a constant pressure of 2 bar (200 kPa). A volumetric flow rate of 140-145 L/min was set for each rig by adjusting the valve on the return line to the header tank using the instantaneous graphic display reading on an Endress Hauser Proline Promag 10W flow meter (pulse output: $\pm$0.5% of reading $\pm$ 2mm/s). Both flow meter outputs were wired to a DT85 DataTaker logger. In addition water quality sensors and logging equipment were also installed in the rigs (Figure 7 c) and are described in section 3.5.

Security to the site had to be upgraded twice after vandalism attacks and a robbery. The final security arrangements consisted of a fence enclosure to the site, a boom gate at the access road and centralised alarms in the control rooms.

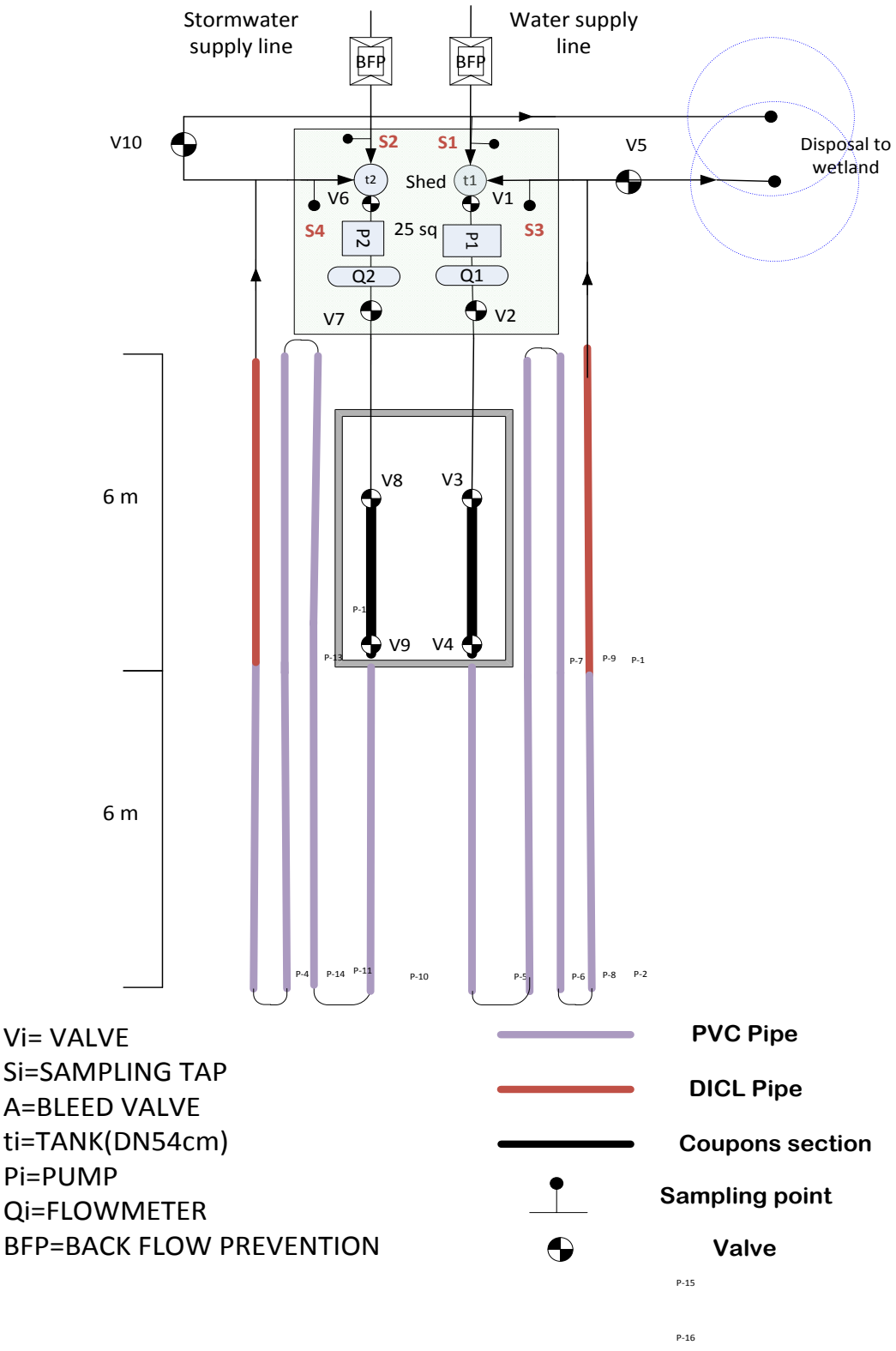


Figure 7. Diagram of experimental twin rig (top view) showing the stormwater and the mains water supply lines. Sampling locations are shown: S1 and S2 for inlet water supply to the rig and S3 and S4 for oulet water after passage through the rig, Material coupons are housed between sections V3-V4 and V8-V9. In-line sensors were placed in the pipe sections preceding the coupons section.

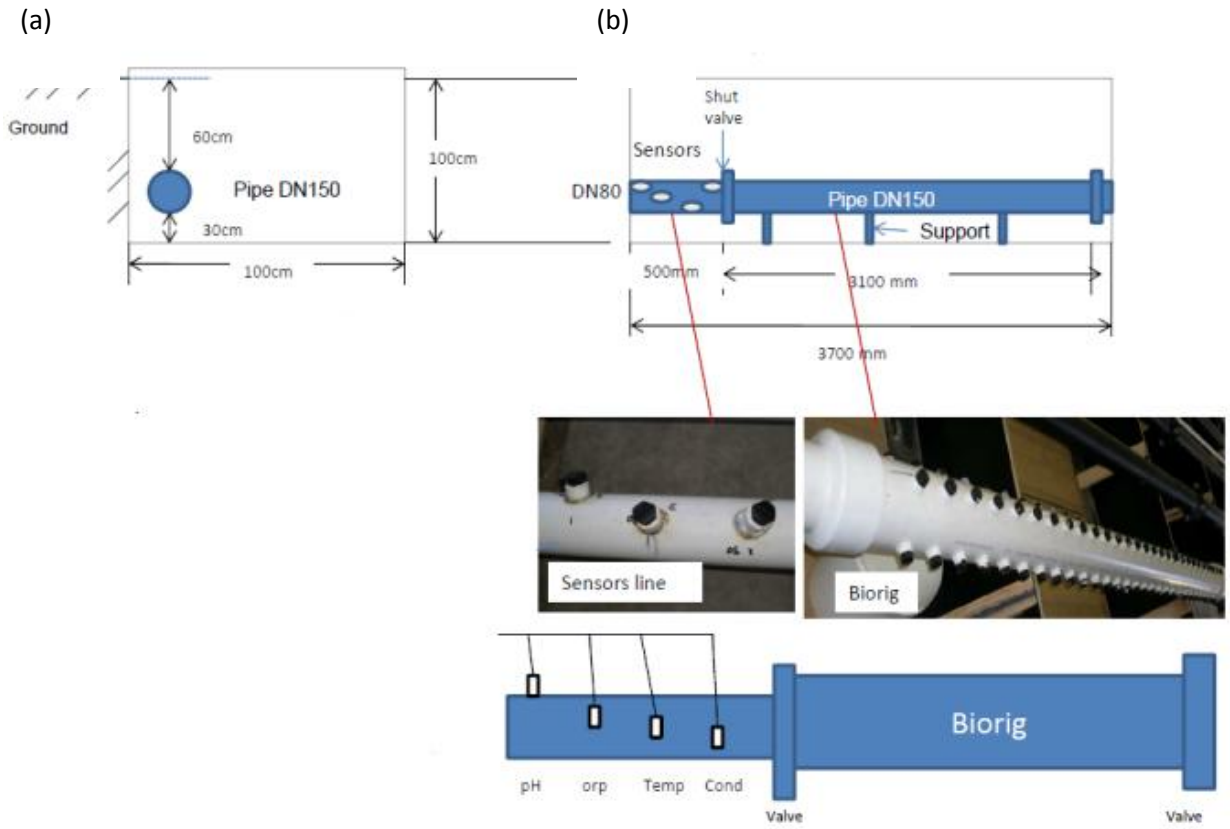


Figure 8. Diagram showing the details of the biorig that houses the material coupons: (a) View from the same direction as the cross-section to the biorig, and (b) View along the biorig length showing the location of sensors and coupons housing.

Figure 9. Photos of experimental rig: (a) Biorig pit and control room; (b) Biorig section showing coupon housing; (c) Rig header tank; (d) Secured (fenced and alarmed) pump shed and site; (e) Flow control instrumentation, (f) Manual boom gate to restrict site access.

3.3 Rig operation

On July 4, 2012 a series of two slug tests using a salt tracer solution were carried out on the stormwater rig to determine the total rig volume. For both tests, 1 kg of NaCl (table salt) was added to the 200L header tank and mixed until dissolved resulting in an initial tank conductivity of 12.5mS/cm in each test. The first test was pumped at a rate of 33L/min and the second test at 11L/min. Peaks in return line conductivity were seen at 17 and 39 minutes respectively. This corresponds to an estimated rig volume (including the header tank) of 580 L.

Stormwater and baseline water were introduced into their respective rigs in 24 h cycles using an automatic timer on the pumps to allow 8 hours off (stagnant) and 16 hours on (recirculation). Recirculation flow rates were set at 145 L/min at a constant 200 kPa. This is equivalent to a velocity of 0.14 m/s in the DN150 biorig pipe section of the rigs, less than the mean velocity of 0.5 m/s within the potable distribution network (C.Hewitson, United Water, pers. comm., 2011). Water was bled from the return line at a rate of approximately 1 L/min over the 16h run triggering inflow of new water at the same rate. This resulted in introducing one entire rig volume of new water after 10 hours of recirculation. However, as the water was recirculated and bled gradually, mixing took place so that the entire turnover of rig water would only occur over several 24 hour cycles.

3.4 Coupons exposure

Removable coupons made of O-PVC, ductile iron cement lined pipe and copper pipes were machined from pipes (dimensions 4.5 cm length x 2cm wide), wiped with ethanol to remove any surface contaminants and installed in the biorigs for exposure. Coupons were installed with the wider coupon surface in the same direction as the flow through the pipe.

Coupons were placed in the rigs and exposed from May 2012 to November 2013. However, operation of the rig was interrupted on two occasions. A short outage occurred from June 12-14, 2012 due to vandalism to the pipe rigs.

A longer outage occurred from October 4 to December 19, 2012, called period 0, due to a break in and theft of equipment from the site and damage to the rig. During this period, coupons were exposed to stagnant water. Following the robbery, the rigs were flushed with the respective water types on December 20, 2012, all coupons were replaced with new ones and the rig recommissioned.

There were a few other occasions when there was a power outage but these were short, typically 1-2 days of non-operation. Therefore, the period of operation of the rig was divided in two periods: (a) period 1 (July to October 2012) and (b) period 2 (December to November 2013). The exposure period for each of the coupons are summarised in Table 4.

3.4.1 Coupon swap

A coupon swap was conducted for selected coupons (exposed since 13 March 2013 or 29 weeks) on 1st October 2013. In the swap, a pair of coupons was removed from each rig, one single replicate was returned to the original rig, whilst the other was swapped with an equivalent coupon from the other rig, e.g. one of the coupons aged in baseline water was transferred to the stormwater rig and vice-versa. All coupons were then removed on the 8th January 2014 (equivalent to 3 months or 99 days additional exposure) and examined. The coupon swap was intended to provide an indication of the impact that a change in water supply type could have on the biofilm developed on the materials.

Table 4. Coupon sampling dates and exposure periods.

Period	Date in	Date out	Approximate exposure (weeks)	Comment
1	23.05.2012	20.08.2012	13	
	23.05.2012	25.09.2012	22	
	20.09.2012	29.10.2012	5	
	Rig interruption from 4.10.2012. Rig resumed on 20.12.2013 Old coupons discarded			
2	20.12.2013	30.01.2013	6	New coupons installed
		13.03.2013	12	
		30.04.2013	19	
		11.06.1013	25	
		22.07.2013	32	
		1.10.2013	35	
	1.10.2013	8.01.2014	43 (including 14 in the alternate water supply)	Coupons 7, 9 and 11 were swapped between rigs

3.5 Water quality monitoring

Inlet and return water temperatures were monitored by T-type mineral insulated metal sheathed stainless steel thermocouples 3mm – TC Measurement and Control (±1%). pH was monitored using SensoreX pH electrodes - flat surface CPVC (±10%). Electrical conductivity was monitored using Thermo Fisher Scientific Alpha Cond 500 connected to a Thermo Fisher Scientific stainless steel conductivity electrode (±5%) and SensoreX ORP electrode sensors (±10%) monitored redox potential. Amplification of pH and ORP sensor signals was via Heaston Electronics self powered pH pre amplifiers (PHAMP-A30). Data was logged at 5 minute intervals using a DT85 DataTaker logger. Remote connection to the logger was made using a NetComm Ltd industrial router (NTC-6908) fitted with a Telstra 3G SIM card. Remote web access to the logger (for data transfer) was via DataTaker deX software through the internet using a dynamic DNS Host (dyndns.com). Continuous monitoring data is summarised in Appendix 1.

In addition to continuous monitoring, samples of stormwater and baseline water from the rig outlets (Figure 7) were collected on ten occasions; two in period 1 and eight in period 2. Sampling of rig water coincided with coupon sampling events (Table 4). Inlet water quality was sampled on two occasions in September 2013 and October 2013 to check against results of earlier rig outlet water quality sampling that showed trends that were not expected from the respective sources (i.e. aquifer recovered stormwater and baseline water). This is discussed in Section 4.2.

Measurements of electrical conductivity (EC), temperature, pH, redox potential (reported as Eh) and dissolved oxygen (DO) were taken using a 90FL-mV field analyser with probes contained in a flow through cell. Residual chlorine was measured using a HACH DR/890 portable colorimeter. These measurements were taken at each water quality sampling event as a check against online measurements and samples sent to the laboratory.

Water quality analysis was undertaken predominantly at the Australian Water Quality Centre (AWQC) or CSIRO Waite Laboratories (Table 5). AWQC has quality system certification under ISO 9001, and NATA technical competence under ISO 17025 and NATA accreditation 1115 for chemical and biological analyses. Appendix 2 lists the AWQC methods and report limits for each parameter. Samples for particle size analyses and UV-visible absorption at 254 nm were analysed at the CSIRO Waite Laboratories using a Malvern Mastersizer 2000 and UV-1600 PC Spectrometer respectively. A slightly reduced suite of parameters was analysed for sample events in September and October 2013, which were used to compare water quality entering the rig to that within the rig itself.

Sample preservation and storage were undertaken according to the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF 2005), including filtration through 0.45 µm filters for soluble metals and nutrients and acidification to pH 2 with HNO_3 for total and soluble metals, with all samples being maintained at 4°C once collected.

Table 5. Summary of water quality parameters analysed in field and AWQC/ CSIRO laboratories.

Analysis type	Parameters
Field parameters	Electrical conductivity, temperature, pH, Redox Potential, Dissolved Oxygen, Residual Chlorine
CSIRO Physicochemical	Particle size analysis, UV_{254} [1]
AWQC Physicochemical	Total dissolved solids (by EC), Suspended solids, Turbidity, pH, True colour Alkalinity
AWQC Major ions	Bicarbonate, Chloride, Sulphate, Fluoride, Calcium, Magnesium, Potassium, Sodium
AWQC Nutrients	Total organic carbon, Dissolved organic carbon, Ammonia, Nitrate + Nitrite, Total Kjeldahl Nitrogen, Total nitrogen, Filterable reactive phosphorus, Total phosphorus, Silica, BDOC[2]
AWQC Faecal indicators[2]	*E. coli,* Thermotolerant coliforms, Bacteriophage
AWQC Total metals	Aluminium, Arsenic, Cadmium, Chromium,, Copper, Iron, Lead, Manganese, Mercury, Nickel, Zinc
AWQC Soluble metals	Aluminium, Arsenic, Boron, Iron, Manganese, Copper[3]

[1] added in Sept 2013; [2] not included in the reduced suite of samples in Sept and Oct 2013; [3] added in Oct 2013

3.6 Modelling chemical interactions

The chemical assessment was based on observations from the exposure test rig in combination with geochemical equilibrium modelling using PHREEQC (Parkhurst and Appelo 1999). This evaluation considered the potential for pipe scale, corrosion and water quality changes due to interaction between various water sources (Table 6) and three pipe materials, cement lined, copper or plastic (PE or PVC). The desktop evaluation assessed eight water sources, while the exposure test rig compared two of these water sources; wetland and aquifer treated stormwater and baseline water (as the control).

Table 6. Water sources evaluated in geochemical assessment of water quality issues.

Water sources	Desktop	Experimental rig
Wetland treated stormwater	✓	✗
Wetland treated stormwater/mains water blend	✓	✗
Wetland treated stormwater/reclaimed water blend	✓	✗
Wetland & aquifer treated stormwater	✓	✓
Wetland & aquifer treated stormwater with chlorination	✓	✗
Wetland & aquifer treated stormwater/mains water blend	✓	✗
Wetland & aquifer treated stormwater/reclaimed water blend	✓	✗
Mains water	✓	✓

3.6.1 Data sources

The desktop study utilised water quality data for the following end-member waters which were then used to calculate blended water quality: wetland treated stormwater; wetland and aquifer treated stormwater; mains water; and reclaimed water (Table 7). The assessment used raw/individual datum for water sources aside from the mains water supply where statistical summary data (average, minimum, maximum) was used.

The experimental study utilised water quality data for samples of mains water and stormwater within the rig sampled on ten occasions between 27 June 2012 and 1 October 2013.

Table 7. End-member water sources and data used within PHREEQC modelling.

Water source	Sample location	Data used
Wetland treated stormwater	WE2	ASTR water quality database (post flushing) 2006-2011 (CSIRO)
Wetland & aquifer treated stormwater	ASR Production wells #9541 & #9542	Parafield ASR wells 2004-2009 (City of Salisbury)
	ASTR - RW1, RW2	ASTR water quality database (post flushing) 2009-2011 (CSIRO)
Mains water (chlorinated)	Little Para System	Metropolitan Adelaide water supply five year statistics 2005-2010 (SA Water)
Reclaimed water (chlorinated)	Bolivar DAF/F plant (post Cl) & Mawson Lakes Greenfield Tank Pre-Mixer (ID 14030)	Bolivar ASR injectant 4th cycle 26/8/08-16/3/09 (CSIRO) & Greenfields AWQC water quality data 2010-11 (SA Water)

3.6.2 Equilibrium modelling

Geochemical equilibrium modelling was undertaken using PHREEQC (Parkhurst and Appelo 1999). Essential to this evaluation is reliable physiochemical data for each water sample. In-situ measurements of pH, dissolved oxygen and redox potential and appropriate sample collection and preservation techniques are essential when redox sensitive species, such as iron or manganese, are considered.

Eh (V SHE) values were converted to pe for use in PHREEQC using the following relationship (eqn 1):

$$Eh\ (V) = 0.059\ pe \text{ at } 25°C \quad \text{(Appelo and Postma, 1999)} \qquad (1)$$

In the initial desktop study, Eh data was available for stormwater, after wetland treatment or wetland and aquifer treatment (ASTR mode) and reclaimed water. When Eh data was not available, a pe of 6 was adopted within the PHREEQC simulations for the mains water and pe of 0 applied for wetland and aquifer treated stormwater (ASR mode). Eh data was available for all samples collected from the experimental rig. Elevated Eh in the mains water rig on 1/10/13 did not allow the model to converge and therefore a pe of 8 was adopted, which was consistent with the general conditions in the rig.

In the desktop study total Cu, Fe and Mn concentrations were used in the input to PHREEQC for calculation of the concentration of Cu(2), Fe(2), Fe(3) and Mn(2) present under the relevant physiochemical conditions. Soluble and total Fe and Mn concentrations were available for samples from the experimental rig and therefore Fe(2) and Mn(2) were assumed to represent soluble concentrations, while the difference between soluble and total concentrations was used to represent either Fe(3) and Mn(3).

Saturation indices of mineral phases were used to examine source water propensity for precipitation or scale to form on pipe surfaces, or for dissolution of the cement lined pipes. A positive saturation index indicates a solution is super-saturated with respect to the mineral phase and will tend toward equilibrium through precipitation of that mineral. Conversely a negative saturation index indicates the solution is sub-saturated and may lead to dissolution of the mineral phase (in this case calcium carbonate mineral representing cement lined pipes). The saturation state indicates the direction a reaction may proceed, but does not mean it will definitely occur.

Saturation indices were calculated and reported for calcium carbonate (SI_{CaCO3}) and (oxy)hydroxides of iron ($SI_{Fe(OH)3}$, $SI_{Goethite}$), manganese (SI_{MnOOH}, $SI_{Mn(OH)2}$) and aluminium ($SI_{Al(OH)3}$). All phases are relevant for precipitation on pipe surfaces, while dissolution is also considered for $CaCO_3$.

3.7 Surface characterisation

The surface characteristics of the coupons after exposure to the two water sources were determined using optical microscopy, colour analysis, x-ray diffraction and elemental analysis.

3.7.1 Coupon sampling for surface characterisation

Surface characterisation was conducted on two replicates collected for each sampling date. Each coupon plug was unscrewed from the pipe rig and placed in a 32 mm x 110 mm cylindrical vial for delivery to the CSIRO Melbourne laboratories to prevent damage to the surface. All samples were retained moist in air-tight containers and refrigerated prior to analysis.

3.7.2 Colour determination

Colour is used as an indicator of changes in surface characteristics. Colour is described as hue (i.e how we perceive an object's colour, e.g. yellow, green, red etc), chroma (the vividness or dullness of a colour) and lightness (the luminous intensity of a colour, e.g. light or dark). The colour on the surface of the coupons was determined in the CIE L*a*b* colour scale using a Konica Minolta CR-300 Chroma meter colorimeter and a white tile reference standard. Colour was denoted by differences in lightness value (ΔL^*), in red/green hue (Δa^*), yellow/blue hue (Δb^*) and the total colour difference ($\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$). The average of five readings across various sections of the surface was taken as the value of each sample.

3.7.3 Dry mass of sediment

Coupons were inserted in a vial with 20mL deionised water and placed in an ultrasonic bath for 2h to remove material attached to the coupon surface. The liquid was then transferred to a pre-weighted beaker and dried at 85°C until a constant weight was determined. The normalised average dry mass was determined in mg/cm^2 of the exposed surface area of the coupons (n=2).

3.7.4 Surface morphology

Coupons were inspected using an Olympus SZX10 optical microscope (magnification up to 126x) with Dyno Capture 2.0 software. In addition, deposits on the surface of selected coupons were also examined using a 3D confocal laser microscope (Olympus OLS4000) with MPFLN10x, 20x and 50x objective lens under XYZ scanning mode.

3.7.5 Elemental composition

Micro x-ray diffraction (micro-XRD) analysis was conducted from representative deposit locations on selected coupons using a Bruker GADDS microdiffractometer at the CSIRO Clayton laboratories. For

samples of copper and cement measurements were conducted directly on the coupons and for PVC samples the sediment was scraped off the coupons for analysis. The excitation radiation was CuKα from a conventional tube source (operating at 40kV, 40mA). The instrument was equipped with a Bruker HiStar area detector, and crossed reflecting Gobel mirror incident beam optics. The beam was collimated to a spot diameter of 0.8mm and the count time in all cases was 120 sec.

X-ray fluorescence (XRF) was determined using a Tracer III-V portable light-elemental XRF analyser from Key Master Inc. The X-ray excitation source was a Rh tube, operating at 20 μA and the instrument had a removable Ti filter.

3.8 Microbial analysis

3.8.1 Sampling for microbial analysis

3.8.1.1 Sampling pilot pipe rigs

Preparation prior to each sampling event included autoclaving (20 min at 121°C) two 250 mL and four 500 mL glass schott bottles. The 250 mL bottles to be used for filtered mains and stormwater required for sampling of biofilm from coupons as well as two times 500 mL of each water type (mains and stormwater) was collected for delivery to the Perth lab.

The coupons (O-PVC, cement lined ductile iron and copper) in the stormwater and mains water pilot rigs (Figures 6-7) were periodically (~ 6 weekly) sampled for microbial analysis. The first set of biofilm coupons were removed for microbial analysis after completion of the installation and initial operating period. Subsequent samplings were conducted during the following seasons (Table 8).

Table 8. Sampling of coupons from the pilot pipe rigs

Sampling days	Season	Approximate length of stay of coupons in the pipe rig (months)	Materials
20.08.2012	winter	3[#]	All
25.09.2012	spring	4	All
29.10.2012	spring	1	Cement lined iron only
30.01.2013	summer	1.5	All
13.03.2013	autumn	3	All
30.04.2013	autumn	5	All
11.06.2013	winter	6	All
22.07.2013	winter	8	All
1.10.2013	spring	9[$]	All

# the first coupons were placed in the pipe rigs 23.5.2012
$ the second round of coupons were placed in the pipe rig 30.1.2013

3.8.1.2 Coupon sampling

Each coupon plug was unscrewed from the pipe rig and stored in a 50 mL centrifuge tube containing either baseline or stormwater (depending on which rig sampled from), the plug lids were tapped to the top of the tube and the tubes were kept upright in plastic racks in small eskies with ice packs. At least 250 mL each of raw water was collected for filtering back at the lab as well, plus the 2 × 500 mL sterile Schott bottles were filled with raw water for delivery to WA.

3.8.1.3 Biofilm sampling

Once back at the lab, 150 mL of stormwater and baseline water were individually filtered using 0.2 µm sterile syringe filters. For each coupon collected, the surfaces were gently rinsed with 1 mL of the filter sterilised site water to remove microbes in bulk solution. Biofilms were detached from the desired coupon surfaces using sterile single use cotton tip applicator buds and the buds with the removed biofilm were placed in a sterile 15 mL centrifuge tubes with 5 mL filter sterilised site water. The surface areas of the individually wiped coupons were recorded. The tubes with the cotton buds were sonicated in a sonicating water bath (Branson 1200) and the cotton buds were then vigorously twisted to release any attached biofilm. The cotton buds were scraped against the lip edges of the tubes to release any remaining biofilm into the tube. For each sampling event eight coupons were sampled and cells from each were collected into a separate tube. Two coupons from each rig (main and stormwater) were sampled for pathogen analysis conducted in Brisbane and two coupons from each rig for other microbial analysis conducted in Perth. Additionally site water was filter sterilised into sterile 50 mL centrifuge tubes and sent to Perth for use as a control in flow cytometric analysis. All samples were kept in an esky with ice bricks and shipped refrigerated to Brisbane and Perth by overnight courier.

3.8.1.4 Sampling stormwater irrigation 'field' pipe

Field samples were collected from an existing PVC irrigation pipeline (~7.5 cm inner diameter) from the Parafield ASTR site (Figure 10) on 14/6/2012. This pipeline supplied the adjacent school oval with recycled stormwater for irrigation. Prior to January 2012, this pipe carried a mix of aquifer recovered stormwater (ASR/ASTR) from Parafield and at times carried water abstracted directly from the Parafield wetland. Since January 2012, all stormwater was supplied from the aquifer (no direct supply form wetland).

As soon as possible after the pipe section was cut from the existing distribution system, biofilm was scraped off with a sterile spatula (e.g. metal spatula wiped with 70% ethanol) into four sterile 50 mL centrifuge tubes. The surface areas that were scraped for each of these tubes were measured to enable the quantification of the cell numbers per surface area.

Water from the distribution pipe was filter sterilised (0.2 µm sterile filter) and 25 mL of the filtered water was added into each of the four tubes. The samples were kept in an esky with ice blocks and two of the tubes were shipped by overnight courier to Brisbane for pathogen analysis and the other two to Perth for other microbiological analysis.

Figure 10. Biofilm present in PVC pipe containing ASR/ASTR stormwater.

Water from the existing stormwater pipe was also collected for physical-chemical analysis. A field analyser (TPS 90FL-mV) was used for determining solution pH, electric conductivity, dissolved oxygen (DO), temperature and redox potential. Free chlorine was measured using a HACH colorimeter (DR/890) and turbidity with a HACH portable meter (2100P). Total alkalinity was determined using an automatic titrator (Orion 960), total carbon (TC), total inorganic carbon (IC), total organic carbon (TOC) and total nitrogen (TN) using a Shimadzu Total Organic Carbon and Total Nitrogen Analyser (TOC-$V_{CSH/CSN}$ + TNM-1). NH_4-N, NO_2-N, PO_4-P were measured using an automated flow injection analyser (Lachat QuikChem 8500 series 2). F^-, Cl^-, Br^-, NO_3^-, SO_4^{2-} were analysed by ion chromatography using a Dionex ICS-2500 system. Ca, K, Mg, Na and S Fe, Mn, P, Si, Sr and Zn were analysed using inductively coupled plasma coupled with optical emission spectrometer (ICPOES) and Ag, Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb and Sb were measured using inductively coupled plasma coupled to mass spectrometer (ICPMS).

3.8.2 Total cell counts by flow cytometry (FCM)

The cell suspension samples for flow cytometry (FCM) were mixed well, then a 500 µL aliquot was removed and filter sterilized with a 0.8/0.2 µm Acrodisc PF Supor Membrane filter (Pall Life Sciences) for use as a control to check background counts and to use as a diluent for diluting the samples if needed. All samples were initially run diluted which involved 200 µL of undiluted sample being stained with 2 µL of stain (SYBR green) for 10 min. This was mixed well then 20 µL was removed and added to 180 µL of filtered sample to give a 1:10 dilution. As a control, aliquots of each sample were also filtered through a 0.8/0.2 µm filter and thereafter stained as described above for FCM.

All FCM experiments were performed using Cell Lab QuantaTM SC Beckman Coulter flow cytometer equipped with an air-cooled 15 mW argon ion laser, emitting at a fixed wavelength of 488 nm. Fluorescent filters and detectors were all standard with green fluorescence collected in the FL1 channel (525 nm), orange fluorescence collected in the FL2 channel (575nm) and red fluorescence collected in the FL3 channel (>670 nm). All parameters were collected as logarithmic signals (Hoefel et al., 2003). Data were analysed using Cell Lab Quanta® SC MPL Analysis software.

3.8.3 Culturable cell counts

For the plate counts the cell suspensions were mixed by vortexing, serially diluted with sterile dechlorinated tap water and plated out with sterile glass spreaders onto plates containing plate count agar (Merck, catalogue number 1.05463.0500) to check for heterotrophs, one series which was incubated at 22°C for 72 h and another series which was incubated at 37°C for 48 h. The samples were also plated out onto chromocult agar (Merck, catalogue number 1.10426.0500) to assess for thermotolerant coliforms which were incubated at 37°C for 24 h, inspected for colonies and counted, and then incubated at 45°C for a further 24 h and inspected for the growth of new colonies.

3.8.4 DNA extraction

For extracting DNA from the samples shipped to CSIRO Brisbane laboratory, the samples were vortexed for 3 min. A 2 mL sub-sample was taken and centrifuged at 6,000 × g for 3 min. The DNA was extracted from the resulting pellet with ZR Soil Microbe DNA MiniPrep™ according to the manufacturer's instruction (ZYMO, USA). The resulting DNA was stored at -80°C and process in a single batch. Each DNA sample was amplified using a universal bacterial PCR assay to confirm successful DNA extraction process (Heuer *et al.*, 1997).

For extracting DNA from the cell suspension samples from swapped coupons shipped to CSIRO Perth laboratory, the samples were sonicated for 5 min and then mixed by vortexing for 30 s. A 5 mL (for field samples) or 4 mL (for pipe rig samples) aliquot of each well mixed sample was centrifuged at 16060 g for 1 min to get a pellet from which DNA was extracted using a Mo Bio Power Soil kit (Mo Bio Laboratories, USA).

The bulk water samples (approximately 500 mL) were filtered onto 0.22 µm Millipore filters. The cells captured on the filters were resuspended into the 1 mL of original water sample. The two replicate concentrated stormwater aliquots were then mixed together and the two concentrated baseline water samples were mixed together. These 2 mL lots were centrifuged at 16060 g for 1 min and resuspended in 500 µl of the supernatant.

3.8.5 Detection of pathogens

3.8.5.1 Positive controls for polymerase chain reaction (PCR)

Aeromonas hydrophila ATCC 7966, *Campylobacter coli* ATCC 43478, *Legionella pneumophila* ATCC 33152, and *Salmonella* serovar Typhimurium ATCC 14028. *C. jejuni* NCTC 11168, *Clostridium perfringens* ATCC 13124, *Pseudomonas aeruginosa* ATCC 27853, *Cryptosporidium parvum* ATCC PRA-67D, *Shigella sonnei* ATCC 29930 were used as positive controls.

3.8.5.2 PCR and melt curve analysis

PCR amplification was performed in 20 µL reaction mixtures using Sso Fast™ EvaGreen® Supermix (Bio-Rad Laboratories, CA, USA). The PCR mixture contained 10 µL of Supermix, 200-300 nM each forward and reverse primer (Table 20 in Appendix 3) and 300 nM probe, DNase- and RNase-free de-ionized water, and 3 µL of template DNA. For each PCR experiment, corresponding positive (i.e., target DNA) and negative (sterile water) controls were included. A quantitative PCR (QPCR) melt curve analysis was performed after each PCR run to differentiate between actual products and primer-dimers, and to eliminate the possibility of false-positive results. The melt curve was generated using 80 cycles of 10 s each starting at 55°C and increasing in 0.5°C intervals to a final temperature of 95°C. The melting temperature (T_m) for each amplicon was determined using the iQ5 software (Bio-Rad).

3.8.5.3 PCR inhibitors

All samples were tested for the potential presence of PCR inhibitors. Ten-and one hundred fold serial dilutions were made for each sample and were tested for the presence of bacteria with universal primer set (Heuer et al., 1997). The cycle threshold (C_T) values obtained from undiluted and serially diluted (10 and 100 fold) samples were compared to check for the presence of PCR inhibitors in the extracted DNA from biofilms.

3.8.5.4 Detection of nontuberculous mycobacteria and Legionella in water samples

QPCR was used to detect nontuberculous *Mycobacteria* (NTM) and *Legionella* spp. from all bulk water and biofilm samples collected for the study.

3.8.5.4.1 NTM primers

Oligonucleotide primers targeting the 16S rRNA gene of NTM were used to amplify gene fragments of 1030 bases to quantify *Mycobacteria* spp. Primers were obtained from GeneWorks (GeneWorks, Australia). The primer sequences and names are detailed in Table 21. in appendix 3 (Wilton and Cousins 1992).

3.8.5.4.2 Amplification conditions for NTM with general NTM primers

QPCR reactions for amplification of NTM using general NTM primers were carried out in 25 µL volumes containing: 12.5 µL Hot Star Taq Master Mix (Qiagen, U.S.), 250 nM MYCGEN-F, 250 nM

MYCGEN-R, 2 µM SYTO9 (Molecular Probes, U.S.), 2 µL DNA template and sterile double distilled H_2O to make up the final 25 µL volume (Wilton and Cousins 1992). Samples were amplified with positive and negative controls. *M. avium* (M8867 8.2); *M. avium* (ATCC 25291 8.2); *M. avium* (NJ9141 8.2) reference strains obtained from Dr Frank Haverkort, (PathWest, WA), were used as positive controls and ddH_2O (Qiagen, U.S.) was used for negative controls. DNA samples were amplified in triplicates and an average of those triplicates was used for analysis. The QPCR amplification was performed in an iQ5 Real-Time PCR Detection System (BIO-RAD, U.S.) under the following conditions: pre-denaturation for 15 minutes at 95 °C, then 50 cycles of denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 62 °C, extension for 1.5 minutes at 72 °C, followed by a 6 second pause at 80 °C for fluorescent dye detection (Wilton and Cousins 1992).

3.8.5.4.3 *Legionella* spp. primers

Oligonucleotide primers targeting the ribosomal binding site or open reading frame region and the peptidylprolyl cis/trans isomerase (PPIase) site of the *mip* gene (Helbig et al., 2003) were used to amplify a gene fragment of 661-715 base to identify *Legionella* spp. The primer sequences and names are detailed in Table 21.

3.8.5.4.4 Amplification conditions for *Legionella* spp.

A modified version of Ratcliffe *et al*'s methods was used to amplify the *mip* gene of *Legionella* spp. (Ratcliff, Lanser et al. 1998). Reactions were carried out in 25 µL volumes containing: 12.5 µL of Hot Star Taq Master Mix (Qiagen, U.S.), 500 nM LEGMIP-F, and 3.75 µM LEGMIP-R, 2 µM of SYTO9 (Molecular Probes, U.S.), 2 µL of DNA template. DNA from *L. pneumophila* (ATCC 33152 A 8.2) and *L. pneumophila (*ATCC 3315 B 8.2) reference strains, obtained from the Dr Frank Haverkort, (PathWest, WA), were used as positive controls and ddH_2O (Qiagen, U.S.) was used for negative controls. Samples were amplified in triplicate and the average values were used in the analysis. The QPCR amplification was performed in an iQ5 Multicolour Real-Time PCR Detection System (BIO-RAD, U.S.) under the following conditions: pre-denaturation for 15 minutes at 95 °C, then 35 cycles of denaturation for 1 minute at 94 °C, annealing for 2 minutes at 58 °C, extension for 2 minutes at 72 °C, followed by a 6 second pause at 80 °C for fluorescent dye detection (Ratcliff, Lanser et al. 1998).

3.8.6 Pyrosequencing and data analysis of 16S rRNA and 18S rRNA genes

The field pipe samples and selected samples (January, March and June 2013) from the pilot pipe rigs were submitted for pyrosequencing. The DNA samples for pyrosequencing (454 sequencing) were mixed with DNAstable Plus in a 1:4 ratio (1 part DNA Stable Plus and 4 parts DNA) to prevent degradation at room temperature and shipped to Molecular Research LP in the USA for 16S rRNA gene and 18S rRNA gene pyrosequencing.

The primers used were 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 530R (5'-TTWGGTTTAATWGTACARCC-3') for the 16S eubacterial sequences and 7F (5'–TTGCCGTCCCAAGCAATGGATG–3') and 507R (5'- GCCACCCCAATGAGGCACAGG-3') for the 18S eukaryotic sequences and were supplied by Molecular Research LP. Amplicon pyrosequencing (bTEFAP) was performed as originally described by Dowd *et al.* (2008). A single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute; after which a final elongation step at 72°C for 5 minutes was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing Roche 454 FLX titanium instruments and reagents and following manufacturer's guidelines. The Q25 sequence data derived from the sequencing process was processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Sequences are depleted of barcodes and primers then short sequences < 200bp, sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp were all removed. The sequences were then

deniosed and chimeras removed. Operational taxonomic units (OTU) were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity) (Dowd *et al.* 2008a, Dowd *et al.* 2008b, Sun *et al.* 2008, Edgar 2010, Capone *et al* 2011, Dowd *et al* 2011, Eren *et al* 2011, Swanson *et al* 2011). OTUs were then taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis, Hugenholtz *et al.* 2006) and compiled into each taxonomic level into both "counts" and "percentage" files. Counts files contain the actual number of sequences while the percent files contain the relative (proportion) percentage of sequences within each sample that map to the designated taxonomic classification. Data was further analyzed using the percentage of sequences within each sample and compared.

3.8.7 Amplification of bacterial 16S rRNA genes and denaturing gradient gel electrophoresis

An initial PCR was performed on the extracted DNA using 27F and 1492R primers with 2 µL of template in a 50 µL reaction volume with HotStarTaq Master Mix (Qiagen) and 1 µL of each primer (25 µM) (Table 22). The amplified was conducted on PTC-200 Thermal Cycler (MJ Research) and the PCR consisted of an initial 15 min denaturing step at 95 °C; 35 cycles of 95 °C for 1 min; 48 °C for 1 min; 72 °C for 2 min; then 72 °C for 10 min. After this, 5 µL the PCR products were visualised on a 1% agarose gel run at 90 V for 40 min and then cleaned up using the Mo Bio Ultra Clean kit. The cleaned products were visualised again on a 1% agarose gel run for 40 min at 90 V. The cleaned products were amplified by PCR again using 1 µL BACV3f+GC and 907R primers (25 µM), 0.5 µL of template in a 50 µL reaction volume and run on the thermocycler. The temperature program for the second PCR consisted of an initial denaturation step of 95 °C for 15 min; 20 cycles of 94 °C for 1 min; 63 °C for 1 min and 72 °C for 1 min. The annealing temperature of 63 °C was decreased by 1 °C per cycle until it reached 53 °C, following 9 cycles with annealing temperature of 53 °C. This was followed by a final extension step at 72 °C for 10 min. The PCR products were visualised again using the same gel run parameters as before.

Denaturing gradient gel electrophoresis was performed using the DCode Universal Mutation Detection System (BIORAD) as per the manufacturer's instructions. A 20 µL aliquot of product was mixed with 4 µL of 5 X DNA Loading Buffer (BIOLINE) and loaded onto a 7 % (w/v) polyacrylamide gel (40 % acrylamide/bis solution, 37.5:1). For the initial gel, the gradient 30 to 80 % (100 % denaturant: 7 M urea, 40 % deionised formamide). A second gel was prepared with 40-60% gradient to achieve a better separation of DNA bands. Gels were run at 150 V (60 °C) over night. Gels were then stained for 10 min with SYBR Gold (1:10,000 dilution, Molecular Probes, Eugene, OR) and visualized with a blue light table and a Gel DocTM 2000 gel documentation system (BIORAD).

Discrete bands were excised from each lane of the second gel using sterile scalpel blades. The bands were resuspended in 40 µL sterile ddH2O. A subsequent PCR step was performed to amplify DNA contained in bands using 1 µL primers BACV3F with no GC clamp and 907R (25 µM) (Table 10) and 1 µL of template in a 50 µL reaction volume. PCR was carried out using the program described above for the first PCR. Products were visualised by agarose electrophoresis and sent to Macrogen (South Korea) for DNA sequencing.

The 16S rRNA gene sequences were edited using ChromasPro software. The closest relatives of the species were identified by comparing the sequences to those in GenBank database (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) program (Altschul *et al.*, 1997).

4 Results and discussion

4.1 Physico- chemical conditions

The summary of the in-line monitoring data (pH, Temperature, EC and ORPis summarised in Appendix 1. The water quality of grab samples is provided in Appendices 3, 4 and 6.

4.1.1 Temperature

Temperatures in the rigs for period 1 (Winter and Spring 2012) and period 2 (Summer, Autumn, Winter and Spring 2013) ranged between 16 to 24.8°C, with a median of 21°C, and between 14 to 36°C, with a median of 24.4°C (Appendix 1).

Seasonal variability affected the water temperature in the rigs. Figure 11 shows the day and night temperatures across the seasons for period 2, with the highest temperature averages in summer (29.8±2.9°C) and the lowest in winter (19.0±1.52°C). During autumn and spring temperature averages ranged in the low to mid twenties and were subject to large variability. Overall, temperature was consistent within a rig across the seasons and was within the normal variability range (Figure 42 a, Appendix 1). The temperature of the grab samples from both rigs ranged from 12.1 ± 1°C in June 2012 and 29.6 ± 1°C in March 2013 (Appendices 3 and 4). Further analysis of diurnal trends is provided in Appendix 6.

In comparison, the minimum and maximum ambient daily temperatures in 2013 were 0.9°C and 44.7°C, respectively (Appendix 5). Thus the buried rig provided some insulation from the ambient, although the temperature maxima and variability exceeded the typical values (≤ 20°C) for buried mains distribution (SA Water 2013a), the temperatures were still within the range observed for water at the customer tap (SA Water 2012). Such temperatures favour bacterial growth (Torvinen *et al.* 2007).

4.1.2 pH

The mean pH in periods 1 and 2 was respectively 6.4±0.2 and 8.2±0.2 for baseline water and 7.0±1.0 and 6.6±0.5 for stormwater (Appendix 1). The pH in the baseline rig during period 2 was neutral to slightly basic (7.6 to 8.7), whilst in the stormwater rig the range of 4.1 to 9.0 was wider particularly due to summer and spring readings (Figure 12). Over a 24h period low pH variability was observed (see Figure 42 b, Appendix 1).

Grab samples for both baseline and stormwater rigs were in the neutral range (within 6.5 to 8.5) recommended by the ADWG guidelines (NHMRC–NRMMC, 2011), shown in Figure 13.

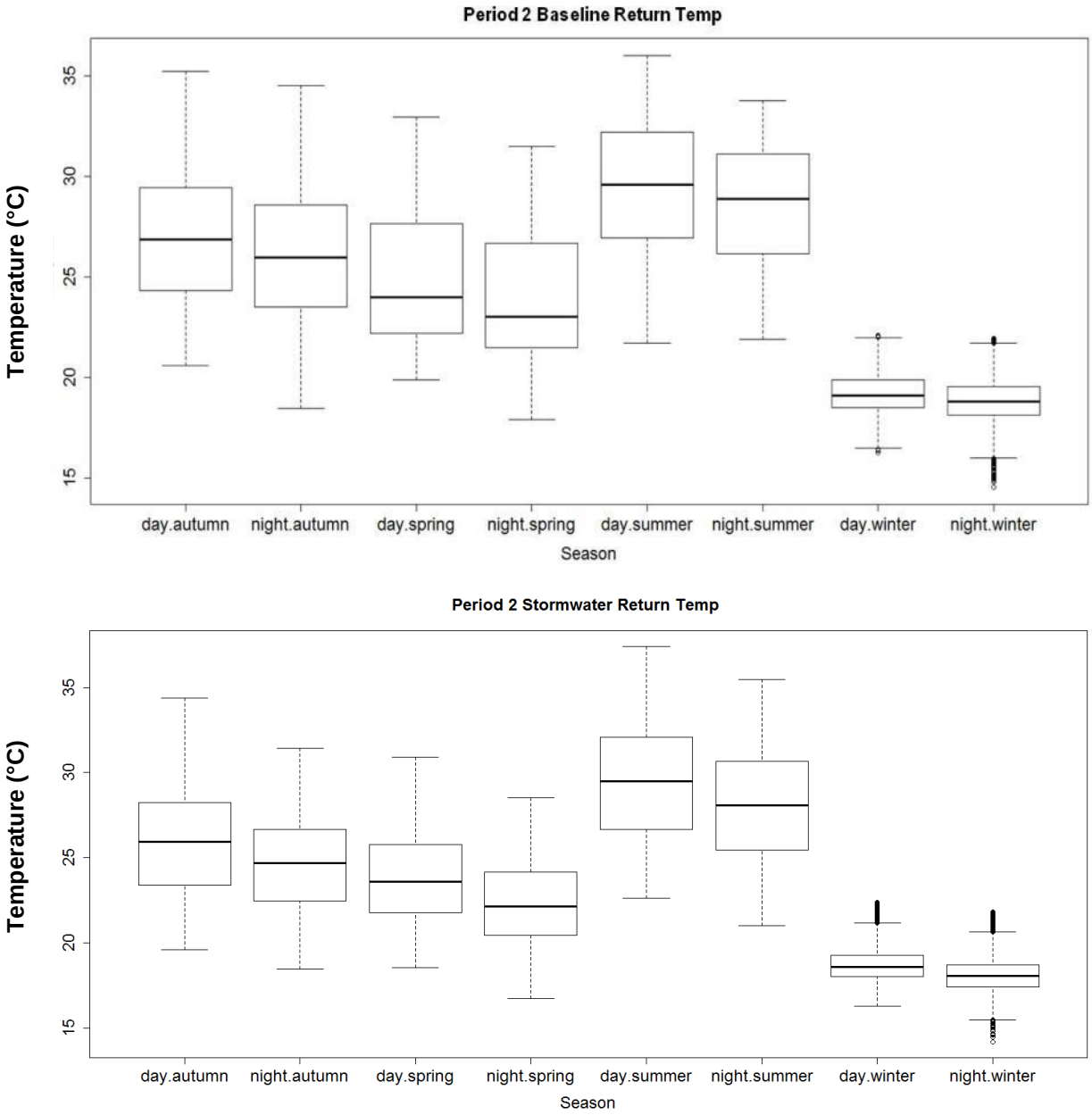


Figure 11. Seasonal temperature characteristics in the rigs for period 2 (December 2012 to October 2013).

