Performance of Large Diameter Residential Drinking Water Wells - Biofilm Growth: Laboratory and Field Testing

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

In the first phase of this project three enhanced large diameter (> 60 cm) residential wells were constructed at a study site in Lindsay, Ontario. Two wells were constructed using concrete tile casing while the other well was constructed using galvanized steel casing. Javor (2010) evaluated various aspects of drinking water well construction and design to determine the susceptibility of residential large diameter drinking water wells to surface water and airborne contamination. One of the purposes of these new installations was to remove the uncertainty with respect to construction methods, age and maintenance that is characteristic of residential drinking water well performance studies. Javor (2010) conducted a field and laboratory study to assess the performance of several design changes that were thought to improve the integrity of large diameter drinking water wells. These experiments were also used to determine whether one design was more prone to atmospheric and/or surface water contamination than another.

During the second phase of this project routine monitoring was continued and data pertinent to assess the performance of the test wells were collected using the same instrumentation. This routine monitoring involved the visual inspection of the wells, collection of well water elevation, collection of soil temperature profile data, collection and analysis of water samples, and collection of cumulative water volumes extracted from the test wells. In addition to the routine monitoring, a ground penetrating radar (GPR) survey was performed in October 2010 to complement the previous data collected during February 2010. Smoke tracer tests were performed under non-frozen and frozen conditions to re-assess the potential pathways of contaminants between the atmosphere and the interior of the test wells.

Bacteriological indicators and high concentrations of two dissolved ions were detected in all test and monitoring wells. The smoke tracer tests demonstrated that pathways for airborne contaminants to enter the test wells exist with similar pathways observed in the winter and the summer. GPR surveys indicated that the bentonite slurry annular sealant was the most homogeneous media. A baseline characterization of the microbial nature of the biofilm performed in three of the test wells (CTH1, ETH1 and ETH3) indicated that the sessile bacteria are more metabolically diverse than suspended bacteria, and that this diversity is even higher in the concrete cased wells. Biofilm characterization performed on concrete, fibreglass and

galvanized steel coupons incubated in two of the test wells (concrete and galvanized steel) showed that bacteria in the concrete cased wells barely colonized on fiberglass and galvanized steel, while bacteria in the galvanized steel cased well did not have difficulty colonizing on any of the casing materials. The results of the biofilm cleaning study indicated that the use of pressure washing combined with chlorination effectively removed biofilm grown on galvanized steel and fibreglass casing materials.

This study investigated various factors that could affect the performance of large diameter drinking water wells. Since the test wells used in this study were under the direct influence of surface water a comparison between the various annular sealants was problematic. However, the three enhanced test wells outperformed the conventional test well. The observations from the smoke tracer tests performed under non-frozen and frozen conditions indicate that the Poly-Lok lid seam is the most prevalent pathway for airborne contaminants to enter a well. Fibreglass may be the preferred choice for large diameter well casing material since fibreglass is corrosion resistant, lightweight, easy to install, has a high strength to weight ratio, and a greater degree of biofilm was able to be removed from fibreglass casing material than from galvanized steel casing material.