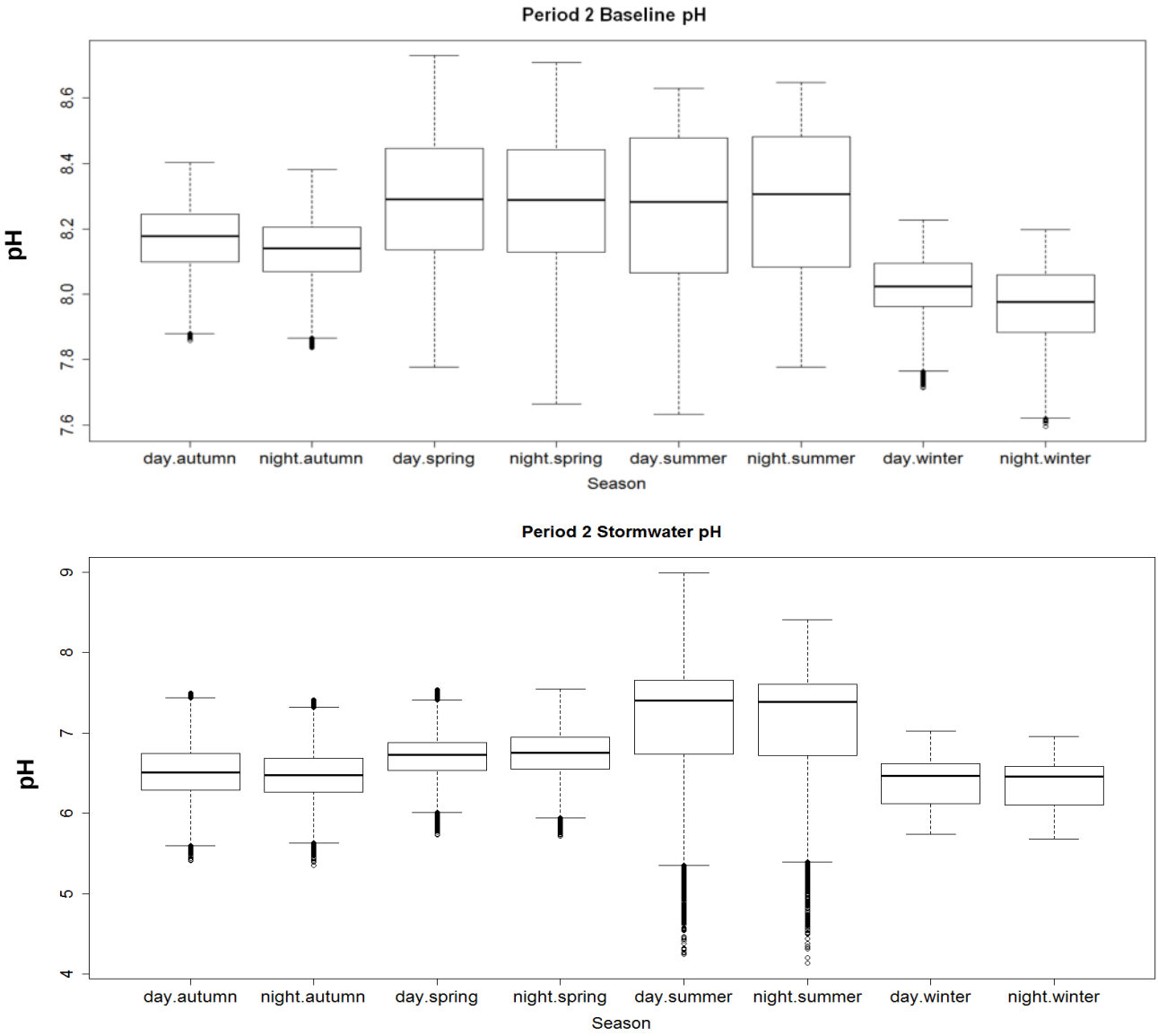


Figure 12. Seasonal pH characteristics in the baseline and stormwater rigs for period 2 (December 2012 to October 2013)

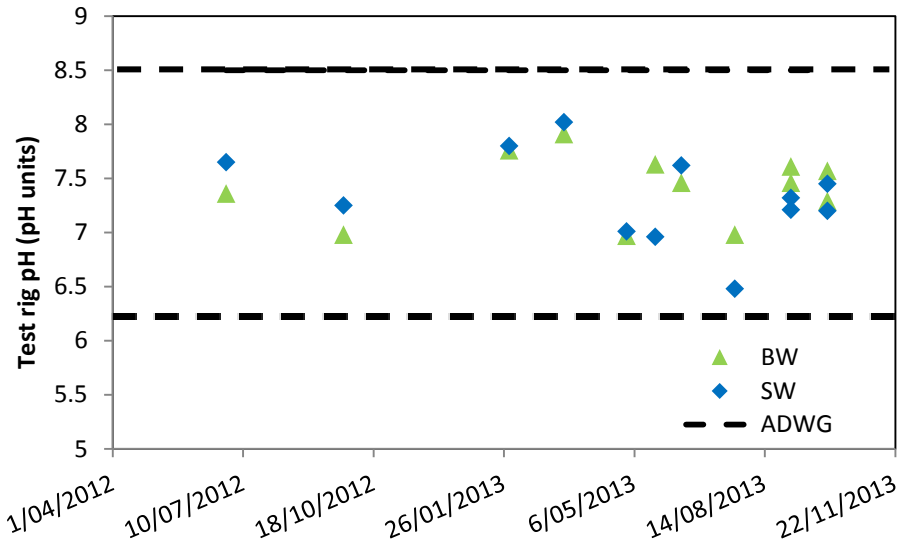


Figure 13. pH measured from grab sampling from the baseline rig (BW) and stormwater rig (SW).

4.1.3 Electrical conductivity and TDS

For MAR operation, salinity is used as an indication of detention time. The salinity of the recovered water is managed by cessation of recovery at a set limit for electrical conductivity which can be measured continuously. In addition, the volume of water that can be recovered under this salinity limit is optimised by leaving a residual of injectant in the aquifer in between recovery cycles which creates a buffer zone, containing a mixture of fresh stormwater injectant and brackish ambient groundwater (Page *et al* 2013).

In the rigs, electrical conductivity (EC) ranged from 232 to 825 µS/cm in the baseline water and 155 to 1367 µS/cm in stormwater for the duration of the experiment (Appendix 1). However, the median EC in period 2 was respectively 490 µS/cm and 642 µS/cm in the baseline and in the stormwater rigs, the latest value is comparable to the median EC after ASR (646 µS/cm; Appendix 3). In contrast, grab samples during the entire experiment showed a slightly lower EC in the stormwater rig (440 µS/cm) than in the baseline rig (530 µS/cm). While the median salinity in both rigs was similar, more variability in EC was observed for stormwater through both continuous monitoring and grab sampling (Figure 14 and Appendix 1).

The EC in the stormwater rig was lower in spring and winter and higher in autumn and summer. This was attributed to the seasonal nature of injection and extraction cycles with more injection over wet months and more extraction in dry months. Stormwater was injected into the aquifer via the Parafield ASR and ASTR systems between May and October 2013. Water recovered over this period is likely to be fresher as less mixing between fresh stormwater and brackish ambient groundwater in the T2 aquifer occurs. During dry months, less injection and more extraction occurs leading to higher salinities of recovered water. The salinity of stormwater recovered from the aquifer is managed by cessation of recovery upon reaching an upper salinity limit.

Total dissolved solids (TDS) calculated from the EC of grab samples was 289$\pm$44 mg/L and 286$\pm$161 mg/L for respectively the baseline and stormwater, which is below the aesthetic ADWG value (NHMRC–NRMMC, 2011) (Figure 15).

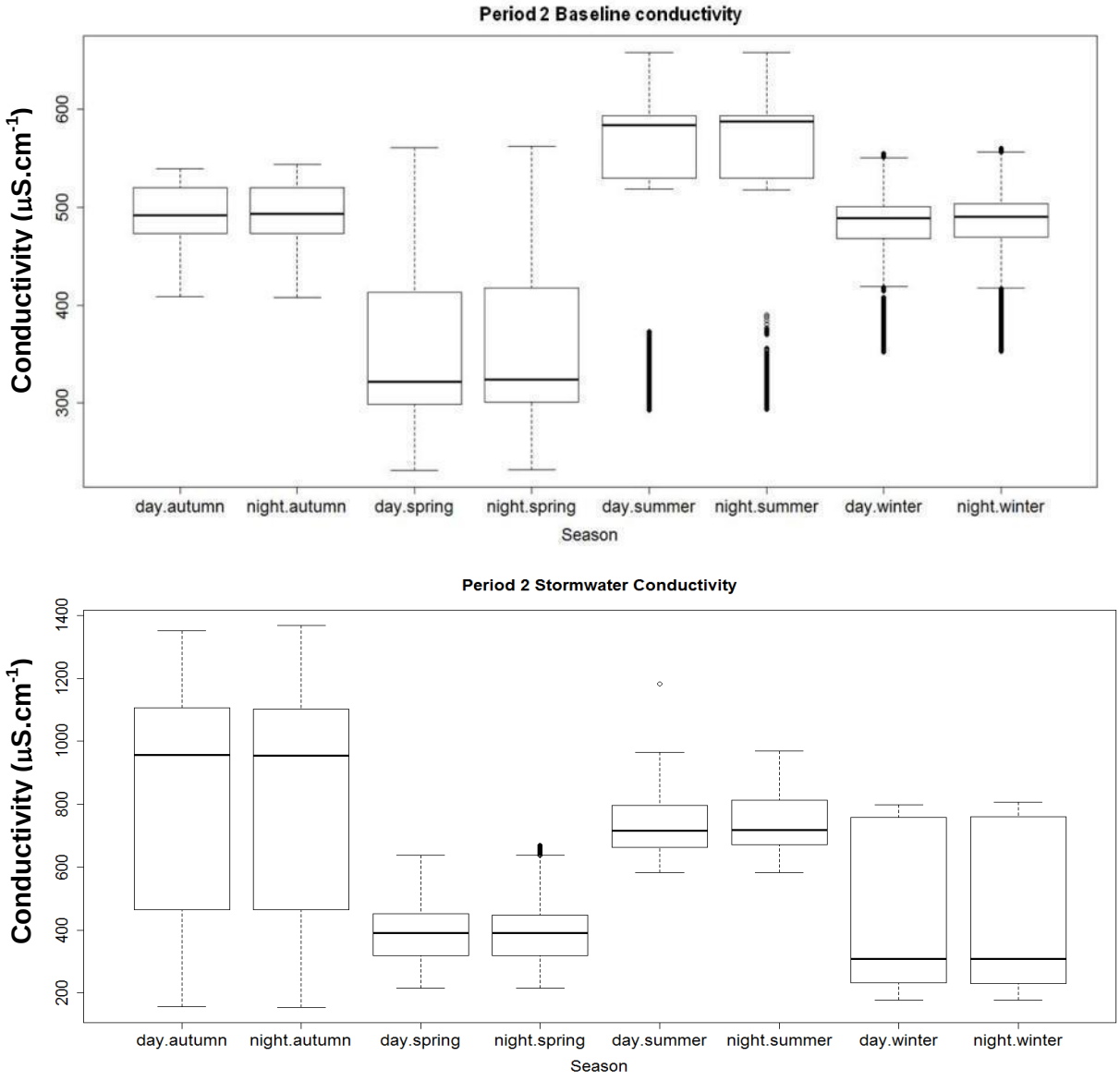


Figure 14. Seasonal changes in electrical conductivity in baseline and stormwater rigs for period 2 (December 2012 to October 2013).

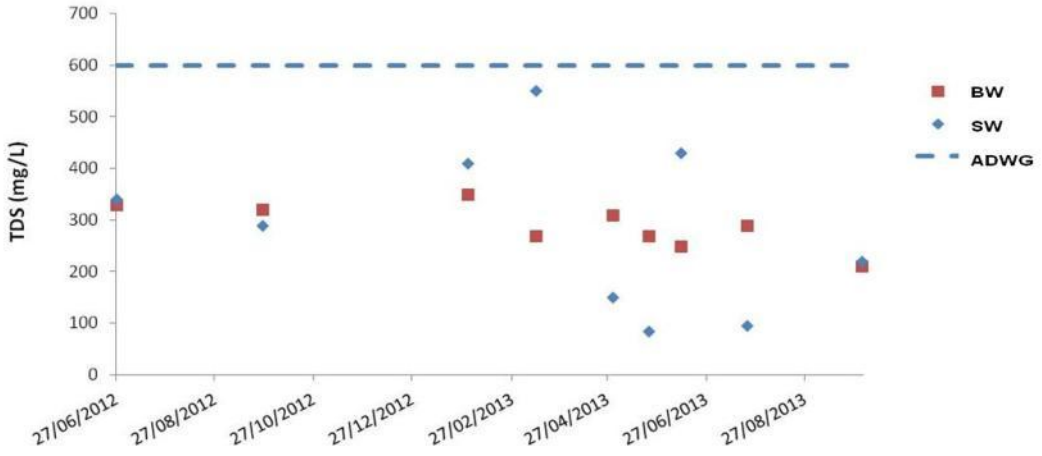


Figure 15. Total dissolved solids of grab samples from exposure rigs for stormwater (SW) and baseline water compared to the Australian Drinking Water Guideline (ADWG).

4.1.4 Dissolved oxygen and free chlorine

The dissolved oxygen (DO) concentrations in the two rigs was similar, ranging from 2 to 9.6 mg/L and 3.7 to 9.1 mg/L for the baseline and stormwater rigs respectively (Appendix 3 and 4). Monitoring a recirculation cycle in both rigs showed no notable change in DO following passage through the rig (Figure 47, Appendix 6). During that interval, DO mean concentrations were 7.1±0.40mg/L (09:00-15:00) and 6.6±1.28 mg/L (09:00-11:00) in the stormwater and baseline rigs respectively.

The stormwater had not been chlorinated. Furthermore, chlorine concentrations recorded in the baseline rig on the 14 Feb 2013 for the baseline water supply and during rig circulation and for grab samples during the study period had a mean residual of 0.03±0.01mg/L (Figure 46, Apendix 6 and Appendix 1), below the recommended 0.2 mg/L ADWG health values (NHMRC–NRMMC, 2011), thus baseline water would be classified as undisinfected in water distribution.

4.1.5 Redox potential

Redox potential was continuously monitored in the rigs and was positive during periods 1 and 2 (Appendix 3 and 4 and Figure 48, Appendix 6). Redox indicators and dissolved oxygen concentrations indicate that conditions within the rigs remained aerobic during those test periods. However, during the shut-down period (period 0) negative redox values occurred, particularly in the stormwater rig, indicating the development of anaerobic or anoxic conditions at the time (Appendix 1 and 6).

4.2 Chemical evaluation

Water quality data sampled from the baseline water and stormwater rigs are presented in Appendix 3. Typical water quality for each source is presented for comparison (Page *et al.* 2013).

The major ion composition of the baseline rig is compared to average quality for treated water from the Little Para reservoir, while the stormwater rig is compared to the median quality recovered from Parafield ASR operation (Figure 16). The major ion composition of stormwater after wetland and aquifer treatment can vary considerably due to the amount of mixing between the wetland treated stormwater and the ambient brackish, groundwater within the T2 aquifer. In addition, the composition of stormwater supplying the rig can also vary depending on the origin and volume of water supplied to the ASR/ASTR holding tank at SA Water.

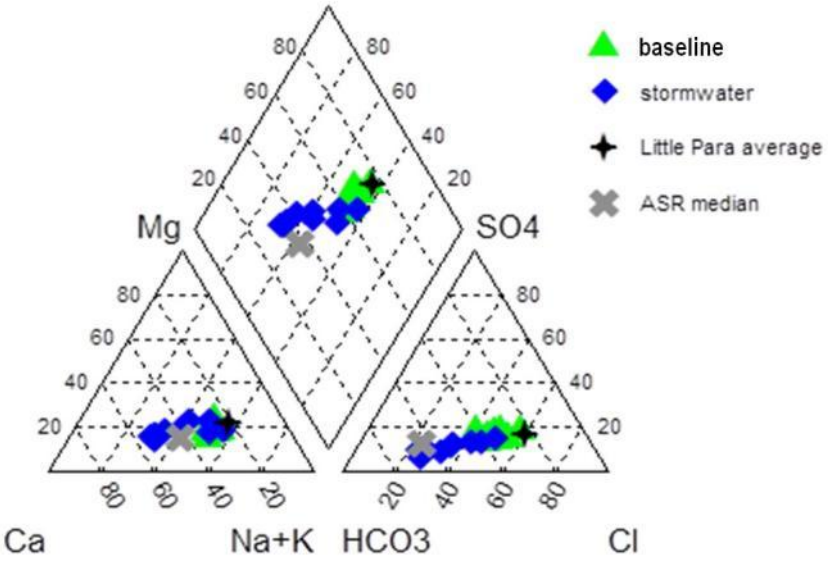


Figure 16. Piper diagram showing the major ion composition within the baseline water and stormwater rigs.

The quality of water circulating through the rig was generally consistent with the quality for the Little Para treated drinking water supply (baseline rig) and wetland and aquifer treated stormwater (stormwater rig), assessed within the desktop evaluation of chemical interactions. Notable exceptions were elevated concentrations of iron and copper (Figure 17) and to a lesser extent, turbidity within the baseline rig and elevated concentrations of dissolved oxygen within the stormwater rig.

The treated stormwater within the rig had a higher dissolved oxygen concentration (4-9 mg/L) (section 4.1.4) than expected for stormwater after storage within an anoxic aquifer (Appendix 5). The concentrations within the rig were also greater than the 95th percentile dissolved oxygen concentration of 2.4 mg/L, measured from the Salisbury ring main which distributes stormwater from the same origin (Page *et al.* 2013), suggesting oxygenation has occurred during transfer of the water from the mixing tank to the rig. The oxygenated condition within the stormwater rig results in many occasions where insoluble iron is dominant, whereas stormwater following aquifer storage is typically dominated by soluble iron species. Page *et al.* (2013) recommends aeration as a treatment step to manage elevated soluble iron concentrations in stormwater after aquifer storage. If this treatment step was adopted it would be reasonable for stormwater to have dissolved oxygen concentrations similar to those observed in the rig. Use of chlorine to oxidise and subsequently remove soluble iron could also be employed, with the dual benefit of providing disinfection while removing iron. Either way, oxidation would be followed by removal of insoluble iron, by filtration or sedimentation, prior to distribution.

A comparison between the quality of baseline water entering and within the rig on 3 September 2013 revealed elevated total iron (0.72 mg/L) and total copper (0.63 mg/L) in the water entering the rig, also coincident with elevated turbidity. A lower soluble iron concentration of 0.013 mg/L confirmed that the elevated levels were within the particulate fraction. While soluble copper was not measured on this occasion, it is likely that the high values are also due to insoluble material. This suggests contamination by particulate iron and copper prior during delivery to the rig. Conversely, another comparison on 1 October 2013 revealed higher iron (0.77 mg/L), copper (0.72 mg/L) and turbidity (12 NTU) within the rig than in the water entering the rig. On this latter occasion, it is likely that accumulated particulate matter, originating from the source water, dislodged from the pipe surface, also adding other metals such as Al, Mn, Ni and Zn and TOC. The high turbidity within the baseline rig on this occasion was due to fine particles with median particle size of 20 μm.

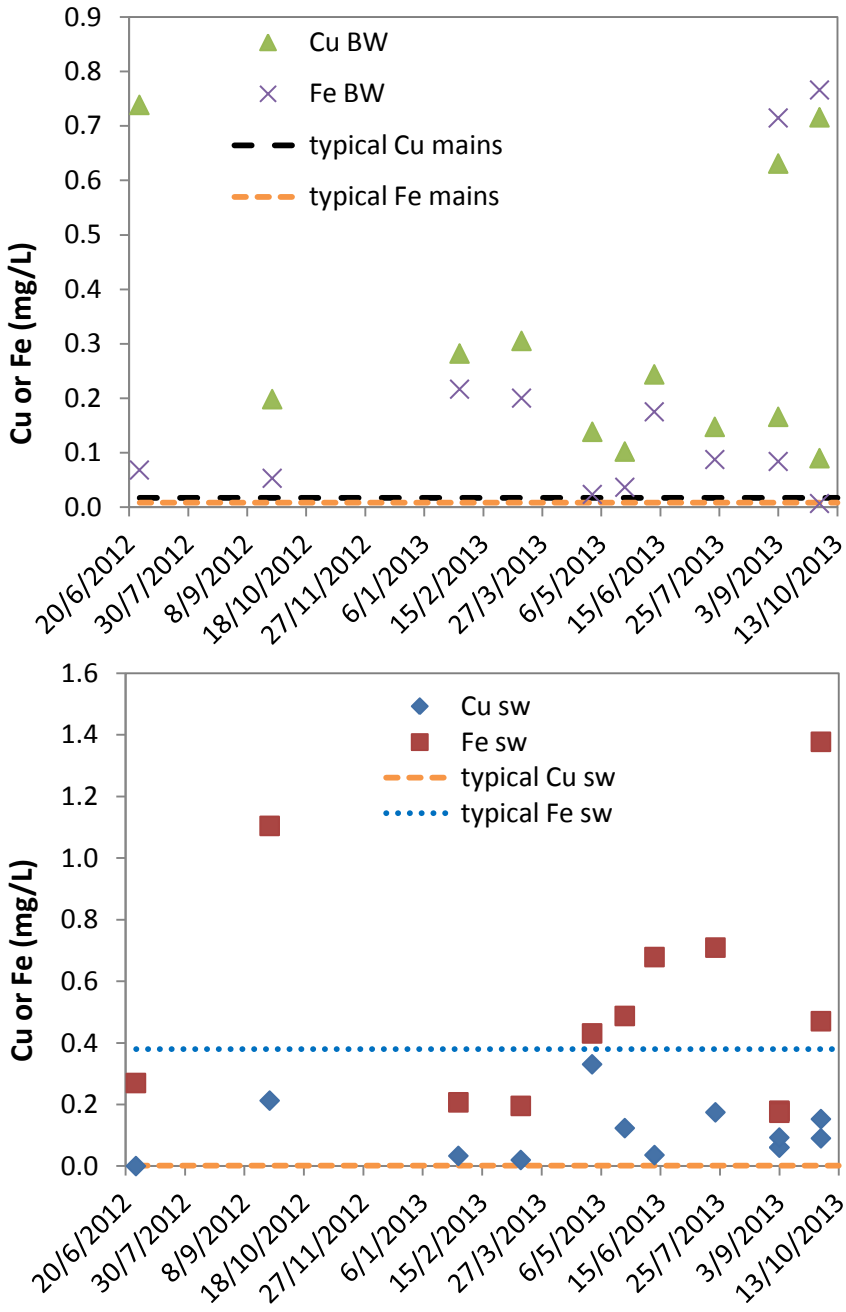


Figure 17. Copper (Cu) and iron (Fe) concentrations in the in the a) baseline water (BW) and b) stormwater (sw) rig; both in comparison to typical concentrations in mains water and stormwater.

4.2.1 Copper

The Australian Drinking Water Guidelines (ADWG) (NHMRC–NRMMC, 2011) advise an aesthetic guideline for pH from 6.5-8.5 to prevent corrosion (at low pH) and excess scale build up (high pH). With this in mind, a pH <6.5 can be considered as the most aggressive toward copper pipes. Disinfection via chlorination prior to reticulation may lead to a slightly more acidic pH. However, this was not shown in the baseline water rig where the chlorine residual was low (<0.03 mg/L) and the pH measured in grab samples remained above 6.5.

Of the eight sources of water assessed, wetland treated stormwater exhibited the potential for the lowest pH, with a minimum value of 6.5. While this meets the lower ADWG value the low alkalinity wetland treated stormwater may also require pH adjustment to manage corrosion if chlorination was to be employed. Additional storage of stormwater in a carbonate aquifer provides pH and alkalinity buffering, which is considered sufficient to buffer any acidity that may result from chlorination prior to distribution.

All waters sampled within the experimental rig maintained pH between pH 6.5 and 8.0 and are therefore not considered a high risk for chemically induced copper dissolution. However, as discussed earlier the water samples collected from the baseline water rig had higher copper concentrations than expected for this source water, likely due to contamination during transit to the rig. As a result the copper concentrations in the stormwater rig (<0.001-0.3 mg/L) were generally lower than in the baseline water rig (0.1-0.7 mg/L), but both remained below the 1 mg/L aesthetic guideline value.

4.2.2 Carbonate

The solution chemistry adopted for the end-member waters within the desktop evaluation indicated little potential for precipitation of calcium carbonate scale within pipes (tendency for precipitation is indicated by super-saturation with respect to calcite and pH>8.5).

In contrast, wetland treated stormwater and reclaimed water, on occasions, can be aggressive and induce calcium carbonate dissolution (tendency for dissolution is indicated by sub-saturation with respect to calcite). The wetland treated stormwater alone, or blended with another source of water, illustrated the greatest potential to induce dissolution of calcium carbonate in cement lined pipes. The aquifer treatment step provides buffering and thus recovered water is near to equilibrium with respect to carbonate minerals and less reactive toward cement pipes.

The calcite saturation index ($SI_{Calcite}$) of water within the rig varied between -0.03 and -1.1 for the baseline water and between 0.6 and -1.9 for the stormwater (Figure 18). Overall the water in the rig was predominantly sub-saturated with respect to carbonate minerals, which could lead to dissolution of a carbonate surface, such as cement lining. The $SI_{Calcite}$ of grab samples collected from the rigs suggested that stormwater after wetland and aquifer treatment was more aggressive than mains water.

Storage of stormwater in an aquifer containing carbonate minerals is expected to produce treated stormwater that is near to equilibrium with carbonate minerals, as shown by the median calcite saturation indices after ASR (-0.3), ASTR (-0.1) and from the Salisbury ring main (-0.4). However, the stormwater rig generally exhibited greater sub-saturation with respect to carbonate minerals than expected for wetland and aquifer treated stormwater. This sub-saturation may be indicative of a brief storage period in the aquifer, which was not sufficient to reach an equilibrium condition or a gain of (biogenic) carbon dioxide. $SI_{Calcite}$ less than -0.6 was coincident with EC below 300 μS/cm, closer to stormwater after the wetland (median EC = 240 μS/cm) than after the wetland and aquifer (median EC after ASR = 646 μS/cm) (Figure 19). This suggests insufficient storage time in the aquifer to reach equilibrium with carbonate minerals. Treated stormwater also exhibited some potential for calcite precipitation, shown by super-saturation with respect to calcite on some occasions.

There was a clear relationship between $SI_{Calcite}$ and pH (measured in the field), suggesting pH could be used as an indicator of reactivity toward carbonate material for both the stormwater and mains water (Figure 20). Above pH 7.7, the stormwater rig illustrated a tendency for calcite to precipitate, which could contribute to calcium carbonate scale. Maintaining pH ≤7.7 is also favourable for the effectiveness of disinfection by chlorination.

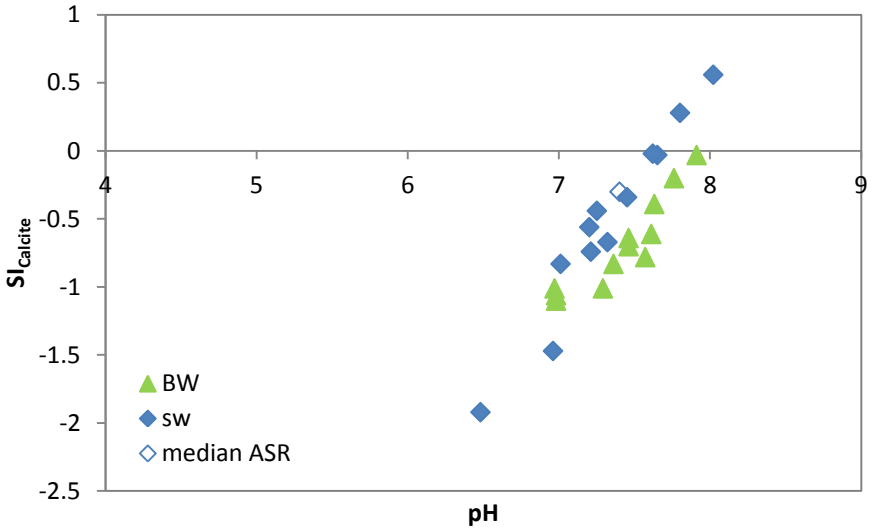


Figure 18. Relationship between the calcite saturation index ($SI_{Calcite}$) and pH in the baseline water (BW) and stormwater (sw) rig.

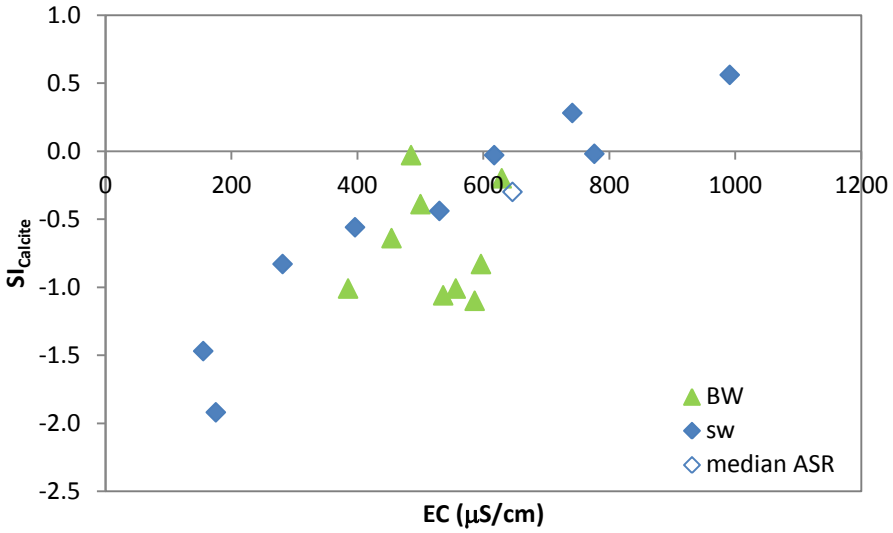


Figure 19. Relationship between the calcite saturation index ($SI_{Calcite}$) and electrical conductivity (EC) in the baseline water (BW) and stormwater (sw) rig. The median ASR quality is shown for comparison to the stormwater rig data.

Biogenic carbon dioxide production within the rig can also lead to sub-saturation. It is feasible that organic carbon in stormwater (see section 4.2.4) could stimulate more biological activity in the stormwater rig than in the baseline rig. The comparisons of water entering the rig and within the rig indicated a tendency toward increased sub-saturation within the rig, shown by a 0.1 to 0.2 unit decrease in the calcite saturation index and a reduction in pH. This increase in sub-saturation could be explained by an addition of carbon dioxide within the rig itself, due to microbial respiration. However, this has a minimal impact on the calcite saturation index of water in comparison to short residence time in the aquifer (as suggested by salinity.

During rig operation a decline in $SI_{Calcite}$ coincided with an increase in cell number, indicating the potential for greater biological activity and the subsequent production of biogenic carbon dioxide. However, the resultant sub-saturation then leads to dissolution of carbonate and loss of mineral surface, which in turn reduces the cell numbers.

Deposition on the surface was evident for both rigs, shown by the presence of crystalline forms of carbonate (calcite and aragonite) not present within deposition on other surfaces (see section 4.3.2.3). This suggests reaction with the carbonate in the cement coupon involving both dissolution and precipitation of carbonate minerals, indicating that concrete pipes would be involved in reaction.

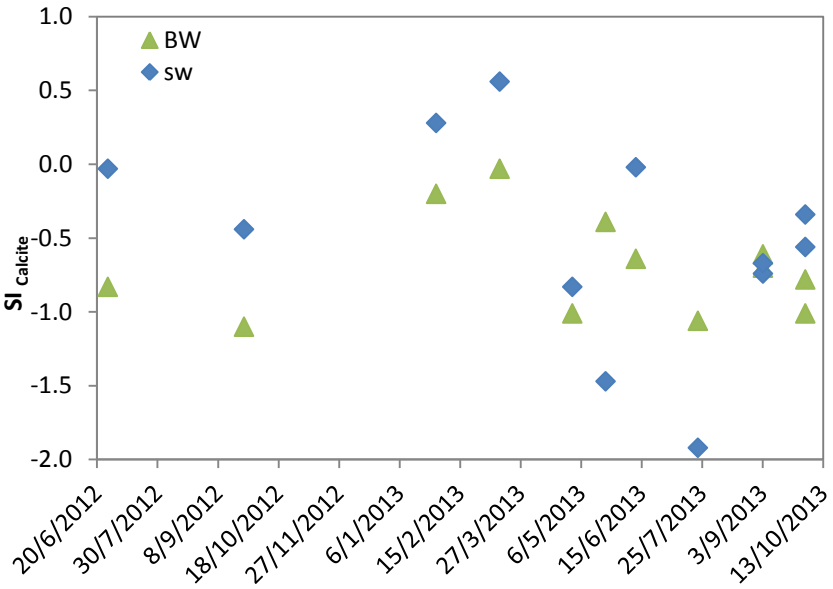


Figure 20. Calcite saturation index ($SI_{Calcite}$) in the baseline water (BW) and stormwater (sw) rig.

4.2.3 Iron, manganese and aluminium

Iron concentrations in the baseline water rig (range 0.02-0.8 mg/L, median 0.09mg/L) were slightly lower than the stormwater rig (range 0.2-1.4 mg/L, median 0.49 mg/L), but both had occasions when the 0.3 mg/L aesthetic guideline value was exceeded.

The comparison between water entering the rig and within the rig revealed an increase in metals (Fe, Mn, along with Cu, Ni, Zn) for both the baseline and stormwater rigs on 1 October 2013. This impacted on both total and soluble concentrations, but was more pronounced for the insoluble fraction. This was also evident for turbidity and TOC and is consistent with physical removal of biofilm from the rig surface.

The potential for iron, manganese and aluminium to precipitate as (oxy)hydroxides was examined as this can contribute to scale build up on pipes. In addition to this soluble iron concentrations are considered for the impact on the aesthetic water quality issue of colour.

Water recovered from the aquifer is anoxic and therefore may require oxygenation to meet the ADWG target of greater than 85% saturation to prevent aesthetic concerns caused by the presence of anaerobic microorganisms. Iron(3) precipitates are likely to form when treated stormwater is reticulated if oxygenation occurs, on its own or within a blend. It is expected that blending with oxygenated water sources (i.e. mains or reclaimed water) or storage and transfer measures during reticulation, such as splash entry into mixing tanks, would provide an opportunity for dissolved oxygen to enter the solution. This was evident in the stormwater rig, where dissolved oxygen concentrations were higher than expected for stormwater immediately after aquifer storage (Appendix 5). Chlorination will also increase the redox state of the solution. Under oxic conditions, Fe(2) will be converted to Fe(3) which is generally insoluble. This will result in reduction in the soluble iron concentration but also precipitation of insoluble iron (oxy)hydroxide or oxides within the reticulation system.

The desktop study revealed that the saturation with respect to amorphous iron hydroxide was variable, but greatest for the wetland treated stormwater than after wetland and aquifer treatment, owing to the lower oxygen concentration and redox state after aquifer storage (Table 9). All blends were super-saturated with

respect to $Fe(OH)_3$ under oxic conditions and all solutions exhibited elevated saturation indices for goethite, which were also comparable to that for mains water (Table 10).

Water within both rigs maintained super-saturation with respect to iron minerals (Appendix 4 and 5) as suggested by the desktop evaluation. This was generally greater in the baseline rig than the average Para treated drinking water quality, as the water entering the rig was elevated in iron concentrations. The likelihood for iron(3) precipitates to form is increased due to the reticulation network itself. The deposition and adhesion of a sediment layer rich in iron was observed on the cement and PVC coupons over time, particularly in the stormwater rig (see section 3.7.3).

Table 9. Mineral saturation indices in selected end-member solutions

Water source	Sample location	Date sampled	pH	pe	Saturation index					
					SI_{CaCO3}	$SI_{Fe(OH)3}$	$SI_{Goethite}$	SI_{MnOOH}	$SI_{Mn(OH)}$	$SI_{Al(OH)3}$
wetland treated stormwater	WE2(1)	7/6/07	6.7	6	-2.0	2.2	7.5	-6.2	-8.8	0.3
	WE2(2)	9/1/08	6.8	6	-1.1	2.3	8.1	-5.0	-7.6	-0.5
	WE2(3)	7/12/09	8.8	6	0.7	3.0	8.7	0.4	-4.2	-3.3
wetland & aquifer treated stormwater	ASTR(1)	22/4/09	7.3	0.6	-0.2	-1.0	4.7	-9.1	-6.8	-2.0
	ASTR(2)	14/9/11	7.5	0.6	-0.2	-0.79	5.0	-8.9	-6.3	-1.7
	ASR(1)	2/12/08(#9542)	7.8	0	-0.05	-0.07	5.6	-8.3	-18	-2.4
	ASR(2)	5/2/08 (#9541)	7.8	0	0.09	-0.1	5.6	-8.3	-6.3	-2.4
mains	average		7.4	6	-0.8	1.0	6.7	-4.9	-8.2	-1.2
reclaimed	DAFF(1)	24/9/08	7.3	10	-0.6	2.6	8.2	0.2	-7.0	-2.0

Table 10. Mineral saturation indices in blended solutions

Water source	Sample location	pH	pe	Saturation index					
				SI_{CaCO3}	$SI_{Fe(OH)3}$	$SI_{Goethite}$	SI_{MnOOH}	$SI_{Mn(OH)2}$	$SI_{Al(OH)3}$
wetland treated stormwater/mains blend	WE2(1)/average	7.0	5.0	-1.5	1.8	7.3	-6.7	-8.5	-0.2
	WE2(2)/average	7.0	5.7	-1.1	2.2	7.9	-5.1	-7.6	-0.7
	WE2(3)/average	8.1	6.1	-0.07	2.8	8.5	-1.5	-5.5	-2.1
wetland treated stormwater/reclaimed blend	WE2(1)/DAFF(1)	7.1	5.6	-1.2	2.6	8.0	-5.1	-7.6	-0.3
	WE2(2)/DAFF(1)	7.0	5.9	-0.9	2.5	8.2	-4.6	-7.4	-1.0
	WE2(3)/DAFF(1)	7.7	9.2	-0.3	3.0	8.6	0.6	-6.2	-2.5
wetland & aquifer treated stormwater/mains blend	ASTR(1)/average	7.3	2.6	-0.5	0.8	6.5	7.3	-7.0	-1.4
	ASTR(2)/average	7.5	2.0	-0.5	0.9	6.6	-7.3	-6.6	-1.4
	ASR(1)/average	7.6	1.9	-0.4	0.9	6.6	-7.3	-6.6	-1.6
	ASR(2)/average	7.6	1.9	-0.3	0.9	6.6	-7.3	-6.7	-1.7
wetland & aquifer treated stormwater/reclaimed blend	ASTR(1)/DAFF(1)	7.3	4.2	-0.4	2.3	7.9	-5.5	-6.9	-2.3
	ASTR(2)/DAFF(1)	7.4	3.7	-0.4	2.3	7.9	-5.7	-6.7	-1.9
	ASR(1)/DAFF(1)	7.5	3.8	-0.4	2.4	8.0	-5.5	-6.7	-2.4
	ASR(2)/DAFF(1)	7.5	3.9	-0.3	2.4	8.0	-5.4	-6.7	-2.4

In contrast to iron, the risk to infrastructure due to precipitation of manganese and aluminium (oxy)hydroxides appears to be quite low indicated by sub-saturation with respect to these phases.

Excessive build up of iron precipitate within the reticulation system may be managed by incorporating aeration and filtration prior to the distribution pipe network.

4.2.4 Organic carbon

Biodegradable dissolved organic carbon (BDOC) within the source water serves as a food source and stimulates biological growth. BDOC within the baseline water rig varied between 0.3 mg/L and 1.3 mg/L (Figure 21), contributing 9-30% of the total organic carbon (TOC) content (3.0-5.3 mg/L TOC). The median values were 0.75 mg/L BDOC and 3.7 mg/L TOC in baseline water. The dissolved organic carbon (DOC) concentrations of 1.6-5.2 mg/L were slightly lower than the typical values reported for the Little Para mains water supply (Page *et al.* 2013), suggesting that the DOC (and BDOC) within mains water may be greater than the concentrations evident during this experiment.

BDOC within the stormwater rig ranged between <0.2 mg/L and 3.6 mg/L , contributing 9-48% of the total organic carbon (TOC) content. The medians were 0.65 mg/L BDOC and 2.6 mg/L TOC, respectively. On two occasions (30 April 2013 and 22 May 2013), stormwater exhibited high BDOC (1.9 and 3.6 mg/L) and TOC (4.7 and 7.5 mg/L). Aside from these two events, stormwater and mains water were comparable in the BDOC provided as a food source to stimulate microbial growth. Elevated TOC concentrations corresponded to a larger particle size.

The origin of this elevated BDOC can be related to the source water storage within the aquifer.
The 95th percentile BDOC concentration for wetland treated stormwater from this catchment is 11 mg/L, while after aquifer storage the 95th percentile is reduced to approximately 2 mg/L (Page *et al*. 2013).

Organic carbon can be elevated in the first water recovered from an aquifer storage system. This water may have been subject to a short storage time with limited time for biodegradation. Furthermore the initial water recovered (<1 ML) from an Aquifer storage and Recovery (ASR) scheme can be impacted by biological activity in the near well zone, producing water quality that is atypical of the bulk of the stored water. The samples with high BDOC concentrations were low in salinity (<400 µS/cm), typical of wetland treated stormwater that has not mixed with the brackish ambient groundwater. As discussed for EC, it is feasible that these samples may have experienced a short residence time in the aquifer as both injection and recovery of stormwater were occurring within this time interval.

The events with elevated BDOC support microbiological growth, which was evident in increased cell numbers during the same time interval for all pipe materials. The two occasions where high BDOC was evident in the stormwater rig also resulted in an increased median particle size, above 1000 µm.

The comparison between water entering the rig and within the rig revealed an increase in TOC for both the mains and stormwater rigs on 1 October 2013 which was not evident in DOC. This suggests physical removal of particular organic matter or biofilm sloughing. This was also evident for turbidity and metals.

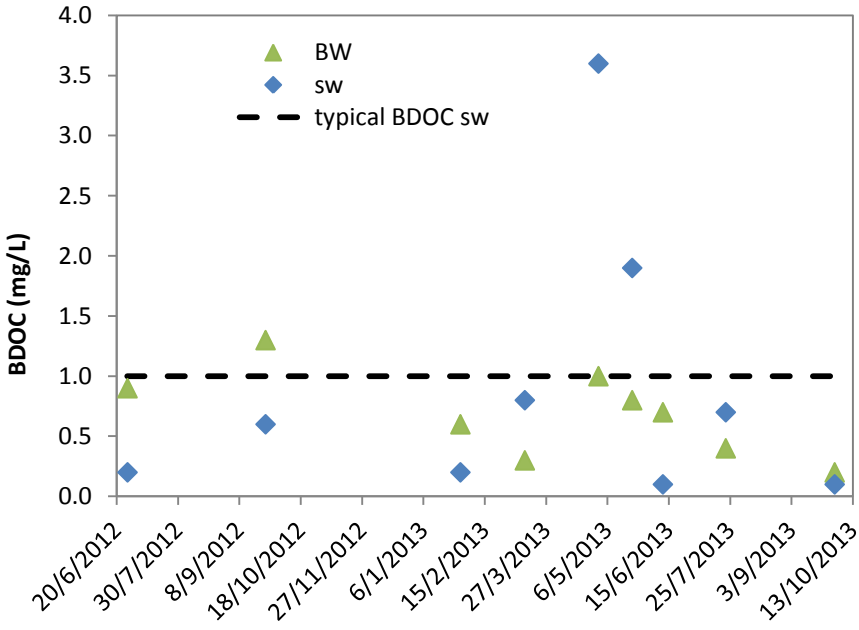


Figure 21. Biodegradable organic carbon (BDOC) in the baseline water (BW) and stormwater (sw) rig in comparison to typical concentration in stormwater (note: no BDOC data available for mains water).

The causes for the unusual high concentration events would appear to be linked to the aquifer operation. A plot of organic carbon and conductivity of water samples taken from the stormwater rig (Figure 22)

showed that when organic carbon was very high in April and May, 2013, whilst conductivity was relatively low (0.2-0.3 mS/cm). No aquifer injection occurred throughout February and March 2013 but 1.6 ML was injected in April following a 23 mm rain event on the 21-22nd. The low salinity of the April 30 rig sample (0.288 mS/cm) indicates this water was 'new' as previous months during summer had seen the conductivity at 0.683-0.889 mS/cm. This also meant that the aquifer residence time for this water had to be less than 8 days. In May 2013, a 54 mm rain event occurred on the 12-16th and a further 129 ML was injected at Parafield. The conductivity of the May 22 rig sample was lower again (0.158 mS/cm) than the last sample in April and would have had a residence time of less than 10 days.