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Table of Contents

List of Figures ix

List of Tables xi

1.0 Introduction 1

1.1 Thesis objectives 4

1.2 Thesis Scope 4

2.0 Biofilms 6

2.1 Biofilm formation 6

2.2 Factors influencing biofilm formation 7

2.3 Biofilm enumeration 8

2.4 Biofilm characterization 10

2.5 Biofilm growth control and removal 11

2.6 Biofilm growth in water wells 12

3.0 Methods and Materials 14

3.1 Water extraction system 14

3.2 Monitoring instrumentation 14

3.3 Water quality 15

3.4 Smoke tracer tests 15

3.5 Geophysical methods 16

3.6 Laboratory biofilm experiments 17

3.6.1 Reactor design 18

3.6.2 Biofilm growth 19

3.6.3 Biofilm enumeration 19

3.6.4 Biofilm removal 20

3.6.4.1 Chlorination ... 20

3.6.4.2 Pressurized water ... 21

3.6.4.3 Chlorination/Pressured washing Combination ... 21

3.6.4.4 Chloramination ... 21

3.6.4.5 Hydrogen Peroxide ... 22

3.7 Biofilm field investigation ... 22

4.0 Results and Discussion ... 30

4.1 Water extraction ... 30

4.2 Water level measurements and precipitation ... 30

4.3 Soil temperature profile ... 31

4.4 Water quality ... 31

4.5 Smoke tracer test ... 34

4.6 Geophysical measurements ... 34

4.7 Preliminary biofilm growth and enumeration ... 37

4.7.1 Trial 1 ... 37

4.7.2 Trial 2 and Trial 3 ... 38

4.8 Biofilm removal ... 38

4.8.1 Chlorination ... 38

4.8.2 Pressurized water ... 39

4.8.3 Chlorination/Pressurized water combination ... 39

4.8.4 Chloramination ... 40

4.8.5 Hydrogen peroxide ... 40

4.8.6 Summary ... 41

4.9 Biofilm field assessment ... 41

5.0 Conclusions and Recommendations ... 64

5.1 Conclusions 64

5.2 Recommendations 66

References 67

Appendix A. Biofilm experimental method. 73

Appendix B. Experimental Data. 80

Appendix C. Denaturing gradient gel electrophoresis (DGGE), heterotrophic plate count (HPC) and Biolog® Ecoplates™S report by Mike Mitzel. 96

Appendix D. Correlations analysis between precipitation events and microbial contamination. 102

List of Figures

Figure 1.1. Field site (Javor, 2010). 5
Figure 1.2. Diagram of engraved areas on coupons. 5
Figure 3.1. Site map (Javor, 2010). 26
Figure 3.2. Smoke tracer test (Javor, 2010). 26
Figure 3.3. Borehole radar data acquisition modes showing typical ray path patterns between transmitter (Tx) and receiver (Rx) positions (Parkin et al., 2000). 27
Figure 3.4. Picture of biofilm reactor with coupons undergoing biofilm incubation. 27
Figure 3.5. Photos of biofilm sampling showing (a) swabbing from coupon, (b) tip collection, and (c) swab used for biofilm and suspended bacteria collection from test wells. 28
Figure 3.6. Photo of fibre glass coupon being power washed. 28
Figure 4.1. Cumulative volume of water pumped from test wells. 43
Figure 4.2. Test well water level measurements from pressure transducers. 44
Figure 4.3. Monitoring well water level measurements from pressure transducers. 44
Figure 4.4. Soil temperature profile. 45
Figure 4.5. TC in (a) CTH1, MW1 and MW3; and in (b) ETH1, ETH2 and ETH3. 45
Figure 4.6. *E. coli* in CTH1, ETH1, ETH2, ETH3, MW1 and MW3. 46
Figure 4.7. Chloride concentrations found in MW1, MW2 and MW3. 46
Figure 4.8. Chloride concentrations found in (a) CTH1, (b) ETH1, (c) ETH2, and (d) ETH3. 47
Figure 4.9. Hardness concentrations found in (a) CTH1, (b) ETH1, (c) ETH2, and (d) ETH3. 48
Figure 4.10. Hardness in MW1, MW2 and MW3. 49
Figure 4.11. Zinc concentrations on the three test wells and MW2. 49
Figure 4.12. Variation of (a) water content and (b) RMS amplitude with depth for test well ETH1. 50
Figure 4.13. Variation of (a) water content and (b) RMS amplitude with depth for test well ETH2. 51
Figure 4.14. Variation of (a) water content and (b) RMS amplitude with depth for test well ETH3. 52

Figure 4.15. Variation of (a) water content and (b) RMS amplitude with depth for test well CTH1. 53
Figure 4.16. Preliminary biofilm growth - (a) Trial 1, (b) Trial 2, and (c) Trial 3. 54
Figure 4.17. Biofilm removal by chlorination treatment from galvanized steel. 55
Figure 4.18. Biofilm removal by pressure washing treatment from galvanized steel. 55
Figure 4.19. Biofilm removal by pressure washing/chlorination treatment from galvanized steel. 56
Figure 4.20. Biofilm removal by pressure washing/chlorination treatment from fibreglass. 56
Figure 4.21. Biofilm removal by chloramination treatment from galvanized steel. 57
Figure 4.22. Biofilm removal by chloramination treatment from fibreglass. 57
Figure 4.23. Biofilm removal by hydrogen peroxide treatment for galvanized steel. 58
Figure 4.24. Biofilm removal by hydrogen peroxide treatment from fibreglass. 58
Figure 4.25. Wells biofilm characterization showing (a) AWCD, (b) metabolic richness and (c) metabolic diversity present in CTH1, ETH1 and ETH3. 59
Figure 4.26. AWCD of biofilm on coupons incubated inside ETH1 and ETH3. 60
Figure 4.27. Metabolic Richness of biofilm on coupons incubated inside ETH1 and ETH3. 60
Figure 4.28. Metabolic diversity of biofilm on coupons placed inside ETH1 and ETH3. 61

List of Tables

Table 3.1. Methods and method detection limits (MDLs) used on water quality analysis. ... 29

Table 4.1. Daily test well pumping program (Javor, 2010). ... 61

Table 4.2. Observations from five smoke tracer tests performed between December 2008 and February 2011. ... 62

Table 4.3. Pre-treatment logarithmic units and LR after treatment. ... 63

Table B.1. CTH1 water quality data. ... 81

Table B.2. CTH1 water quality data (cont.). ... 82

Table B.3. ETH1 water quality data. ... 83

Table B.4. ETH1 water quality data (cont.). ... 84

Table B.5. ETH2 water quality data. ... 85

Table B.6. ETH2 water quality data (cont.). ... 86

Table B.7. ETH3 water quality data. ... 87

Table B.8. ETH3 water quality data (cont.). ... 88

Table B.9. MW2 water quality data. ... 89

Table B.10. MW1 water quality data. ... 90

Table B.11. MW3 water quality data. ... 91

Table B.12. TC and *E. coli* (Victoria County Health Unit and CAWT Labs). ... 92

Table B.13. TC and *E. coli* (Victoria County Health Unit and CAWT Labs) (cont.). ... 93

Table B.14. Biofilm growth Trial 1, 2 and 3 ... 94

Table B.15. Biofilm removal results ... 95

1.0 Introduction

In the Province of Ontario about 25% of the population rely on groundwater resources, with almost all the rural population depending entirely on the extraction of groundwater from private wells (Goss et al., 1998; OFEC et al., 2001; Simpson, 2004). Private water wells include small diameter (drilled) and large diameter (dug or bored) wells (Gibb, 1973; OFEC et al., 2001). Drilled wells have typical diameters between 10 and 20 cm and use steel or PVC casings (OFEC et al., 2001; Simpson, 2004). These wells are used in aquifers with a high hydraulic conductivity (sand and gravel) capable of yielding water at the desired extraction rate (Gibb, 1973). Presently, dug/bored wells use prefabricated concrete or corrugated galvanized steel pipe with diameters larger than 60 cm and are usually not deeper than 9 m. Older large diameter wells were dug by hand and cased with brick, stone or wood, while recent large diameter wells are constructed using excavation equipment (Simpson, 2004).. Large diameter drinking water wells are necessary when the aquifer to be exploited has a low hydraulic conductivity. Since these aquifers cannot yield water as fast as it is withdrawn, the volume available in these large diameter wells is used to store water for periods of high water demand (Gibb, 1973; Simpson, 2004).

Residential large diameter wells are considered highly vulnerable water supplies (Conboy and Goss, 2000; Simpson, 2004). These wells have a higher risk of contamination from surface waters than drilled wells especially when they are located in areas with a shallow water table (Conboy and Goss, 1999; Goss et al., 1998). Determining water well contamination due to improper annular sealant is hard if the groundwater is under the direct influence of surface water (GUDI). A GUDI is groundwater that rapidly shifts in water characteristics (i.e. microbiological quality and/or ions concentration, etc.). In 1998 a study by Goss et al. found that 33% of 1292 private wells (majority of dug wells) in Ontario presented microbial contamination, but this study did not specify whether the contamination source was septic systems, or animal manure or biofilm within the wells (Goss et al., 1998).