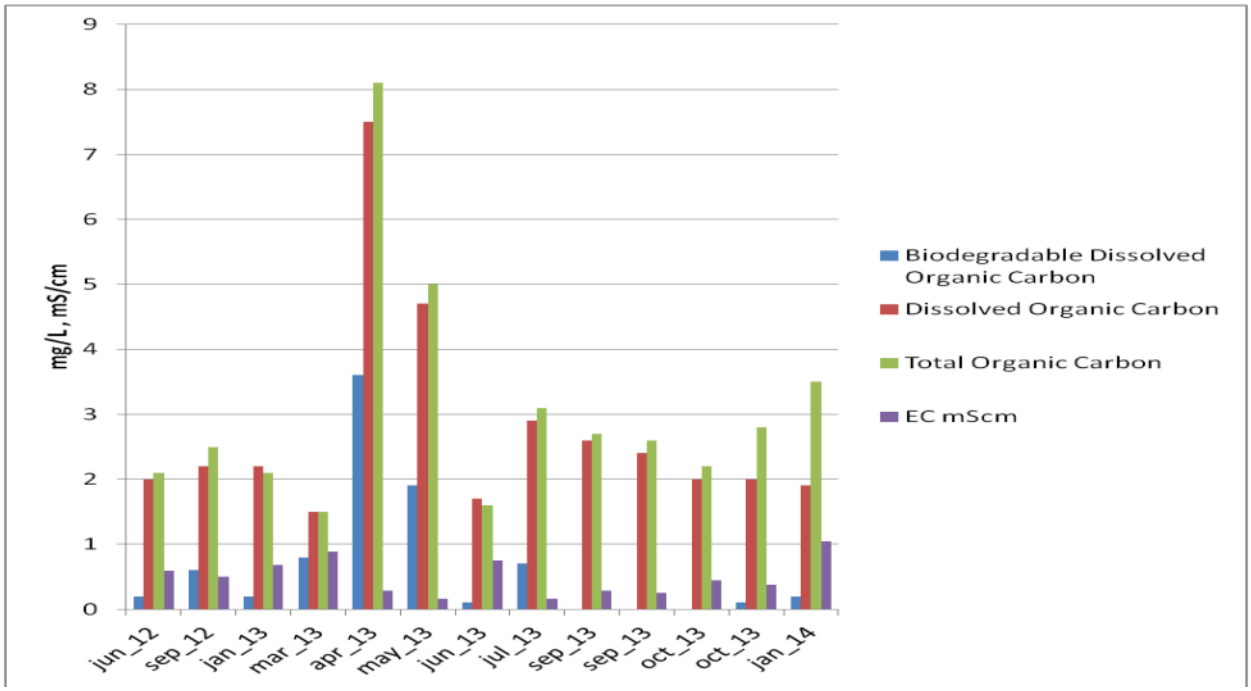


Figure 22. Organic carbon and conductivity of samples from the stormwater rig.

4.2.5 Nitrogen and Phosphorus

Nutrient concentrations in the rigs varied. Overall, the stormwater rig had similar or lower concentrations of nitrogenous compounds than baseline water, but a higher concentrations of phosphorus based nutrients (Figure 23). There are no ADWG values for total nitrogen or total phosphorus. The aesthetic guideline value for ammonia is 0.5 mg/L and the health based guideline values for nitrate and nitrite are 50 mg/L and 3 mg/L , respectively.

Total nitrogen (TN) for baseline water (range 0.27 – 0.61mg/L, median 0.45mg/L) and stormwater (range 0.18-0.42mg/L, median 0.32mg/L) were similar, but the nitrogen composition differed between the two rigs. Stormwater total nitrogen concentrations were comparable to those reported after aquifer storage (ASR median 0.18 mg/L, 95th percentile 0.77mg/L) but lower than for the Salisbury ring main (median 0.28 mg/L, 95th percentile 1.1mg/L) (Page *et al*. 2013). It was also noted that the peak in stormwater TN and TKN occurred on 30/4/13 and 22/5/13, corresponding to the dates of high BDOC (see section 4.2.4).

TKN ranged between 0.16-0.43mg/L and 0.10 -0.38mg/L in the baseline and stormwater rigs, which corresponded to 69±10% of the TN in baseline water (range 58 to 86%) and 82±20% in stormwater (range 47 to 100%). Baseline water TKN was generally comparable with concentrations reported for Little Para, while stormwater TKN was considerable lower than the 95th percentile after aquifer storage and within the ring main.

Ammonia-N concentrations in both rigs were significantly lower than the aesthetic guideline (0.4 mg/L) and the median concentrations for the ASR (0.094 mg/L), ASTR (0.14 mg/L), the ring main (0.25 mg/L) and the Little Para (0.23 mg/L), with baseline and stormwater rigs concentrations between <0.005 to 0.011 mg/L and <0.005 to 0.076 mg/L, respectively. Whilst the sum of nitrate-N and nitrite-N for the two rigs (max. 0.196 mg/L) was mostly higher than the 95th percentile after aquifer storage (ASR 0.052 mg/L; ASTR 0.023 mg/L) and the ring main (0.06 mg/L), due to nitrification under oxic conditions within the rig. But too low to pose any human health risks (WHO guidelines for nitrite 3mg/L as NO_2^- and nitrate 50mg/L as NO_3^- (World Health Organization 2011).

In baseline water, the maximum total phosphorus (TP) was 0.018 mg/L on the 27/6/2013, but typically it was below 0.006 mg/L. Whilst in stormwater TP varied from 0.017 to 0.083 mg/L. It was also noticed that the concentration of TP increased after passage through the stormwater rig, but not the FRP. Such concentrations were typically below the 95th percentile of stormwater in the ring main (0.15mg/L).

Concentrations of reactive phosphorus in the stormwater rig were low (<0.003 – 0.025mg/L, median 0.017mg/L) and typically within the range expected from ASR and ASTR treatment.

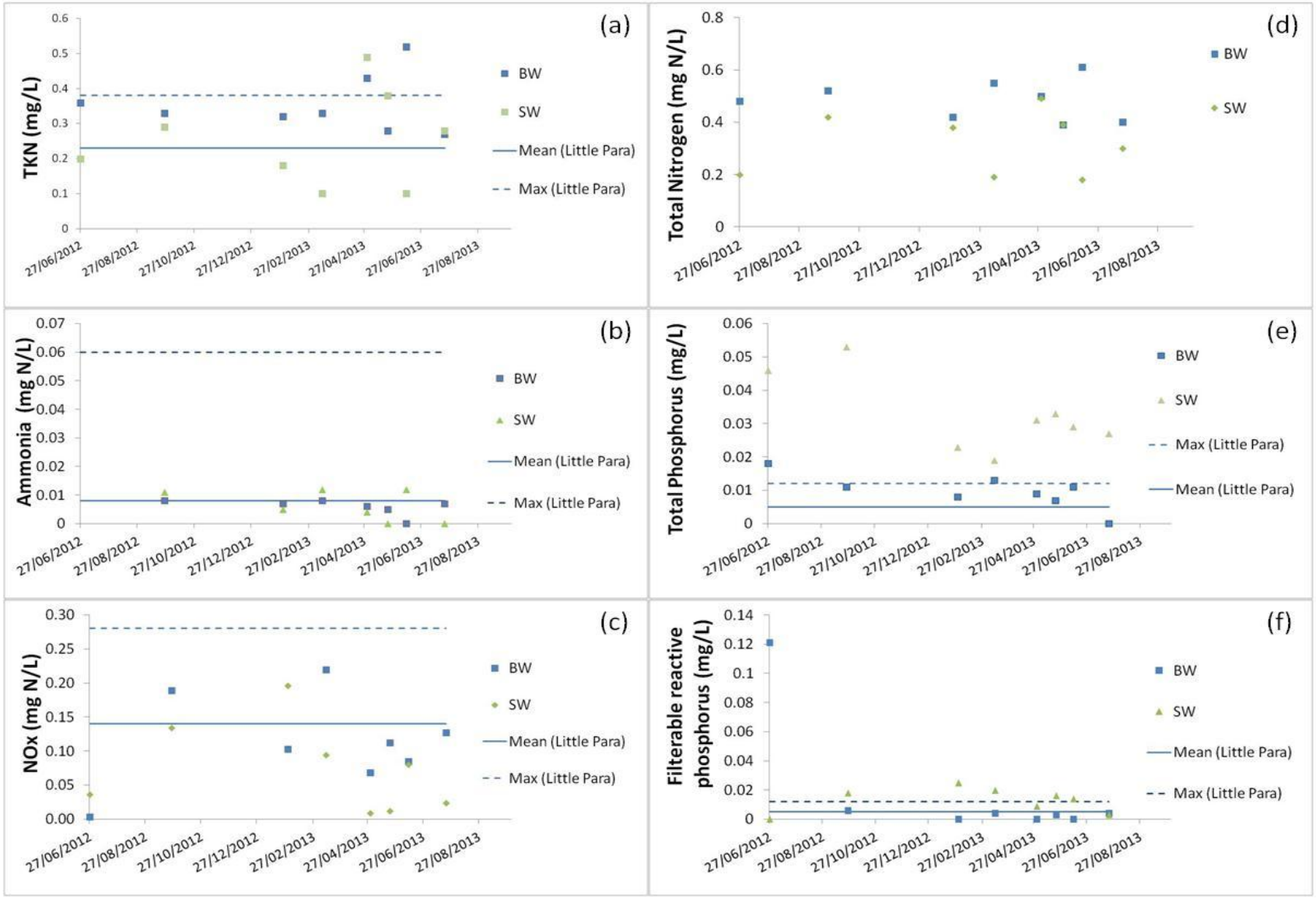


Figure 23. Nutrient concentrations in the baseline water (MW) and stormwater (SW) rig in comparison to the typical concentration in Little Para dam: (a) Total Kjehldahl nitrogen (TKN), (b) Ammonia, (c) Nitrate and nitrite (NO_x), (d) Total nitrogen, (e) Total phosphorus, (f) Reactive phosphorus.

4.2.6 Colour and turbidity

Stormwater circulating within the experimental rig exhibited variable true colour, from 2 to 94 HU and a median of 12HU, while the baseline water rig remained below 5 HU (Figure 24). The colour of water sampled in the rig was the same as that of water entering the rig on the same day. An earlier desktop evaluation reported that treated stormwater is likely to exceed the ADWG value of 15 HU due to elevated iron concentrations (Page *et al.* 2009). Mean turbidity in the baseline water and stormwater water supplies were 1.82 $\pm$2.06 NTU and 3.37 $\pm$2.80 NTU, respectively. However, the 95th percentiles, respectively 5.4 NTU and 7.8 NTU, exceeded the 5 NTU ADWG aesthetic value (Figure 25).

The experimental evaluation showed a greater influence from dissolved organic carbon concentration than soluble iron concentration on true colour (
Figure 26). This suggests colour cannot be managed by treatment of soluble iron alone, such as via oxidation to convert soluble Fe(2) to insoluble Fe(3) alone. The true colour within the stormwater rig exceeded the 95th percentile value from the Salisbury ring main, due to elevated concentrations of both soluble iron and dissolved organic carbon. Notably, stormwater exceeded the true colour limit of 15 HU for only the fresher samples, where electrical conductivity was less than 300 μS/cm (Figure 26). This is lower than the median quality for stormwater after storage in the T2 aquifer such as after ASR (650 μS/cm), ASTR (550 μS/cm) or from the Salisbury ring main (430 μS/cm). Again, this may be influenced by inadequate storage time in the aquifer.

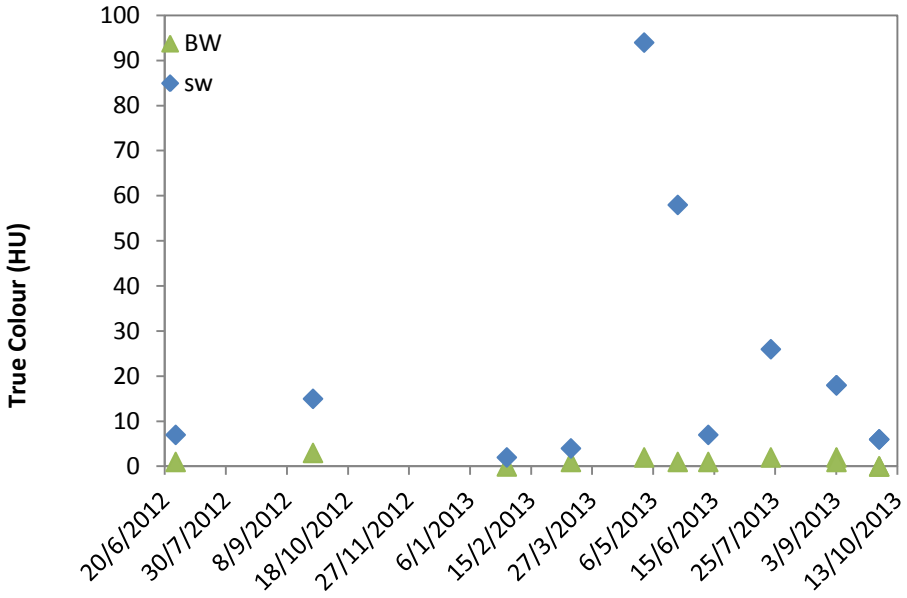


Figure 24 True colour in the baseline water (BW) and stormwater (sw) rig.

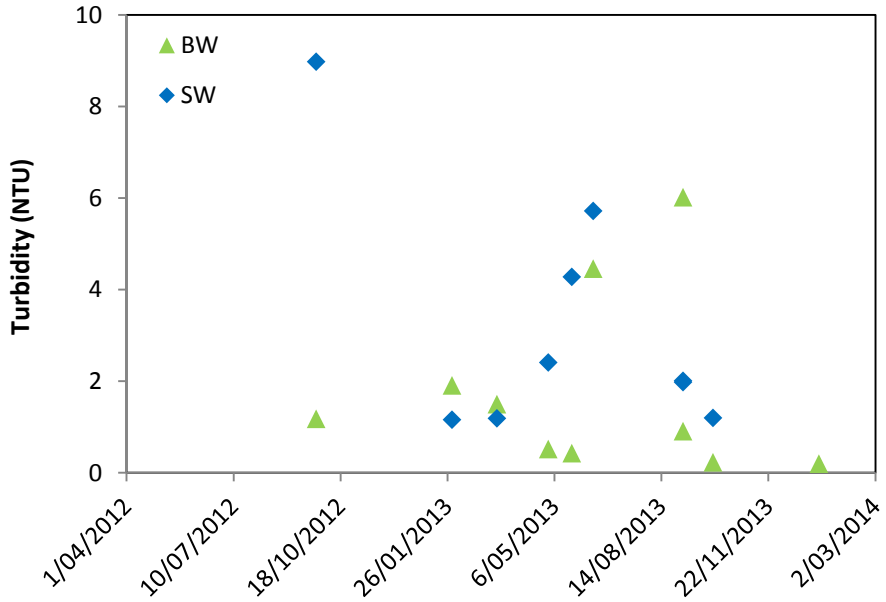


Figure 25. Turbidity in the baseline water (BW) and stormwater (sw) rig

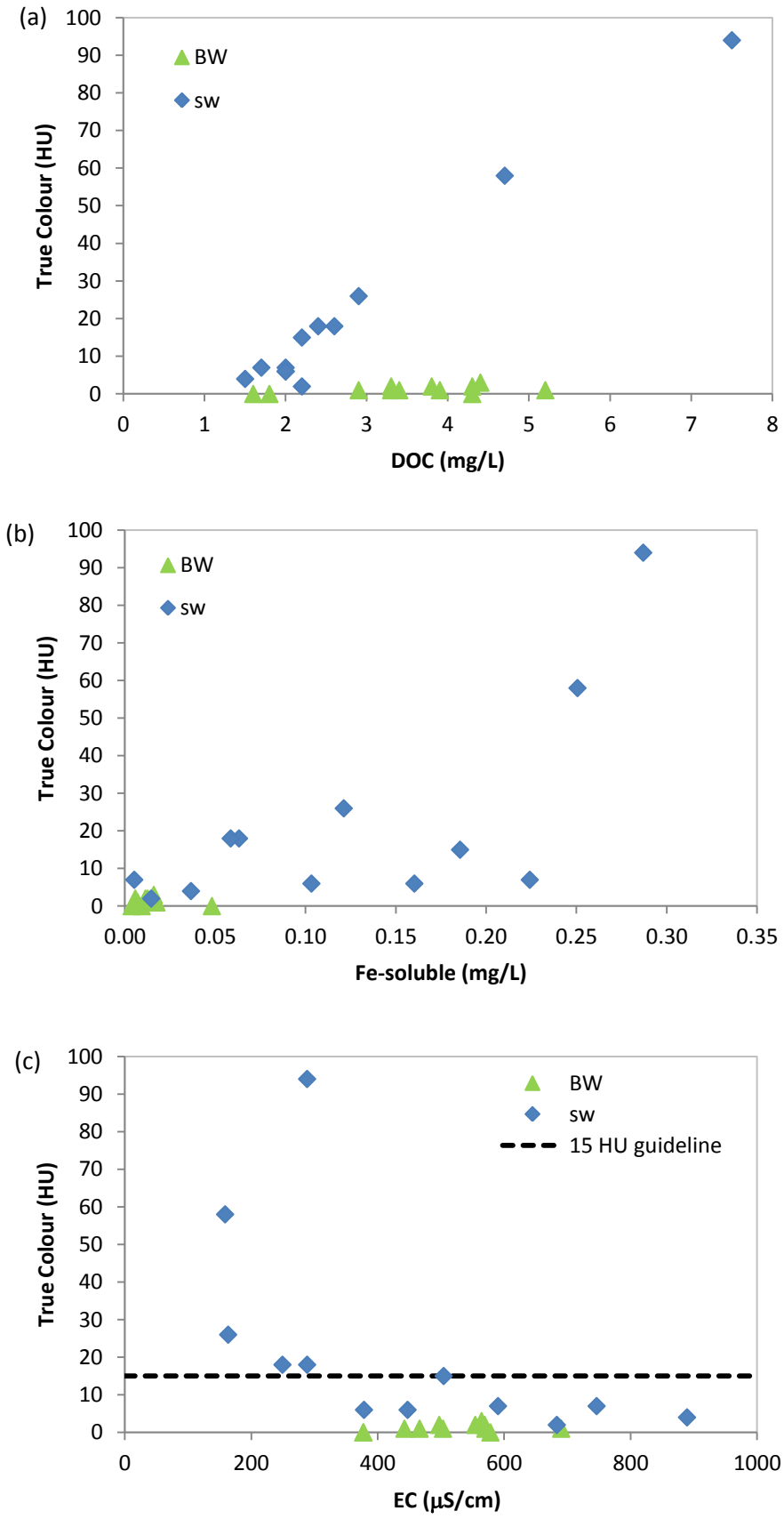


Figure 26 Relationship between soluble true colour and (a) dissolved organic carbon (DOC); (b) soluble iron (Fe-soluble); and (c) electrical conductivity (EC) in the experimental rig .

4.3 Surface characterisation

4.3.1 Surface appearance

The appearance of coupons before and after exposure is compared in Figure 27. All three materials (copper, cement and PVC) changed with exposure time and with water source, however there were indications that type of change and hence the mechanisms responsible differed for each material type. Sediment deposition and changes in surface morphology were observed on the coupons as exposure time increased.

4.3.1.1 Continuous exposure to a single water supply

In cement and PVC, there was a gradual deposition of sediment on the surface of the coupons resulting in red to light brown deposits (shown in Figure 27). The sediment layer was visible to the naked eye after week 12 (13/03/13).

The rate of sediment deposition was faster in coupons exposed to stormwater than in baseline water. In stormwater the sediment layer grew from small patches to full coverage of the coupon surface by week 31, whilst in baseline water the sediment deposits were minimal and interdispersed.

Cement coupons in baseline water darkened and changed coloration after 5 weeks exposure, but there was less sediment deposition and it did not result in full sample coverage. Whilst, in stormwater the layer of sediment gradually increased in thickness and spread until the coupon surface was covered.

Likewise PVC coupons underwent similar changes in baseline and stormwater. The sediment was a brown powdery material, shown in Figure 30. Deposits tended to be dispersed and shallow over the smooth PVC surface, but thicker on the rougher cement surface (Figure 30). However, as exposure time increased (after 19 weeks) both PVC and cement samples were fully covered in sediment.

Differences in the topography of the deposits on each material were also confirmed with 3D laser microscopy, which showed homogeneously distributed deposits on the surface of PVC coupons, whilst on cement coupons, in addition to sediment deposition, changes in the cement surface topography and morphology was also observed and was attributed to reactions between the cement material and water (section 4.3.2). The sediment layer on the surfaces of the stormwater coupons was generally darker and thicker than their counterparts on the baseline water coupons.

Copper coupons displayed discoloration and changes in surface morphology, evidence of copper oxidation, but showed the least sediment accumulation, as exposure time increased. Even samples that had been exposed for over 12 weeks showed minimal sediment residue (Figure 27). The appearance of the copper coupons exposed to both water sources was similar for the first 10 weeks, however subsequent exposure resulted in stormwater coupons developing a darker coloration than coupons exposed in baseline water.

The sediment deposits on PVC and cement and the discoloration of the copper were intensified for coupons collected after 12weeks. These changes may have been influenced by higher concentrations of biodegradable organic carbon (BDOC) in the stormwater rig after 10 weeks (on 30/04/13 and 22/5/13), stimulating biological growth. Stormwater samples collected during collection of coupons exposed for 12 and 19 weeks (period 2) had BDOC concentrations of 3.6 mg/L and 1.9 mg/L, whereas BDOC in the baseline water rig at the same time remained less than 1 mg/L. This would have favoured biofilm formation.

This leads to the hypothesis that the attachment of the sediment layer is facilitated by the formation of a biofilm layer on the coupons' surface. The microorganisms in the biofilm then facilitate the attachment of sediment. Copper which has anti-microbial properties delays the biofilm development and was less

susceptible to sediment deposition, instead showed different oxidation processes in baseline and stormwater.

The dry weight of the surface residues removed from selected coupons varied during the exposure period for the three material types and tended to be slightly higher for stormwater for PVC and copper, however for cement no clear difference was verified, yet samples also had high standard deviation values as shown in Figure 28. The maximum residue variation for copper, PVC and cement in baseline water was 0.4, 1.1 and 4.6 mg.cm^{-2} and in stormwater 1.31, 1.01 and 2.28 mg.cm^{-2}, respectively and occurred after extended exposure (min. 25 weeks), but tapered off afterwards.

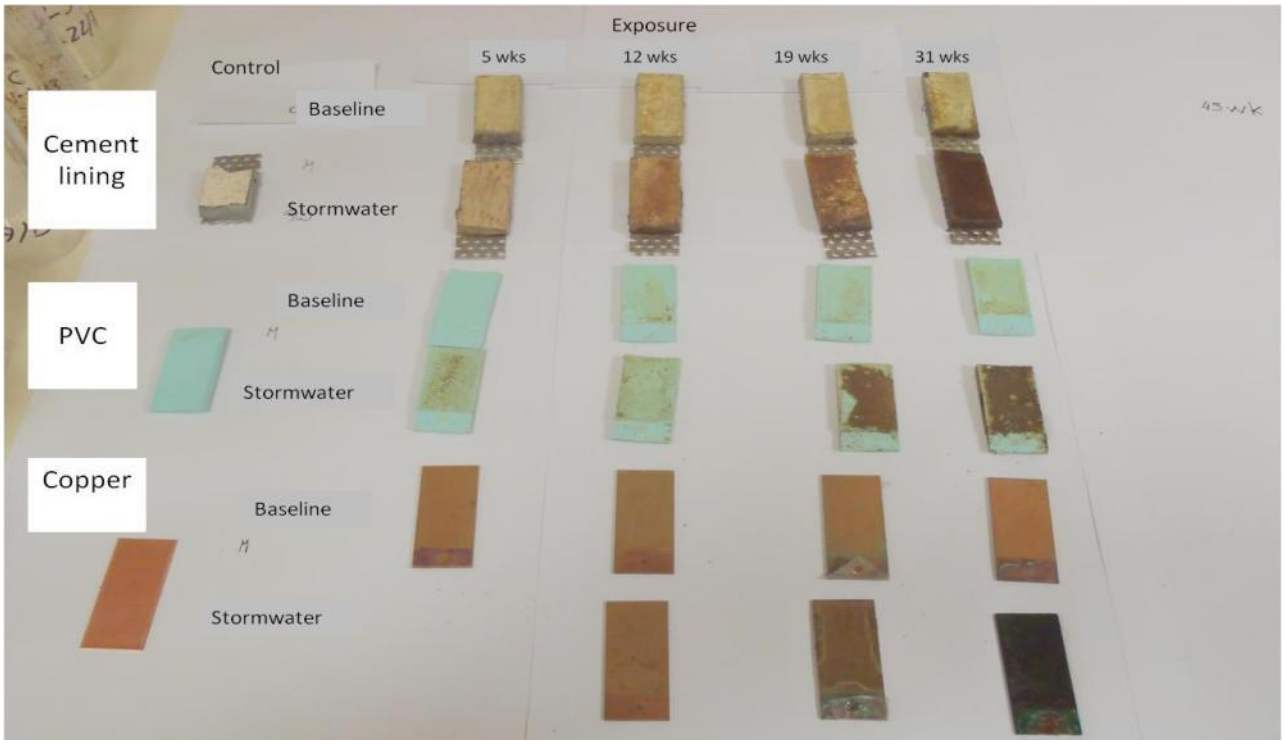


Figure 27. Appearance of cement, copper and PVC coupons after 31 weeks exposure to baseline and stormwater .

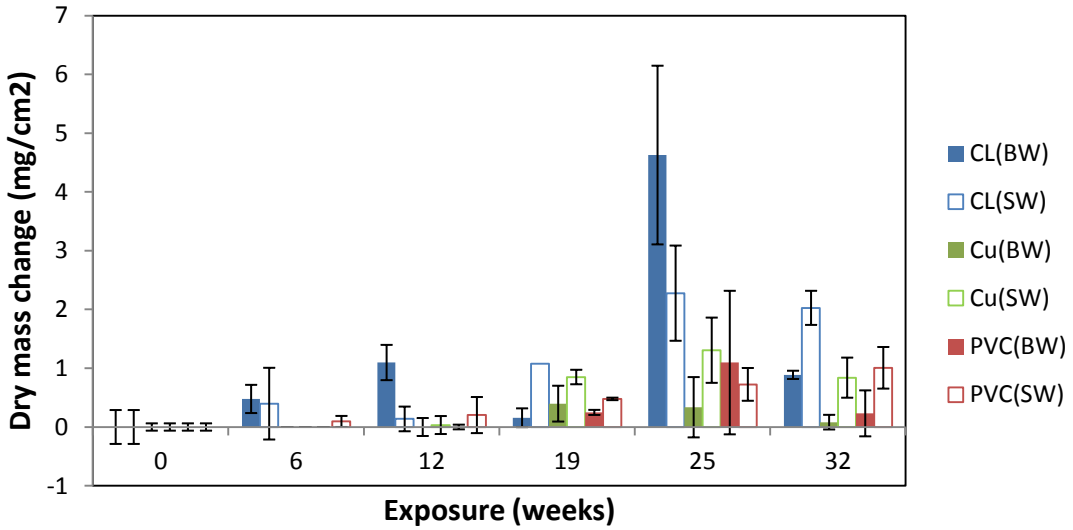


Figure 28. Change in mass removed from coupons of cement (CL,), copper (Cu) and PVC exposed to baseline water (BW) and stormwater (SW) using sonication for 2h.

4.3.1.2 Change of water supply type (coupon swap)

The appearance of the swapped pairs of coupons is shown in Figure 29. The control coupons were aged in the same water type (either baseline or stormwater) for the whole period, whilst the swap coupons were the replicates that were aged in the other water source for the final 3 months. The swap coupons when originally removed on the 1st Oct 2013 had the same appearance as their control counterpart.

Coupons that had originally been aged in stormwater had a visible layer of brown sediment over their surfaces (even the copper coupons). After the swap into baseline water, the brown sediment layer which covered the stormwater coupons diminished, particularly for the PVC (Figure 29, a.2). Coupons that had originally been aged in baseline water and were swapped into stormwater showed traces of the initial sediment deposition on PVC and cement (Figure 29, b.2), however the copper appearance did not change. The control samples had not changed either. This would suggest that a change in the water supply from stormwater water to baseline will result in dissolution of accumulated sediment, which could be attributed to the breakdown of the biofilm due to the change of water quality, given the lower concentration of iron in bulk baseline water and despite the low chlorine residual.

Figure 29. Appearance of cement, copper and PVC coupons with and without the swap from baseline to stormwater and from stormwater to baseline after exposure for 43 weeks. Legend: Stormwater (SW) and baseline water (BW).

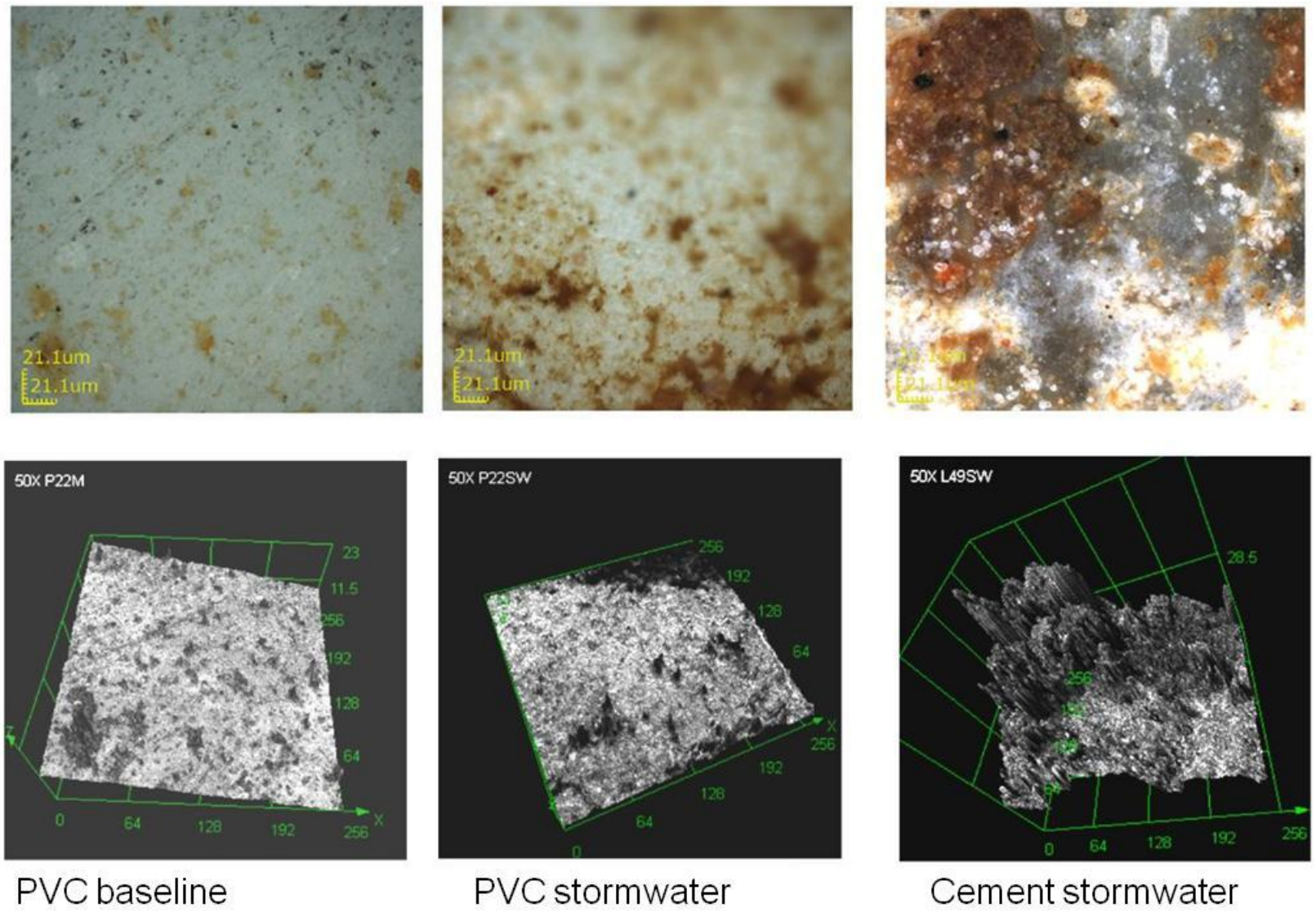


Figure 30. Three-D confocal laser images of sediment deposits on the surface of PVC and cement coupons after exposure in baseline water and stormwater after 7months (19 weeks).

4.3.2 Elemental composition of surface layer

As previously mentioned in section 4.3.1, deposits on the coupons' surfaces took the form of a light to dark brown deposits. Analysis of the deposits on copper, cement and PVC coupons by XRD and XRF was conducted to determine the elemental composition of the deposits and to verify if other surface changes could be verified. The exposed samples had been exposed for 19 weeks (removed on 30.04.2013). The XRD results are shown in Figure 31. The XRF results for copper, PVC and cement are shown in Appendix 8.

4.3.2.1 PVC coupons

PVC is amorphous and shows no crystalline diffraction peaks in XRD. Samples of the sediment residue were also analysed with XRD. The XRD traces, both in-situ and removed material, from coupons exposed to stormwater (P24SW) and baseline water (P23M) showed no crystalline diffraction peaks (i.e. the sample was amorphous) (Figure 27). In addition, there was a relatively high background indicating the presence of fluorescence from iron content.

XRF analysis indicated the presence of Cl in the PVC control (due to PVC composition) and the presence of Zn, Ni and Ti which are attributed to additives used in PVC manufacture (shown in Figure 49 a, Appendix 8). In samples exposed to baseline and stormwater (respectively P23M and P24SW), both spectra showed the presence of Fe in the deposits, whilst the relatively greater intensities of Cl, Zn, and Ni in the spectrum from the baseline sample indicated the deposit to be far thinner in baseline water than in the stormwater sample (Figure 49 b, c). The presence of copper was also observed in the deposits from both coupons, and this was not seen in the control coupon.

In summary, the conclusion is that the deposits on all coupons are predominantly an amorphous iron containing material. The presence of a small amount of copper in the deposits on coupons P23M, P24SW and L24M (cement) indicates pick up of corrosion products from elsewhere in the system. Neither of the samples examined showed traces of minerals derived from soil or sand contamination. In addition, results confirmed the greater thickness of the sediment layer on stormwater coupons.

4.3.2.2 Copper coupons

The diffraction patterns of all three surfaces were similar. The unexposed copper coupon had a small peak at 2 theta app 45.2 deg (Figure 31 a), which corresponds to the position for the Cu (111) Kβ peak.

XRD patterns from the copper samples exposed to baseline water (C23M) and stormwater (C22SW) showed the same crystalline diffraction peaks arising from the copper substrate, in addition crystalline diffraction peaks also showed cuprite (Cu_2O) and copper oxide (Figure 31 b, c), indicating signs of oxidation (Figure 31)

Fluorescence analysis indicated that after exposure to baseline and stormwater, thin deposits were also detected on both these coupons (C23M and C22SW), and whilst Cu dominated the spectra, the presence of Fe was also confirmed in both deposits shown in Figure 50, Appendix 8.

4.3.2.3 Cement lining coupons

The control cement coupon was a mixture of two crystalline forms of calcium carbonate, calcite and aragonite (a crystal polymorph of $CaCO_3$ with lower dynamic stability), shown in Figure 31 a. XRD traces from coupons exposed to baseline (L24M) and stormwater (L24SW) were virtually identical and showed diffraction peaks corresponding to calcite ($CaCO_3$) and aragonite. This is consistent with diffraction from

the carbonation products of portlandite (portland cement); portlandite is known to react with carbon dioxide to form a hard shell of calcium carbonate (Figure 31 b, c).

However both coupons had an elevated background scatter compared to the untreated coupon, indicating the presence of surface deposits that were likely to be amorphous. There were also differences in the relative heights of the calcite/aragonite peaks between those coupons (particularly the aragonite peak at 2-theta app 29.2 deg) indicating the crystal orientation of grains within the coupons was likely to be random.

An XRD trace measured from a darker brown sediment spot on sample L24SW(sediment) showed no diffraction peaks, indicating that brown deposit was likely to be amorphous and also sufficiently thick in that region to mask the diffraction from the cement substrate.

XRF analysis of the cement control showed the presence of Fe and Ca shown in Figure 51 (a). After exposure to baseline and stormwater, both spectra showed substantial iron in the samples, along with calcium from the cement substrate (Figure 51 b and c). The baseline coupon (L24M) showed considerable peaks from copper, which were not seen in the stormwater equivalent coupon, which as previously discussed in section 4.2.1 comes from the baseline water supply. The iron peaks were higher in the stormwater sample (L24SW) indicating a thicker coating of deposits on this sample.

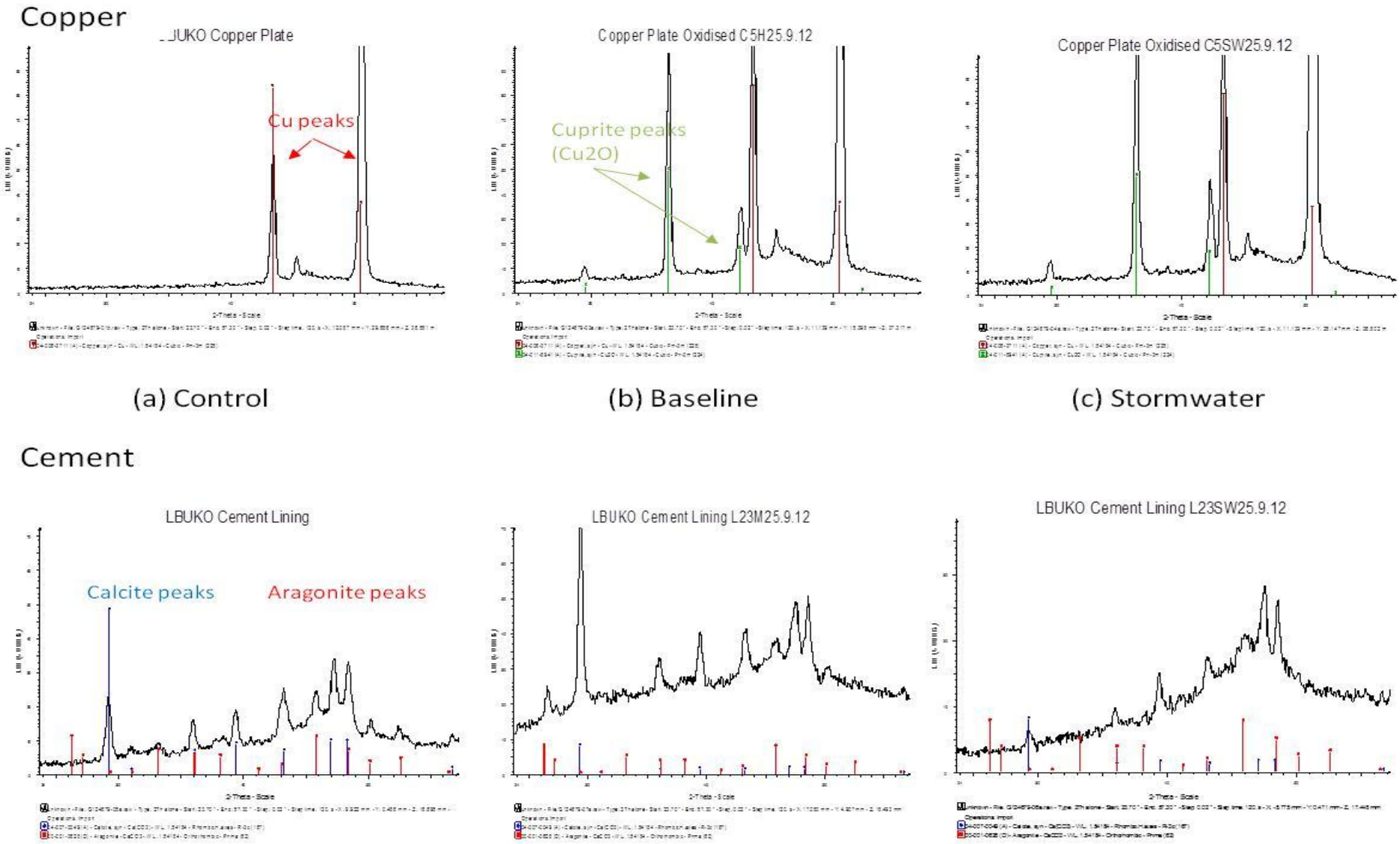


Figure 31. XRD Analysis of surface and sediment on copper and cement coupons: (a) unexposed, exposed to (b) baseline water and (c) stormwater after 19 weeks.

4.3.3 Discoloration of coupons

Discoloration of the coupon materials recorded in CIE L*a*b* colour coordinates was adopted to determine visual changes and is shown in Figure 32 and Figure 33. These are expressed as lightness value (ΔL*), in red/green hue (Δa*), yellow/blue hue (Δb*) and the total colour difference, which combines effect of the previous parameters (previously mentioned in section 3.7.2).

Coupons underwent on-going discoloration with exposure. Coupons exposed to both water sources became darker and/or discoloured either because of surface changes and/or the sediment deposition onto the samples. However, the rate of change varied with material type.

Figure 31 and 33 show that discoloration was more severe after 10 weeks exposure to stormwater compared to baseline water, except for the copper samples. The samples from that period correspond to the dates when high organic concentrations in stormwater were verified (section 4.2.4)

4.3.3.1 Copper

The total colour difference (ΔE) in Figure 32a showed that copper experienced rapid discoloration in the initial 5 to 10 weeks of exposure (increase by +25 units) from both water sources, but afterwards the rate of discoloration decreased. The copper darkened with exposure as seen in the reduction of (L*), reduction in the redness (*a) and the marked transition from yellow to green for the coordinate (*b) (Figure 33 a,b,c). These are attributed to the oxidation of the copper surface. Overall, copper samples had the least differentiation between samples exposed to baseline and stormwater. However, the more severe change in the b* coordinate towards green from 20 to 30 weeks exposure between the samples supports the observations that the corrosion mechanisms and/or microbial populations are likely to differ upon exposure to the two water sources. Following the swap the differences in colour between the reference and swapped samples were not as distinct.

4.3.3.2 Cement

Cement coupons also discoloured with exposure. Rapid discoloration shown as total colour change (ΔE), reduction in lightness and increase in b* occurred in both water sources at a similar rate in the initial 5 weeks, afterwards coupons in stormwater continued to discolour at a slower rate (Figure 32). In baseline water the total colour difference (ΔE) increased by 30 units in the initial 10 weeks and remained constant with extended exposure.

Coupons in stormwater increased of redness (+a*) by 140% in 20 weeks, and afterwards experienced a reduction returning to hues close to the original sample, which could indicate sediment slough off. On the other hand, samples exposed to baseline water retained the same red hue as the control sample up to 20 weeks (a*= +5.28$\pm$0.13), followed by discolouration (a*=+5.0$\pm$2.3) (Figure 33e). For the b* axis (yellow/blue), the intensity of the yellow hue increased in stormwater (Δb* = 98%), coupons in baseline water also changed colour but changes were not as severe and there was larger variability in colour intensity compared to stormwater (Figure 33 f). Colour on the coupons was not homogeneous and the variability within a single sample and between samples was also the highest among materials as observed in the standard deviation.

4.3.3.3 PVC

PVC discoloration occurred at a slower pace than the other 2 materials and discoloration was evidenced up to 10 weeks exposure, after that time the total colour difference for the samples plateaud. PVC is considered the least reactive of the materials tested regarding interaction with water quality due to its

chemical stability and is also less prone to biofilm formation than cement (Niquette *et al* 2000). Thus discoloration for PVC is indicative of sediment deposition.

Coupons in baseline water suffered slight discoloration ($\Delta E \leq$ 17 units). The L* and a* coordinates of samples in baseline water varied by less than 10 units same over time, however in the yellow/ blue axis (b* coordinate) the hue changed from blue to yellow as sediment residues attached (Figure 33 g, h, i). In stormwater the changes were more pronounced with reduction in lightness L*, increase of a* and b* coordinates from green towards red and blue towards yellow and a more marked total colour difference ($\Delta E \leq$ 61 units) , this was caused by the thicker sediment layer accumulated on the coupons and caused darkening of the samples.

There were marked colour differences between the swapped coupons and their replicates subject to continuous exposure in the same water source. The original stormwater- exposed coupon maintained the same discoloration trend as the earlier samples. However, for the sample transferred into baseline water, there was less sediment and the colour was equivalent to samples exposed to baseline water only. Meanwhile, the coupon initially exposed to baseline water and subsequently to stormwater showed traces of in sediment deposition and discoloration.

Overall, the colour changes were observed on the three materials indicating changes in surface characteristics. In PVC colour darkening was due to increased deposition of a sediment layer on the material surface. In cement, sediment deposition was also observed but variability in colour readings was significant, potentially indicating sediment slough-off. Finally in copper, colour changes were attributed to surface oxidation and sediment adhesion was minimal. Thus the results support that the microbial species that populate the three materials are likely to differ.

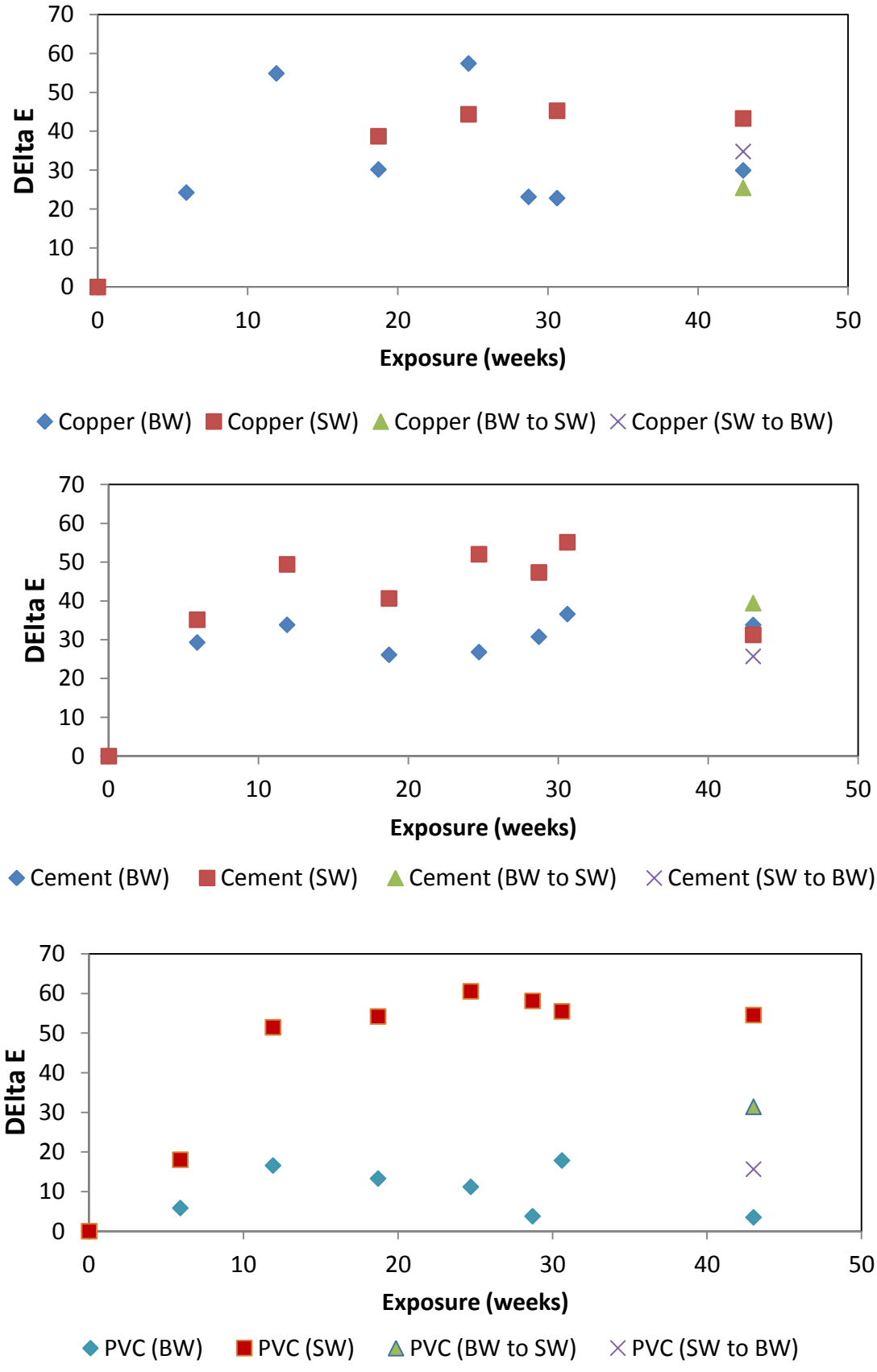


Figure 32. Total colour difference (Delta E) of copper, cement and PVC coupons exposed to baseline and stormwater in experimental rig from Nov.2012 to December 2013. Legend: Baseline water (BW), Stormwater (SW), Coupon exposed to baseline water for 29 weeks followed by exposure to stormwater (BW to SW), and Coupon exposed to stormwater for 29 weeks followed by exposure to baseline water (SW to BW).