In addition to an acute health risk, microbial contamination in residential large diameter wells could lead to a chronic health risk if biofilm is formed. Microbial contamination is very likely to

lead to biofilm formation since biofilm grow on almost any surface as long as water and minute nutrients are present (Coghlan, 1996; Dreeszen, 2003). Biofilms can shelter pathogens and opportunistic pathogens which can cause diseases in individuals with a compromised immune system (USEPA, 1992).

Scientific knowledge suggests that biofilm will still grow even if the surface is not permanently wet (Buckingham-Meyer et al., 2007) which is the situation for some well portions of the large diameter well due to water level fluctuations. Biofilm formation is encouraged even more if the colonized surface releases biodegradable compounds that could be utilized as food for any form of bacteria.

Planktonic bacteria (in suspension), will try to arrange as a biofilm because these micro communities are able to exploit available nutrients much more efficiently. As part of a biofilm, a species of bacteria will use other species' waste as food making these communities very efficient systems with regard to food supply (Coghlan, 1996; Jefferson, 2004; Potera, 1996). Also the resistance of bacteria to environmental stress (temperature change, pH change, and disinfectants) increases when they are part of a biofilm. Previous investigations have found that biofilm cells require antimicrobial agents up to 1500 times more concentrated compared to what would be needed to kill planktonic cells of the same kind (Coghlan, 1996; Jefferson, 2004; Potera, 1996). This resistance is believed to be acquired due to up-regulation of genes that generate diversity in these communities, and also to gene transfer favoured by the proximity of cells (Costerton, 2007).

This thesis builds on a previous research effort performed by Javor (2010). This previous research focused on evaluating large diameter drinking water well designs, installation methods, and structural integrity. As part of this previous study an innovative and comprehensive infrastructure system was installed at a secure field study site located on the Fleming College campus in Lindsay, Ontario, 90 km northeast of Toronto (Figure 1.1). The infrastructure at this site consisted of four (4) large diameter test holes (depth < 3 m) which have been designed to mimic large diameter residential drinking water wells. Three (3) of these test holes were constructed using existing best practices, while the fourth test hole was constructed using historical practices. To represent daily residential water use (approximately 1000 L/day), an

environmentally sustainable water extraction system was installed using solar panels and low-voltage, high-efficiency pumps. Site instrumentation includes an array of pressure transducers, flow meters, sample collection facilities, a detailed thermocouple nest, a rain gauge, and geophysical access tubes. There are also three previously installed drilled monitoring wells which provide reference information.

Well Regulation 903 states that wells "(a) made to test or to obtain information in respect of ground water or an aquifer, and (b) are not used or intended for use as a water source for agriculture or human consumption" are considered "test holes" (Ontario Ministry of the Environment, 1990). Although the studied wells are all classified as "test holes", in this research work they are referred to as "test wells".

The work performed by Javor (2010) contained a minor element that dealt with biofilm growth and removal. Javor (2010) grew bacteria on different casing materials coupons, each with two (2) equal engraved areas (Figure 1.2), using water from Laurel Creek (an urban stream that runs through the City of Waterloo) as both the bacteria seed and the nutrient source. Javor (201) used one of the areas on the coupon to determine the initial conditions of bacteria before treatment, and the other area was used to determine the efficiency of the different biofilm removal methods utilized. Javor (2010) did not enumerate biofilm and assumed that the quantity of bacteria grown on both areas marked on the well material coupons were the same, and that the number of biofilm bacteria developed on every coupon within a "batch" was the same. Biofilm bacteria determination was conducted by removing the biofilm using a sterilized swab followed by biofilm bacteria resuspension. Once the biofilm bacteria were resuspended a negative/positive (qualitative) adenosine triphosphate (ATP) method was used to determine the amount of biofilm bacteria present on the swabbed portion of a coupon. This biofilm bacteria determination method was performed before and after treatment with the different biofilm removal methods.

The main findings from Javor's (2010) effort were:

- Galvanized steel and fibreglass surfaces were easier to clean than a concrete surface when using chemical and physical biofilm removal methods; and
- Pressure washing was a good method to physically remove biofilm.

1.1 Thesis objectives

This scope of work captured in this thesis addresses two objectives:

(1) biofilm growth and removal; and

(2) performance monitoring of test wells over two freeze/thaw cycles.

1.2 Thesis Scope

To satisfy Objective 1, both laboratory experiments and field trials were performed. Considering the findings from Javor (2011), the goals of this laboratory effort were:

- To grow biofilm under similar *in situ* conditions as those observed in the large diameter test wells at the study site;
- To confirm that the number of biofilm bacteria grown on both areas of the coupons are within an acceptable range (< 1 log unit);
- To determine if the sampling and enumeration method previously used by Javor (2009) could be improved; and
- To compare the performance of different biofilm cleaning alternatives (i.e. chorine, pressure washing, chloramine, hydrogen peroxide).

The field component extends from the laboratory studies, and lessons learned at both these scales will provide a better understanding of biofilm removal issues.

The scope of work associated with Objective 2, involves the collection of well performance data (primarily water quality) over two additional freeze/thaw cycles; the evaluation of airborne path ways employing smoke tests; and the evaluation of the integrity of the sealing materials using a non-destructive method (ground penetrating radar (GPR)).

Relevant background information is included in Chapter 2 followed by methods and materials in Chapter 3, and the results and discussion in Chapter 4. Chapter 5 contains major findings and outlines recommendation for future studies. Detailed procedures for biofilm growth, cleaning and analysis are provided in Appendix A. Water quality data obtained from the monitoring wells and large diameter test wells are found in Appendix B.

Figure 1.1. Field site (Javor, 2010).

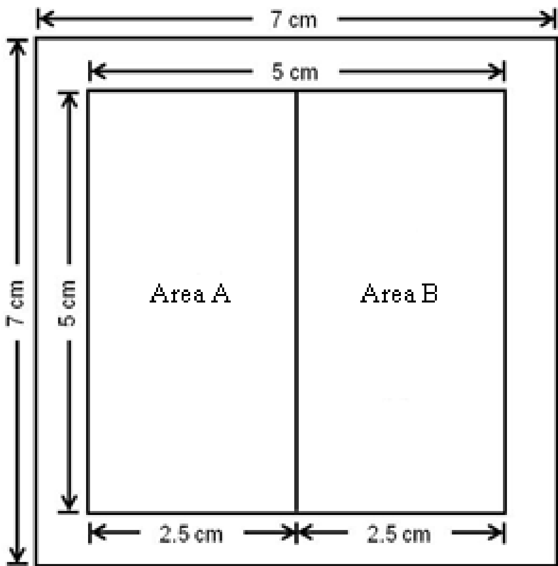

Figure 1.2. Diagram of engraved areas on coupons.

2.0 Biofilms

Biofilm, the natural habitat for most existing bacteria (Costerton, 2004; Potera, 1996), are complex structures in which it is common to find different species of bacteria helping each other to exploit available nutrients. Biofilm formation usually starts with biodegradable compounds being attached to a surface, followed by the colonization of a species of bacteria that feeds from these compounds. The bacterial species that starts colonization of a virgin surface is known as the primary colonizer (Dreeszen, 2003).

Biofilms composition includes between 75 and 95% biofilm matrix (Geesey et al., 1994). The biofilm matrix is a mix of compounds that include acid polysaccharides, polymers of sugars, and DNA from disintegrated cells. Biofilms can indistinctly be formed by either aerobic and/or anaerobic bacteria (Potera, 1996). Biofilm cells and matrix are usually organized in patterns consisting of channel networks that emulate a circulatory system with channels used to distribute nutrients and to transport waste (Coghlan, 1996; Costerton, 2007).