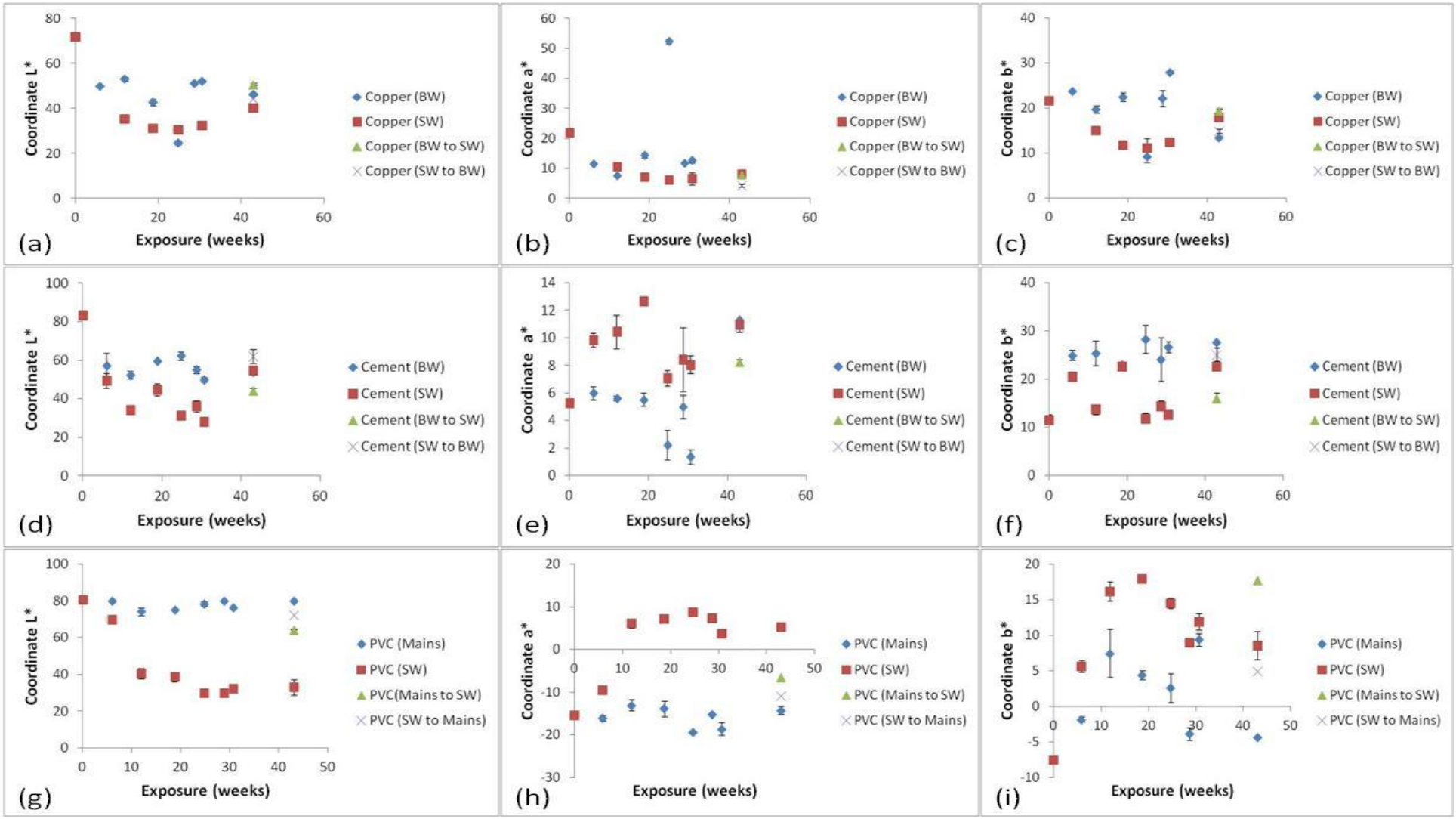


Figure 33. Colour coordinates CIE L*a*b* for copper, cement and PVC coupons exposed to baseline and stormwater in experimental rig from Nov.2012 to January 2014.Legend: Baseline water (BW), Stormwater (SW), Coupon exposed to baseline water for 29 weeks followed by exposure to stormwater (BW to SW), and Coupon exposed to stormwater for 29 weeks followed by exposure to baseline water (SW to BW).

4.4 Microbial communities

4.4.1 Microbial communities in the pipe rigs

4.4.1.1 Total cell numbers

Total cell counts provide an indication of the microbial density (for both living and deceased microorganisms) in biofilm. The total cell counts on pilot rig coupons exposed to baseline water or stormwater as determined by flow cytometry are shown in Figure 34. The cell counts were in the order of 10^5 to 10^7 cells/cm^2 and showed some variation over the duration of the study. For most sampling times (except two sampling times), the cell counts were higher for the stormwater exposed than the baseline water exposed copper and PVC coupons. For cement lining the difference was not as consistent. There was no clear difference in the cell numbers between the different materials for either baseline water or stormwater.

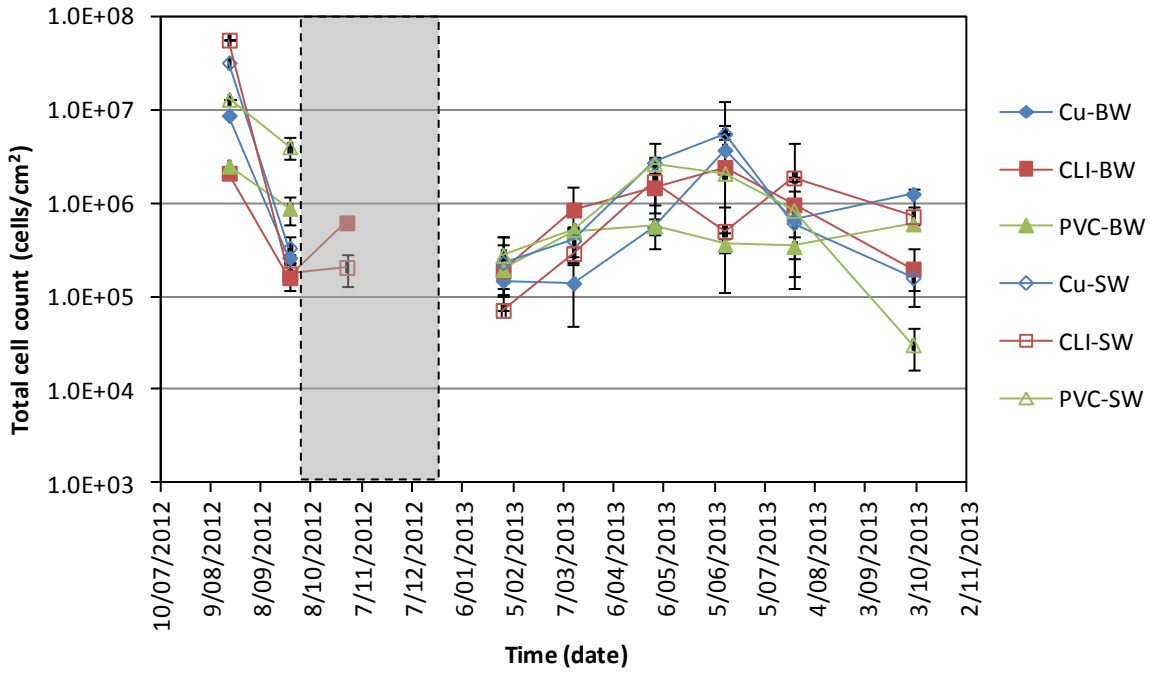


Figure 34. Total cell counts on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (BW) or stormwater (SW) as determined by flow cytometry. The grey area shows the outage period (24.10-20.12.2012) when the rigs were not operated due to stolen pumps.

4.4.1.2 Culturable cell numbers

Culturable cell counts on pilot rig coupons exposed to baseline water or stormwater as determined by plate counting with various incubation temperatures are shown in Figures 35-37. The culturable cell counts determined at 22°C were in the order of 10^1 to 10^6 cells/cm^2, culturable cell counts at 37 °C were 10^1 to 10^5 cells/cm^2 and thermotolerant coliform cell counts at 37/45 °C were 10^1 to 10^3 cells/cm^2. None of the coupon materials stood out as having consistently higher or lower culturable cell numbers than the other materials. Neither was there a consistent difference in culturable counts at 22°C or 37°C between coupons exposed to baseline water and stormwater. However, the number of thermotolerant coliforms was larger in the stormwater system than in baseline water system.

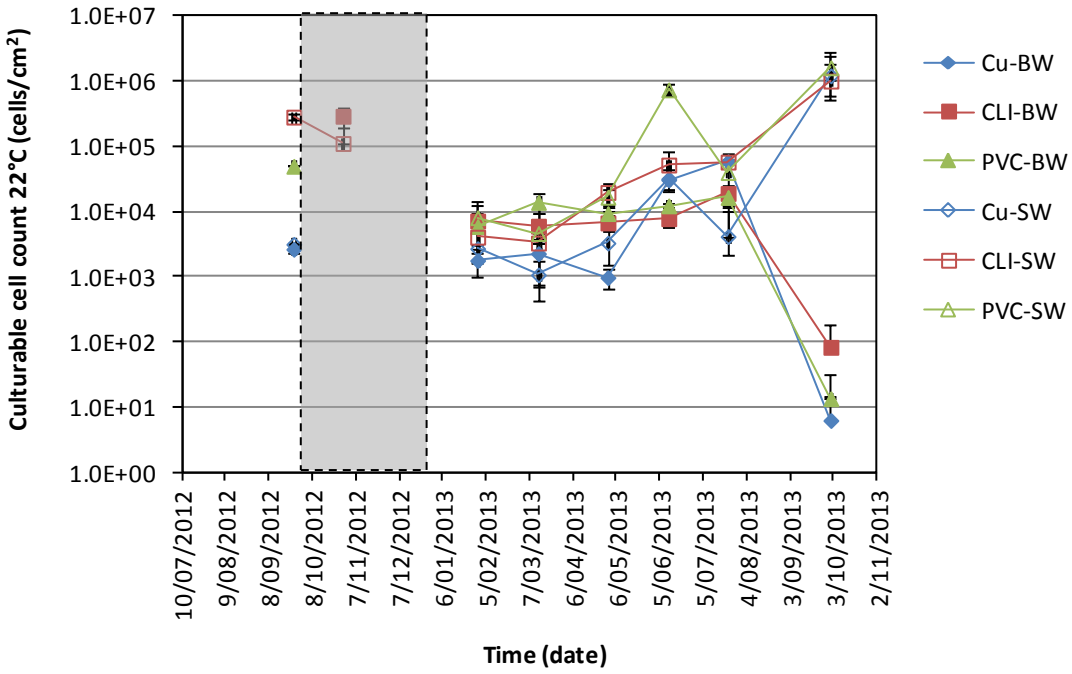


Figure 35. Culturable cell counts on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (BW) or stormwater (SW) as determined with plate counting and incubation at 22 °C. The grey area shows the outage period (24.10-20.12.2012) when the rigs were not operated due to stolen pumps.

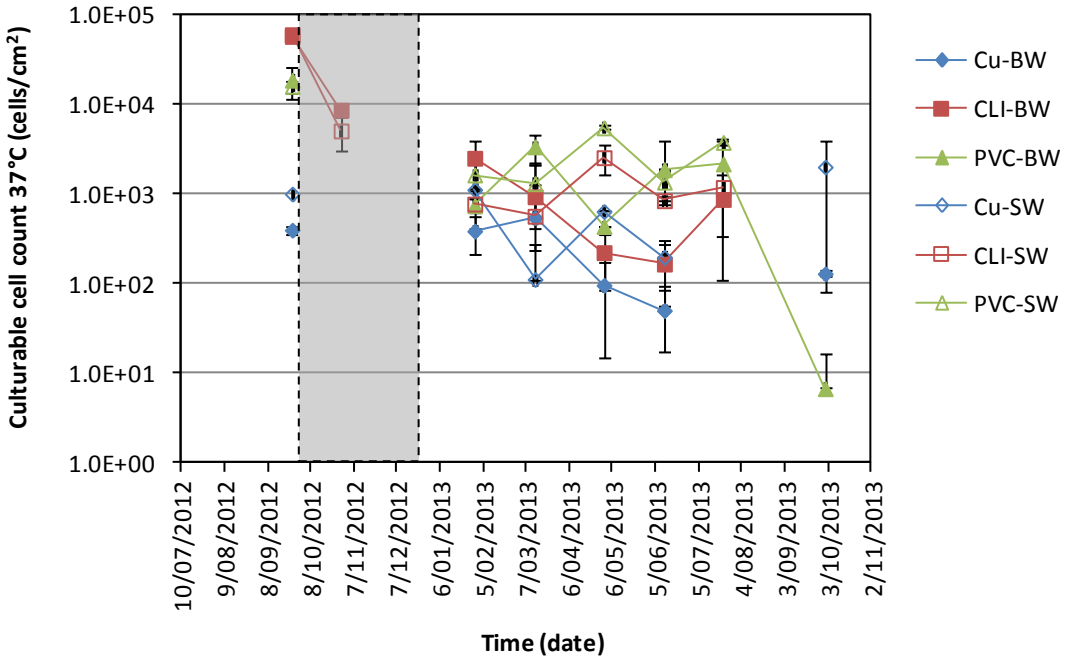


Figure 36. Culturable cell counts on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (BW) or stormwater (SW) as determined with plate counting and incubation at 37 °C. The grey area shows the outage period (24.10-20.12.2012) when the rigs were not operated due to stolen pumps.

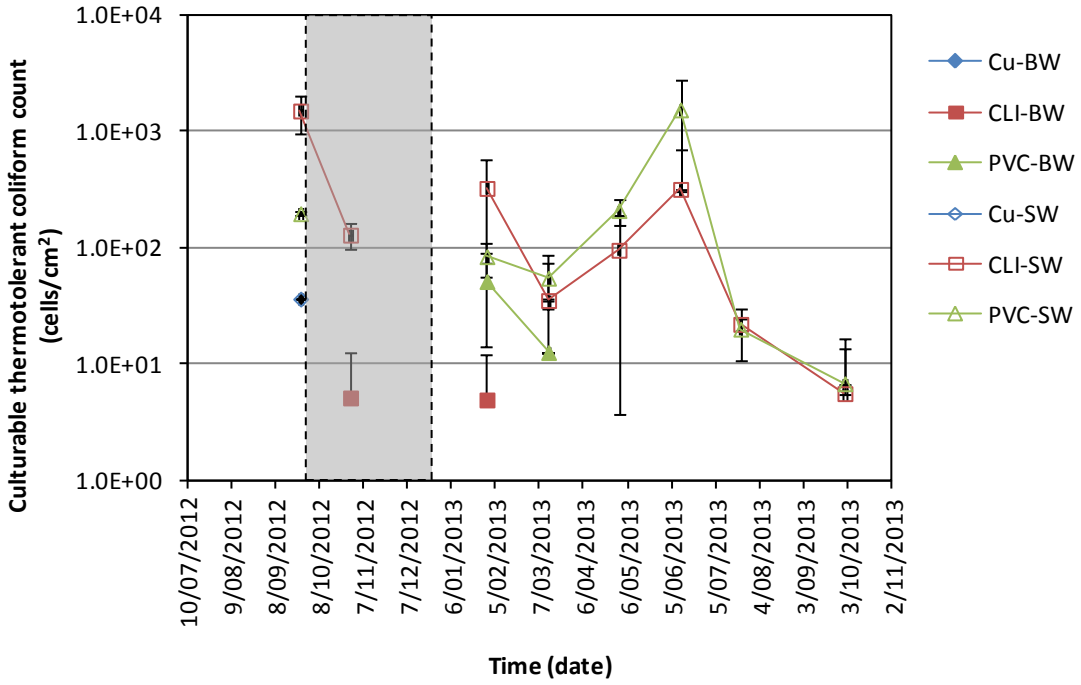


Figure 37. Culturable thermotolerant coliform counts on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (BW) or stormwater (SW) as determined with plate counting and incubation at 37/45 °C. Most of the counts on copper coupons were below detection limit. The grey area shows the outage period (24.10-20.12.2012) when the rigs were not operated due to stolen pumps.

4.4.1.3 Pathogens

DNA extracted from the pilot rig coupons exposed to baseline water or stormwater was tested for the presence of bacterial DNA with universal bacterial primers. All samples were positive for the presence of bacteria (Tables 14 and 15). Extracted DNA was also screened for the presence of PCR inhibitors by 10 and 100 fold dilution of the extracted DNA. No PCR inhibition was observed from the C_T values of real time PCR with universal bacterial primers as C_T values increased with the dilution of DNA. As a result DNA without dilution was used for the real time PCR detection of enteric pathogens and opportunistic pathogens.

Biofilms grew on all the coupons placed in both baseline and stormwater during this study. Cement and PVC material coupons in the stormwater had higher bacterial 16S rRNA gene copy numbers in the biofilm as evident from the lower C_T values as compared to copper coupons exposed to stormwater or coupons exposed to baseline water (Tables 14 and 15). The extracted DNA from the biofilms were tested for the presence of spore forming bacteria *C. perfringens* and *C. difficile* and samples were found to be negative. Similarly, all samples were negative for the presence of *S enterica*, *Campylobacter* spp. and *Cryptosporidium*. One single sample extracted from copper coupon in baseline water after 6 months of incubation was positive for human adenovirus however, C_T value of 40 suggest very low numbers in the biofilm. In the same sample *P. aeruginosa* and *A. hydrophila* were also detected. *A. hydrophila* was occasionally also found in other samples but primarily in the baseline water. *P. aeruginosa* was regularly detected in the biofilms from all three coupon types in both baseline and stormwater. *P. Aeruginosa* is a major cause of hospital infections (Percival and Walker 1999)

Table 11.PCR detection of enteric pathogens and opportunistic pathogens in biofilms from copper, cement and PVC coupons exposed to baseline water.

Sample ID	Date	Bacteria (C_T values)	*Clostridium difficile*	*Clostridium* perfringens	*Aeromonas hydrophila*	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Campylobacter* spp.	Human Adenovirus	*Cryptosporidium parvum*
Cement lined iron										
ML3	29/10/2012	1 (24.19)	0	0	0	0	0	0	0	0
ML4	29/10/2012	1 (24.06)	0	0	0	0	0	0	0	0
ML15	30/01/2013	1 (20.62)	0	0	0	0	0	0	0	0
ML16	30/01/2013	1 (26.58)	0	0	0	0	0	0	0	0
ML9	13/03/2013	1 (27.03)	0	0	0	0	0	0	0	0
ML10	13/03/2013	1(26.19)	0	0	0	0	0	0	0	0
ML21	30/04/2013	1(24.29)	0	0	0	**1**	0	0	0	0
ML22	30/04/2013	1(25.65)	0	0	0	**1**	0	0	0	0
ML27	11/06/2013	1(24.19)	0	0	0	0	0	0	0	0
Copper										
MC15	30/01/2013	1(27.03)	0	0	0	0	0	0	0	0
MC16	30/01/2013	1(26.19)	0	0	0	0	0	0	0	0
MC9	13/03/2013	1(22.27)	0	0	0	**1**	0	0	0	0
MC10	13/03/2013	1(24.69)	0	0	0	**1**	0	0	0	0
MC21	30/04/2013	1(25.06)	0	0	**1**	**1**	0	0	0	0
MC22	30/04/2013	1(25.36)	0	0	0	**1**	0	0	0	0
MC27	11/06/2013	1(25.59)	0	0	**1**	**1**	0	0	**1**	0
MC28	11/06/2013	1(24)	0	0	0	**1**	0	0	0	0
PVC										
MP15	30/01/2013	1(25.7)	0	0	0	**1**	0	0	0	0
MP16	30/01/2013	1(26.76)	0	0	0	**1**	0	0	0	0
MP9	13/03/2013	1(24)	0	0	0	**1**	0	0	0	0
MP10	13/03/2013	1(21.82)	0	0	0	**1**	0	0	0	0
MP21	30/04/2013	1(23.83)	0	0	**1**	**1**	0	0	0	0
MP22	30/04/2013	1(24.4)	0	0	0	**1**	0	0	0	0
MP27	11/06/2013	1(21.08)	0	0	0	**1**	0	0	0	0
MP28	11/06/2013	1(23.21)	0	0	0	**1**	0	0	0	0

M = Baseline water, L = cement lined iron coupon, C = copper coupon, P = PVC coupon, 1 = detection and 0 = no detection

Table 12. PCR detection of enteric pathogens and opportunistic pathogens in biofilms from copper, cement and PVC coupons exposed to stormwater.

Sample ID	Date	Bacteria (C_T values)	*Clostridium difficile*	*Clostridium* perfringens	*Aeromonas hydrophila*	*Pseudomonas aeruginosa*	*Salmonella enterica*	*Campylobacter* spp.	Human Adenovirus	*Cryptosporidium parvum*
Cement lined iron										
SWL3	29/10/2012	1(20.62)	0	0	0	0	0	0	0	0
SWL4	29/10/2012	1 (26.58)	0	0	0	0	0	0	0	0
SWL15	30/01/2013	1(19.79)	0	0	0	**1**	0	0	0	0
SWL16	30/01/2013	1(22.75)	0	0	0	**1**	0	0	0	0
SWL9	13/03/2013	1 (20.06)	0	0	0	**1**	0	0	0	0
SWL10	13/03/2013	1(17.79)	0	0	0	**1**	0	0	0	0
SWL21	30/04/2013	1(16.85)	0	0	0	**1**	0	0	0	0
SWL22	30/04/2013	1(18.25)	0	0	0	**1**	0	0	0	0
SWL27	11/06/2013	1(17.97)	0	0	0	**1**	0	0	0	0
Copper										
SWC15	30/01/2013	1(24.06)	0	0	0	**1**	0	0	0	0
SWC16	30/01/2013	1(26.95)	0	0	0	0	0	0	0	0
SWC9	13/03/2013	1(24.05)	0	0	0	**1**	0	0	0	0
SWC10	13/03/2013	1(24.78)	0	0	0	**1**	0	0	0	0
SWC21	30/04/2013	1(23.85)	0	0	0	**1**	0	0	0	0
SWC22	30/04/2013	1(23.1)	0	0	0	**1**	0	0	0	0
SWC27	11/06/2013	1(21.68)	0	0	0	**1**	0	0	0	0
SWC28	11/06/2013	1(21.94)	0	0	0	**1**	0	0	0	0
PVC										
SWP15	30/01/2013	1(23.84)	0	0	0	**1**	0	0	0	0
SWP16	30/01/2013	1(20.81)	0	0	0	**1**	0	0	0	0
SWP9	13/03/2013	1(18.88)	0	0	0	**1**	0	0	0	0
SWP10	13/03/2013	1(19.53)	0	0	0	**1**	0	0	0	0
SWP21	30/04/2013	1(18.75)	0	0	0	**1**	0	0	0	0
SWP22	30/04/2013	1(17.84)	0	0	0	**1**	0	0	0	0
SWP27	11/06/2013	1(17.23)	0	0	0	**1**	0	0	0	0
SWP28	11/06/2013	1(18.66)	0	0	0	**1**	0	0	0	0

SW = Stormwater, L = cement lined iron coupon, C = copper coupon, P = PVC coupon, 1 = detection and 0 = no detection

4.4.1.4 Detection of nontuberculous mycobacteria (NTM)

All four bulk water samples and 24/25 biofilm samples from the pilot scale stormwater and baseline water pipe rigs were positive for *Mycobacteria* spp. by QPCR. No temperature data were available for the samples and no chlorine was present in the systems. NTM were present regardless of season, water sample type (baseline versus stormwater) and material type (Tables 16 and 17).

Table 13. Detection of NTM in bulk water of stormwater and baseline water pipe rigs.

Season (sampling time)	Water type	Positive result	C_T average
Summer (January 2013)	Stormwater	1/1	40.60
	Baseline water	1/1	42.24
Autumn (March 2013)	Stormwater	1/1	39.08
	Baseline water	1/1	41.09
Total positive		4/4	

Table 14. Detection of NTM in stormwater and baseline water pipe rig biofilms and field pipe scrapings.

Season (sampling time)	Water type	Material	Positive result	C_T average
Summer (January 2013)	Baseline water	PVC	2/2	44.93
		Cement lined iron	2/2	40.56
		Copper	2/2	45.75
	Stormwater	PVC	2/2	39.05
		Cement lined iron	2/2	39.37
		Copper	2/2	41.10
Autumn (March 2013)	Baseline water	PVC	2/2	45.02
		Cement lined iron	2/2	42.55
		Copper	2/2	42.39
	Stormwater	PVC	2/2	41.00
		Cement lined iron	2/2	41.93
		Copper	1/2	41.25
Winter (June 2012)	Stormwater	PVC (field pipe*)	2/2	36.39
Total positive			25/26	

*'Field pipe' refers to samples taken from stormwater irrigation pipeline at ASTR site (see Section 3.8.1.4).

NTM can cause disease in the lungs, skin, soft tissue, joints, bursa, tendon sheaths, bones and lymph nodes (Falkinham 1996; Hsu 1981; O'Brien, Geiter & Snider 1987; Wallace *et al.* 1983; Wolinsky 1979). The most important human pathogens include: *M. kansasii, M. genavense, M. marinum, M. simiae, M. scrofulaceum, M. szulgai, M. avium, M. haemophilum, M. intraceullare, M. malmoense, M. ukcerans, M. xenopi, M. abscessus, M. chelonae, M. fortuitum* and *M. smegmatis* (Horsburgh *et al*. 1986). NTM can be typically prevented by disinfection.

4.4.1.5 Detection of Legionella spp. in stormwater

One stormwater bulk water sample and 50% (n = 13) of biofilm samples from stormwater and baseline water pipe rigs and field stormwater pipe were positive for *Legionella* spp. by QPCR (Tables 18 and 19). The positive stormwater bulk sample was collected in autumn (March) (Table 18). Overall, *Legionella* spp. were more common in the stormwater samples compared to the baseline water samples. Low numbers precluded statistical analyses but more stormwater pipe rig biofilm samples were positive for *Legionella* spp. (8/12) than the baseline biofilm samples (3/12) and the C_T values were generally lower for stormwater than for baseline water indicating greater abundance of *Legionella* in the biofilms exposed to stormwater. More samples were positive in summer (January) (7/12) versus autumn (March) (4/12). Interestingly,

copper pipeline material showed no positive detections for *Legionella* spp. *Mycobacteria* and *Legionella* spp. are known to be harboured within amoebae in biofilms (Marciano-Cabral *et al*., 2010). Both bulk water and biofilm gave positive detection of NTM, but *Legionella* and amoebae were more often co-detected in biofilm than in bulk water.

Table 15. Detection of *Legionella* spp. *mip* gene in bulk water of stormwater and baseline water pipe rigs.

Season (sampling time)	Water type	Positive result	C_T average
Summer (January 2013)	Stormwater	0/1	ND
	Baseline water	0/1	ND
Autumn (March 2013)	Stormwater	1/1	31.13
	Baseline water	0/1	ND
Total positive		1/4	

ND = no detection after 35 cycles.

Table 16. Detection of *Legionella* spp. *mip* gene in stormwater and baseline water pipe rig biofilms and field pipe scrapings.

Season (sampling time)	Water type	Material	Stormwater biofilm	
			Positive result	C_T average
Summer (January 2013)	Baseline water	PVC	1/2	33.07
		Cement lined iron	2/2	31.10
		Copper	0/2	ND
	Stormwater	PVC	2/2	31.08
		Cement lined iron	2/2	33.24
		Copper	0/2	ND
Autumn (March 2013)	Baseline water	PVC	0/2	ND
		Cement lined iron	0/2	ND
		Copper	0/2	ND
	Stormwater	PVC	2/2	31.82
		Cement lined iron	2/2	32.14
		Copper	0/2	ND
Winter (June 2012)	Stormwater	PVC (field pipe*)	2/2	26.04
Total positive			13/26	

*'Field pipe' refers to samples taken from stormwater irrigation pipeline at ASTR site (see Section 3.8.1.4).
ND = no detection after 35 cycles

4.4.1.6 Microbial communities

The bacterial and eukaryotic communities on the coupons of the pilot rigs were analysed by pyrosequencing. The relative abundance of various bacterial families (with ≧1 % abundance) in the samples retrieved in January, March and June 2013 are shown in Figures 53 to 55, respectively and percent values in Appendices 8-10. The relative abundance of various eukaryotic families (with ≧1 % abundance) in the samples obtained in January, March and June 2013 are shown in Figures 56-58, respectively, and percent values in Appendices 11-13. For the sampling time in January the DNA from the replicate samples were combined for analysis whereas the replicate samples from March and June were analysed separately. Notable differences were observed between the bacterial communities on various materials, communities exposed to baseline water and stormwater, and communities observed at different sampling times.

Bacterial families

January 2013

In January 2013, the total number of families with ≧1 % relative abundance were 10 for copper coupons in both baseline water and stormwater pipe rigs, 4 and 5 for cement lined iron coupons in baseline water and stormwater rigs, respectively, 2 and 4 for PVC coupons in baseline water and stormwater rigs, respectively, and 7 and 13 for bulk baseline water and stormwater, respectively (Table 20).

The most dominant bacterial family on copper coupons for both baseline water and stormwater in January 2013 was Nitrosomonadaceae representing 29.1 and 22.1 % of all bacteria in baseline water and stormwater pipe rigs, respectively (Appendix 9). Burkholderiaceae was the dominant family on both cement lined iron and PVC coupons in the baseline water rig with 71.7% and 91.5% abundances, respectively, whereas Comamonadaceae (55.4%) and Pseudomonadaceae (88.1%) were the dominant families on cement lined iron and PVC in stormwater rig, respectively. Burkholderiaceae was also the most dominant family in both bulk baseline water (39.9%) and stormwater (14.6%).

March 2013

The total number of families with ≧1 % relative abundance was higher for both biofilm and bulk water samples in March 2013 as compared to the January 2013 samples. In March 2013 the total number of families with ≧1 % abundance were 14-19 and 16-18 for copper coupons in baseline water and stormwater pipe rigs, respectively, 14-15 and 14-18 for cement lined iron coupons in baseline water and stormwater rigs, respectively, 13-16 and 16-18 for PVC coupons in baseline water and stormwater rigs, respectively, and 18 and 22 for bulk baseline water and stormwater, respectively (Table 18).

The most dominant bacterial family on copper coupons for baseline water in March 2013 was Nitrosomonadaceae representing 21.1-24.4% of all bacteria (Appendix 10). On copper coupons exposed to stormwater Nitrosomonadaceae (12.8-14.3%), Rhodocyclaceae (11.0-15.2%), Sinobacteraceae (11.3-12.0%), Cytophagaceae (9.2-12.5%) showed the highest relative abundances. For cement lined iron exposed to baseline water Nitrosomonadaceae (20.1-21.7%), Comamonadaceae (14.1-22.8%) and Nitrospiraceae (12.1-17.1%) showed highest abundances. The variation in the relative abundances of dominant bacterial families between the two cement lined iron coupons exposed to stormwater was very high. While the community on one coupon was very dominated by Flavobacteriaceae (53.7%) the other coupon was dominated by Nitrospiraceae (19.8%). Nitrospiraceae was also the dominant family on plastic coupons exposed to both baseline water (13.3-16.2%) and stormwater (15.6-20.2%). The most abundant family in bulk baseline water and stormwater were Sphingomonadaceae (11.0%) and Sinobacteraceae (15.9%), respectively.

June 2013

The total number of families with ≧1 % relative abundance was lower for both biofilm and bulk water samples in June 2013 as compared to the March 2013 samples (Appendix 11). In June 2013 the total number of families with ≧1 % abundance were 5-10 and 5-6 for copper coupons in baseline water and stormwater pipe rigs, respectively, 7-8 and 10-12 for cement lined iron coupons in baseline water and stormwater rigs, respectively, 6 and 5-17 for PVC coupons in baseline water and stormwater rigs, respectively, and 9 and 12 for bulk baseline water and stormwater, respectively (Table 20).

The most dominant bacterial family on copper coupons for baseline water in June 2013 was Bradyrhizobiaceae representing 31.2-78.7% of all bacteria, while cement lined iron and PVC coupons exposed to baseline water were dominated by Comamonadaceae with 53.5-72.4% and 66.0-77.2%

abundances, respectively. Copper, cement lined iron and PVC coupons exposed to stormwater were all dominated by Flavobacteriaceae with 79.0-81.1%, 44.7-52.7% and 31.6-63.3% abundances, respectively. The most dominant family in both bulk baselinewater and stormwater was Comamonadaceae with 58.5% and 37.8% abundances, respectively.

Table 17. Summary of the number of bacterial families with ≧1 % relative abundance on pipe rig coupons made of various materials and exposed to baseline water (MW) or stormwater (SW) and in bulk baseline water and stormwater.

	Biofilm						Bulk water	
	Copper		Cement lined iron		PVC			
Sampling time	BW	SW	BW	SW	BW	SW	BW	SW
January 2013	10	10	4	5	2	4	7	13
March 2013	14-19	16-18	14-15	14-18	13-16	16-18	18	22
June 2013	5-10	5-6	7-8	10-12	6	5-17	9	12

Eukaryotic taxa

January 2013

In January 2013 the total number of eukaryotic taxa with ≧1 % relative abundance were 9 for copper coupons in baseline water and 11 in stormwater in the pipe rigs, 12 and 14 for cement lined iron coupons in baseline water and stormwater rigs, respectively, 13 and 3 for PVC coupons in baseline water and stormwater rigs, respectively, and 1 and 4 for bulk baseline water and stormwater, respectively (Table 21).

The most dominant eukarotic taxa on copper coupons for both baseline water and stormwater in January 2013 belonged to Fungi representing 45.7% and 20.6 % of all eukaryotes in baseline water and stormwater pipe rigs, respectively (Appendix 12). Fungi were the dominant taxa on both cement lined iron (50.7% and 30.0% for baseline water and stormwater, respectively) and PVC baseline water coupons (31%). PVC coupons in the stormwater rig were colonised with 89.0% Heteromitiea (amoeboid and flagellated protozoans). Fungi were also the most dominant taxa in bulk baseline water (96.5%) and stormwater (43.2%), but stormwater had high levels of Paraphysomonadaceae (algae) at 36.0%.

March 2013

The total number of eukaryotic taxa with ≧1 % relative abundance (shown in Appendix 13) was similar or slightly higher for both copper and cement lined iron biofilm samples in March 2013 (5-12 for Cu in baseline water, 12-16 for Cu in stormwater, 10-19 for cement lined iron in baseline water and 9-14 for cement lined iron in stormwater) as compared to the January 2013 samples (9 for Cu in baseline water, 11 for Cu in stormwater, 12 for cement lined iron in baselinewater, and 14 for cement lined iron in stormwater (Table 19). March PVC samples showed a greater increase in eukaryotic taxa (18-25 for PVC in baseline water and 11-12 for PVC in stormwater) as compared to the January samples (13 for PVC in baseline water and 3 for PVC in stormwater). March bulk baseline water and stormwater samples also had increased number of eukayotic taxa with ≧1% relative abundance, 21 and 9 respectively (Table 21).

The most dominant eukaryotic taxa on copper coupons for baseline water in March 2013 was Dipodascaceae (yeast) representing 39.0%, Acaulosporaceae (Fungi) 25.0% and Cercomonadidae (amoebae and flagellates) at 21.0% (Figure 41). On copper coupons exposed to stormwater these were Araeolaimida (free living nematodes) (29.0%), Blastocladiaceae (Fungi) (17.0%), Acaulosporaceae (Fungi) (13.1%), Thaumatomonadida (biflagellated heterotrophic flagellates) (13.1%) and Fungi (13.0%). For cement lined iron exposed to baseline water Ulvales (green algae) (58.2%) and Monoblepharidales (Fungi) (37.0%) showed highest abundances for the two replicates. The stormwater cement lined iron coupons both had the highest percentage for the taxa Monhysterida (nematodes) (30.2% and 44.4%). The baseline water PVC coupons were colonised with Pyramimonadales (green algae) at 14.8% and 38.8%, respectively. PVC

stormwater coupons had Monhysterida (nematodes) (34.7% and 31.9%) and Thaumatomonadida (biflagellated heterotrophic flagellates) (17.2% and 15.8%) as the most abundant taxa present. The most abundant family in bulk baseline water and stormwater were Chrysolepidomonadaceae (microalgae) (18.7%) and Araeolaimida (free living nematodes) (39.7%), respectively.

June 2013

The total number of taxa with ≧1 % relative abundance was lower for both biofilm and bulk water samples in June 2013 as compared to the March 2013 samples (Figure 57, Appendix 13). In June 2013 the total number of families with ≧1 % abundance were 9-11 and 9-9 for copper coupons in baseline water and stormwater pipe rigs, respectively, 7-9 and 7-10 for cement lined iron coupons in baseline water and stormwater rigs, respectively, 7-18 and 8-10 for PVC coupons in baseline water and stormwater rigs, respectively, and 10 and 7 for bulk baseline water and stormwater, respectively (Table 21).

The most dominant eukaryotic taxa on coupons exposed to baseline water in June 2013 was Fungi representing 43.3-50.5% (Cu), 13.8-67.9% (cement lined iron) and 23.7-27.3% (PVC) of all taxa. Only one of the replicate cement lined iron coupons in baseline water pipe rig had Chromulinaceae (microalgae) (29.9%) as the most abundant taxa. The communities on the two replicate copper stormwater coupons were different from each other. One of the coupons had Hartmannellidae (amoebae) (24.2%) and Cercozoa (amoebae and flagellates) (30.7%) as most abundant, while the other had Eimeriidae (protists)(40.3%) as most abundant. One of the cement lined iron coupons exposed to stormwater had Didymellaceae (Fungi) and Cecozoa (amoebae and flagellates) (42.5% and 35.2%, respectively) and the other coupon had Fungi (27.3%) as most abundant. One of the PVC coupons exposed to stormwater was colonised by Hypocreales (Fungi) (31.7%), Fungi (23.0%) and Cercozoa (amoebae and flagellates) (21.7%) and the other coupon by Fungi (34.9%) and Stramenopiles (algae) (28.8%) as most abundant. The most dominant family in bulk baselinewater was Fungi (34.9%) and bulk stormwater was Eimeriidae (56.7%).

Table 18. Summary of the number of eukayotic families with ≧1 % abundance on pipe rig coupons made of various materials and exposed to baseline water (MW) or stormwater (SW) and in bulk baseline water and stormwater.

	Biofilm						Bulk water	
	Copper		Cement lined iron		PVC			
Sampling time	BW	SW	BW	SW	MW	SW	MW	SW
January 2013	9	11	12	14	13	3	1	4
March 2013	5-12	12-16	10-19	9-14	18-25	11-12	21	9
June 2013	9-11	9-9	7-9	7-10	7-18	8-10	10	7

4.4.2 Microbial communities in field stormwater pipe

The water quality parameters observed for the field samples taken from the existing PVC irrigation pipeline in June 2011 are listed in Appendix 15. Microbiological analysis of the biofilm showed the presence of ample microbial cells (6.6-9.9*10^6 cells/cm^2). In addition, microbial eukaryotes, i.e. amoebae, were detected in the stormwater biofilm samples (data not shown). Profiling of the eubacterial species present in the biofilm by DGGE showed the presence of several bacterial genera based on percent similarity of the 16S rRNA gene (Table 19). The identified groups also represent various metabolic capabilities (Table 19).

Table 19. Bacteria identified in existing stormwater pipe with denaturing gradient gel electrophoresis of 16S rRNA genes.

Closest genera	% Similarity	Possible metabolism
Nitrosospira sp.	73-93	Autotrophic ammonia oxidation
Bacillus sp.	72	Heterotrophic nitrification/denitrification
Burkholderia sp.	69-74	Heterotrophic (some pathogenic)
Pantoea sp.	74	(some plant pathogens)
Methylosoma sp.	94	Methane/methanol oxidation, N-fixation
Sphingobacteriales sp.	76	(some pathogenic)
Dokdonella sp.	70	Heterotrophic
Herbaspirillum sp.	72	Heterotrophic N-fixation
Leptothrix sp.	75	Fe^{2+} and Mn^{2+} oxidation
Methylobacillus sp.	75	Methane/methanol oxidation
Uliginosibacterium sp.	75	Heterotrophic
Arcicella sp.	71	Heterotrophic
Halanaerobium sp.	71	Heterotrophic

The bacterial and eukaryotic communities in the existing stormwater pipe biofilms were also characterised using pyrosequencing. The total number of bacterial families with ≧1 % relative abundance in existing field stormwater pipe biofilms was 18 and 24 for the two replicate samples shown in Figures 38 and 39. The dominant family in both samples was Clostridiaceae with 20.1-14.3% abundance. The next dominant family was Rhodocyclaceae (7.0-8.7%). A total of 17 of the families with ≧1 % relative abundance were not detected in the pipe rig samples. These families were Chlorobiaceae, Clostridiaceae, Crenotrichaceae, Desulfobacteraceae, Desulfobulbaceae, Halomonadaceae, Methylococcaceae, Myxococcaceae, Pirellulaceae, Polyangiaceae, Pseudonocardiaceae, Rhodothermaceae, Ruminococcaceae, Streptomycetaceae, Syntrophaceae, Thermoanaerobacterales, Thermodesulfovibrionaceae.

Desulfobacteraceae, Desulfobulbaceae and Thermodesulfovibrionaceae families contain sulphate reducing bacteria, which can generate H_2S which is malodorous and corrosive and hence may decrease the quality of water and result in infrastructure deterioration.

The total number of eukaryotic families with ≧1 % relative abundance in existing field stormwater pipe biofilms was 21 and 19 for the two replicate samples. The dominant taxa in both samples were Calcinea (calcareous sponge) with 26.2-30.7% abundance (Figure 43). The next dominant family was Perkinsidae (protozoans) (9.7-8.8%). A total of 13 of the taxa with ≧1 % relative abundance were not detected in the pipe rig samples. These taxa were Acantharian, Anurofeca, Calcinea, Capsaspora, Codonosigidae, Desmidiales, Ochromonadaseae, Perkinsidae, Pucciniomysotina, Ustilaginomycotina, Vampyrellidae, and Vexilliferidae.

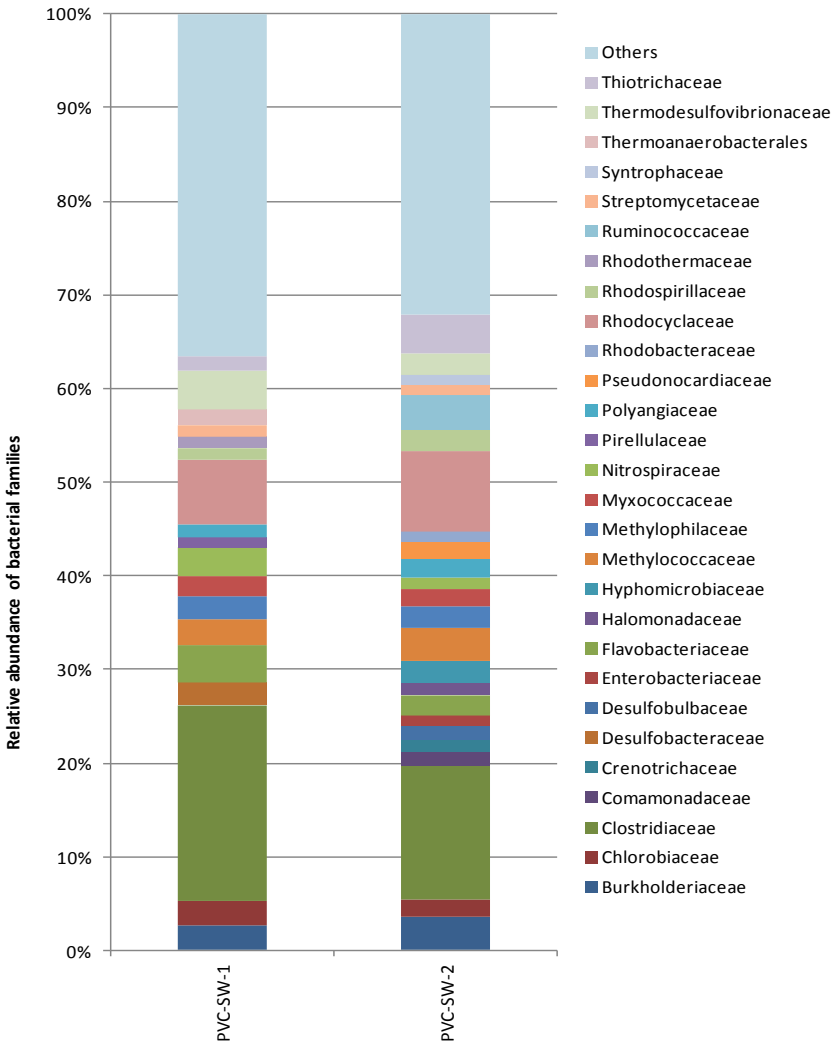


Figure 38. Relative abundance of bacterial families detected in the two samples from the field PVC stormwater pipe June 2012. The DNAs from the replicate samples were analysed separately. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into “Others”. The percent values for each of the families are shown in Appendix 15.