2.1 Biofilm formation

Scientific knowledge suggests that most bacteria will form biofilm communities in order to increase survival chances. However, if the biofilm bacteria are starving, they will mutate to their planktonic phenotype gaining the ability to go into a semi-hibernation mode of growth called ultramicrobacteria (UMB). As a UMB they will leave the community looking for a more suitable environment; the dormant bacteria will reactivate when nutrients become readily available (Costerton, 2007). Once active, the planktonic bacteria will find a suitable surface commencing a new biofilm, which will grow as long as nutrients remain available (Pedersen, 1990).

Many primary colonizers have been shown to undergo mobile post adhesion behaviour shortly after the cell has attached to the surface. Once attached, the planktonic cells will modify their gene patterns to the sessile mode of growth. This phenotype provides bacteria with the capacity to release polysaccharides and other matrix components including proteins that are absent in planktonic cells. This matrix facilitates bacteria with the ability to modify their connection with the colonized surface and other bacteria (Costerton, 2007). During the adhesion-behaviour change the bacterium associates with other cells of its kind and with other metabolically

cooperative species, forming aggregates and structures emulating archaic cities (Coghlan, 1996). If conditions in the microniche change, the cell will modify its phenotype, and it may even change its position in the community to improve its chance for survival. These changes can also lead to the displacement of entire communities, retaining their spatial associations and metabolic integration. These sub-communities will eventually find another suitable surface becoming sessile again (Costerton, 2007).

Biofilms can be formed from individual species if they have enzymes that bond easily to a specific surface (Costerton, 2007). However, planktonic bacteria are not the only precursors for biofilm formation since biofilms will also form from aggregates detached from other biofilms which could be trapped within surface irregularities (Costerton, 2007). Surface smoothness plays an important role when biofilm is being detached by water flow with smoother surfaces yielding higher biofilm detachment (Pedersen, 1990).

2.2 Factors influencing biofilm formation

The survival of bacteria increases when they are part of a biofilm. Bacteria are led to arrange as a biofilm because a biofilm community is able to exploit available nutrients much more efficiently than planktonic cells. The fact that a bacteria species can use waste as food makes the biofilm community a very efficient system with regard to food supply (Coghlan, 1996; Jefferson, 2004; Potera, 1996). Another reason why bacteria exist mostly as biofilm is because as part of these communities their capacity to resist abrupt environmental changes increases. Some researchers have found that biofilm cells require antimicrobial agents 10 to 1500 times more concentrated compared to what would be needed to kill planktonic cells of the same kind (Coghlan, 1996; Jefferson, 2004; Potera, 1996). This resistance is believed to be acquired due to up-regulation of genes that generate diversity in these communities, and also to gene transfer favoured by the proximity of cells (Costerton, 2007).

Scientific knowledge suggests that in addition to nutrient availability there are some other factors affecting biofilm growth in wells. Temperature, pH and surface water infiltration are some of these factors. Infiltrating surface water washes down soil nutrients through the vadose zone to the saturated zone and becomes available to bacteria. Studies have shown that in the intake of some drinking water systems, bacterial occurrences have been observed to increase when

fertilizer application was followed by a rain event (USEPA, 1992). Other studies have determined that the amount and variety of coliforms detected in water, and even their reproduction rate, change as water temperature changes (USEPA, 1992).

Studies have shown that biofilms will grow on any surface as long as water and sufficient nutrients are present (Coghlan, 1996; Dreeszen, 2003; Pedersen, 1990), suggesting that scarcity of nutrients will only inhibit biofilm growth but it will inevitably establish as long as nutrients are present in the water. A laboratory study evaluating biofilm formation on two inert materials (lead stabilized PVC (hydrophobic) and glossy stainless steel (hydrophilic)) found no difference between the amounts of bacteria grown suggesting that the wettability of inert materials had no effect on biofilm formation (Pedersen, 1990). Biofilm formation is encouraged if the surface of the colonized material releases biodegradable compounds that could be used as food for any form of bacteria (i.e., iron, steel, PVC).