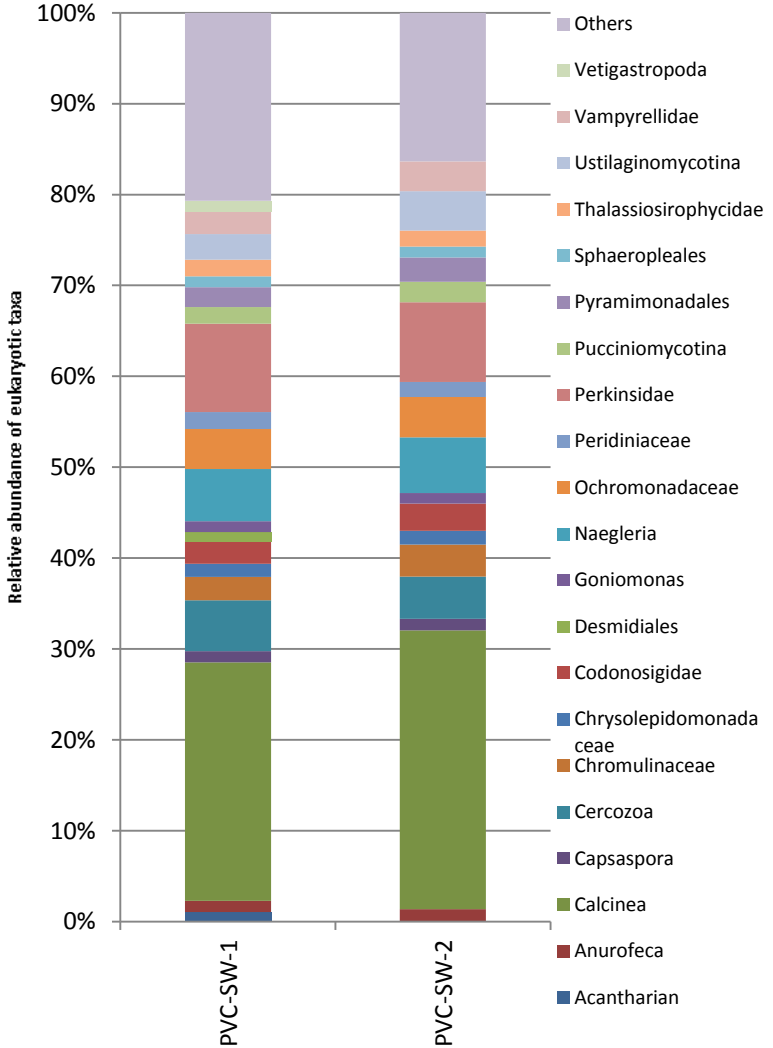


Figure 39. Relative abundance of eukaryotic taxa detected in the two samples from the field PVC stormwater pipe in June 2012. The DNAs from the replicate samples were analysed separately. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into “Others”. The percent values for each of the families are shown in Appendix 16.

4.5 Discussion

4.5.1 Microbial analysis

Cell counts were somewhat higher for the stormwater exposed than the baseline water exposed copper and PVC coupons during most of the study, however the difference was not consistent for cement lined concrete coupons. None of the coupon materials stood out as having consistently higher or lower culturable cell numbers than the other materials. Neither was there a consistent difference between coupons exposed to baseline water and stormwater. When quantifying bacterial 16S rRNA genes using QPCR, cement lined iron and PVC coupons in the stormwater had higher 16S rRNA gene copy numbers as compared to copper coupons exposed to stormwater or coupons exposed to baseline water. It is to be noted that some bacterial species and genera may harbour larger number of 16S rRNA copies in their genome than others.

A summary for detection of bacterial families and eukaryotic taxa on various coupon materials, pipe rigs exposed to baseline water or stormwater and bulk water sources is shown in Appendices 16 and 17. Some of the detected families (representing 1% or more of the total bacterial or eukaryotic community) were observed only in biofilms exposed to either baseline water or stormwater. Likewise some of the families were only detected on some of the materials, but not others, or either in bulk baseline water or stormwater. This indicates that both the source water and pipe material influence the entire microbial community (bacterial and eukaryotic) in the pipe wall biofilms.

A total of 19 bacterial families and 43 eukaryotic taxa were common for the biofilms of both stormwater and baseline water pipe rigs (Figure 40). Additionally 14 unique bacterial families and 22 unique eukaryotic taxa were detected in the stormwater pipe rig biofilms, but were not detected in baselinewater pipe rig biofilms. Similarly 15 unique bacterial families and 30 unique eukaryotic taxa were detected in baseline water pipe rig biofilms that were not detected in the stormwater piperig biofilms.

A total of 13 bacterial families and 8 eukaryotic taxa were common for the bulk stormwater and bulk baseline water (Figure 40).Additionally 18 unique bacterial families and 11 unique eukaryotic taxa were detected in the bulk stormwater that were not detected in bulk baseline water. Similarly 11 unique bacterial families and 22 unique eukaryotic taxa were detected in bulk baseline water, but were not detected in bulk stormwater.

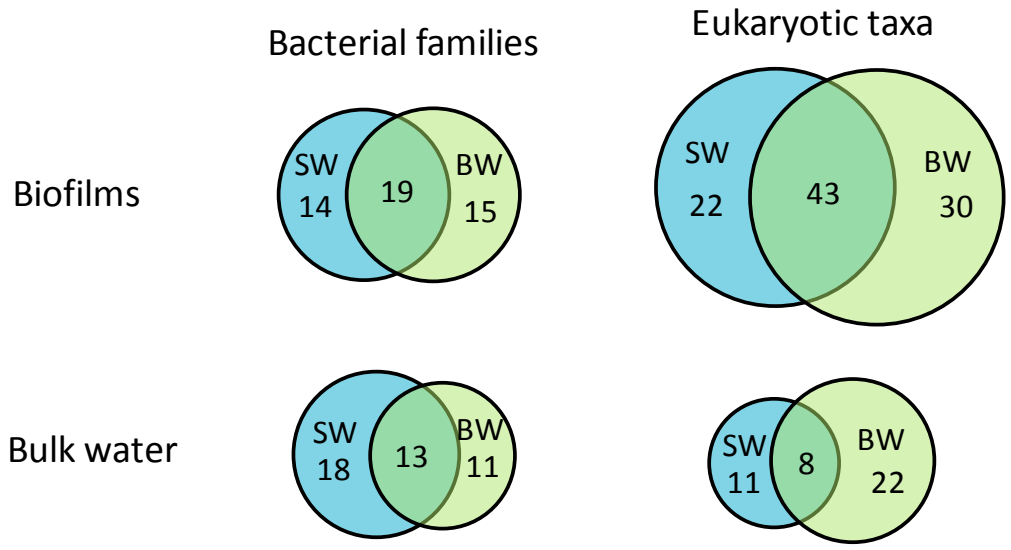


Figure 40. . Unique and shared bacterial and eukaryotic taxa between the coupon biofilms exposed to stormwater (SW) and baseline water (BW).

A total of 23 bacterial families and 28 eukaryotic taxa were common for copper, cement lined iron and PVC coupons in the pipe rigs when looking at the combined communities from both stormwater and baseline water pipe rigs (Figure 41). Nine, two and one bacterial families were unique for copper, cement lined iron and PVC coupons, respectively, and 14, 19 and 11 eukaryotic taxa were unique for copper, cement lined iron and PVC coupons, respectively.

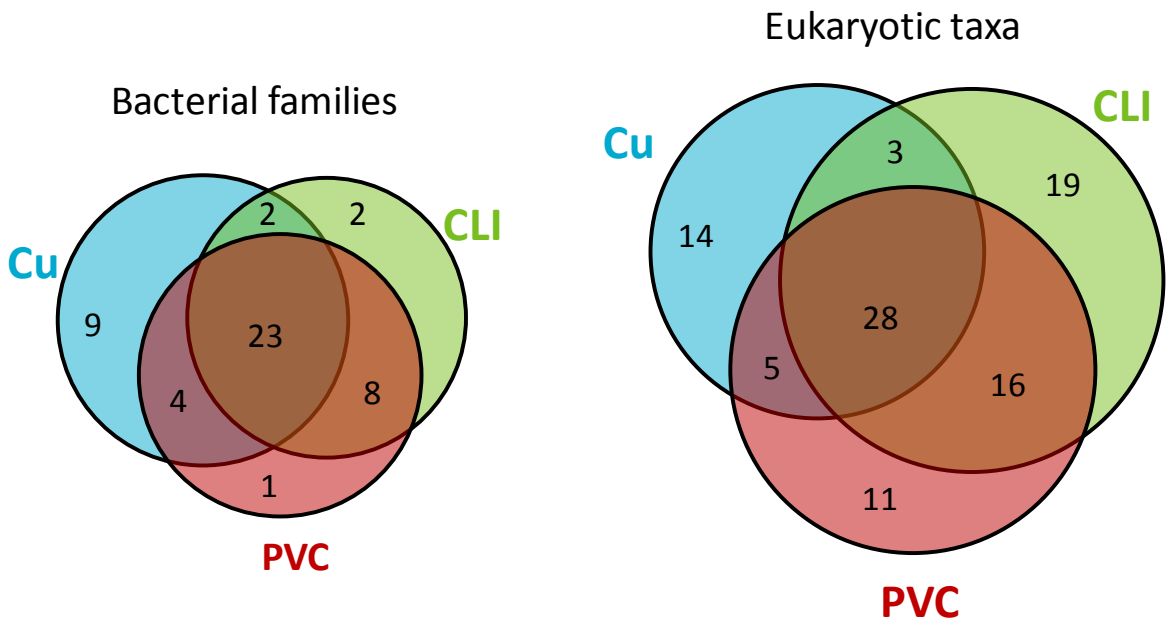


Figure 41. Unique and shared bacterial and eukaryotic taxa between the biofilms developed on copper, cement lined iron (CLI) and PVC coupons.

Bacterial families that were only observed in biofilms exposed to stormwater included Acidithiobacillaceae, Acidobacteriaceae, Chlorobiaceae, Chromatiaceae, Clostridiaceae, Coxiellaceae, Crenotrichaceae, Desulfobacteraceae, Desulfobulbaceae, Desulfurellaceae, Flavobacteriaceae, Halomonadaceae, Holosporaceae, Hydrogenophilaceae, Hyphomicrobiaceae, Hyphomonadaceae, Methylococcaceae, Methylophilaceae, Micrococcaceae, Myxococcaceae, Oxalobacteraceae, Pirellulaceae, Polyangiaceae, Pseudomonadaceae, Pseudonocardiaceae, Rhodothermaceae, Rikenellaceae, Ruminococcaceae, Streptomycetaceae, Synergistaceae, Syntrophaceae, Thermoanaerobacterales, and Thermodesulfovibrionaceae (Appendix 18). The significance of these for being human pathogens or affecting pipe materials and water quality is discussed below.

Bacterial families detected in biofilms exposed to baseline water (but not to stormwater) included Acidimicrobiales, Alicyclobacillaceae, Armatimonadaceae, Beijerinckiaceae, Caldilineaceae, “Candidatus Solibacter”, Caulobacteraceae, Chitinophagaceae, Erythrobacteraceae, Gallionellaceae, Phycisphaeraceae, Planctomycetaceae, Planococcaceae and Xanthomonadaceae (Appendix 18).

Dominant genera in the bacterial families observed with ≧1% relative abundance either in the pipe rigs or field stormwater pipe samples and some characteristics of each genera are shown in Appendix 18. Both aerobic and anaerobic bacteria were observed in baseline water and stormwater biofilms and metabolically the communities were diverse with chemolitotrophs, chemoorganotrophs, photolitotrophs and photoorganotrophs. As the biofilms are not exposed to light in the pipes, the phototrophic bacteria are likely to originate from the source water rather than exhibit phototrophic activity in the pipes (Appendix 20).

Bacterial genera (and families) which harbour potential human pathogens and which were observed with ≧1% relative abundance in biofilms exposed to both stormwater and baseline water included *Afipia* (Bradyrhizobiaceae), *Arcobacter* (Campylobacteraceae), *Escherichia*, *Shigella* (Enterobacteraceae), *Legionella* (Legionellaceae) and *Acinetobacter* (Moraxellaceae). Additionally the following genera (and families) which harbour potential human pathogens were observed only in biofilms exposed to stormwater with ≧1% relative abundance: *Burkholderia* (Burkholderiaceae), *Clostridia* (Clostridiaceae), *Flavobacterium* (Flavobacteriaceae), *Massilia* (Oxalobacteraceae), *Pseudomonas* (Pseudomonaceae), *Streptomyces* (Streptomycetaceae). No additional families harbouring potential human pathogens were observed in the baseline water biofilms that would not have been observed in the storm water biofilms with ≧1% relative abundance. The results indicate that the stormwater may increase the number of families with potential human pathogens in water distribution networks as compared to baseline water (Appendix 19).

Families harbouring dissimilatory sulfate reducing bacteria (Desulfobacteriaceae, Desulfobulbaceae, Nitrospira (*Thermodesulfovibrio*)) were observed with ≧1% relative abundance only in the pipe rig biofilms exposed to stormwater. A family with dissimilatory sulfur reducing bacteria (Desulfurellaceae) were observed with ≧1% relative abundance only in the existing field stormwater pipe. Under anaerobic conditions sulfate and sulfur reducing bacteria generate H_2S, which is corrosive and malodorous, and hence can deteriorate pipe materials and cause odour problems. Therefore the biofilms supported by stormwater may shorten the life of pipe materials susceptible to corrosion and decrease water quality (Appendix 19).

The biofilms exposed to baseline water harboured aerobic iron oxidising bacteria belonging to the family Galionellaceae and genus *Sideroxydans* with ≧1% relative abundance, whereas the following families (with iron oxidising genera) were detected in the biofilms exposed to stormwater with ≧1% relative abundance: Acidithiobacillaceae (*Acidithiobacillus*), Burkholderiaceae (*Leptothrix*), Crenotrichaceae (*Crenothrix*) and Hydrogenophilaceae (*Thiobacillus*). Additionally iron oxidising genus *Rhodovulum* from family Rhodobacteraceae was observed in biofilms from both stormwater and baselinewater systems. Iron oxidising bacteria may contribute to the deposition of ferric iron precipitates in the distribution systems and discoloured water events caused by ferric iron precipitates (Appendix 20 and 21).

A number of the bacterial genera detected by pyrosequencing in both baseline water and stormwater systems can facilitate nitrogen cycling by contributing to nitrogen fixation, nitrite oxidation, and denitrification (reduce nitrate or nitrite) (Appendix 20). Additionally, ammonia oxidising bacteria were observed using the DGGE in the existing stormwater pipe (Table 21). Nitrogen fixing bacteria may provide a source of nitrogen to the overall microbial community, even if the source water nitrogen concentrations were low.

Eukaryotic taxa that were only observed in biofilms exposed to stormwater included Acantharian, Anurofeca, Calcinea, Capsospora, Codonaosigidae, Desmidiales, Ochromonadaceae, Perkinsidae, Pucciniomycotina, Ustilaginomycotina, Vampyrellidae and Vestigastropoda (Appendix 19).

Eukaryotic taxa detected in biofilms exposed to baseline water (but not to stormwater) included Acanthamoebidae, Acrosiphoniales, Agaricomycotina, Ambisporaceae, Asparagaceae, Biddulphiophycidae, Catenariaceae, Chromadorida, Colpodea, Ectocarpales, Euglyphida, Hydruraceae, Labyrinthuloides, Laminariales, Maxillopoda, Orchitophryidea, Peritrichia, Saccharomycetales, Sebacinaceae, Streptophyta and Ulvales (Appendix 19)

Dominant genera in the eukaryotic taxa observed with ≧1% relative abundance either in the pipe rigs or field stormwater pipe samples and some characteristics of each genera are shown in Appendix 21. A diverse range of eukaryotic organisms were observed in baseline water and stormwater biofilms. The communities contained phototrophic algae, heterotrophic fungi, heterotrophic yeast, ciliates, amoebae and nematodes, which indicate a dynamic system capable of supporting heterotrophic eukaryotes as well as bacterial and eukaryotic grazers. As the biofilms are not exposed to light in the pipes, the phototrophic

algae are likely to originate from the source water rather than exhibit phototrophic activity in the pipes (Appendix 21).

Eukaryotic genera which harbour potential human pathogens and which were observed with ≧1% relative abundance in biofilms exposed to both stormwater and baseline water included several free living amoebae from *Acathamoebe*, *Hartmannella* and the *Naegleria* genera. These genera contain human pathogens as well as non-pathogens which are known to harbour pathogen bacteria (Appendix 21).

Biofilms are considered as main source of microorganism in the water distribution pipes fed with adequately treated water (LeChevallier *et. al.,* 1987). A number of factors such as temperature, residual chlorine concentration, dissolved organic matter and hydraulic conditions are reported to influence presence of occurrence of viable microorganism in drinking water and biofilms (LeChevallier *et. al.,* 1996; Codony *et. al.,* 2005). The level of free chlorine has been reported to control the concentration of microorganisms in water and development of biofilms in pipelines (Codnoy *et al.,* 2005). In the biofilms, microorganisms are reported to be 10-1000 times more resistant to the effects of antimicrobial agents (Processor *et al.,* 1987). Furthermore, penetration of disinfectants such as chlorine into biofilms containing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have been reported to be only 20% of the bulk media's concentration (De Beer *et. al.,* 1994). Substantially higher prevalence of coliform bacteria in the distribution net work that maintained free chlorine levels of <0.2 mg/liter or monochloramine levels of <0.5 mg/liter have been reported as compared the systems with higher disinfectant residuals (LeChevallier *et. al.,* 1996). Consequently, maintenance of adequate disinfectant concentration in the pipe networks is expected lower the chances of biofilm build up.

4.5.2 Pathogen detection in biofilm samples

Biofilms are ubiquitous and occur in all water supply and plumbing systems. There has been increasing evidence of presence and prolonged survival of enteric pathogens in the biofilms especially in the drinking water distribution networks (Huq *et al.,* 2008; Sha *et al.,* 2011, Wingender and Flemming 2011). Microorganism of public health significance such as enteric virus (e.g., adenoviruses, rotaviruses and noroviruses), protozoa (e.g., *Cryptosporidium parvum*), bacterial pathogens (e.g., *Salmonella etercica* and *Campylobacter* spp.) and opportunistic pathogens of environmental origin (e.g., *Legionella* spp., *Pseudomonas aeruginosa* and *Aeromonas hydrophilla*) may become integrated into pre-existing biofilms. Longer persistence time of *Campylobacter* spp., *Salmonella enteirca* has been previously reported in biofilms (Buswell *et al*., 1998).

Biofilm samples from different coupon materials were assayed for the presence of enteric pathogens and opportunistic pathogens by direct molecular detection using real time PCR. *S. enterica* and *Campylobacter* spp. are known to occur in aquatic environments especially in surface water impacted by storm runoff (Sidhu *et al.* 2012; Dyke *et al.,* 2009) and reported to survive in the biofilms (Buswell *et al.*, 1998). In this study, we tested for the presence of *Salmonella enterica* and *Campylobacter jejuni* and both were not detected in the baseline water (n=13) and stormwater biofilms (n=13). The *Campylobacter* spp. specific primer set used in this study are reported to detect common species such as *C. jejuni*, *C. coli* and *C. lari* which are common cause of campylobacteriosis in humans (Lund *et al.,* 2004). It is possible that other *Campylobacter* spp. or related species may be present in the biofilms as the metagenomic analysis has detected the presence of genera *Arcobacter*.

Spore forming Gram-positive, anaerobic *Clostridia* commonly occur in soil, water and decomposing organic matter. A few species are opportunistic pathogens e.g. *Clostridium perfringens, Clostridium difficile*, *Clostridium tetani* and *Clostridium botulinum*. *C. perfringens* are considered good surrogates for protozoan parasites such as *Cryptosporidium parvum* and *Giardia lamblia* due to relatively similar size and resistance to inactivation (Payment *et al.*, 1993; Teunis and Havelaar, 2002). In this study, *C. perfringens* and *C. difficile* both spore forming bacteria were not detected in biofilms from baseline or stormwater. However, metagenomic analysis of the biofilms has revealed the occurrence of genera *Clostridia*. Therefore, it is likely that species other than *C. difficile* and *C. perfringens* may be present in the biofilms. The presence of *C.*

perfringens was reported in few drinking water biofilms from Darwin in Australia (Storey and Kaucner 2009).

Opportunistic bacterial pathogens such as *Aeromonas* spp., *Legionella* spp., *Mycobacterium* spp. and *Pseudomonas* spp. naturally occur in aquatic and soil environments (Wingender and Flemming 2011). In this study, *P. aeruginosa* was commonly detected in biofilms from baseline water (8/13 samples, 61%) and stormwater (12/13 samples, 92%). *P. aeruginosa* was detected in the biofilms after a couple of months on cement whereas, on PVC and copper coupons this microorganism was detected in the first sampling event and then each time biofilms were sampled in both baseline and stormwater biofilms (Tables 12 and 13). Detection of *P. aeruginosa* in the biofilms is not unexpected as pseudomonads are found in the aquatic environment and reported to commonly occur in Australian drinking water supplies (Storey and Kaucner 2009). *A. hydrophila* was detected in baseline water supply biofilms only on copper and PVC coupons (3/13 samples, 23%) whereas, *A. hydrophila* was not detected in the stormwater biofilms. *A. hydrophila* has been reported in the drinking water biofilms (7.7%) in a study from the United States (Chauret *et al.,* 2001). The presence of *P. aeruginosa* and *A. hydrophila* in the biofilms indicate a potential for regrowth and contamination of the water distribution systems. This suggests that chlorination of the piped water may be required to reduce biofilm formation and potential contamination of water supply from the dislodged biofilms. Biofilm reduction in turn would also reduce sediment attachment to the pipe material. In addition, *Legionella* and NTM species were also detected in both stormwater and baseline water samples (biofilm and bulk water), except on copper coupons, which were negative for *Legionella spp*. The biofilms with the greatest number of NTM and *Legionalla* present, as indicated by QPCR, were the field pipe scraping from the existing recycled stormwater system. This further indicates that these pathogens are readily capable of colonising the pipe wall biofilms.

Enteric viruses including human adenovirus and polyomavirus are stable in the environment and have been reported to be present in surface water (Hamza *et al*., 2009), stormwater (Rajal *et al.,* 2007) and drinking water (Lee and Kim 2002). In the present study, adenovirus was only detected in one out of 26 samples, in biofilm on copper coupon placed in the baseline water. The sample tested positive for the presence of adenovirus DNA however, it was just on the threshold of detection limit which suggests very low prevalence of adenovirus in the biofilms.

The infectious stages of *Cryptosporidium* and *Giardia* (oo)cysts can remain viable outside human hosts in aquatic environments for months and are resistant to chlorine and chloramine (Fraise *et al.,* 2008). Biofilms in the drinking water distribution network could be potential reservoirs of *Cryptosporidium* and *Giardia* (oo)cysts. In a study from England, ongoing recoveries of oocysts from a drinking water distribution system after a cryptosporidiosis outbreak was suggested to be due to the release of surviving oocysts from biofilms (Howe *et al.,* 2002). In this study, *Cryptosporidium parvum* was not detected from any of the 26 biofilm samples. This suggests potentially low prevalence of *Cryptosporidium* oocysts in the biofilms.

The results of this study suggests low occurrence of enteric pathogens in the baseline and stormwater biofilms developed over 10 months period. The presence of *P. aeruginosa* which is known to produce biofilms suggests that there is potential for the development of biofilms in the non chlorinated stormwater distribution systems due to the presence of high nutrient load. Enteric pathogens may become integrated into these pre-existing biofilms.

4.5.3 Water quality and aesthetics

Water discoloration and turbidity were the main aesthetic parameters evaluated in this study. An earlier desktop evaluation reported that treated stormwater is likely to exceed the 15 HU guideline value for true colour due to elevated iron concentrations (Page *et al.* 2009). As observed in this investigation colour in the stormwater was highly variable (2 - 94HU), whilst baseline water remained below 5HU. However, colour did not vary between water entering or within the rig. Instead it was determined that dissolved organic carbon from the source water was more influential on colour than iron. High DOC events are likely to be

associated with short aquifer residence time, hence they could be managed through control of aquifer residence time which results in natural attenuation of DOC.

Median turbidity in stormwater was 4.3 NTU and varied between 0.8 to 13NTU, with the highest reading associated with passage through the rig on the 1.10.2013 (also accompanied by increase in metals and TOC) and attributed to slough off of biofilm from internal pipe surfaces. It would be expected that disinfection would assist in preventing the build up of biofilm and hence could also positively impact water aesthetics.

The iron concentration stormwater (median 0.48mg/L total Fe) was often higher than the ADWG, as expected and would result in aesthetic changes, as observed. However, the baseline water (median 0.089mg/L total Fe) also had instances when the ADWG was exceeded. Those events could have been caused by release of sediment or corrosion products trapped in the water distribution network.

Although the microbial analysis revealed potential for sulphate-reducing bacteria in the stormwater, conditions within the rig were oxic during the study period. Odours had not been measured in the investigation, however during water sampling events no unpleasant smells such as H_2S were detected. In actual networks, sulphate reducing bacteria could thrive in stagnant water or anoxic conditions, such as in dead ends or following long residence times, causing odours. Disinfectant residual will also help manage such populations.

4.5.4 Infrastructure influences and impacts

The bulk of the experimental rig was made of PVC, hence water quality changes within the rig are indicative of the behaviour of an unchlorinated PVC network. Analysis of the materials (copper, cement and PVC) indicated that the PVC had the great propensity for sediment adhesion and accumulation in the rig when exposed to non-chlorinated stormwater. Conversely, the sediment accumulated was also easily dispersed when the water supply was exchanged, indicating ease of sediment removal in baseline water. However, whether the extent to which the sediment dissolution observed in a single sample swap would be reproduced in a larger network needs to be verified. Furthermore the factors contributing to the sediment dissolution would need to be investigated, e.g. what influence does the lower Fe content or the minimal chlorine residual have on the biofilm and the sediment resuspension.

Whilst concrete for its surface roughness was expected to more easily harbour biofilm, total cell numbers could not differentiate if stormwater favoured biofilm development. Cement surface coupons exposed to both water sources displayed some signs of carbonate reactivity in water. Sediment accumulation on the coupons initially appeared marginally faster on coupons exposed to baseline water, but discoloration was marginally stronger in stormwater, however over time there was large variability in the results and given the small number of replicates, no clear distinction in sediment trends could be determined for the cement using the colour and mass data, even after the swap. However, the mass of material removed from cement coupons using sonication was larger than on the other two materials, but this is likely to contain a mix of sediment, biofilm and carbonate residues. Such results are in agreement with the high standard deviation and variability observed in the biofilm total cell counts (section 4.5.1).

For copper both water sources resulted in oxidation of the surface, however it was less susceptible to sedimentation accumulation and after extended exposure differences in discoloration of the samples were observed, no marked differentiation was made between the two sources. Although there is recognition that microbial populations differ and corrosion occurred, the amount of copper in the rigs was too small to allow the occurrence of major aesthetic events such as blue-green water.

4.5.5 Treatment requirements and operation

Overall, the investigation confirms the predictions from the preliminary risk assessment (section 2.7) and the need for pre-treatment prior to injection into distribution water networks as recommended by *Page et al.* (2013). In the case of stormwater, pre-oxidation (e.g. aeration or chlorination) followed by removal of

the insoluble iron using methods such as filtration would reduce significantly the risk of sediment formation and colour. Water quality results also confirmed the predictions from the preliminary risk assessment (section 2.7) regarding colour associated with iron and the low risk from manganese.

Microbial identification has also shown the need for disinfection and a disinfectant residual is recommended to manage biofilm development and to reduce the risk of pathogens growth within the network, as would be expected.

The investigation also alerted to the impact that inadequate residence in the aquifer could have on the overall water quality of the treated stormwater and the characteristics of the system. High organic content episodes in the stormwater supply were identified as a strong influence on colour and also seen to provide food for microbial communities.

At this point we have not investigated how much of the organic content could be reduced as a consequence of iron removal and chlorination, or if additional oxidation would be required. The use of certain disinfectants could also result in the formation of disinfection by-products, such as THM, which need to be monitored in potable end uses. Hence we would recommend further research to examine the impact of subsequent treatment on BDOC and water aesthetics compared to the baseline, and the formation of any residual disinfection by-products (when intended for potable end use) .

5 Conclusions

The interactions of baseline water and wetland and aquifer treated stormwater on water distribution infrastructure were evaluated by monitoring water quality and material coupons in experimental rigs for a from 25 May 2012 to 10 January 2014. Continuous monitoring was conducted for a period of 10 months from December 2012 to October 2013. Coupons were made of copper, PVC and cement and were representative of the range found in the water distribution network.

Baseline water was sourced directly from the Water distribution system, whilst stormwater was sourced from the Salisbury City council scheme and was a blend of ASR/ASTR recovered water. The two water sources received no additional treatment other than aeration upon entry into the rigs by dropping into a storage tank.

The total cell counts on the pilot rig coupons were in the order of 10^5 to 10^7 cells/cm^2 and showed some variation over the duration of the study. For most sampling times (except two sampling times), the cell counts were higher for the stormwater exposed than the baseline water exposed copper and PVC coupons. For cement lined iron the difference was not as consistent. There was no clear difference in the cell numbers between the different materials for either baseline water or stormwater. The total cell counts in the existing stormwater pipes were in the range of 10^6 cells/cm^2. When quantifying bacterial 16S rRNA genes using QPCR, cement lined iron and PVC coupons in the stormwater had higher 16S rRNA gene copy numbers as compared to copper coupons exposed to stormwater or coupons exposed to baseline water.

The culturable cell counts determined at 22°C were in the order of 10^1 to 10^6 cells/cm^2, culturable cell counts at 37 °C were 10^1 to 10^5 cells/cm^2 and thermotolerant coliform cell counts at 37/45 °C were 10^1 to 10^3 cells/cm^2. None of the coupon materials stood out as having consistently higher or lower culturable cell numbers than the other materials. Neither was there a consistent difference in culturable counts at 22°C or 37°C between coupons exposed to baseline water and stormwater. However, the number of thermotolerant coliforms was larger in the stormwater system than in baseline water system.

Environmental conditions (temperature and pH) in the test rigs (median 24.4°C, standard deviation 4.7°C) allow microbial growth. The experimental conditions in the rigs differed slightly from those in water supply

distribution. In particular, buried water mains undergo less temperature fluctuation from seasonal climate variability and temperatures in the ground would have remained below 20°C. However, temperatures at customer taps can be as high as 37°C.

Nutrients

BDOC in baseline water rig had a median of 0.75mg/L (range 0.3 mg/L to 1.3 mg/L), contributing up to 30% of the TOC. In the stormwater rig, the BDOC median was 0.65mg/L (range <0.2 mg/L to 3.6 mg/L), contributing up to 48% of TOC. The maxima were equivalent to only 25% and 69% of maximum BDOC in the Little Para mains water supply. Both water sources provide suitable nutrients for bacterial activity (> 0.16mg/L BDOC and >10 μg/L $PO_4^{-2,}$). Passage of water through the rig caused increase in Total Organic Carbon (TOC), but not of BDOC. The concentration of total nitrogen was 45% lower in stormwater than in baseline water.

Importantly, the potential risk for biofilm development and water aesthetics incidents appears to be closely linked to the source water quality. Residence time in the aquifer is one of the factors that can determine water quality. However, short residence time and inadequate mixing in the aquifer could result in stormwater with nutrients (DOC and TP) and particulate content higher than baseline water, which could contribute to biofilm formation and water discoloration; and lead to sub-saturation of carbonate minerals which increases the water reactivity towards cement.

Biofilm

The total cell counts on the pilot rig coupons were in the order of 10^5 to 10^7 cells/cm^2 and showed some variation over the duration of the study. For most sampling times (except two sampling times), the cell counts were higher for the stormwater exposed than the baseline water exposed copper and PVC coupons. For cement lined iron the difference was not as consistent. There was no clear difference in the cell numbers between the different materials for either baseline water or stormwater. The total cell counts in the existing stormwater pipes were in the range of 10^6 cells/cm^2. When quantifying bacterial 16S rRNA genes using QPCR, cement lined iron and PVC coupons in the stormwater had higher 16S rRNA gene copy numbers as compared to copper coupons exposed to stormwater or coupons exposed to baseline water.

The culturable cell counts determined at 22°C were in the order of 10^1 to 10^6 cells/cm^2, culturable cell counts at 37 °C were 10^1 to 10^5 cells/cm^2 and thermotolerant coliform cell counts at 37/45 °C were 10^1 to 10^3 cells/cm^2. None of the coupon materials stood out as having consistently higher or lower culturable cell numbers than the other materials. Neither was there a consistent difference between coupons exposed to baseline water and stormwater.

Considerable differences were observed in the bacterial and eukaryotic communities formed on the rig coupons made of various materials and exposed to baseline water and stormwater using pyrosequencing. Moreover, seasonal variations were observed in both bacterial and eukaryotic community composition and diversity.

Pathogens

A number of bacterial families and genera harbouring potential human pathogens were detected, by PCR and pyrosequencing, in both baseline water and stormwater systems, with larger numbers of genera observed in the latter indicating a potentially increased risk of exposure to pathogens with stormwater. The stormwater system also harboured sulfate and sulfur reducers which may cause pipe deterioration and odour problems, and a greater diversity of iron oxidisers which may contribute to iron deposits and discoloured water events. A number of bacterial genera that contribute to nitrogen cycling were observed in both baseline water and stormwater systems.

Operating conditions in the rig were aerobic during the trial period, however severe sediment accumulation or long residence times (stagnant water), could lead in principle to the development of localised anoxic pockets that would favour anoxic or anaerobic bacteria development (e.g. sulphate reducing bacteria) that

could impact water aesthetics and cement corrosion. In addition, sediment could also shield microorganisms from disinfectant residuals in bulk water and allow biofilm development.

A number of eukaryotic taxa containing bacterial grazers (amoebae and nematode) were detected in both baseline water and stormwater systems, indicating that the biofilm communities are quite dynamic and the abundance of bacteria is able to support higher level eukaryotes. The data also indicates that both baseline water and stormwater systems support amoebae genera of known concern which are pathogenic or contribute to bacterial pathogenisis.

Salmonella enterica, *Campylobacter* spp. *Cryptosporidium parvum*, *Clostridium perfringens* and *Clostridium difficile* were not detected in the stormwater or baseline water pipe rig biofilm samples with PCR based methodology. Human adenovirus was detected in one copper coupon biofilm sample from baseline water pipe rig in low numbers (on the threshold of detection limit).

Presence of *Legionella*, NTM, *Pseudomonas aeruginosa* and *Aeromonas hydrophila* in the biofilms indicate a potential for regrowth and contamination of the water distribution systems. This suggests that chlorination of the piped water may be required to limit the chances of biofilm formation and potential contamination of water supply from dislodged biofilm.

Source water quality recorded in the rigs was suited to support microbial growth, based on nutrient and organic content (BDOC and TOC), the later was comparable to levels from the little Para mains water supply. Baseline water quality in the rigs was also comparable to that of the Little Para mains water, except for elevated iron, copper and turbidity.

Water quality

Aeration pre-treatment had been previously recommended for removal of excess iron in the aquifer treated stormwater (Page *et al* 2011). Chlorination would also increase the redox state of the solution. This investigation shows the impact of aeration on the fate of iron. Under oxic conditions, Fe(2) was converted to Fe(3) which is generally insoluble and caused precipitation of insoluble iron (oxy)hydroxide or oxides within the reticulation system. Stormwater had mean and 95th percentile concentrations of 1.05$\pm$1.66 mg Fe/L and 3.65 mg Fe/L, which exceed the 0.3mg /L Fe aesthetic ADWG concentration.

Water within the baseline rig maintained super-saturation with respect to iron minerals. This was generally greater than the average Little Para treated drinking water quality, as the water entering the rig was elevated in iron concentrations and hence the likelihood for iron(3) precipitates to form is increased due to the reticulation network itself. Baseline water quality in the rig was compared to that of the Little Para mains water and was verified to have higher total iron, copper and turbidity readings, with mean values of 0.16$\pm$0.21 mg /L Fe, 0.006$\pm$0.003mg/L Mn and 2.80$\pm$4.11 NTU. The 95th percentile for baseline water, 0.49 mg/L Fe, also exceeded the ADWG.

The mean concentrations of manganese for baseline and stormwater were below the aesthetic ADWG value (0.1mg/L Mn), being 0.004$\pm$0.003 mg/L Mn and 0.049$\pm$0.053 mg/L Mn; whilst the respective 95th percentiles were 0.010 mg/L Mn and 0.151 mg/L Mn.

The settling of solids was confirmed by the deposition of iron oxides on coupons aged in the rigs and was more severe in stormwater, and was particularly perceptible on PVC coupons, but the weight of sediment deposits was the highest on cement. In contrast, aluminium and manganese precipitates were not detected during the trial.

In the rigs, all three materials (PVC, cement and copper) were subject to surface changes. Cement showed the largest variability for total cell counts and observations during exposure; and despite PVC's lower surface roughness compared to cement, it was susceptible to sediment deposition with prolonged exposure. Copper coupons, which have biocidal properties, exhibited the least sediment attachment, but

showed signs of oxidation resulting in surface discoloration in both baseline water and stormwater. Therefore, it can be concluded that attachment of insoluble iron on the material surfaces was facilitated by the growth of biofilm. A coupon aged for 29 weeks in stormwater and later introduced in baseline water experienced dissolution of the sediment layer, indicating ease of attached sediment removal with a change in water source.

An increase in the concentration of dissolved copper occurred in the baseline water rig and was attributed to the water supply line from the Greenfield site to the experimental rigs. The stormwater line adopted the same materials, but no copper increase was observed, which indicates less copper corrosion in stormwater compared to baseline water.

Colour

The stormwater rig displayed more variability in aesthetic properties than the baseline water rig, particularly for colour (range 2 - 94 HU, median 12 HU), compared to baseline water (range $\leq$1 – 3 HU, median 1HU). There were indications of slough off of biofilm from the surfaces of both rigs as verified in the increase in metal (Al, Cu, Fe,Mn, Ni, Zn) and turbidity on 1/10/2013, but these did not increase water colour. Iron was the main metal to pose a risk to aesthetics guidelines, however, despite of the formation of insoluble iron and its deposition in the stormwater rig internal surfaces, the analysis indicated a greater influence of dissolved organic carbon in the source water on colour, with exccedance of the ADWG 15HU colour guideline associated with dates when the stormwater supply was highly coloured.

Hence, the potential risk for biofilm development and water aesthetics incidents appears to be more closely linked to the source water quality, which in turn relates to the residence time and recovery period of stormwater in/from the aquifer. Instances when too short residence times and inadequate mixing in the aquifer resulted in elevated nutrient (BDOC and P) and particulate content in the recovered stormwater (higher than baseline water), and could have contributed to rapid microbial development and biofilm formation, water discoloration and sub-saturation of carbonate minerals (which would usually not occur following longer aquifer treatment) which increases the water reactivity towards cement.

Implications to water distribution operation

In summary, the research examined a worst case scenario of undisinfected water sources within a buried distribution network subject to seasonal water supply and temperature variability. The non-chlorinated stormwater can support a vast range of microorganisms that may shorten the life of pipe materials susceptible to iron and sulphate corrosion and decrease water quality. The biofilm also harboured opportunistic pathogens (e.g. *P. Aeruginosa, Legionella* and NTM species) commonly found in distribution systems, which in combination with the nutrients in stormwater, could grow and contaminate the water distribution system. Surrgogates for protozoan parasites have not detected, but other species from the same genera were detected. *Cryptosporidium* and *Giardia* (oo)cysts which are viable outside human hosts in aquatic environments for months and are resistant to chlorine and chloramines were also not detected.

The iron content and the microbial diversity in stormwater were the major parameters that could generate water quality and public health risks in the distribution system. Iron oxidation products could contribute to water aesthetic changes and to sediment deposition on pipe surfaces, including PVC and cement. Copper was not as susceptible to sedimentation. Biofilm formation and slough-off from a PVC distribution network was also verified, however it was observed that colour was influenced not only by iron sediment, but by the BDOC in the source water. Pathogens identified in the stormwater rig were similar to those detected in the baseline rig. Biofilms counts in the two water sources were similar, differences and a wider microbial diversity were verified in stormwater (including sulfate and sulfur reducers, iron) which can evolve dynamically if conditions within water distribution permit.

The aquifer treatment has been shown to produce lower median nutrient levels compared to baseline water in this, and also in other studies (Page *et al* 2013), provided residence time in the aquifer is managed. Furthermore, the rig has also shown that short residence times in the aquifer had detrimental

consequences to the water quality in the rig. Thus, highlighting the benefits of observing a minimum detention time in the aquifer.

The type of intended end-uses for recovered stormwater, either potable or non-potable, will be the main determinant of the level of treatment prior to water distribution , with greater rigour required for potable end uses. In addition, the mode of supply for potable supplies, e.g. if stormwater was injected into a reservoir and blended with other supplies (indirect use) or pre-treatment followed by direct injection into a network, will also influence the treatment steps adopted in each particular case, as direct injection would require stricter monitoring and control of the short and long-term recovered water quality variability to ensure the consistency in water quality in the distribution network.

Therefore to prevent health risks and aesthetics impacts on the distribution network the recommendations are:

- Monitoring and verification of residence time in the aquifer to control the nutrient concentrations in the recovered stormwater; this influences nutrient availability, salinity and alkalinity of the stormwater, and thus could influence the growth of opportunistic pathogens and also the stormwater reactivity towards cement. A predictable residual BDOC is required to manage disinfection residuals and prevent disinfection by-products. This is particularly important if potable end uses are intended for the stormwater.
- Oxidation and filtration of the stormwater for iron removal prior to injection into water distribution systems, to remove excess iron, which can react with residual chlorine, lead to sediment formation and contribute to water discoloration. This can be achieved via aeration or alternatively via chlorination or similar methods. Filtration could also contribute to removal of larger microorganisms;
- Followed by disinfection and the maintenance of a disinfection residual in the network for biofilm and pathogen management, as would be expected.

Such maintenance requirements are expected to be just marginally greater than for current mains water systems.

6 Recommendations for future research

The findings also raise new research questions. The experimental conditions in the rigs differed slightly from those in water supply distribution. Buried water mains would be subject to less temperature fluctuation due to diurnal and seasonal climate variability. Velocities in water distribution would also reach higher values and are more transient depending on households' water demand, which would lead to variable velocity profiles and impact resuspension of sediment and potentially biofilm slough-off. Disinfection of stormwater would be likely to reduce microbial diversity, delay biofilm formation and alter its composition.

There is need to verify the efficacy of the any proposed treatment on biofilm reduction and the survival of the microbial species within a rig over time. Treatment and a disinfectant residual would reduce the biofilm formation, but it may not necessarily eliminate it. The evaluation of the potential for recontamination by seeding opportunistic pathogens into the rig would help elucidate this risk and also help determine the optimal disinfectant type and residual concentrations. However, the use of disinfectants could also result in the formation of disinfection by-products due to reaction with NOM, such as THMs.

Corrosion and odour are influenced by the bacteria species and by the water quality in the pipe. The research here presented was conducted under oxic conditions, but stormwater microbial activity and water quality deterioration under anoxic conditions which simulate dead end pockets was not evaluated.

Suggestions for further improvement of the experimental set-up and additional verification would include:

- Thermal control to achieve a narrower temperature range (allow a maximum of 20°C) in the rig, through insulation of the biorig section, would be likely to impact microbial development and more closely resemble buried distribution infrastructure temperatures;
- Pre-treatment of the stormwater for iron removal and disinfection with pre-selected methods, followed by injection into a rig to examine microorganism survival and biofilm (and compare to the results from this report); and
- Challenge testing of the established biofilm in a rig with disinfectant residual to examine the potential risk for pathogen survival and re-contamination.
- Examination of variable flow regimens on the detachment of biofilm and resuspension of sediment for distribution network maintenance in a stormwater system with residual chlorine.
- If potable end uses were to be considered monitoring of disinfection by-products (e.g. THMs) would also be required for compliance with the ADWG.

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Appendix 1. Summaries of continuous monitoring data from baseline and stormwater rigs

Summary by Period (1, 0 & 2)

	Period 1						Period 0						Period 2					
	n	mean	sd	median	min	max	n	mean	sd	median	min	max	n	mean	sd	median	min	max
Baseline Water																		
inlet temp. ˚C	11665	21.6	1.4	21.5	17.9	24.8	14993	22.8	1.8	22.6	18.9	27.2	103622	25.1	4.7	25.0	14.3	36.0
return temp. ˚C	11665	20.9	1.7	21.0	16.0	24.6	14993	22.4	2.9	22.0	16.8	30.8	103622	24.7	4.7	24.4	14.6	36.0
pH	11665	6.4	0.2	6.5	5.4	6.8	14990	6.7	0.2	6.6	4.9	7.9	103622	8.2	0.2	8.2	7.6	8.7
ORP mV	11665	251.5	15.7	251.2	215.0	329.9	14993	138.4	155.4	213.6	-220.7	284.6	103622	348.3	104.8	329.5	96.1	699.5
EC µS/cm	10651	520.5	70.7	540.9	302.9	825.1	14993	569.7	7.1	569.2	543.0	670.0	103573	466.2	97.6	489.9	231.6	657.8
Stormwater																		
inlet temp. ˚C	11665	21.6	1.4	21.6	16.8	25.5	14993	23.7	2.1	23.5	19.6	33.4	103622	23.7	4.9	23.1	13.7	37.5
return temp. ˚C	11665	21.4	1.4	21.4	17.2	25.3	14993	23.8	2.4	23.7	18.2	30.4	103622	23.8	4.6	23.5	14.2	37.4
pH	9940	7.0	1.0	7.2	4.2	9.0	14555	5.6	0.6	5.7	4.0	7.1	95316	6.6	0.5	6.6	4.1	9.0
ORP mV	11665	380.3	24.6	377.0	296.1	452.5	14993	-229.3	333.6	-380.6	-540.3	325.1	103622	375.2	49.7	384.7	83.4	503.8
EC µS/cm	10651	484.5	70.9	505.1	225.3	575.3	14993	472.4	15.1	471.9	400.9	587.0	103573	606.8	293.3	642.3	155.2	1367.7

Summary by season.

	Summer (Period 0)						Summer (Period 2)					
	n	mean	sd	median	min	max	n	mean	sd	median	min	max
Baseline Water												
inlet temp. ˚C	5616	23.7	1.4	23.6	20.4	27.2	24435	29.8	2.9	29.7	22.2	36.0
return temp. ˚C	5616	24.0	2.8	24.0	18.7	30.8	24435	29.0	3.2	29.3	21.7	36.0
pH	5613	6.7	0.4	6.6	4.9	7.9	24435	8.3	0.2	8.3	7.6	8.7
ORP mV	5616	-37.3	106.9	-21.8	-220.7	135.9	24435	258.6	35.9	254.5	96.1	466.1
EC µS/cm	5616	575.8	7.0	575.0	560.7	670.0	24388	529.9	111.1	585.3	292.6	657.8
Stormwater												
inlet temp. ˚C	5616	24.7	1.9	24.5	20.9	33.4	24435	29.1	3.9	29.3	19.2	37.5
return temp. ˚C	5616	24.7	2.2	24.7	19.7	30.4	24435	28.9	3.5	28.8	21.0	37.4
pH	5615	5.8	0.5	5.9	4.1	7.1	16129	7.1	0.7	7.4	4.1	9.0
ORP mV	5616	-500.9	131.3	-532.5	-540.3	87.7	24435	315.6	51.3	311.8	83.4	392.6
EC µS/cm	5616	484.7	16.4	482.9	438.8	587.0	24388	741.4	93.1	716.4	583.4	1367.7

	Autumn (Period 2)					
	n	mean	sd	median	min	max
Baseline Water						
inlet temp. ˚C	26495	26.8	3.6	26.6	17.5	35.5
return temp. ˚C	26495	26.5	3.5	26.3	18.5	35.2
pH	26495	8.2	0.1	8.2	7.8	8.4
ORP mV	26495	315.9	15.7	320.3	276.1	346.3
EC µS/cm	26493	492.3	27.2	492.9	407.7	544.0
Stormwater						
inlet temp. ˚C	26495	25.1	3.3	25.1	17.1	34.3
return temp. ˚C	26495	25.3	3.0	25.4	18.5	34.4
pH	26495	6.5	0.4	6.5	5.4	7.5
ORP mV	26495	399.6	30.2	393.9	328.7	503.8
EC µS/cm	26493	824.7	355.4	956.1	155.2	1367.7

	Winter (Period 2)					
	n	mean	sd	median	min	max
Baseline Water						
inlet temp. ˚C	26494	19.4	1.4	19.4	14.3	22.8
return temp. ˚C	26494	19.0	1.1	18.9	14.6	22.1
pH	26494	8.0	0.1	8.0	7.6	8.2
ORP mV	26494	339.9	7.3	339.3	323.4	370.8
EC µS/cm	26494	481.4	40.2	490.1	352.1	560.1
Stormwater						
inlet temp. ˚C	26494	18.1	1.4	18.1	13.7	22.4
return temp. ˚C	26494	18.4	1.2	18.3	14.2	22.4
pH	26494	6.4	0.3	6.5	5.7	7.0
ORP mV	26494	374.7	33.6	379.6	301.7	437.6
EC µS/cm	26494	478.6	257.7	308.3	178.1	806.6

	Spring (Period 1)						Spring (Period 0)						Spring (Period 2)					
	n	mean	sd	median	min	max	n	mean	sd	median	min	max	n	mean	sd	median	min	max
Baseline Water																		
inlet temp. ˚C	11665	21.6	1.4	21.5	17.9	24.8	9377	22.2	1.7	22.0	18.9	26.5	26198	24.7	3.0	23.9	17.5	33.0
return temp. ˚C	11665	20.9	1.7	21.0	16.0	24.6	9377	21.5	2.5	20.9	16.8	29.0	26198	24.4	3.0	23.5	17.9	33.0
pH	11665	6.4	0.2	6.5	5.4	6.8	9377	6.6	0.1	6.6	6.4	6.8	26198	8.3	0.2	8.3	7.7	8.7
ORP mV	11665	251.5	15.7	251.2	215.0	329.9	9377	243.6	47.2	264.0	10.5	284.6	26198	473.3	132.7	488.3	220.0	699.5
EC µS/cm	10651	520.5	70.7	540.9	302.9	825.1	9377	566.1	3.9	566.6	543.0	574.0	26198	365.2	94.0	323.1	231.6	562.1
Stormwater																		
inlet temp. ˚C	11665	21.6	1.4	21.6	16.8	25.5	9377	23.2	1.9	22.9	19.6	29.0	26198	22.8	2.7	22.6	16.3	30.9
return temp. ˚C	11665	21.4	1.4	21.4	17.2	25.3	9377	23.3	2.3	23.2	18.2	29.2	26198	23.1	2.6	22.9	16.7	30.9
pH	9940	7.0	1.0	7.2	4.2	9.0	8940	5.4	0.6	5.3	4.0	6.8	26198	6.7	0.4	6.7	5.7	7.6
ORP mV	11665	380.3	24.6	377.0	296.1	452.5	9377	-66.7	311.5	-97.4	-509.2	325.1	26198	406.5	18.8	410.4	362.2	450.8
EC µS/cm	10651	484.5	70.9	505.1	225.3	575.3	9377	465.0	7.8	464.7	400.9	480.9	26198	390.9	95.2	390.2	214.9	669.3

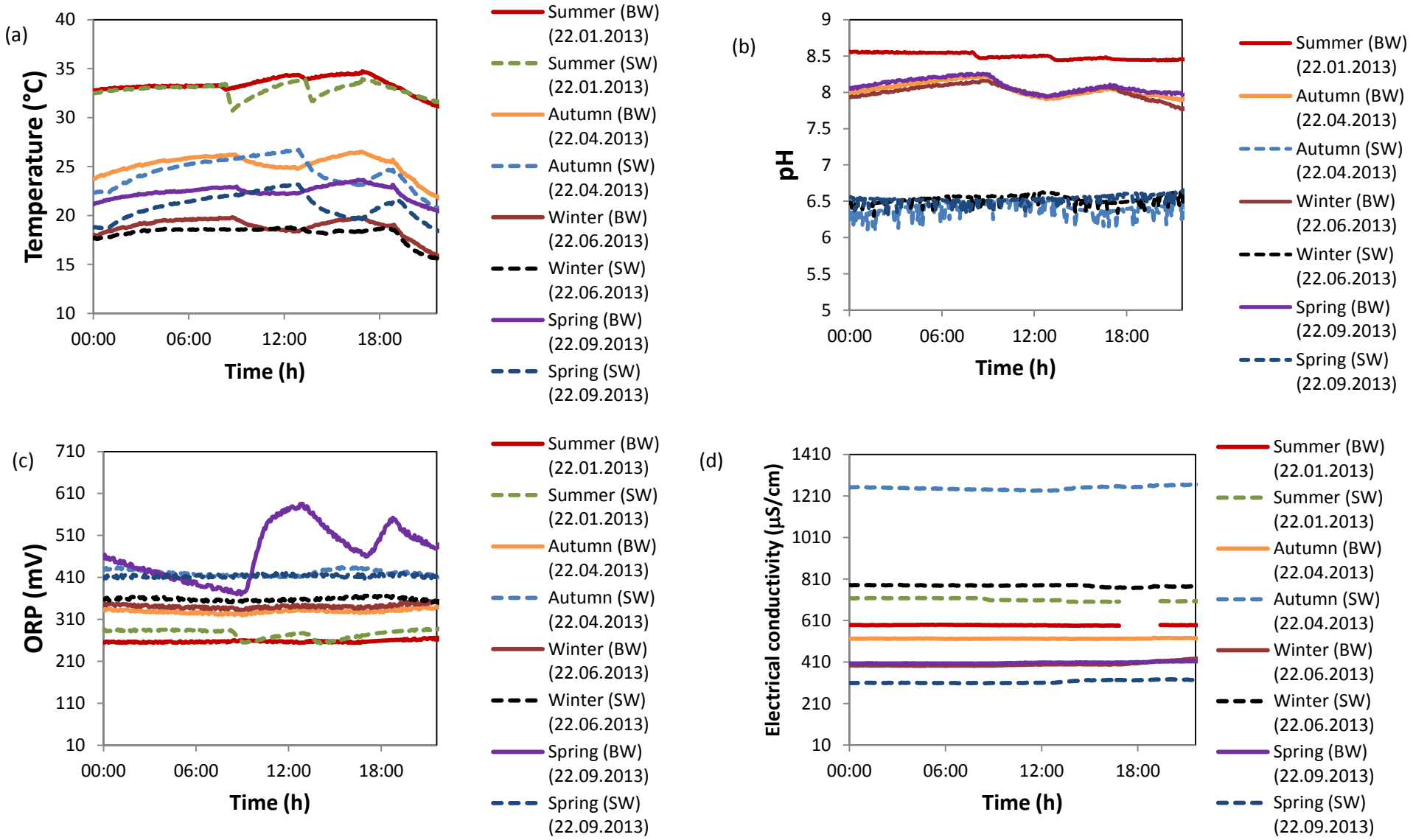


Figure 42. Physico-chemical observations over 24h for the biorigs supplied with stormwater (SW) and baseline water (BW) rigs measured on 22 Jan, 22 April, 22 June and 22 September 2013: (a) Temperature, (v) pH, (c) Oxi-reduction potential (ORP), (d) Electrical conductivity.

Appendix 2. Australian Water Quality Centre (AWQC) method details

	Detection Method	Method reference*	Limit of reporting
Physicochemical			
pH	pH Electrode	APHA 4500-H B	0.1 (no units)
EC	EC Electrode	APHA 2520 B	1.0 (µS/cm and mg/L)
TDS	Total dissolved solids calculate from EC	APHA 2520 B	1 mg/L
Alkalinity	Derived from HCO_3	APHA 2320 B	0 mg/L
Turbidity	Nephelometric Turbity Meter	APHA 2130 B	0.1 (NTU)
Suspended solids	GF/C Filtration	APHA 2540 D	1 (mg/L)
UV_{254} (filtered)	Spectrometric @ 254 nm (Filtered)	T0120-01	0.001
True Colour	Spectrometric @ 456 nm (Filtered)	T0029-01 W09-023 Bennett and Drikas (1982)	1 (HU)
Major ions			
Calcium	0.45um filtration, acidification to 1% HNO3, ICP-ES	USEPA 200.8	0.1 mg/L
Magnesium	Acid digestion, acidification to 1% HNO3, ICP-ES	USEPA 200.8	0.3 mg/L
Potassium	0.45um filtration, acidification to 1% HNO3, ICP-ES	USEPA 200.8	1 mg/L
Sodium	0.45um filtration, acidification to 1% HNO3, ICP-ES	USEPA 200.8	0.5 mg/L
Chloride	Automated ferricyanide colorimetric method	APHA 4500-Cl E	4 (mg/L)
Bicarbonate	Potentiometric titration from pH 8.3 to end-point pH 4.5	APHA 2320 B	1 (mg/L)
Sulphate	0.45um filtration, acidification to 1% HNO3, ICP-ES	APHA Inductively coupled plasma method APHA 3120 B	0.5 (mg/L)
Fluoride	Auto Selective Ion Electrode	APHA 4500-F- C	0.1 mg/L
Bromide	Ion Chromatography	USEPA 300.0 (1993)	0.1 mg/L
Nutrients			
Total Nitrogen	Calculation	Based on Technicon method 376-75W and APHA 4500-NO_3^- F	0.05 (mg/L)
Total Kjeldahl Nitrogen	Kjeldahl digestion followed by automated colorimetric	Based on Technicon method 376-75W; Equivalent to APHA 4500-NH3 G and 4500-Norg B	0.05 (mg/L)
Ammonia	Automated colorimetric	Based on APHA 4500-NH_3 H	0.005 (mg/L)
NO_3 + NO_2 -N	Automated colorimetric cadmium reduction	APHA 4500-NO_3^- F	0.005 (mg/L)
Total Phosphorous	H_2SO_4/K_2SO_4/HgO digestion, automated colorimetric	Technicon Method 376-75W, Technicon Method 155-71W: Equivalent to APHA 4500-P E	0.005 (mg/L)
Ortho-phosphate	Filtered, 0.45 um; automated colorimetric	APHA 4500-P F	0.005 (mg/L)
TOC	Persulphate oxidation - OI analytical TOC analyser	W09-023	0.3 (mg/L)
DOC	Persulphate oxidation - OI analytical TOC analyser	T0158-09 W09-023	0.3 (mg/L)
BDOC		Biodegradation by surface fixed biofilm	0.2 (mg/L)

	Detection Method	Method reference*	Limit of reporting
		with DOC measurements made over 10 days	
Metals (total unless specified)			
Aluminium (soluble), Arsenic (total & soluble), Antimony, Barium, Beryllium, Boron, Cadmium, Chromium, Cobalt, Copper (total & soluble), Lead, Lithium, Mercury, Molybdenum, Nickel, Selenium, Silver, Thallium, Vanadium	ICP-MS, Ag Varian Zeeman AAS (furnace)	USEPA 200.8, Ag APHA Electrothermal atomic absorption method APHA 3113 B	As Cu Li 0.001, B 0.02, Sb Ba Be Cd Co Pb Mo Ni Tl Hg 0.0005, Cr Se V 0.003, Ag 0.0002 mg/L
Chromium (VI)	Colorimetric	T0530-51	0.01mg/L
Aluminium, Iron and Zinc	Total - ICP 1 (High Level)	APHA Inductively coupled plasma method APHA 3120 B	Al 0.02, Fe 0.03, Zn 0.01 mg/L
Boron (soluble)	Membrane Filtration ICP 1	APHA Inductively coupled plasma method APHA 3120 B	0.04 mg/L
Iron (soluble), Lead (soluble) & Manganese (soluble)	Membrane Filtration ICP2	APHA Inductively coupled plasma method APHA 3120 B	Fe 0.005, Pb 0.01, Mn 0.001 mg/L
Manganese	Manganese - ICP 2 (Low Level)	APHA Inductively coupled plasma method APHA 3120 B	0.001mg/L
Faecal indicators			
Ecoli	Membrane filtration	AS/NZS 4276.7:2007	0 / 100mL (cfu)
Thermotolerant coliforms	Membrane filtration	AS/NZS 4276.7:2007	0 / 100mL (cfu)
Bacteriophage	Double Layer Plate Technique	Grabow 1998	0/10mL

*Analytical methods used by the Australian Water Quality Centre laboratory.

Appendix 3. Detailed PCR methodology

Table 20. Primer and probe sequences for real-time PCR assays for the detection of human pathogens and opportunistic pathogens. F = forward primer; R = reverse primer; P = probe. The probe fluorophores have been shown with bold red font.

Pathogens	Primer sequence (5'-3')[a]	Genes	References
Bacteria	F-AAC GCG AAG AAC CTT AC R-CGG TGT GTA CAA GCC GGG AAC G	16S rRNA	Heuer et al., 1997
A. hydrophila	F-CAA GAA CAA GTT CAA GTG GCC A R-ACGAAGGTGTGGTTCCAGT	*aerA*	Wang et al., 2003
C. difficile	F - GAA AGT CCA AGT TTA CGC TCA AT R - GCT GCA CCT AAA CTT ACA CCA	*tcdB*	van den Berg et al., 2007
C. perfringens	F - GCA TGA GTC ATA GTT GGG ATG ATT R - CCT GCT GTT CCT TTT TGA GAG TTA G	*plc*	Lee et al., 2006
P. aeruginosa	F-ATG GAA ATG CTG AAA TTC GGC R- CTT CTT CAG CTC GAC GCG ACG	*oprL*	De Vos et al., 1997
Campylobacter spp.	F-CAC GTG CTA CAA TGG CAT AT R-GGC TTC ATG CTC TCG AGT T P-**FAM** CAG AGAA CAA TCC GAA CTG GGA CA **BHQ1**	16S rRNA	Lund et al., 2004
Adenovirus	F-GCC ACG GTG GGG TTT CTA AAC TT R- GCC CCA GTG GTC TTA CAT GCA P-**FAM** TGC ACC AGA CCC GGG CTC AGG AGG TAC TCC GA **BHQ1**	*Hexon*	Heim et al., 2003
Cryptosporidium parvum	F-CAA ATT GAT ACC GTT TGT CCT TCT G R-GGC ATG TCG ATT CTA ATT CAG CT P-**HEX** TGC CAT ACA TTG TTG TCC TGA CAA ATT GAAT **BHQ1**	COWP	Guy et al., 2003

Table 21. Primer names and sequences used to quantify NTM and *Legionella* spp.

Primer name	Primer sequence	Target organism	References
Forward amplification primer MYCGEN-F	5'AGAGTTTGATCCTGGCTCAG 3'	*Mycobacteria* spp.	Wilton & Cousins 1992
Reverse amplification primer MYCGEN-R	5'-TGCACAGGCCACAAGGGA 3'	*Mycobacteria* spp.	Wilton & Cousins 1992
Forward amplification primer LEGMIP-F (27-mer)	5'-GGG RAT TVT TTA TGA AGA TGA RAY TGG 3'	*Legionella* spp.	(Ratcliff, Lanser et al. 1998)
Reverse amplification primer LEGMIP-R (23-mer)	5'-TCR TTN GGDCCD ATN GGN CCD CC 3'	*Legionella* spp.	(Ratcliff, Lanser et al. 1998)

Table 22. List of bacterial primers used for amplifying 16S rRNA genes for DGGE (Kaksonen et al. 2014)

Primer	Amplification target	Sequence
27F	Bacterial	5’- GAG TTT GAT CCT GGC TCA G -3'
1492R[#]	Universal	5’- ACG G5T ACC TTG TTA CGA CTT -3’
BacV3f*	Bacterial	5’- CCT ACG GGA GGC AGC AG -3’
907R	Universal	5’- CCG TCA ATT CMT TTG AGT TT -3’

[#]Mixed base codes: 5 = deoxyribose inosine modification (universal base) (GeneWorks Pty Ltd)
*Primer BACV3f+GC had a GC rich clamp (5’-CGCCCGCCGCGCGCGGCGGGCGGGGCGGG-3’) in the 5’-end.

Appendix 4. Baseline rig water quality during experimental operation.

		Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline inlet	Baseline rig	Baseline inlet	Baseline rig	Little Para	
	Period	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**		
	Date	**27/6/12***	**25/9/12**	**30/1/13**	**13/3/13**	**30/4/13**	**22/5/13***	**11/6/13**	**22/7/13**	**3/9/13***	**3/9/13**	**1/10/13**	**1/10/13**	**Ave**	**Max**
	Coupon exposure (wks)	**-**	**22**	**6**	**12**	**19**	**-**	**25**	**31**	**-**	**-**	**35**	**35**		
	ADWG[†]														
Physical characteristics															
Dissolved oxygen (mg/L)	>85% [a]	5.0	9.6	4.9	4.6	3.9	2.0	8.5	8.2	6.8	5.9				
Electrical conductivity (µS/cm)		690	564	578	466	554	503	442	497	569	570	377	377		
pH	6.5-8.5 [a]	7.36	6.98	7.76	7.91	6.97	7.63	7.46	6.98	7.61	7.46	7.57	7.29	7.4	7.9
Eh (mV SHE)		334	526	412	359	481	465	486	478	462	158	960	904		
Temperature (° C)		12.1	20.4	25.9	29.6	25.0	22.0	20.3	17.5	19.2	22.5	17.8	21.2	20	32
Free chlorine residual (mg/L)	5 [h], 0.6 [a]				0.04	0.03	0.02	0.03					0.03		
Total chlorine (mg/L)					0	0	0	0					0		
PSD10 (µm)			0.09	36.9	446	393	69.9	8.9	38.7			121	3.6		
PSD50 (µm)			0.33	402	660	525	524	345	368			182	19.6		
PSD90			227	603	941	656	950	572	514			268	374		
Turbidity (NTU)	5 [a]	0.63	0.66	1.6	2.0	0.32	0.38	1.1	1.2	**6.2**	0.87	0.23	**12**	0.16	**1.4**
UV absorbance @254nm filtered (/cm)										0.092	0.048	0.019	0.077		
Total Dissolved Solids (by EC; mg/L)	600 [a]	330	320	350	270	310	270	250	290	320	320	210	210	356	420
Suspended Solids (mg/L)		<1	1	2	4	<1	<1	2	<1				16		
Turbidity (lab) (NTU)	5 [a]	0.63	0.66	1.6	2	0.32	0.38	1.1	1.2	**6.2**	0.87	0.11	**12**	0.16	1.4
True Colour (HU)	15 [a]	1	3	<1	1	2	1	1	2	2	1	<1	<1	1	10
Major Ions (mg/L)															
Alkalinity (as $CaCO_3$)		90	82	83	91	88	89	82	92	64	64	55	56	69	91
Bicarbonate		110	100	102	111	107	109	100	112	78	79	68	68	84	111

		Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline inlet	Baseline rig	Baseline inlet	Baseline rig	Little Para	
	Period	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**		
	Date	**27/6/12***	**25/9/12**	**30/1/13**	**13/3/13**	**30/4/13**	**22/5/13***	**11/6/13**	**22/7/13**	**3/9/13***	**3/9/13**	**1/10/13**	**1/10/13**	**Ave**	**Max**
	Coupon exposure (wks)	**-**	**22**	**6**	**12**	**19**	**-**	**25**	**31**	**-**	**-**	**35**	**35**		
	ADWG†														
Sulfate	500 [h], 250 [a]	48	44	45	44	39	37	29	40	54	54	34	34	47	55
Chloride	250 [a]	91	105	110	66	96	76	70	83	114	113	62	64	127	151
Fluoride	1.5 [h]	0.91	0.81	1.0	0.93	0.98	0.79	0.93	0.86				0.83	0.84	1.0
Calcium		26	28	31	25	27	27	25	29	28	28	22	22	25	33
Magnesium		14	17	14	10	13	11	9	12	13	13	7	7	16	20
Potassium		7.3	5.6	6.1	6.1	6.4	5.2	4.5	6.1	5.6	5.6	2.9	3.0	4.1	6.2
Sodium	180 [a]	54	60	66	48	60	48	44	61	65	65	39	40	75	90
Nutrients (mg/L)															
Nitrate + Nitrate as N		0.003	0.19	0.10	0.22	0.068	0.11	0.085	0.13	0.17	0.19	0.11	0.12	0.14	0.28
Ammonia as N	0.4 [a]		0.008	0.007	0.008	0.006	0.005	<0.005	0.007	0.011	0.008	<0.005	0.006	0.008	0.06
TKN		0.36	0.33	0.32	0.33	0.43	0.28	0.52	0.27	0.28	0.26	0.16	0.24	0.23	0.38
Nitrogen - Total		0.48	0.52	0.42	0.55	0.50	0.39	0.61	0.40	0.45	0.45	0.27	0.36		
Phosphorous – Filterable Reactive		0.018	0.006	<0.003	0.004	<0.003	0.003	<0.003	0.004	0.005	0.004	0.005	0.005	0.005	0.012
Phosphorus - Total		0.121	0.011	0.008	0.013	0.009	0.007	0.011	<0.005	0.01	0.007	0.01	0.031	0.005	0.012
Biodegradable Dissolved Organic Carbon		0.9	1.3	0.6	0.3	1.0	0.8	0.7	0.4				0.2		
Dissolved Organic Carbon		5.2	4.4	4.3	3.4	4.3	3.9	2.9	3.8	3.3	3.3	1.8	1.6		
Total Organic Carbon		5.3	4.5	4.4	3.5	4.5	3.7	3.0	3.7	3.6	3.4	1.6	2.0		
SUVA [s] (L/m mg)										2.8	1.5	1.0	4.8		
C/N [c]		14	13	13	10	10	14	6	14	12	13	11	7		
Silica - Reactive	80 [a]	3	4	3	3	2	4	2	5	6	6	3	3	3	7
Metals (mg/L)															
Aluminum - Soluble	0.2 [a]	0.029	0.029	0.069	0.082	0.052	0.051	0.004	0.037	0.033	0.032	0.051	0.050	0.040	0.084
Aluminium - Total		0.062	0.058	0.091	0.146	0.061	0.056	0.148	0.073	0.274	0.055	0.059	0.386		
Arsenic - Soluble	0.01 [h]	0.004	0.0003	0.0007	0.0005	0.0005	0.0005	0.002	0.0004				<0.0003		
Arsenic - Total		0.004	0.0003	0.0007	0.0005	0.0004	0.0005	0.0006	0.0004				0.0004	0.0009	0.002
Boron - Soluble	4 [h]	0.049	0.035	0.037	0.061	0.044	0.09	0.121	0.080				0.089	0.046	0.093
Cadmium - Total	0.002 [h]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003				<0.0001	0.0004	0.0006

		Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline rig	Baseline inlet	Baseline rig	Baseline inlet	Baseline rig	Little Para	
Period		**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**		
Date		**27/6/12***	**25/9/12**	**30/1/13**	**13/3/13**	**30/4/13**	**22/5/13***	**11/6/13**	**22/7/13**	**3/9/13***	**3/9/13**	**1/10/13**	**1/10/13**	**Ave**	**Max**
Coupon exposure (wks)		**-**	**22**	**6**	**12**	**19**	**-**	**25**	**31**	**-**	**-**	**35**	**35**		
ADWG†															
Chromium - Total	0.05 as Cr (VI) [h]	<0.0001	0.0002	0.0009	0.0002	0.0002	0.0002	0.0004	0.0002				0.0005	0.0025	<0.003
Copper - Soluble	2 [h], 1 [a]											0.079	0.13		
Copper – Total	2 [h], 1 [a]	0.74	0.20	0.28	0.31	0.14	0.10	0.24	0.15	0.63	0.17	0.090	0.72	0.017	0.065
Iron - Soluble	0.3 [a]	0.007	0.016	0.048	0.0062	0.0057	0.015	0.017	0.011	0.013	0.013	0.0037	0.0092		
Iron - Total	0.3 [a]	0.069	0.053	0.22	0.20	0.024	0.037	0.18	0.088	**0.72**	0.084	0.0071	**0.77**	0.0083	0.028
Lead - Total	0.01 [h]	0.0023	0.0008	0.0009	0.0012	0.0003	0.0002	0.0018	0.0006				0.0061	0.0006	<0.01
Manganese - Soluble	0.5[h], 0.1 [a]	0.0005	0.0014	0.0093	0.0006	0.0010	0.0010	0.0038	0.0009				0.0003		
Manganese - Total	0.5[h], 0.1 [a]	0.0046	0.0029	0.0095	0.0018	0.0013	0.0011	0.0025	0.0016	0.0092	0.0016	0.0009	0.0104	0.00027	0.005
Mercury - Total	0.001 [h]	0.00005	0.00003	<0.00003	<0.00003	<0.00003	<0.00003	<0.00003	<0.00003				0.00007		
Nickel - Total	0.02 [h]	0.0015	0.0009	0.0009	0.0009	0.0008	0.0006	0.0009	0.0015	0.001	0.0008	0.0003	0.0018	0.0007	0.005
Zinc - Total	3 [a]	0.030	0.010	0.035	0.014	0.0089	0.0065	0.012	0.012	0.0053	0.011	0.0014	0.022	0.0056	0.022
$SI_{Calcite}$		-0.8	-1.1	-0.2	-0.03	-1.0	-0.4	-0.6	-1.1	-0.6	-0.7	-0.8	-1.0	-0.8	
$SI_{Fe(OH)3}$		2.3	1.5	2.0	1.9	0.9	1.4	2.3	1.9	3.0	1.8	0.8	2.9	1.0	
$SI_{Goethite}$		7.7	7.2	7.9	7.9	6.8	7.1	8.0	7.5	8.7	7.6	6.4	8.6	6.7	
SI_{MnOOH}		-5.8	-5.2	-2.1	-3.8	-4.2	-2.6	-2.3	-4.3	-1.7	-2.9	-2.6	-2.4	-4.9	
$SI_{Mn(OH)2}$		-8.8	-9.1	-6.7	-7.8	-9.2	-7.9	-7.8	-9.2	-7.0	-8.1	-8.0	-7.5	-8.2	
$SI_{Al(OH)3}$		-0.99	-0.99	-1.6	-1.8	-0.9	-1.4	-2.3	-0.8	-1.5	-1.5	-1.2	-1.1	-1.2	

*no coupons sampled on this date; †NHMRC–NRMMC, 2011; [h] health guideline value; [a] aesthetic guideline value; [s] UV_{254}/DOC; [c] DOC/TKN; **bold** indicates value exceeds ADWG

Appendix 5. Stormwater rig water quality during experimental operation.

	Period	SW rig 1 27/6/12*	SW rig 1 25/9/12	SW rig 2 30/1/13	SW rig 2 13/3/13	SW rig 2 30/4/13	SW rig 2 22/5/13*	SW rig 2 11/6/13	SW rig 2 22/7/13	SW inlet 2 3/9/13*	SW rig 2 3/9/13	SW inlet 2 1/10/13	SW rig 2 1/10/13	ASR Median	ASR 95th %ile	ASTR Median	ASTR 95th %ile	Ring main Median	Ring main 95th %ile
	Coupon exposure (wks) ADWG†	-	22	6	12	19	-	25	31	-	-	35	35						
Physical characteristics																			
Dissolved oxygen (mg/L)	>85% [a]	3.8	9.1	4.3	4.5	6.9	3.7	5.3	8.5	4.6	5.9			0.1	3.9	0.1	1.4	0.4	2.4
Electrical conductivity (µS/cm)		590	504	683	889	288	158.3	746	163.1	288	249	447	378	646	801	546	616	425	592
pH	6.5-8.5 [a]	7.65	7.25	7.8	8.02	7.01	6.96	7.62	6.48	7.21	7.32	7.45	7.20	7.4	7.6	7.5	8.2	7.3	7.5
Eh (mV SHE)		282	483	362	439	459	496	451	486	447	452	710	490	-129	-42	5	207	88	401
Temperature (° C)		14.4	21.1	26.4	29.5	26.4	22.6	20.3	17.6	19	21.3	16.9	20.5	18	19	19	20	19.8	23
Free chlorine residual (mg/L)	5 [h], 0.6 [a]	0.01			0	0	0	0				0	0						
Total chlorine (mg/L)		0			0	0	0	0				0	0						
PSD10			5.7	6.2	9.4	640	1014	6.9	10.5			12.6	6.3	13	31	0.1	16	2.5	6.4
PSD50			14.2	15.4	17.9	1050	1338	21.3	56			265	16.4	3	7	0.2	178	7.9	201
PSD90			32.6	146	30.6	1598	1664	166	1393			436	36.6	17	23	4.2	298	29	478
Turbidity (NTU)	5 [a]	1.3	**5.9**	1.3	0.8	2.2	4.0	4.0	**5.8**	1.8	1.8	0.99	**13**						
UV absorbance @254nm filtered (/cm)										0.10	0.11	0.094	0.14						
Total Dissolved Solids (by EC; mg/L)	600 [a]	340	290	410	550	150	85	430	96	160	140	250	220	240	710	300	344	245	350
Suspended Solids (mg/L)		2	5	4	2	<1	3	1	4				7						
Turbidity (lab) (NTU)		1.3	5.9	1.3	0.8	2.2	4	4	5.8	1.8	1.8	0.99	13	1.1	16	0.7	5.9	1.8	4
True Colour (HU)	15 [a]	7	15	2	4	**94**	**58**	7	**26**	**18**	**18**	6	6	1.1	**16**	**21**	**36**	14	**59**
Major Ions (mg/L)																			
Alkalinity (as $CaCO_3$)		156	142	166	177	93	51	162	55	97	87	128	119	145	201	157	213	142	154
Bicarbonate		191	173	203	216	113	62	198	68	118	106	156	145	165	228	192	260	174	188
Sulfate	500 [h], 250 [a]	33	28	45	71	13	6.9	46	8.1	<1.5	7.2	23	16	24	62	24	42	27	36
Chloride	250 [a]	75	65	109	176	25	13	125	16	23	24	59	47	35	146	63	84	51	84
Fluoride	1.5 [h]	0.58	0.45	0.32	0.44	0.2	0.14	0.56	0.11				0.26	0.28	0.41	0.30	0.60	0.37	0.63

	Period	SW rig 1	SW rig 1	SW rig 2	SW rig 2	SW rig 2	SW rig 2	SW rig 2	SW rig 2	SW inlet 2	SW rig 2	SW inlet 2	SW rig 2	ASR		ASTR		Ring main	
		27/6/12*	25/9/12	30/1/13	13/3/13	30/4/13	22/5/13*	11/6/13	22/7/13	3/9/13*	3/9/13	1/10/13	1/10/13	Median	95th %ile	Median	95th %ile	Median	95th %ile
	Coupon exposure (wks) ADWG†	-	22	6	12	19	-	25	31	-	-	35	35						
Physical characteristics																			
Calcium		45	38	44	47	30	15	41	18	28	0.1	37	37	39	47	45	69	36	42
Magnesium		13	14	14	20	4.8	2.8	20	3.2	4.6	4.6	11	8.3	8.3	15.2	10	18	10	19
Potassium		4.9	4.6	5.3	5.5	4.9	3.0	4.8	3.1	2.2	2.5	3.5	3.2	3.6	6.5	4.2	5.2	4.1	5.7
Sodium	180 [a]	48	47	80	115	19	8.7	80	11	14	16	38	28	42	120	42	61	36	63
Nutrients (mg/L)																			
Nitrate + Nitrate as N		0.036	0.134	0.196	0.094	0.009	0.012	0.08	0.024	0.012	0.045	0.007	0.033	<0.005	0.052	<0.005	0.023	0.01	0.06
Ammonia as N	0.4 [a]		0.011	0.005	0.012	0.004	<0.005	0.012	<0.005	0.044	0.013	0.076	0.021	0.094	0.32	0.14	5.75	0.08	0.19
TKN		0.2	0.29	0.18	0.1	0.49	0.38	0.1	0.28	0.24	0.22	0.25	0.29	0.17	0.77	0.32	5.69	0.25	1.1
Nitrogen - Total		0.2	0.42	0.38	0.19	0.49	0.39	0.18	0.3	0.25	0.27	0.26	0.32	0.18	0.77	0.32	5.69	0.28	1.1
Phosphorous – Filterable Reactive		<0.003	0.018	0.025	0.02	0.009	0.016	0.014	0.003	0.013	0.013	0.019	0.02	0.02	0.03	0.02	0.03	0.02	0.08
Phosphorus - Total		0.046	0.053	0.023	0.019	0.031	0.033	0.029	0.027	0.02	0.017	0.032	0.083	0.029	0.11	0.03	0.23	0.03	0.15
Biodegradable Dissolved Organic Carbon		0.2	0.6	0.2	0.8	3.6	1.9	<0.2	0.7				<0.2	1	1.3	0.4	1.77	1.1	5.4
Dissolved Organic Carbon		2	2.2	2.2	1.5	7.5	4.7	1.7	2.9	2.6	2.4	2	2	2.3	3.3	3.9	6.43	2.8	10
Total Organic Carbon		2.1	2.5	2.1	1.5	8.1	5	1.6	3.1	2.7	2.6	2.2	2.8	2.7	5.4	4.2	9.29		
SUVA [s] (L/m mg)										4.0	4.5	4.7	7.1						
C/N [c]		10	8	12	15	15	12	17	10	11	11	8	7						
Silica - Reactive	80 [a]	8	7	7	9	3	3	9	3	3	3	6	5	5.5	7.9	8	10		
Metals (mg/L)																			
Aluminum - Soluble	0.2 [a]	0.011	0.002	<0.001	0.001	0.011	0.061	0.044	0.037	0.009	0.012	0.003	0.004		<0.01	<0.001	0.002	0.004	0.037
Aluminium - Total		0.014	0.049	0.002	0.002	0.017	0.138	0.01	0.114	0.031	0.036	0.009	0.052	0.04	0.13	<0.01	0.034	0.009	0.108
Arsenic - Soluble	0.01 [h]	0.0026	0.0015	0.0024	0.0023	0.0012	0.0012	0.0003	0.0006				0.0008						
Arsenic - Total		0.0026	0.0023	0.0029	0.003	0.0016	0.0012	0.0037	0.0017				0.0037	0.003	0.006	0.002	0.003	0.002	0.003
Boron - Soluble	4 [h]	0.083	0.067	0.106	0.128	0.069	0.037	0.109	0.028				0.044	0.062	0.17	0.08	0.09	0.07	0.11
Cadmium - Total	0.002 [h]	0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001				<0.0001		<0.0001		<0.0001	<0.0001	0.0006

	Period	SW rig 1	SW rig 1	SW rig 2	SW rig 2	SW rig 2	SW rig 2	SW rig 2	SW rig 2	SW inlet 2	SW rig 2	SW inlet 2	SW rig 2	ASR		ASTR		Ring main	
		27/6/12*	25/9/12	30/1/13	13/3/13	30/4/13	22/5/13*	11/6/13	22/7/13	3/9/13*	3/9/13	1/10/13	1/10/13	Median	95th %ile	Median	95th %ile	Median	95th %ile
	Coupon exposure (wks) ADWG†	-	22	6	12	19	-	25	31	-	-	35	35						
Physical characteristics																			
Chromium - Total	0.05 as Cr (VI) [h]	<0.0001	0.0008	0.0001	<0.0001	0.0004	0.0006	<0.0001	0.0004				0.0005						
Copper - Soluble	2 [h], 1 [a]											0.0408	0.0333						
Copper – Total	2 [h], 1 [a]	0.0005	0.21	0.034	0.020	0.33	0.12	0.036	0.18	0.061	0.093	0.090	0.1531	<0.001	0.054	<0.0001	0.008	0.001	0.008
Iron - Soluble	0.3 [a]	0.22	0.19	0.015	0.037	0.29	0.25	0.0051	0.12	0.063	0.059	0.16	0.10	**1.3**	**2.8**	0.3	**5.7**	0.22	**0.87**
Iron - Total	0.3 [a]	0.27	**1.1**	0.21	0.20	**0.43**	**0.49**	**0.68**	**0.71**	0.18	0.17	**0.47**	**1.4**	**0.38**	**3.7**	**0.36**	**5.5**	**0.38**	**1.31**
Lead - Total	0.01 [h]	0.0001	0.001	0.0003	<0.0001	0.0006	0.0012	0.0003	0.0007				0.0012	<0.0005	0.004	<0.0001	0.003	<0.0001	0.0008
Manganese - Soluble	0.5[h], 0.1 [a]	0.030	0.012	0.0015	0.002	0.0295	0.031	0.0008	0.0064				0.009	0.08	**0.14**	0.06	**0.27**	0.025	**0.137**
Manganese - Total	0.5[h], 0.1 [a]	0.030	0.057	0.0077	0.008	0.031	0.033	0.023	0.029	0.053	0.024	0.036	**0.12**	0.041	**0.15**	0.06	**0.31**	0.026	**0.14**
Mercury - Total	0.001 [h]	0.00004	0.00004	<0.00003	<0.00003	<0.00003	<0.00003	0.00008	0.00006				<0.00003						<0.00003
Nickel - Total	0.02 [h]	0.0004	0.0012	0.0012	0.0003	0.0015	0.0015	0.0003	0.0008	0.0008	0.0009	0.0005	0.0013	0.002	0.006	0.0003	0.002	0.0005	0.0011
Zinc - Total	3 [a]	0.0044	0.0085	0.0043	0.0035	0.036	0.050	0.0094	0.020	0.021	0.020	0.0054	0.015	0.008	0.09	<0.003	0.017	0.004	0.041
$SI_{Calcite}$		-0.03	-0.4	0.3	0.6	-0.8	-1.5	-0.02	-1.9	-0.7	-0.7	-0.3	-0.6	-0.3		-0.1		-0.4	
$SI_{Fe(OH)3}$		2.1	2.9	2	1.8	1.7	2.2	2.9	2.5	2.1	2	2.8	3.1	-3.3		1.9		2.3	
$SI_{Goethite}$		7.6	8.7	8	7.8	7.7	8	8.7	8.1	7.8	7.8	8.4	8.8	2.3		7.6		8	
SI_{MnOOH}		-4.7	-2.3	-3.8	-1.8	-3	-2.4	-3	-5.2	-2.3	-2.3	2.6	-1.3	-11		-9		-8.5	
$SI_{Mn(OH)2}$		-6.5	-7.6	-7.6	-7.1	-7.6	-7.7	-8	-9.3	-7	-7.1	-6.8	-6.7	-6.8		-6.5		-7.2	
$SI_{Al(OH)3}$		-1.8	-2.4	-5.5	-3.8	-1.7	-0.7	-2.1	-0.5	-1.6	-1.7	-2.3	-2	-2.6		-3.2		-2.11	

*no coupons sampled on this date; †NHMRC–NRMMC, 2011; [h] health guideline value; [a] aesthetic guideline value; [s] UV_{254}/DOC; [c] DOC/TKN; **bold** indicates value exceeds ADWG

Appendix 6. Ambient temperature at the Parafield airport weather station.

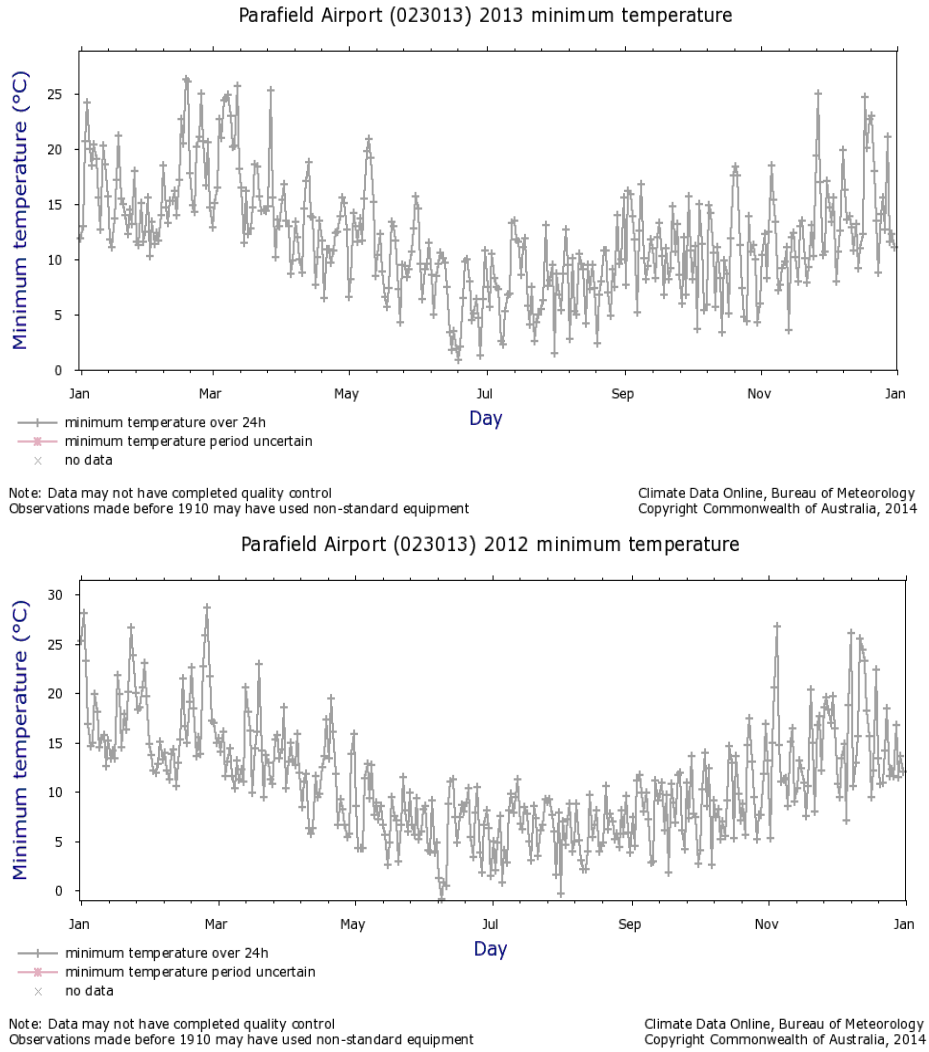
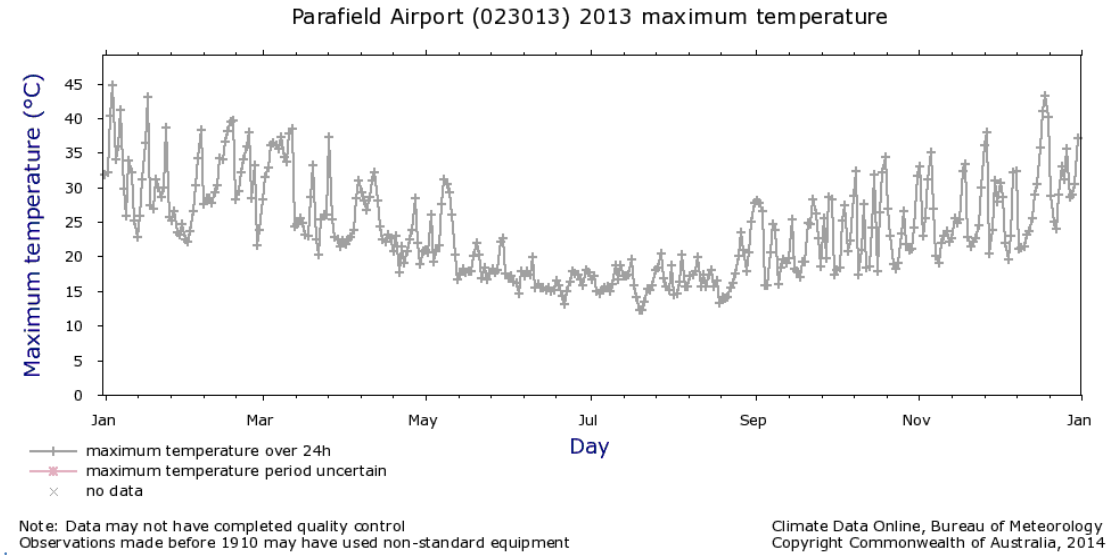


Figure 43. Minimum ambient temperatures at the Parafield airport weather station for 2012 and 2013.



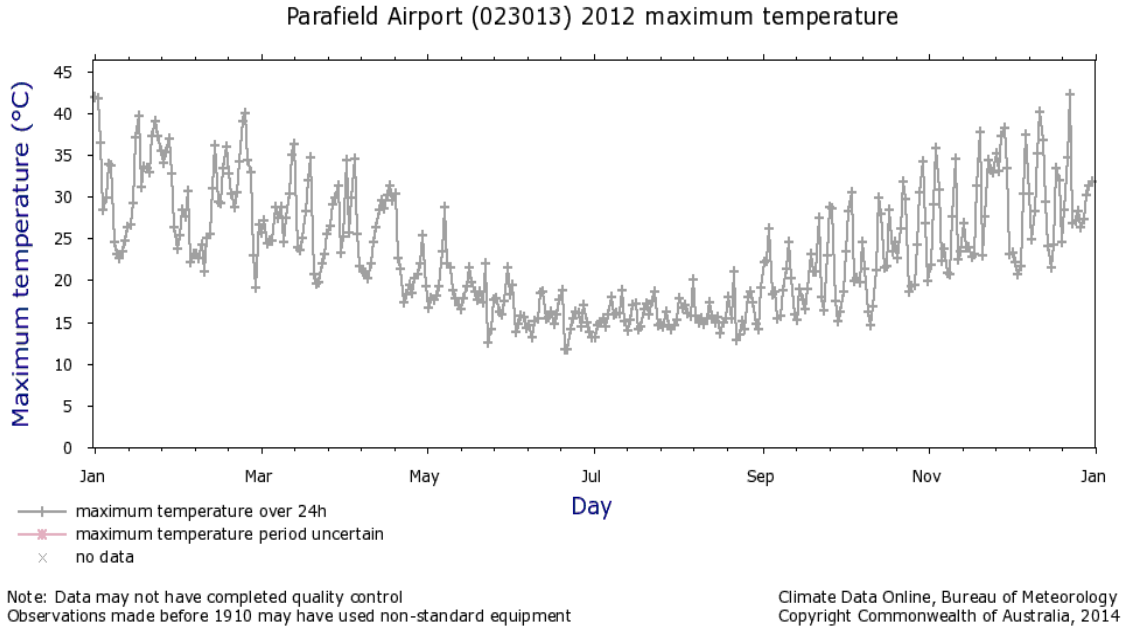


Figure 44. Maximum ambient temperatures at the Parafield airport weather station for 2012 and 2013.

Appendix 7. Analysis of water quality changes in experimental rigs

Temperature

The water heating rate within the rigs was measured during the summer period on the 14 February 2013 at 30 minute intervals from 9am to 3pm during recirculation in the rigs. During the test period, the temperature gradually increased at respective rates of 0.26°C/hour (recorded from 9 to 11am only for stormwater) and 0.56°C/hour at the baseline rig as shown in Figure 45.

Diurnal temperature variability recorded over 24 h using in-line sensors was previously exemplified in Figure 43, and showed the temperature of baseline and stormwater entering the rig on selected dates. Temperatures in Summer (22 January 2013) were the highest exceeding 30°C on that day, whilst in winter (22 June 2013) temperatures remained below 20°C. Temperatures withint a day varied by less than 5°C over 24h.

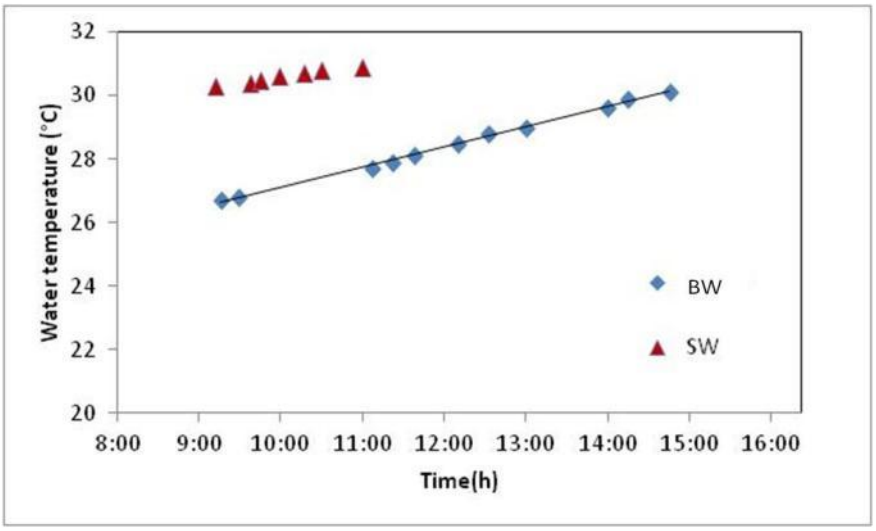


Figure 45. Verification of temperature change rate during water recirculation in the rig supplied with baseline water (BW) and stormwater water (SW) measured with grab samples on 14 Feb 2013.

Free Chlorine and Dissolved oxygen

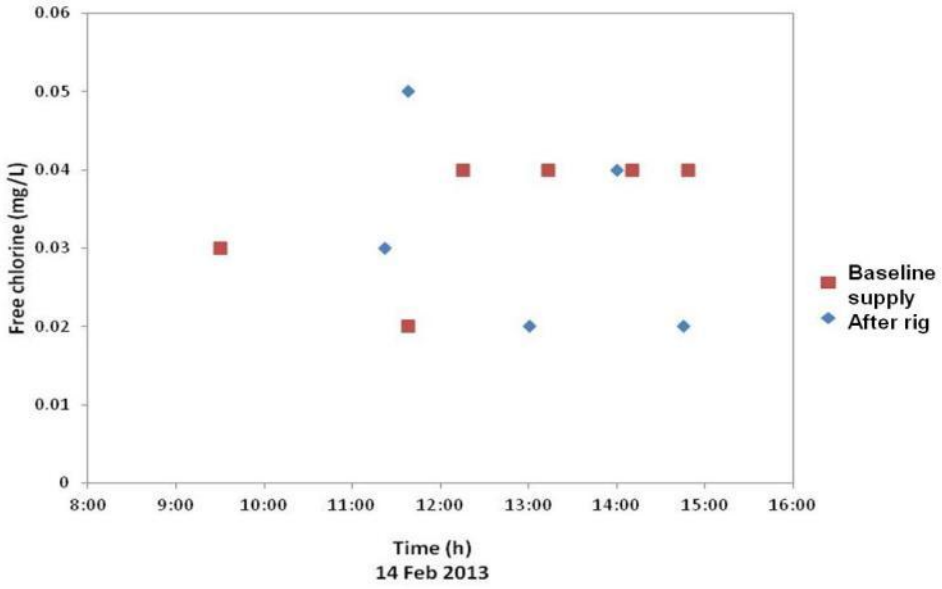


Figure 46. Free chlorine in the baseline water supply before and after passage through the rig determined using grab samples on the 14 Feb.2013.

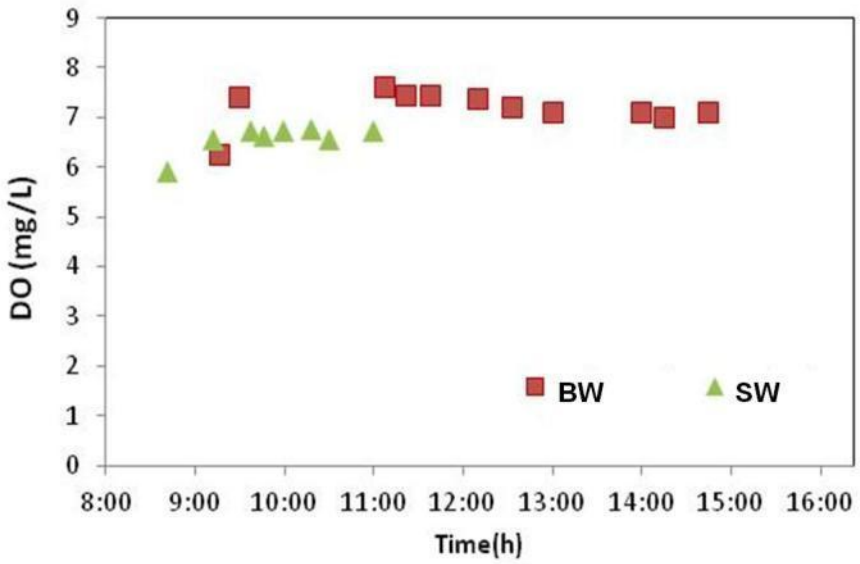


Figure 47. Dissolved oxygen over a recirculation cycle in the baseline water (BW) and Stormwater (SW) rigs measured on the 14 Feb.2013 using grab samples.

Redox potential

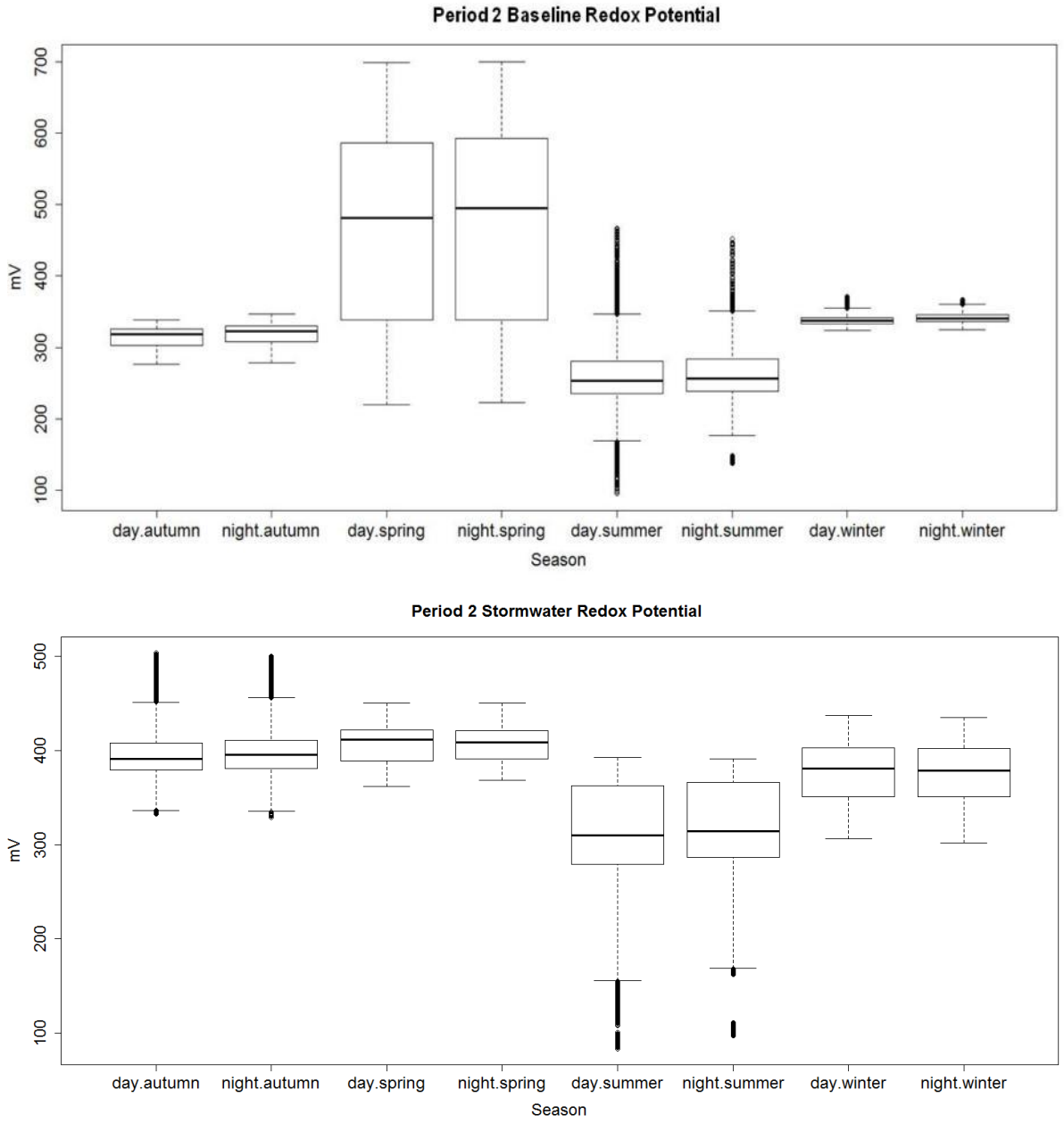


Figure 48. Summary of seasonal variability of redox potential during operation of the rig for period 2 (December 2012 to October 2013).

Appendix 8. Results of composition analysis using XRD and XRF

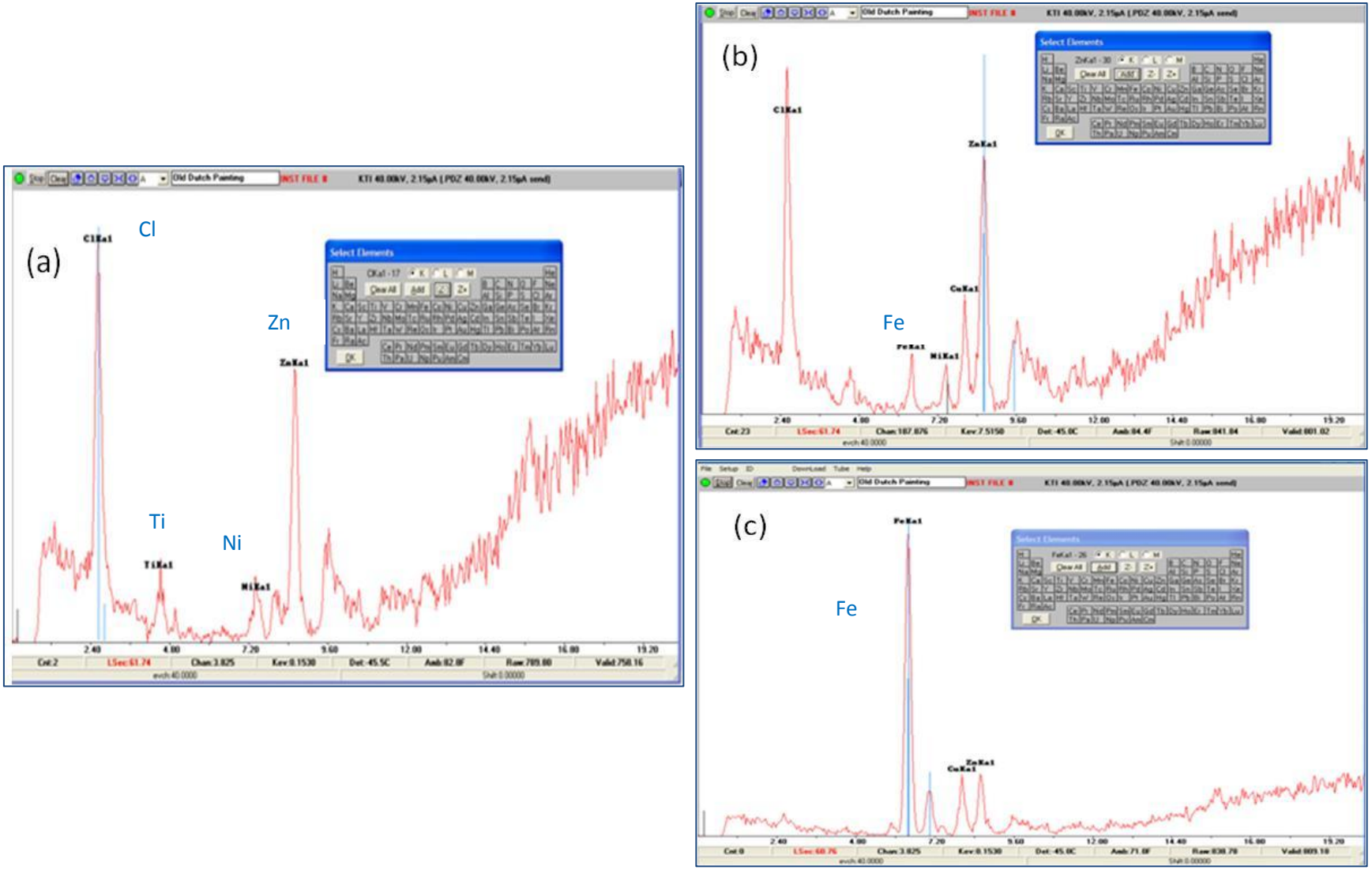


Figure 49. XRF trace for PVC coupons after 19 weeks exposure to baseline and stormwater: (a) PVC Control, (b) PVC sample P23M (Baseline water), (c) PVC sample P24SW (Stormwater),

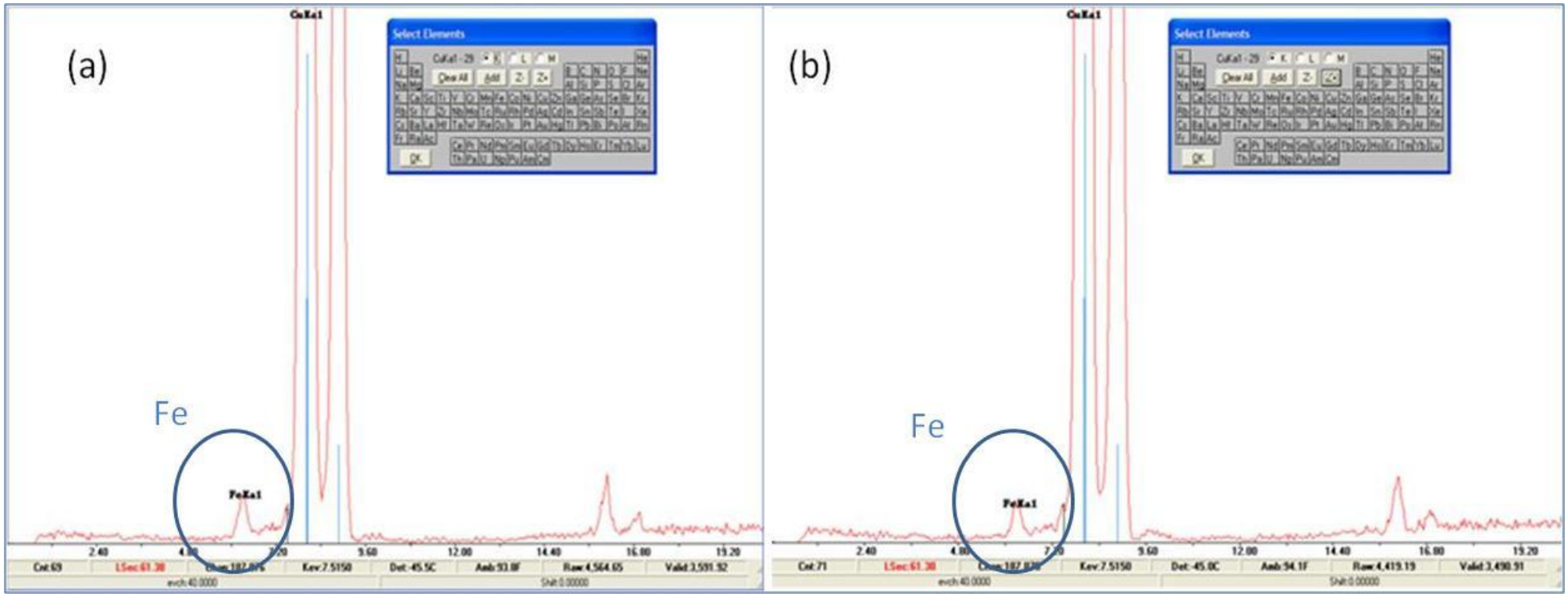


Figure 50. XRF trace for copper coupons after 19 weeks exposure to baseline water and stormwater: (a) Baseline water (sample C23M), (b) Stormwater (sample C22SW).

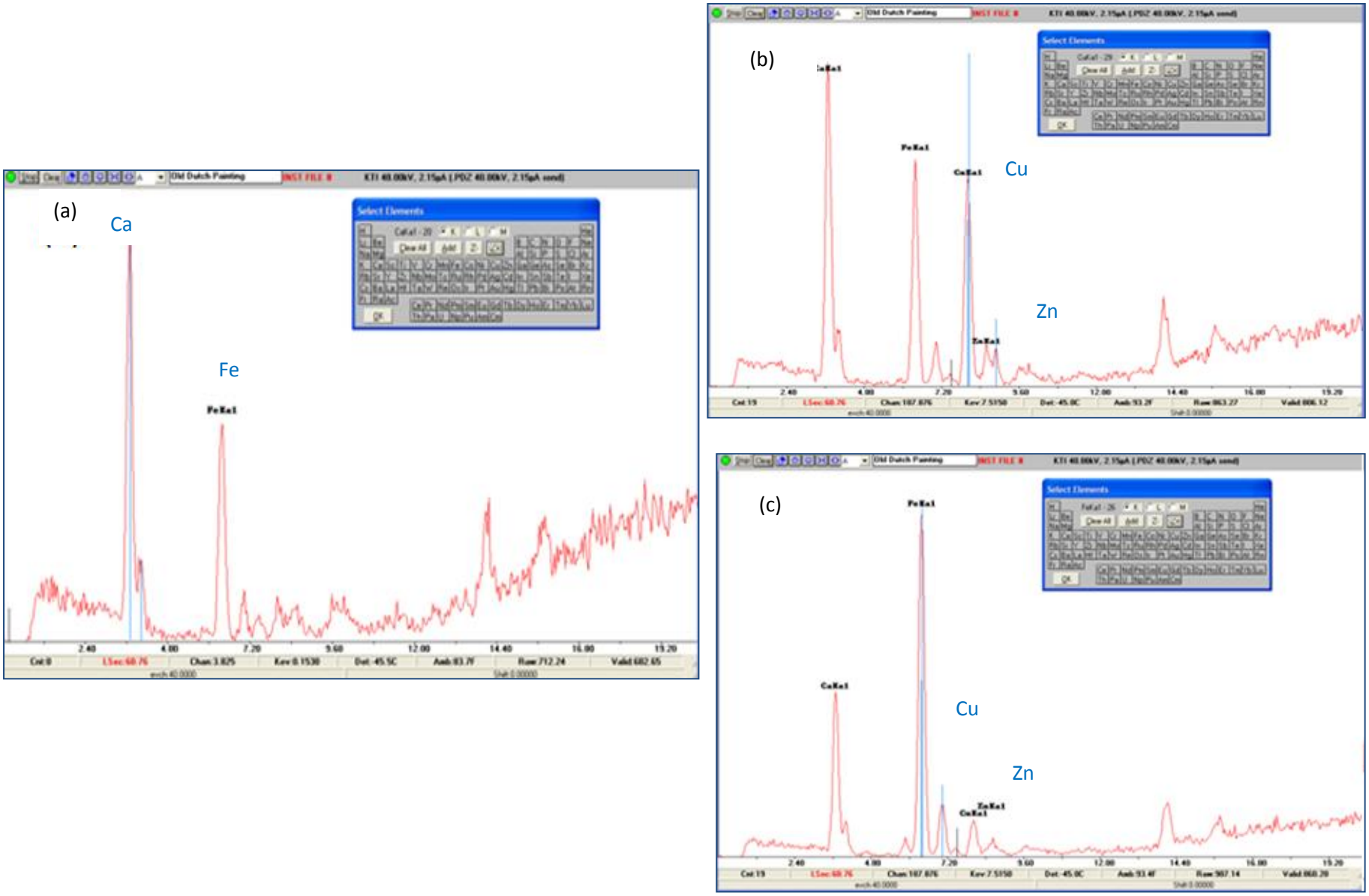


Figure 51. XRF trace for cement coupons after 19weeks exposure in baseline and stormwater (a) Cement control, (b) Cement sample L24M (Baseline water), (b) Cement sample L24SW (stormwater).

Appendix 9. Relative abundance of bacterial families on pilot rig coupons in January 2013

The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into “Others”. Cu = copper, CLI = cement lined iron, BW= baseline water, SW = stormwater.

Family	Cu-BW	Cu-SW	CLI-BW	CLI-SW	PVC-BW	PVC-SW	Bulk BW	Bulk SW
Acetobacteraceae	1.7							
Burkholderiaceae			71.7	4.0	91.5		39.9	14.6
Caulobacteraceae							1.0	
Chitinophagaceae	1.6							2.8
Chromatiaceae								2.2
Comamonadaceae		1.4	13.3	55.4	6.1	2.1	2.0	13.5
Cytophagaceae	5.9	12.5						1.0
Gallionellaceae	1.4							
Gemmatimonadaceae		1.1						
Geodermatophilaceae							5.3	
Legionellaceae								4.4
Methylophilaceae							3.6	10.7
Micrococcaceae				5.6		1.4		
Nitrosomonadaceae	29.1	22.1	1.8					4.0
Nitrospiraceae								1.2
Opitutaceae		3.6	4.0					
Oxalobacteraceae				22.8				
Parvularculaceae		1.6						
Phycisphaeraceae	1.1							
Planctomycetaceae	5.8							
Planococcaceae	3.7							
Pseudomonadaceae						88.1		
Rhodobacteraceae	1.7	1.8					1.0	4.0
Rhodocyclaceae		18.1						
Rhodospirillaceae								8.9
Sinobacteraceae		12.4		1.4				3.3
Sphingomonadaceae	1.8	1.3				2.8	6.0	2.1
Others	46.2	24.1	9.2	10.8	2.4	5.6	41.2	27.2
Number of families with ≧1 % relative abundance	10	10	4	5	2	4	7	13

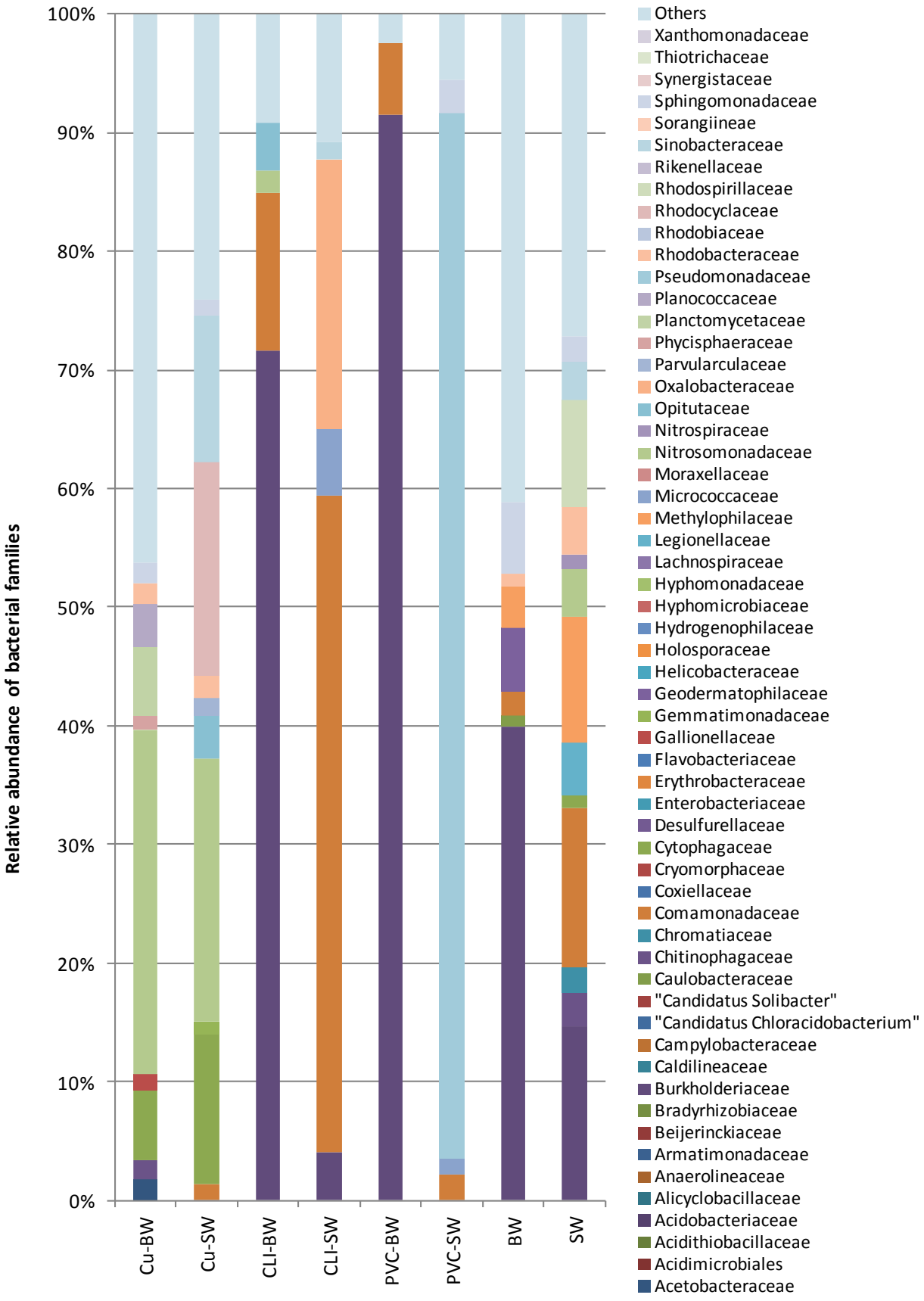


Figure 52. Relative abundance of bacterial families on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (BW) or stormwater (SW), and in bulk BW and SW samples in January 2013. The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into "Others". Note that not all of the listed families were observed at each sampling time.

Appendix 10. Relative abundance of bacterial families on pilot rig coupons in March 2013

The DNAs from replicate coupons were analysed separately. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into "Others". Cu = copper, CLI = cement lined iron, BW = baseline water, SW = stormwater.

Family	Cu-BW-1	Cu-BW-2	Cu-SW-1	Cu-SW-2	CLI-MW-1	CLI-BW-2	CLI-SW-1	CLI-SW-2	PVC-BW-1	PVC-BW-2	PVC-SW-1	PVC-SW-2	Bulk BW	Bulk SW
Acetobacteraceae				1.5			12.8	3.6			11.0	11.4		1.1
Acidimicrobiales		2.8								1.1				
Acidithiobacillaceae			3.5	2.1			2.6	2.1			1.9	3.3		2.4
Acidobacteriaceae			8.6	5.6			1.7	1.2			1.3	2.4		
Alicyclobacillaceae	1.1													
Anaerolineaceae			3.5	4.1	1.5		5.0	3.8	2.1	2.6	2.5	4.4		2.1
Armatimonadaceae	1.4	1.0							1.6	3.2			2.1	
Bradyrhizobiaceae														1.4
Caldilineaceae		1.1												
Candidatus_ hloracidobacterium	7.2	7.0		1.5	5.1	3.4			2.7	2.2			1.6	
Candidatus_solibacter	3.4	4.9			3.5	2.9			3.3	3.9			2.7	
Caulobacteraceae														1.7
Chitinophagaceae	3.2	2.2								1.1				
Chromatiaceae											13.7	2.6		
Comamonadaceae	8.9	13.9	3.6	4.8	14.1	22.8	1.6		6.5	7.3		1.2	7.5	9.1
Coxiellaceae							1.0							
Cytophagaceae			12.5	9.2		1.3	6.6	2.7			3.9	6.9		1.6
Desulfurellaceae							2.8	1.6			2.0	3.5		
Enterobacteriaceae							7.5	3.3			6.6	6.4		1.7
Flavobacteriaceae							2.5	53.7			10.5			1.2
Gallionellaceae					2.9	1.5				1.0			3.1	2.8
Gemmatimonadaceae	1.8	1.9	2.7	2.1	1.6	1.5	2.8	1.2	3.0	2.5	1.8	3.5	1.4	3.0
Helicobacteraceae														4.3
Holosporaceae							1.6					1.4		1.8
Hydrogenophilaceae			1.8	1.2								1.6		
Hyphomicrobiaceae													1.2	1.6
Hyphomonadaceae			1.2											
Lachnospiraceae													4.2	
Methylophilaceae														3.2
Moraxellaceae		1.1		1.1										
Nitrosomonadaceae	24.4	21.1	12.8	14.3	20.2	21.7	2.5	1.6	10.0	9.1	1.7	2.4	5.1	1.1
Nitrospiraceae	8.6	6.3	8.9	6.4	17.1	12.1	19.8	5.0	16.2	13.3	20.2	15.6	6.7	3.6
Opitutaceae		1.0		1.2										
Parvularculaceae					1.1	1.2			1.0	1.2			1.3	
Planctomycetaceae					1.9				3.0	1.6			3.6	
Rhodobacteraceae	4.6	5.7	2.2	2.4	2.4	2.1			3.2	3.7			2.0	
Rhodobiaceae													6.4	
Rhodocyclaceae		1.0	11.0	15.2	1.4		4.0	2.3			3.5	4.7		4.7
Rhodospirillaceae	2.4	1.5	2.2	1.7	1.4	1.9	1.9	1.2	2.0	3.2	1.4	2.1	2.8	5.6
Sinobacteraceae		1.2	12.0	11.3		1.3	8.5	5.9			6.3	7.8	5.0	15.9
Sorangiineae														3.8
Sphingomonadaceae	1.8	1.7	2.1	1.4	1.4	1.6	1.5		2.7	2.6	1.1	1.8	11.0	4.6
Synergistaceae			1.3											
Thiotrichaceae	6.6	5.8												
Xanthomonadaceae	3.1	3.5			3.3	2.0							2.3	
Others	21.8	15.4	10.2	12.7	21.1	22.7	13.1	10.8	42.6	40.4	10.5	17.2	30.2	21.6
Number of families with ≧1 % relative abundance	14	19	16	18	15	14	18	14	13	16	16	18	18	22

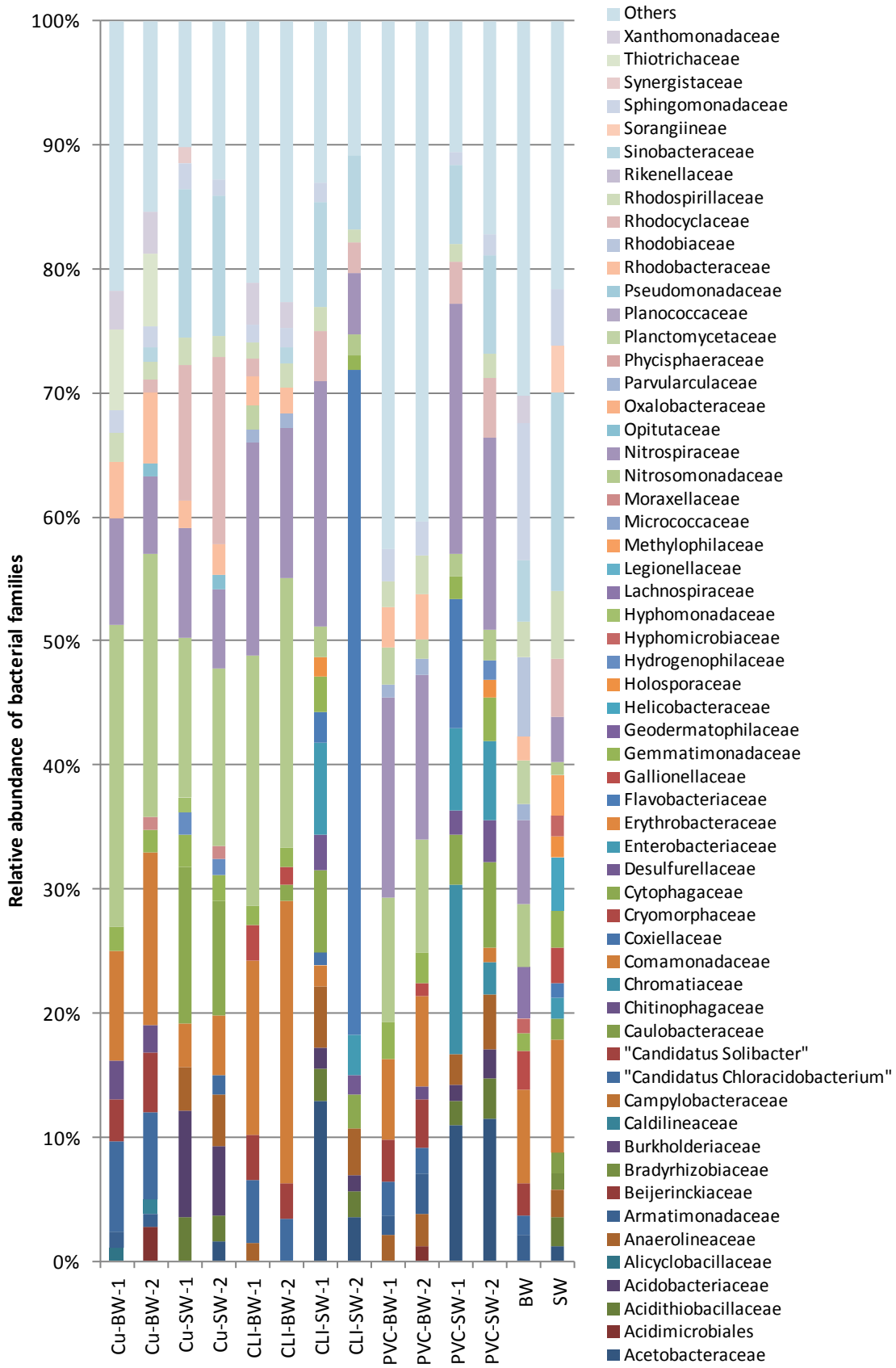


Figure 53. Relative abundance of bacterial families on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (MW) or stormwater (SW), and in bulk MW and SW samples in March 2013. The DNAs from replicate coupons were analysed separately. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into “Others”. Note that not all of the listed families were observed at each sampling time.

Appendix 11. Relative abundance of bacterial families on pilot rig coupons in June 2013

The DNAs from replicate coupons were analysed separately. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into “Others”. Cu = copper, CLI = cement lined iron, BW = baseline water, SW = stormwater.

Family	Cu-BW-1	Cu-BW-2	Cu-SW-1	Cu-SW-2	CLI-BW-1	CLI-BW-2	CLI-SW-1	CLI-SW-2	PVC-BW-1	PVC-BW-2	PVC-SW-1	PVC-SW-2	Bulk BW	Bulk SW
Acetobacteraceae							1.3				5.0			
Acidimicrobiales	1.5													
Anaerolineaceae											1.2			1.2
Beijerinckiaceae	1.1													
Bradyrhizobiaceae	31.2	78.7	1.1	2.1										
Burkholderiaceae					1.1								17.7	
Campylobacteraceae														10.0
Caulobacteraceae						2.2				1.8				
Chitinophagaceae														2.7
Chromatiaceae								1.5			1.6			
Comamonadaceae			1.5	2.2	72.4	53.5	3.4	5.7	77.2	66.0	3.2	7.6	58.5	37.8
Cryomorphaceae														1.2
Cytophagaceae	1.1		1.7			1.3	2.5	2.3		1.4	9.3		4.1	
Desulfurellaceae							2.1	1.2			3.7			
Enterobacteriaceae	1.0						2.6	1.6			4.4			
Erythrobacteraceae	30.6	7.8				1.0			1.9				3.1	
Flavobacteriaceae			81.1	79.0			44.7	52.7			31.6	63.3		14.0
Gallionellaceae	2.6													
Gemmatimonadaceae					1.3				1.9		1.4		1.8	
Helicobacteraceae														5.4
Hydrogenophilaceae														3.2
Hyphomonadaceae											1.1			
Methylophilaceae														7.7
Moraxellaceae					9.1	27.6	2.6	3.5		17.6	2.3	1.5		1.2
Nitrosomonadaceae	7.1	1.5		1.7	3.6	2.4			2.9	1.6	2.0		2.0	
Nitrospiraceae							1.1				4.8			
Oxalobacteraceae							3.3	9.3			1.9	12.9		
Pseudomonadaceae							24.6	11.3			10.6	7.0		
Rhodobacteraceae			4.1		1.7	1.1			1.8				1.5	
Rhodocyclaceae							1.5				2.1			
Rikenellaceae							2.1	1.3						
Sinobacteraceae											1.8			
Sphingomonadaceae	7.3	1.9	6.9	11.1	3.1	3.8			5.3	2.8			2.4	3.5
Thiotrichaceae														2.9
Xanthomonadaceae	4.8	3.3											1.0	
Others	11.6	6.8	3.7	3.9	7.7	7.2	8.3	9.5	9.1	8.8	11.9	7.8	7.9	9.3
Number of families with ≧1 % relative abundance	10	5	6	5	7	8	12	10	6	6	17	5	9	12

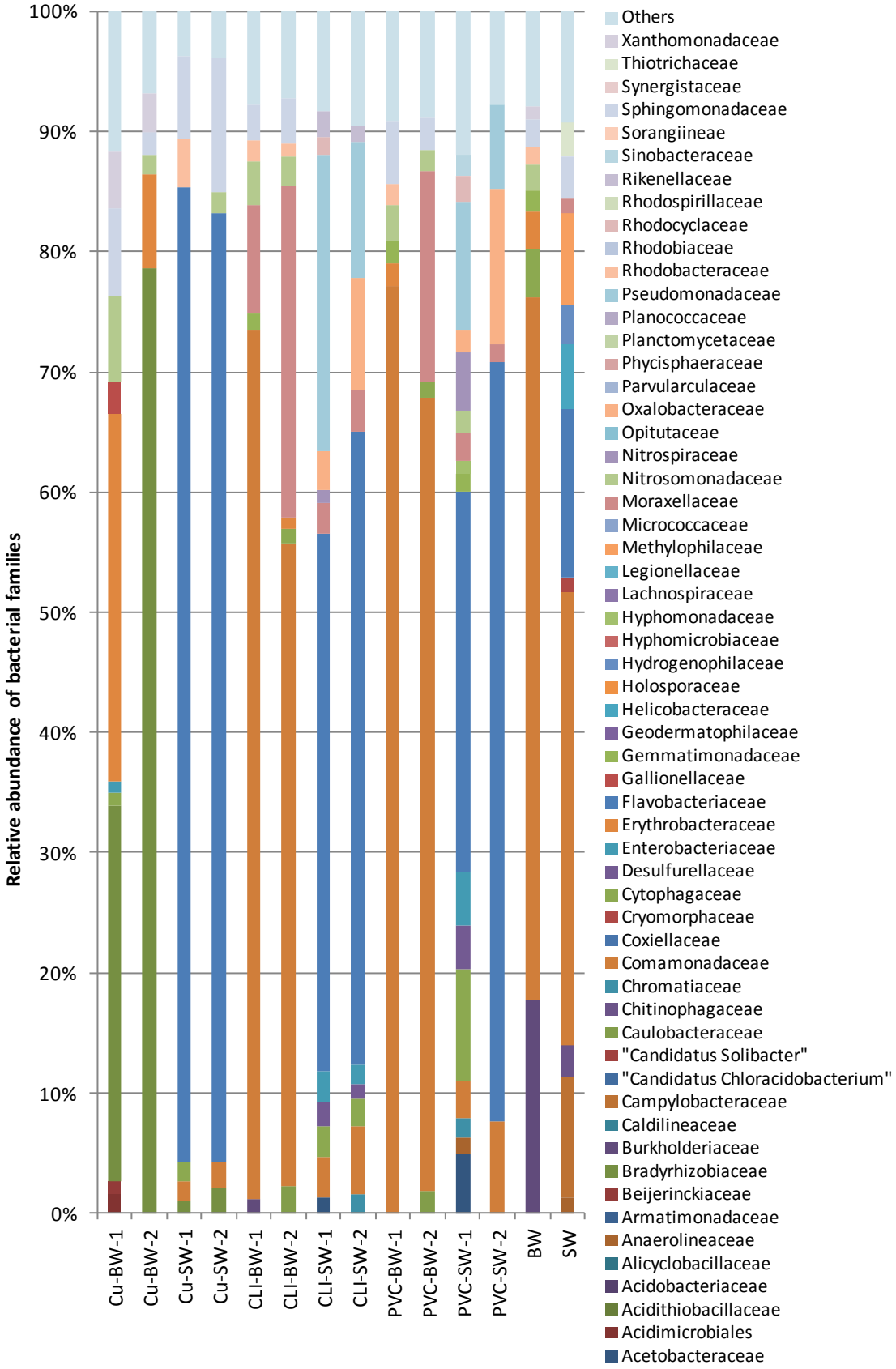


Figure 54. Relative abundance of bacterial families on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (MW) or stormwater (SW), and in bulk MW and SW samples in June 2013. The DNAs from replicate coupons were analysed separately. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into “Others”. Note that not all of the listed families were observed at each sampling time.

Appendix 12. Relative abundance of eukaryotic taxa on pilot rig coupons in January 2013

The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into “Others”. Cu = copper, CLI = cement lined iron, BW = baseline water, SW = stormwater.

Taxa	Cu-BW	Cu-SW	CLI-BW	CLI-SW	PVC-BW	PVC-SW	Bulk BW	Bulk SW
Agaricomycotina	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0
Alveolata	3.0	11.6	2.4	1.2	5.0	8.7	0.0	0.0
Bodo	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.7
Bryophyta	0.0	5.9	0.0	0.0	0.0	0.0	0.0	0.0
Cercozoa	0.0	0.0	4.9	0.0	1.7	0.0	0.0	0.0
Cf._acremonium	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0
Chytridiales	0.0	9.7	0.0	1.1	0.0	0.0	0.0	0.0
Colpodea	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0
Echinamoebidae	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0
Eucoccidiorida	6.3	1.7	3.3	1.0	2.7	0.0	0.0	0.0
Flamella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fungi	45.7	8.1	50.7	5.8	31.7	0.0	96.5	0.0
Fusarium	9.2	3.5	5.8	30.5	4.1	1.0	0.0	43.3
Heteromitidae	11.7	1.9	6.0	9.8	8.9	89.3	0.0	0.0
Invertebrate	10.3	0.0	2.6	3.2	6.4	0.0	0.0	0.0
Lobosea	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0
Nematoda	0.0	0.0	0.0	3.1	0.0	0.0	0.0	0.0
Paraphysomonadaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.0
Pezizomycotina	0.0	20.6	11.9	29.9	23.7	0.0	0.0	4.6
Pyramimonadales	1.3	0.0	1.3	0.0	1.4	0.0	0.0	0.0
Salpingoeca	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0
Ttaphrinomycotina	0.0	1.2	0.0	1.1	1.1	0.0	0.0	0.0
Tracheophyta	4.0	8.0	2.3	2.5	9.5	0.0	0.0	0.0
Tubulinida	5.2	23.6	2.2	0.0	0.0	0.0	0.0	0.0
Other	3.2	4.2	4.7	4.5	1.4	0.9	3.5	0.5
Number of families with ≧1 % relative abundance	9	11	12	14	13	3	1	4

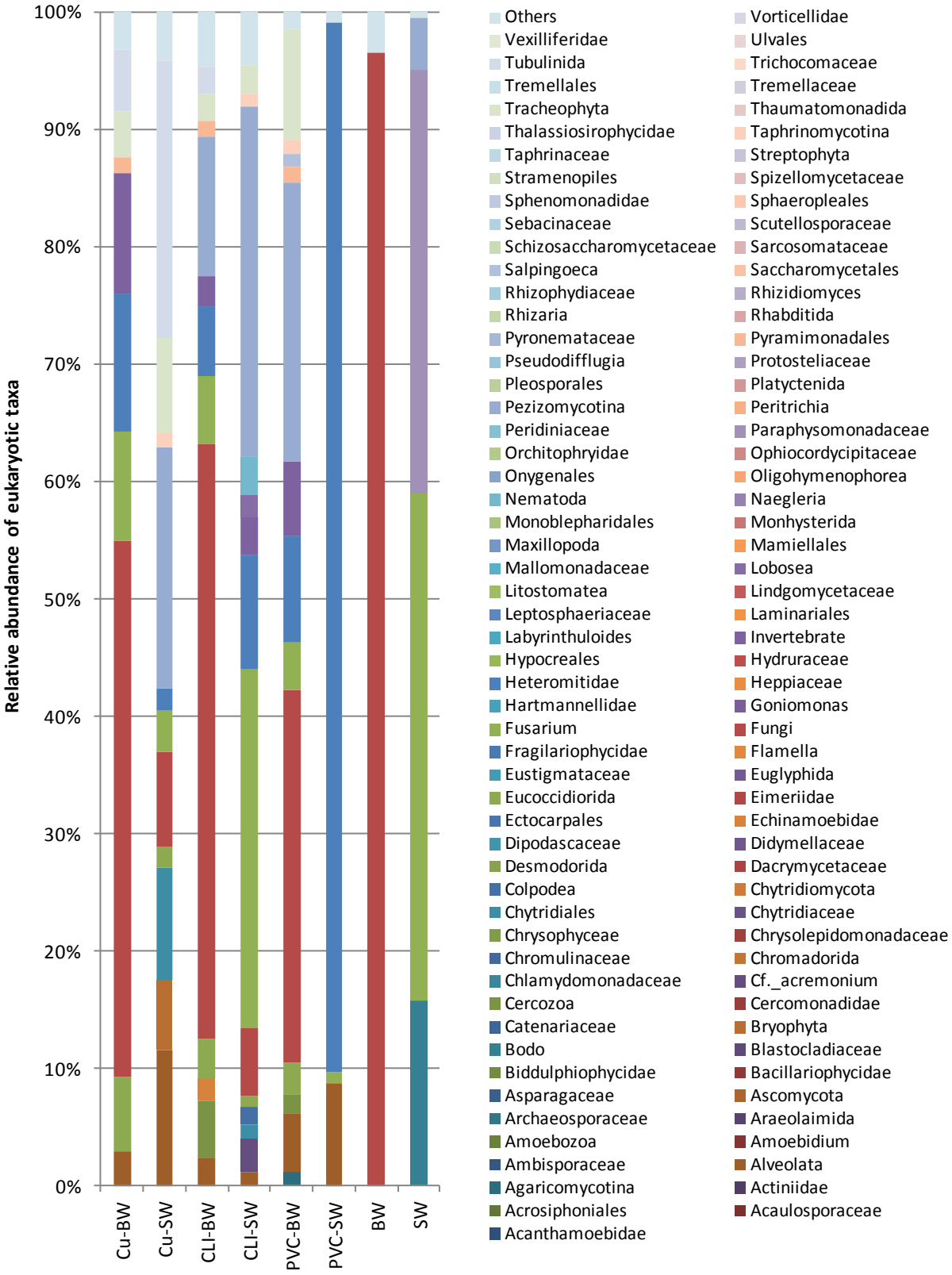


Figure 55. Relative abundance of eukaryotic taxa on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (MW) or stormwater (SW), and in bulk MW and SW samples in January 2013. The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into "Others".

Appendix 13. Relative abundance of eukaryotic taxa on pilot rig coupons in March 2013

The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into “Others”. Cu = copper, CLI = cement lined iron, BW = baseline water, SW = stormwater.

Taxa	Cu-BW-1	Cu-BW-2	Cu-SW-1	Cu-SW-2	CLI-BW-1	CLI-BW-2	CLI-SW-1	CLI-SW-2	PVC-BW-1	PVC-BW-2	PVC-SW-1	PVC-SW-2	Bulk BW	Bulk SW
Acaulosporaceae		25.4		13.0					1.2					
Acrosiphoniales						5.9								
Ambisporaceae					1.6									
Araeolaimida				29.8										39.7
Archaeosporaceae							1.3							
Ascomycota		1.1					1.4		2.4	4.6		1.6	1.5	
Bacillariophycidae					2.6	8.6			1.1	2.0				
Biddulphiophycidae						1.3								
Blastocladiaceae			17.1											
Catenariaceae									1.3					
Cercomonadidae		21.0			1.4		2.8	1.5	5.8	2.5	1.1	1.8	2.1	
Chromadorida						1.3								
Chromulinaceae					1.8					1.1			4.4	
Chrysolepidomonadaceae					4.0		2.6	1.3	2.1	3.4	3.2	3.6	18.7	
Chytridiaceae			3.2											
Chytridiales			1.1											
Dacrymycetaceae					1.1				4.9	3.6				
Desmodorida														1.5
Dipodascaceae	39.0	1.9	1.4						1.2	1.1				
Echinamoebidae		8.2			1.6				8.0	2.0			4.7	
Ectocarpales						5.3								
Euglyphida									3.7					
Eustigmataceae							1.5					1.3		
Fragilariophycidae			1.6											
Fungi			13.4				3.0	2.5	2.8		5.1	1.7	1.1	
Goniomonas												1.8		2.4
Heppiaceae		9.8		6.0										
Hydruraceae													4.0	
Hypocreales				6.1			2.8	1.3			1.6	2.7		1.8
Labyrinthuloides						1.5								
Laminariales						1.5								
Lindgomycetaceae					10.8					1.3			4.9	
Litostomatea													1.6	1.3
Mallomonadaceae													1.5	
Mamiellales			1.9											
Maxillopoda						7.8								
Monhysterida							30.3	44.4			34.7	31.9		21.0
Monoblepharidales					37.0				3.3	4.9	1.5		13.2	
Naegleria			6.1											
Oligohymenophorea				6.3			5.6	10.4			6.1	13.1		8.6
Onygenales							2.5							
Platyctenida				1.2										
Protosteliaceae							2.5							
Pseudodifflugia											1.0			
Pyramimonadales		7.3	11.5	11.3	3.5				14.8	38.8			8.7	
Pyronemataceae	9.5				2.4				2.0				1.7	
Rhabditida				2.0										2.7
Rhizidiomyces							7.0	14.4			7.8	10.2		
Rhizophydiaceae			1.6	2.0	1.1				2.5					
Saccharomycetales									2.0	2.0			1.3	
Schizosaccharomycetaceae	39.5		7.2	7.4	1.4				2.3				1.7	
Scutellosporaceae					1.3				6.4	6.1			1.7	
Sebacinaceae		1.7							2.9					
Sphaeropleales			3.9											
Sphenomonadidae														
Spizellomycetaceae		2.4			2.6		6.3	3.2	6.1	2.3	7.8	2.2	2.8	

Appendix 13 (continued). Relative abundance of eukaryotic taxa on pilot rig coupons in March 2013

The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into "Others". Cu = copper, CLI = cement lined iron, BW = baseline water, SW = stormwater.

Taxa	Cu-BW-1	Cu-BW-2	Cu-SW-1	Cu-SW-2	CLI-BW-1	CLI-BW-2	CLI-SW-1	CLI-SW-2	PVC-BW-1	PVC-BW-2	PVC-SW-1	PVC-SW-2	Bulk BW	Bulk SW
Taphrinaceae	1.7		2.3	2.3	8.6				4.6	2.0			5.0	
Thalassiosirophycidae						1.5								
Thaumatomonadida			13.1	7.1	1.5		16.7	10.0	1.6	2.0	17.2	15.8	1.6	8.7
Tracheophyta	6.7		1.3							9.8			5.3	
Tremellaceae		1.2							1.2					
Tremellales		1.8			5.7				2.6	2.0			1.9	
Trichocomaceae		11.6	5.4		1.4				2.4					
Ulvales						58.2								
Others	3.6	6.7	7.9	5.6	8.8	7.2	13.8	10.9	10.8	8.5	12.8	12.2	10.7	12.3
Number of families with ≧1 % relative abundance	5	12	16	12	19	10	14	9	25	18	11	12	21	9

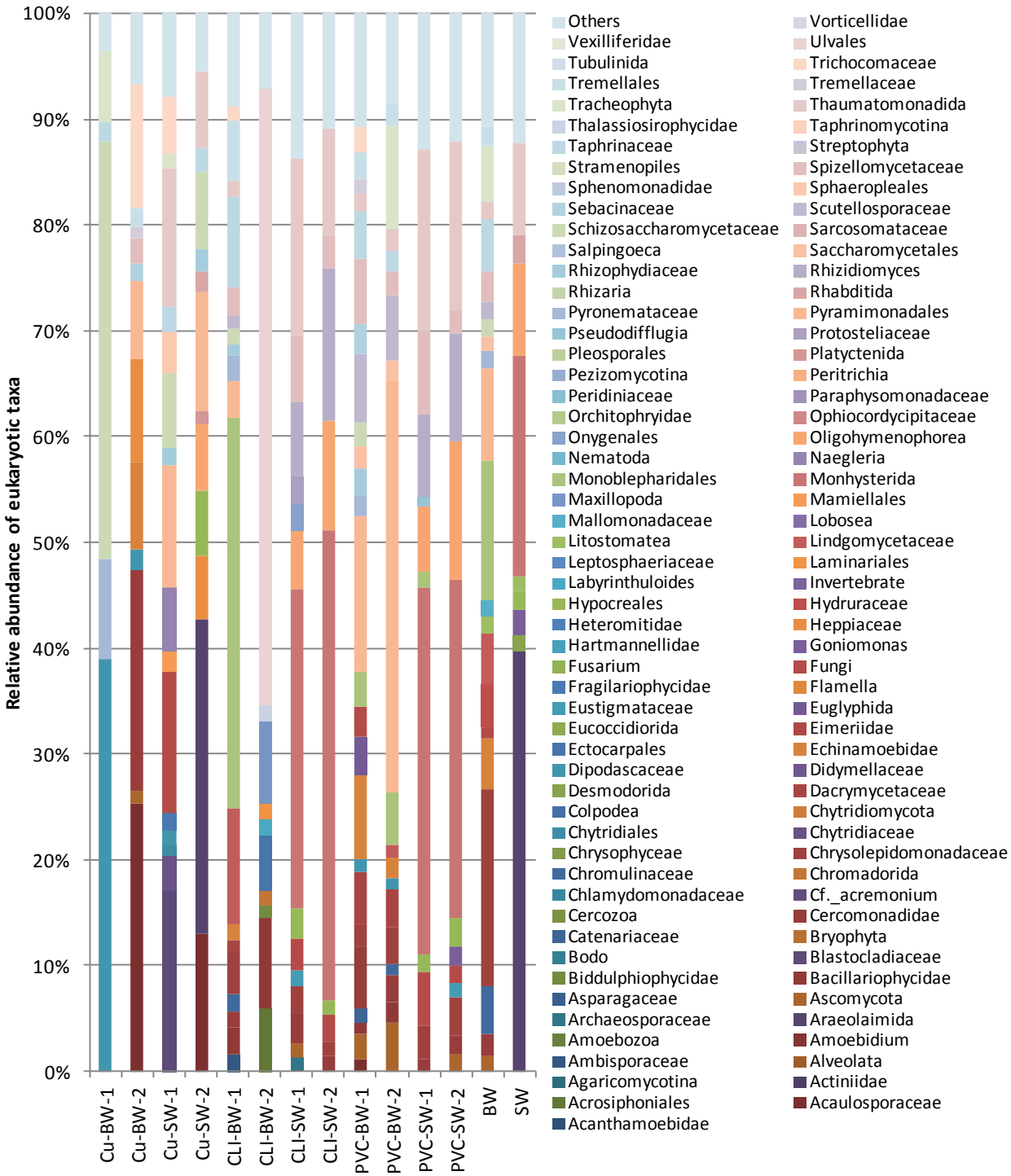


Figure 56. Relative abundance of eukaryotic taxa on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (MW) or stormwater (SW), and in bulk MW and SW samples in March 2013. The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into "Others".

Appendix 14. Relative abundance of eukaryotic taxa on pilot rig coupons in June 2013

The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into "Others". Cu = copper, CLI = cement lined iron, BW = baseline water, SW = stormwater.

Taxa	Cu-BW-1	Cu-BW-2	Cu-SW-1	Cu-SW-2	CLI-BW-1	CLI-BW-2	CLI-SW-1	CLI-SW-2	PVC-BW-1	PVC-BW-2	PVC-SW-1	PVC-SW-2	Bulk BW	Bulk SW
Acanthamoebidae						1.1								
Actiniidae								1.3						
Alveolata									6.4				9.6	4.2
Amoebidium					3.8			12.1						
Amoebozoa				1.4					1.0			3.6	1.4	
Ascomycota									1.3					
Asparagaceae		1.7												
Bodo					15.7			6.3				1.3		
Cercozoa		6.3	30.7			3.2	35.2	1.2	2.1	1.5	21.7			
Chlamydomonadaceae											1.2			
Chromulinaceae					29.9			14.5	5.9					
Chrysophyceae					10.2				2.3					
Chytridiomycota													1.5	
Didymellaceae			11.6	15.9	2.5		42.5		1.2	54.8			2.3	
Eimeriidae	23.4	11.7	6.0	40.3	7.3	1.2	3.4	18.1	2.3		3.4	6.5	1.7	56.7
Fungi	43.3	50.5	11.8	21.6	13.8	67.9	5.7	27.1	27.3	23.7	23.0	39.4	34.9	17.8
Hartmannellidae	1.3	7.1	24.2	10.1							2.0			6.9
Heteromitidae	2.1	1.4				11.0		3.2	4.5	3.8	2.5	5.2		
Hydruraceae					2.7				16.4					
Hypocreales	2.6		3.1	1.9			1.3		1.4		31.7			
Leptosphaeriaceae							1.8			1.4				
Naegleria						1.9								
Nematoda									1.5					
Oligohymenophorea									4.1					
Ophiocordycipitaceae											1.8			
Orchitophryidae		1.4												
Peridiniaceae				1.4										
Peritrichia									4.3					
Pleosporales				1.4			2.8		1.2	3.6				
Rhizaria	1.7	1.3				9.1		2.8		3.9	2.2	4.9		
Sarcosomataceae	17.3		1.6	1.7									15.5	1.7
Sphenomonadidae		2.1	2.2										9.8	1.3
Stramenopiles					2.2			4.9	3.0			28.8	3.9	
Streptophyta	1.5	2.8												
Tracheophyta	3.4	8.7	1.4								1.6			5.4
Vexilliferidae									3.8			3.7		
Vorticellidae													1.3	
Others	3.3	4.9	7.6	4.3	11.9	4.6	7.2	8.4	10.3	7.3	8.8	6.6	18.1	6.0
Number of families with ≧1 % relative abundance	9	11	9	9	9	7	7	10	18	7	10	8	10	7

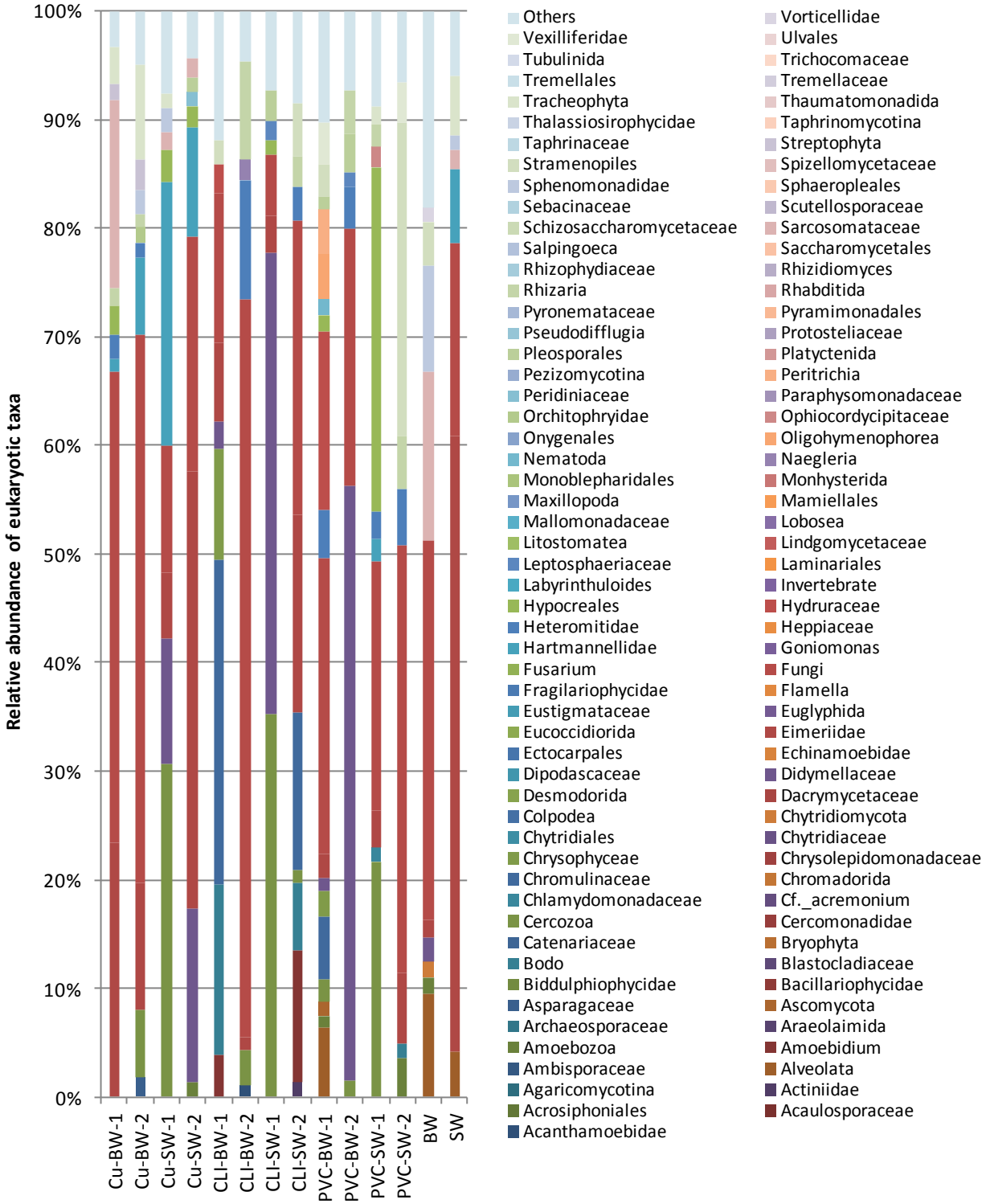


Figure 57. Relative abundance of eukaryotic taxa on pilot rig coupons made of copper (Cu), cement lined iron (CLI) and PVC and exposed to baseline water (BW) or stormwater (SW), and in bulk BW and SW samples in June 2013. The DNAs from replicate coupons were combined for the analysis. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into "Others".

Appendix 15. Field water conditions in existing stormwater pipe

Table 23. Field water conditions in existing stormwater pipe sampled for microbial analysis.

Water quality from irrigation pipeline	Unit	Value
Electrical conductivity	µS/cm	252-259
pH	-	7.14
Dissolved oxygen	mg/L	3.42
Temperature	°C	12.9
Redox potential (Ag/AgCl)	mV	-71
Turbidity	NTU	6.0-6.7
Total alkalinity	meq/L	1.3
TC	mg/L	24
IC	mg/L	16
TOC	mg/L	7.9
Free Cl_2	mg/L	0.09
NH_4-N	mg/L	0.23
NO_x-N	mg/L	0.0248
NO_2-N	mg/L	0.0067
PO_4-P	mg/L	0.0213
F^-	mg/L	0.096
Cl^-	mg/L	33
Br^-	mg/L	0.074
NO_3^-	mg/L	0.084
SO_4^{2-}	mg/L	2.8
Ca	mg/L	19.9
K	mg/L	4.24
Mg	mg/L	4.29
Na	mg/L	15
S	mg/L	1.51
Ag	µg/L	0.04
Al	µg/L	9.6
As	µg/L	0.85
B	µg/L	<0.1
Cd	µg/L	0.01
Co	µg/L	0.24
Cr	µg/L	0.28
Cu	µg/L	2.0
Fe	mg/L	3.2
Mn	mg/L	0.101
Mo	µg/L	0.06
Ni	µg/L	0.7
P	mg/L	0.12
Pb	µg/L	0.60
Sb	µg/L	0.14
Se	mg/L	<0.05
Si	mg/L	1.43
Sr	mg/L	0.102
Zn	mg/L	0.0999

Appendix 16. Relative abundance of bacterial families detected in the two samples from the field PVC stormwater pipe in June 2012

The DNAs from the replicate samples were analysed separately. Only known families representing 1% or more of the total bacterial community have been listed and other sequences are grouped into “Others”.

Family	PVC-SW-1	PVC-SW-2
Burkholderiaceae	2.6	3.6
Chlorobiaceae	2.6	1.8
Clostridiaceae	20.9	14.3
Comamonadaceae		1.6
Crenotrichaceae		1.2
Desulfobacteraceae	2.4	
Desulfobulbaceae		1.5
Enterobacteriaceae		1.1
Flavobacteriaceae	4.1	2.2
Halomonadaceae		1.3
Hyphomicrobiaceae		2.4
Methylococcaceae	2.8	3.5
Methylophilaceae	2.3	2.3
Myxococcaceae	2.3	1.8
Nitrospiraceae	3.0	1.2
Pirellulaceae	1.2	
Polyangiaceae	1.3	2.0
Pseudonocardiaceae		1.8
Rhodobacteraceae		1.1
Rhodocyclaceae	7.0	8.7
Rhodospirillaceae	1.3	2.3
Rhodothermaceae	1.3	
Ruminococcaceae		3.7
Streptomycetaceae	1.2	1.1
Syntrophaceae		1.1
Thermoanaerobacterales	1.7	
Thermodesulfovibrionaceae	4.2	2.3
Thiotrichaceae	1.4	4.0
Others	36.6	32.2
Number of families with ≧1 % relative abundance	18	24

Appendix 17. Relative abundance of eukaryotic taxa detected in the two samples from the field PVC stormwater pipe in June 2012

The DNAs from the replicate samples were analysed separately. Only known families representing 1% or more of the total eukaryotic community have been listed and other sequences are grouped into “Others”.

	PVC-SW-1	PVC-SW-2
Acantharian	1.0	
Anurofeca	1.2	1.4
Calcinea	26.2	30.7
Capsaspora	1.2	1.3
Cercozoa	5.6	4.6
Chromulinaceae	2.6	3.5
Chrysolepidomonadaceae	1.5	1.5
Codonosigidae	2.4	3.0
Desmidiales	1.0	
Goniomonas	1.2	1.2
Naegleria	5.8	6.1
Ochromonadaceae	4.4	4.5
Peridiniaceae	1.8	1.7
Perkinsidae	9.7	8.8
Pucciniomycotina	1.8	2.3
Pyramimonadales	2.2	2.7
Sphaeropleales	1.2	1.2
Thalassiosirophycidae	1.8	1.8
Ustilaginomycotina	2.8	4.4
Vampyrellidae	2.4	3.3
Vetigastropoda	1.2	
Others	20.7	16.3
Number of families with ≧1 % relative abundance	21	18

Appendix 18. Summary for detection of bacterial families in pipe rigs and field samples

Green colour indicates families detected in pilot rigs only, red colour families detected in field stormwater samples only and blue colour families detected in both pilot rigs and field samples. Cu = copper, CLI = cement lined iron, PVC = polyvinylchloride, BW = baseline water, SW = stormwater.

	Materials			Biofilms		Bulkwater	
Family	Cu	CLI	PVC	BW	SW	BW	SW
Acetobacteraceae							
Acidimicrobiales							
Acidithiobacillaceae							
Acidobacteriaceae							
Alicyclobacillaceae							
Anaerolineaceae							
Armatimonadaceae							
Beijerinckiaceae							
Bradyrhizobiaceae							
Burkholderiaceae							
Caldilineaceae							
Campylobacteraceae							
Candidatus							
Candidatus Solibacter							
Caulobacteraceae							
Chitinophagaceae							
Chlorobiaceae							
Chromatiaceae							
Clostridiaceae							
Comamonadaceae							
Coxiellaceae							
Crenotrichaceae							
Cryomorphaceae							
Cytophagaceae							
Desulfobacteraceae							
Desulfobulbaceae							
Desulfurellaceae							
Enterobacteriaceae							
Erythrobacteraceae							
Flavobacteriaceae							
Gallionellaceae							
Gemmatimonadaceae							
Geodermatophilaceae							
Halomonadaceae							
Helicobacteraceae							
Holosporaceae							
Hydrogenophilaceae							
Hyphomicrobiaceae							
Hyphomonadaceae							
Lachnospiraceae							
Legionellaceae							

Appendix 18 (continued). Summary for detection of bacterial families in pipe rigs and field samples

Green colour indicates families detected in pilot rigs only, red colour families detected in field stormwater samples only and blue colour families detected in both pilot rigs and field samples. Cu = copper, CLI = cement lined iron, PVC = polyvinylchloride, BW = baseline water, SW = stormwater.

	Materials			Biofilms		Bulkwater	
Family	Cu	CLI	PVC	BW	SW	BW	SW
Methylococcaceae							
Methylophilaceae							
Micrococcaceae							
Moraxellaceae							
Myxococcaceae							
Nitrosomonadaceae							
Nitrospiraceae							
Opitutaceae							
Oxalobacteraceae							
Parvularculaceae							
Phycisphaeraceae							
Pirellulaceae							
Planctomycetaceae							
Planococcaceae							
Polyangiaceae							
Pseudomonadaceae							
Pseudonocardiaceae							
Rhodobacteraceae							
Rhodobiaceae							
Rhodocyclaceae							
Rhodospirillaceae							
Rhodothermaceae							
Rikenellaceae							
Ruminococcaceae							
Sinobacteraceae							
Sorangiineae							
Sphingomonadaceae							
Streptomycetaceae							
Synergistaceae							
Syntrophaceae							
Thermoanaerobacterales							
Thermodesulfovibrionaceae							
Thiotrichaceae							
Xanthomonadaceae							

Appendix 19. Summary for detection of eukaryotic taxa in pipe rigs and field samples

Green colour indicates families detected in pilot rigs only, red colour families detected in field stormwater samples only and blue colour families detected in both pilot rigs and field samples. Cu = copper, CLI = cement lined iron, PVC = polyvinylchloride, BW = baseline water, SW = stormwater.

	Materials			Biofilms		Bulkwater	
Taxa	Cu	CLI	PVC	BW	SW	BW	SW
Acanthamoebidae							
Acantharian							
Acaulosporaceae							
Acrosiphoniales							
Actiniidae							
Agaricomycotina							
Alveolata							
Ambisporaceae							
Amoebidium							
Amoebozoa							
Anurofeca							
Araeolaimida							
Archaeosporaceae							
Ascomycota							
Asparagaceae							
Bacillariophycidae							
Biddulphiophycidae							
Blastocladiaceae							
Bodo							
Bryophyta							
Calcinea							
Capsaspora							
Catenariaceae							
Cercomonadidae							
Cercozoa							
Cf._acremonium							
Chlamydomonadaceae							
Chromadorida							
Chromulinaceae							
Chrysolepidomonadaceae							
Chrysophyceae							
Chytridiaceae							
Chytridiomycota							
Codonosigidae							
Colpodea							
Dacrymycetaceae							
Desmidiales							
Desmodorida							
Didymellaceae							
Dipodascaceae							
Echinamoebidae							
Ectocarpales							
Eimeriidae							
Eucoccidiorida							
Euglyphida							
Eustigmataceae							
Fragilariophycidae							
Fungi							
Fusarium							
Goniomonas							
Hartmannellidae							
Heppiaceae							
Heteromitidae							
Hydruraceae							
Hypocreales							
Invertebrate							
Labyrinthuloides							
Laminariales							
Leptosphaeriaceae							
Lindgomycetaceae							

Appendix 19 (continued). Summary for detection of eukaryotic taxa on various coupon materials, pipe rigs exposed to baseline water or stormwater and bulk water sources

Green colour indicates families detected in pilot rigs only, red colour families detected in field stormwater samples only and blue colour families detected in both pilot rigs and field samples. Cu = copper, CLI = cement lined iron, PVC = polyvinylchloride, BW = baseline water, SW = stormwater.

	Materials			Biofilms		Bulkwater	
Taxa	Cu	CLI	PVC	BW	SW	BW	SW
Litostomatea							
Lobosea							
Mallomonadaceae							
Mamiellales							
Maxillopoda							
Monhysterida							
Monoblepharidales							
Naegleria							
Nematoda							
Ochromonadaceae							
Oligohymenophorea							
Onygenales							
Ophiocordycipitaceae							
Orchitophryidae							
Paraphysomonadaceae							
Peridiniaceae							
Peritrichia							
Perkinsidae							
Pezizomycotina							
Platyctenida							
Pleosporales							
Protosteliaceae							
Pseudodifflugia							
Pucciniomycotina							
Pyramimonadales							
Pyronemataceae							
Rhabditida							
Rhizaria							
Rhizidiomyces							
Rhizophydiaceae							
Saccharomycetales							
Salpingoeca							
Sarcosomataceae							
Schizosaccharomycetaceae							
Scutellosporaceae							
Sebacinaceae							
Sphaeropleales							
Sphenomonadidae							
Spizellomycetaceae							
Stramenopiles							
Streptophyta							
Taphrinaceae							
Taphrinomycotina							
Thalassiosirophycidae							
Thaumatomonadida							
Tracheophyta							
Tremellales							
Trichocomaceae							
Tubulinida							
Ulvales							
Ustilaginomycotina							
Vampyrellidae							
Vetigastropoda							
Vexilliferidae							
Vorticellidae							

Appendix 20. Dominant bacterial genera in each of the families with ≧1% relative abundance and characteristics of the genera

Family	Genera	Characteristics
Acetobacteraceae	Acidiphilium	Aerobic, acidophilic chemoorganotroph
	Rhodovastum	Acidophilic, chemoorganotrophic facultative phototrophs growing anaerobically in light and aerobically in darkness
Acidimicrobiales	<1%	
Acidithiobacillaceae	*Acidithiobacillus*	Aerobic/facultatively anaerobic, acidophilic, chemoautotrophic iron and/or sulfur oxidisers
Acidobacteriaceae	<1%	
Alicyclobacillaceae	*Tumebacillus*	Aerobic, grow chemoorganotrophically on complex carbon substrates and chemolithoautotrophically on inorganic sulfur compounds
Anaerolineaceae	<1%	
Armatimonadaceae	*Armatimonas*	Aerobic chemoorganotroph
Beijerinckiaceae	<1%	
Bradyrhizobiaceae	*Afipia*	Chemoorganotroph, human pathogens
	Bradyrhizobium	Common legume-root nodulating, microsymbiotic nitrogen fixing bacteria found in soil
Burkholderiaceae	*Burkholderia*	Human/animal/plant pathogens, aerobic, some degrade chlororganic pesticides and polychlorinated biphenyls
	Cupriavidus	Obligately aerobic chemoorganotrophs
	Leptothrix	Oxidise iron, manganese and organic compounds
	Limnobacter	Oxidise thiosulfate
	Methylibium	Aerobic heterotrop
Caldilineaceae	<1%	
Campylobacteraceae	*Arcobacter*	Aerobic/facultatively anaerobic, some species fix nitrogen and some have been associated with human and animal diseases humans
Candidatus Chloracidobacterium	<1%	
Candidatus Solibacter	<1%	
Caulobacteraceae	*Brevundimonas*	Chemoorganotroph, some reduce nitrate
	Phenylobacterium	Grows on chloridazon, antipyrin, and pyramidon
Chitinophagaceae	*Hydrotalea*	Aerobic heterotrophs
	Lacibacter	Aerobic heterotrophs
Chlorobiaceae	*Chlorobium*	Phototrophic green sulphur bacteria
Chromatiaceae	*Rheinheimera*	Aerobic chemoheterotroph
Clostridiaceae	*Clostridium*	Obligate anaerobic chemoorganotrophs, some human pathogens
Comamonadaceae	*Alicycliphilus*	Aerobic, denitrifying chemoorganotroph
	Acidovorax	Aerobic chemoorganotrophic/litoautotrophic, some denitrifiers
	Albidiferax	Iron reducing chemoorganotroph
	Brachymonas	Aerobic chemoorganotrophic, denitrifying
	Hydrogenophaga	Aerobic chemoorganotrophic/chemolithoautotrophic, denitrifiers
	Limnohabitans	Aerobic/facultatively anaerobic chemoorganotrophic
	Polaromonas	Aerobic chemoorganotroph
Coxiellaceae	<1%	
Crenotrichaceae	*Crenothrix*	Iron oxidising bacteria (common nuisance in water pipes and springs)
Cryomorphaceae	*Fluviicola*	Strictly aerobic heterotroph
Cytophagaceae	*Arcicella*	Aerobic chemoorganotroph
	Flexibacter	Strictly aerobic chemoorganotroph
Desulfobacteraceae	<1%	Sulfate reducing bacteria, generate H_2S which is malodorous and corrosive
Desulfobulbaceae	<1%	Sulfate reducing bacteria, generate H_2S which is malodorous and corrosive
Desulfurellaceae	<1%	Sulfur reducing bacteria, generate H_2S which is malodorous and corrosive
Enterobacteriaceae	*Escherichia*	Facultatively anaerobic chemoorganotrophs, some pathogenic
	Shigella	Facultatively anaerobic chemoorganotrophs, some pathogenic
Erythrobacteraceae	<1%	
Flavobacteriaceae	*Flavobacterium*	Found in soil and fresh water, several species cause disease in freshwater fish
Gallionellaceae	*Sideroxydans*	Iron oxidising bacteria
Gemmatimonadaceae	<1%	
Geodermatophilaceae	*Blastococcus*	Aerobic or microaerophilic chemoorganotroph
Halomonadaceae	*Chromohalobacter*	Strictly aerobic chemoorganotroph
Helicobacteraceae	*Sulfuricurvum*	Oxidise sulfur compounds and use nitrate as electron acceptor
Holosporaceae	*Holospora*	Symbiotic bacteria of cialiate Paramecium bursaria
Hydrogenophilaceae	*Thiobacillus*	Autotrophic, oxidise iron

Appendix 20 (continued). Dominant bacterial genera in each of the families with ≧1% relative abundance and characteristics of the genera

Family	Genera	Characteristics
Hyphomicrobiaceae	*Hyphomicrobium*	Facultatively methylotrophic bacteria, grow well on methanol, monomethylamine, chloromethane and some other C1 compounds
	Rhodoplanes	Facultative phototrophs growing anaerobically in the light or aerobically in darkness. Anaerobic growth in darkness by nitrate respiration is also possible
Hyphomonadaceae	<1%	
Lachnospiraceae	<1%	
Legionellaceae	*Legionella*	Possible pathogens
Methylococcaceae	*Methylomonas*	Methanotrophs (obtain carbon and energy from methane)
Methylophilaceae	*Methylobacillus*	Strictly aerobic chemoorganotroph, obligately methylotrophic (only methanol and methylamine support growth)
	Methylophilus	Aerobic chemoorganotroph;
	Methylotenera	Aerobic, obligate methylamine utilizer
Micrococcaceae	*Arthrobacter*	Common soil bacterium, some species can reduce Cr^{6+} and degrade 4-chlorophenol and various other aromatic compounds
Moraxellaceae	*Acinetobacter*	Strictly aerobic chemoorganotrophic, some human pathogens;
	Perlucidibaca	Facultatively anaerobic chemoorganotrophic, reduced nitrate
Myxococcaceae	*Anaeromyxobacter*	Facultatively anaerobic chemoorganotroph, reduction of uranium, ferric iron, manganese, nitrate and nitrite (to ammonia), nitrous oxide (to dinitrogen)
Nitrosomonadaceae	<1%	
Nitrospiraceae	*Nitrospira*	Oxidise nitrite to nitrate
	Thermodesulfovibrio	Sulfate reducer
Opitutaceae	*Opitutus*	Obligate anaerobes, utilise mono-, di- and polysaccharides, reduce nitrate to nitrite
Oxalobacteraceae	*Herbaspirillum*	Nitrogen fixing microaerobic chemoorganotroph;
	Massilia	Aerobic chemoorganotroph, some isolated from human patients
Parvularculaceae	*Parvularcula*	Aerobic, reduce nitrate to nitrite, chemoheterotrophic, moderately halophilic
Phycisphaeraceae	<1%	
Pirellulaceae	<1%	
Planctomycetaceae	*Pirellula*	Aerobic chemoorganotroph
Planococcaceae	*Planococcus*	Aerobic chemoorganotroph
Polyangiaceae	*Sorangium*	Aerobic chemoorganotroph
Pseudomonadaceae	*Pseudomonas*	Aerobic, some are human/plant pathogens, some can degrade aromatic compounds
Pseudonocardiaceae	*Saccharopolyspora*	Chemoorganotroph
Rhodobacteraceae	*Rhodobacter*	Photolitotroph, facultatively anaerobic, fixes nitrogen
	Rhodovulum	Phototrophic iron oxidiser
Rhodobiaceae	*Rhodobium*	Photoheterotroph
Rhodocyclaceae	*Azoarcus*	Nitrogen fixing, microaerophilic chemoorganotroph, some reduce nitrate
	Azospira	Nitrogen fixing, microaerophilic chemoorganotroph
	Methyloversatilis	Chemoorganotrophic
	Thauera	Facultatively anaerobic chemoorganotroph, reduces nitrate and selenate
	Zoogloea	Aerobic chemoorganotrophic
Rhodospirillaceae	*Azospirillum*	Nitrogen fixing, nitrate reducing chemoorganotroph;
	Nisaea	Nitrate/nitrite reducing chemoorganotrophs
Rhodothermaceae	*Rhodothermus*	Thermophilic aerobic chemoorganotroph
Rikenellaceae	*Alistipes*	Human gastrointestinal microbiota
Ruminococcaceae	*Oscillospira*	Ruminal bacterium
Sinobacteraceae	<1%	
Sorangiineae	<1%	
Sphingomonadaceae	*Blastomonas*	Aerobic chemoorganotroph
	Novosphingobium	Strictly aerobic chemoorganotroph, degrades aromatic compounds, reduced nitrate
	Sphingomonas	Strictly aerobic chemoorganotroph
	Zymomonas	Facultatively anaerobic chemoorganotroph
Streptomycetaceae	*Streptomyces*	Chemoorganotrophs, some are human/plant pathogens,
Synergistaceae	*Aminobacterium*	Anaerobic chemoorganotroph
Syntrophaceae	*Syntrophus*	Anaerobic chemoorganotroph

Appendix 20 (continued). Dominant bacterial genera in each of the families with ≧1% relative abundance and characteristics of the genera

Family	Genera	Characteristics
Thermoanaerobacterales	*Thermoanaerobacterium*	Thermophilic anaerobic chemoorganotroph, reduces thiosulfate to elemental sulfur
Thermodesulfovibrionaceae	*JGI lists as part of Nitrospiraceae but no NCBI listing*	Sulfate reducing bacteria, generate H_2S which is malodorous and corrosive
Thiotrichaceae	*Achromatium*	Aerobic or microaerophilic sulfur-oxidisers, facultatively autotrophic, chemoorganotrophic or mixotrophic
	Thiothrix	Aerobic or microaerophilic sulfur-oxidisers, facultatively autotrophic, chemoorganotrophic or mixotrophic
Thiotrichaceae	<1%	
Xanthomonadaceae	*Arenimonas*	Obligately aerobic chemoorganotrophs
	Aquimonas	Obligately aerobic chemoorganotrophs
	Thermomonas	Aerobic, nitrate reduction, chemoorganotroph

Appendix 21. Dominant genera in each of the eukaryotic taxa with ≧1% relative abundance and characteristics of the genera

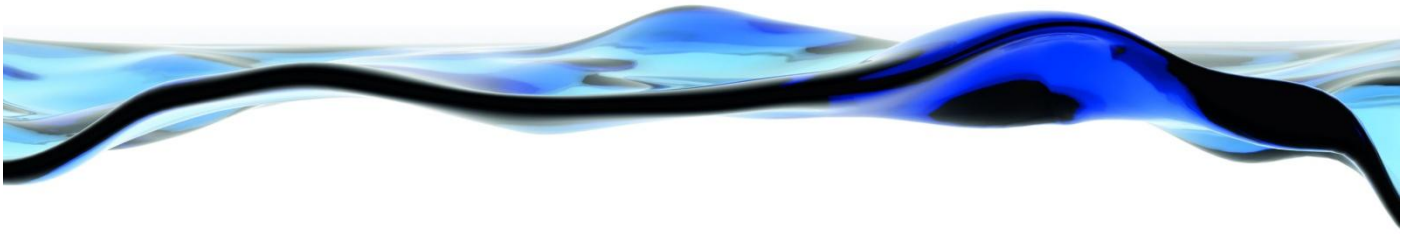
Taxa	Genera	Notes
Alveolata no rank	*Spirotrichea*	Parasitic Apicoplexa (plasmodium) and free living heterotrophs Ciliophora (ciliates)
	Orchitophryidae	
	Eucoccidiorida	
	Peritrichia	
	Colpodella	
	Colpodidium	
	Dinophyceae	
	Halteria	
	Kahliella	
	Peridinium	
	Spirostomum	
Ascomycota no rank	*Ascomycota*	Heterotrophic yeast Fungi
	Pleosporales	
	Eladia	
	Kirschsteiniothelia	
	Penicillium	
	Phoma	
	Pezizomycotina	
Acanthamoebidae family	*Acanthamoeba*	Bacterial grazers. Free living protozoan- some species pathogenic and known to harbour pathogenic organisms (i.e. *Legionella*)
Acaulosporaceae family	*Acaulospora*	Heterotrophic Fungi
Acrosiphoniales order	*Urospora*	Photosynthetic algae
Actiniidae family	*Epiactis*	Sea anemones
Ambisporaceae family	*Ambispora*	Fungi
Ameiridae family	*Ameira*	Crustaceans
Amoebidiaceae family	*Amoebidium*	Protozoa
Amobidium genus	Unclassified Ichthyophonida	Parasitic protists
Amoebozoa	*Neoparamoeba*	Bacterial grazers. Amoeboid Protozoa - Contain the genus Balmuthia, Entaoemba, which contain known pathogens
	Flamella	Amoebae
Archaeosporaceae family	*Archaeospora*	Heterotrophic Fungi
Asparagaceae family	*Bulbine*	Plant
Bacillariophycidae family	*Epithemia*	Diatoms
	Cylindrotheca	Diatoms
Biddulphiophycidae	*Bellerochea*	Diatoms
Blastocladiaceae family	*Allomyces*	Fungi
Bodonidae family	*Bodo*	Heterotrophic Fungi
Catenariaceae family	*Catenaria*	Fungi
Cercomonadidae family	*Bodomorpha*	Cercozoans
	Cavernomonas	Cercozoans
	Paracercomonas	Cercozoans
Cercozoa no rank	*Protaspis*	Bacterial grazers. Amoebae and Flagellates
	Pseudodifflugia	Cercozoans
	Trachelocorythion	Cercozoans
Chaetonotidae family	*Chaetonotus*	Gastrotrichs
Chlamydomonadaceae family	*Chlamydomonas*	Green algae
Chromulinaceae family	*Chromulina*	Photosynthetic golden algae
	Spumella	Golden algae
Chrysolepidomonadaceae family	*Chrysophyceae*	Photosynthetic golden algae
	Chrysolepidomonas	Golden algae
Chrysophyceae	*Chrysosphaera*	Golden algae
Chytridiales order	*Entophlyctis*	Chytrids
Chytridiomycota phylum	*Chytriomyces*	Chytrids
Colpodea class	*Colpoda*	Ciliates
Dacrymycetaceae family	*Dacrymyces*	Fungi
Dipodascaceae family	*Galactomyces*	Fungi
	Geotrichum	Fungi
Echinamoebidae family	*Echinamoeba*	Amoebae
Ectocarpaceae family	*Ectocarpus*	Brown algae
Euglyphida order	*Euglypha*	Cercozoans

Appendix 21 (continued). Dominant genera in each of the eukaryotic taxa with ≧1% relative abundance and characteristics of the genera

Taxa	Genera	Notes
Fragilariophycidae class	*Climacosphenia*	
Fungi kingdom	*Fungi*	Heterotrophic Fungi
	Mortierella	Fungi
	Scutellospora	Fungi
	Smittium	Fungi
	Trichosporon	Fungi
Hartmannellidae family	*Hartmannella*	Bacterial grazers. Free living amoebae know to harbour pathogenic bacteria (i.e. *Legionella*)
	Nolandella	Amoebae
	Vermamoeba	Amoebae
Heppiaceae family	*Heppia*	Bacterial grazers Amoeboid and flagellate protozoans
Heteromitidae family	*Heteromitidae*	Cercozoans
Hydruaceae family	*Hydrurus*	Golden algae
Hypocreales order	*Fusarium*	Ascomycetes
	Ophiocordyceps	Ascomycetes
Laminariales order	*Costaria*	Brown algae
Leptosphaeriaseae family	*Leptosphaeria*	Ascomycetes
Litostomatea class	*Loxophyllum*	Ciliates
Mallomonadaceae famile	*Mallomonas*	Flagellates
Mamiellales order	*Mantoniella*	Green algae
Maxillopoda class	*Itunella*	Crustaceans
Monhysterida order	*Monhystera*	Bacterial and eukaryotic grazers, Nematodes
	Theristus	Nematodes
Monoblepharidales order	*Hyaloraphidium*	Monoblepharidomycetes
Naegleria genus	*Naegleria*	Bacterial grazers, free living amoebae including pathogens
Nematoda phylum	*Nematoda*	Bacterial and eukaryotic grazers, Nematodes
	Domorganus	Nematodes
	Funaria	Nematodes
No hit	invertebrate_environmental_sample	
No hit	*Javania*	Stony corals
No rank	*Alveolata*	Alveolates
No rank	*Haptophyceae*	Haptophytes
No rank	*Stramenopiles*	Heterokonts
No rank	*Rhizaria*	Bacterial grazers Amoebae and Flagellates
No rank	*Cercozoa*	Bacterial grazers Amoebae and Flagellates
No rank but Acanthamoeba is listed as a lower genus	*Amoebozoa*	Bacterial grazers Amoebae
Oligohymenophorea class	*Cyclidium*	Ciliates
	Epicarchesium	Ciliates
	Epistylis	Ciliates
	Stokesia	Ciliates
Onygenlaes order	*Coccidioides*	Ascomycetes
Peritrichia subclass	*Zoothamnium*	Parasitic Apicoplexa and free living heterotrophs Ciliophora (ciliates)
Phylum level	*Ascomycota*	Ascomycetes
Phylum level	*Chytridiomycota*	Heterotrophic Fungi
Phylum level	*Streptophyta*	Green plants
Platyctenida order	*Coeloplana*	Stenophores
Pleosporales order	*Lindgomyces*	Ascomycetes
Protosteliaceae family	*Schizoplasmodium*	Bacterial grazers Amoebae
Pyramimonadales order	*Pterosperma*	Photosynthetic algae
	Pyramimonas	Green algae
Pyronemataceae family	*Lamprospora*	Ascomycetes
Rhabditida order	*Teratocephalus*	Bacterial and eukaryotic grazers. Nematodes
Rhizophydiaceae family	*Rhizophydium*	Chytrids
Saccharomycetales order	*Candida*	Fungi (yeasts)

Appendix 21 (continued). Dominant genera in each of the eukaryotic taxa with ≧1% relative abundance and characteristics of the genera

Taxa	Genera	Notes
Salpingoecidae family	*Salpingoeca*	Choanoflagellates
Schizosaccharomycetaceae family	*Schizosaccharomyces*	Heterotrophic yeast
Sebacinaceae family	*Sebacina*	Basidiomycetes
Sphaeropleales order	*Hylodesmus*	Green algae
Sphenomondidae family	*Petalomonas*	Euglenoids
Spizellomycetaceae family	*Spizellomyces*	Heterotrophic Fungi
	Triparticalcar	Chytrids
Stramenopiles no rank	*Labyrinthuloides*	Photosynthetic algae
	Ochromonas	Golden algae
	Paraphysomonas	Golden algae
	Pinnularia	Diatoms
	Pleurosigma	Diatoms
	Pseudostaurastrum	Algae
	Navicula	Diatoms
	Rhizidiomyces	Fungi
	Unclassified Pythiaceae	Oomycetes
	Nitzschia	Diatoms
Streptophyta phylum	*Citrus*	Plants
	Spirogyra	Plants
	Zygnema	Plants
	Tracheophyta	Plants
Taphrinaceae family	*Taphrina*	Heterotrophic Fungi
Taphrinomycotina subphylum	*Saitoella*	Heterotrophic Fungi
Thalassiosirophycidae no rank	*Thalassiosira*	Photosynthetic algae
Tracheophyta no rank	*Aponogeton*	Plants
	Ephedra	Plants
	Huperzia	Plants
	Tofieldia	Plants
	Zea	Plants
Tremellaceae family	*Tremella*	Heterotrophic Fungi
Tremellales order	*Kockovaella*	Bacidiomycetes
Ulvales	*Acrochaete*	Photosynthetic green algae
	Ochlochaete	Green algae
	Ulva	Green algae
Unclassified amoebae (Tubulinea no rank)	unclassified_lobosea	Bacterial grazers. amoebae
Vorticellidae family	*Carchesium*	Parasitic Apicoplexa and free living heterotrophs Ciliophora (ciliates)
	Vorticellides	Ciliates
Pezizomycotina subphylum	*Sarcosomataceae*	Heterotrophic Fungi
Family level	*Eimeriidae*	Apicomplexans
Family level	*Asparagaceae*	Plants
Family level	Unclassified Oocystaceae	Green algae
Genus level	*Goniomonas*	Cryptomonads



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