2.3 Biofilm enumeration

When disinfectant efficacy is tested it is strongly recommended to produce biofilms under similar conditions to the environment where the disinfectant is going to be applied (Buckingham-Meyer et al., 2007). This will ensure that biofilm produced in the laboratory will have similar properties to the ones under investigation (Buckingham-Meyer et al., 2007). In drinking water research, biofilms are usually grown on coupons inside annular reactors (AR) which are capable of simulating the different sections of a drinking water distribution system before their quantification (Gagnon and Slawson, 1999).

Biofilm quantification can be classified as non-destructive (biofilm still attached to the colonized surface) or destructive which requires a sample of biofilm to be removed from the surface (Nivens et al., 1995). Nivens et al. (1995) compared several non-destructive analytical techniques (microscopic, spectrochemical, electrochemical and piezoelectric techniques) useful to monitor microbial biofilms, and determined that even though these techniques could provide online measurements that would help to better understand biofilms, it was necessary to implement more than one technique since one by itself will not provide enough information.

The most common form of quantification is destructive quantification. These techniques have shown to be challenging since they comprise two processes that compromise reliable results

when quantifying biofilm bacteria; biofilm detachment and resuspension (Gagnon and Slawson, 1999).

Detachment and resuspension are the main sources of error when enumerating bacteria. If the biofilm sample to be tested is not completely removed, not all of the bacteria will be quantified. Camper (1985) found that during resuspension, if biofilm bacteria are not re-suspended properly there could be an underestimation of microorganisms by a factor of 1,500 to 15,000. Gagnon and Slawson (1999) compared three methods used to remove lab-grown biofilm from coupons (utility knife, swabbing and stomaching) and four methods for cell resuspension (tissue blender, vortex, stomacher and sonicator), and found that stomaching was the best method for cell detachment and resuspension from coupons since it produced a higher number of bacteria (Gagnon and Slawson, 1999). Unfortunately this method is only good to quantify biofilm grown on coupons and hence not useful for field investigations.

Once biofilm cells are resuspended, two common methods for enumeration are Heterotrophic Plate Count (HPC) (Camper et al., 1985; Gagnon and Slawson, 1999; Hallam et al., 2001; Jackson et al., 2001; LeChevallier et al., 1988), and Adenosine Tri-Phosphate (ATP) determination (Hallam et al., 2001; Van der Kooij and Veenendaal, 1994; Vanhaecke et al., 1990). In the first method, 5 series of 10-fold dilutions from an aliquot of the resuspended bacteria solution are prepared. An aliquot (usually 100 µL) of each of the five different dilutions is then plate spread in individual Petri dishes with the agar of choice (usually R2A agar). After incubation at 25 ^{o}C, bacterial colonies are counted and reported as colony forming units (CFU). When using ATP determination for biofilm quantification, ATP from the cells of an aliquot of the resuspended bacteria solution is extracted with the help of an extractant reagent. Once the ATP is in solution Luciferase/Luciferin (rL/L) is added producing luminescence. The luminescence, which is directly proportional to the amount of ATP content, is immediately measured using a luminometer as relative luminescence units (RLU). A standard curve of ATP concentrations vs RLU provides the amount of ATP initially contained in the sample.

Another, less common method that assesses microbial activity on surfaces was developed by the Thames Water Authority in 1979. This method, called mean dissolved oxygen difference (MDOD), consists of determining biological activity by measuring the dissolved oxygen of a

water sample before and after a coupon with biofilm grown on it is submerged in the water sample with a known initial dissolved oxygen concentration (Colbourne and Brown, 1979). Consistent with the stomaching method the MDOD method is only good to quantify biofilm grown on coupons.

2.4 Biofilm characterization

The operation of water and waste water processes in which biofilms are involved includes two important parameters, biofilm composition and activity (Lazarova and Manem, 1995). Literature suggests that biofilm characterization could provide a clearer understanding of biofilm formation and its resistance to disinfectants (Whiteley et al., 2001). Whiteley et al. (2001) found that biofilm population increases with species diversity and that resistance to disinfectants was independent of biofilm cell density. Biofilm characterization provides the opportunity to investigate the effect of the environment and its water quality (i.e. well water) on the microbial composition of biofilm (Lear and Lewis, 2009).

Biofilm characterization techniques include Denaturing Gradient Gel Electrophoresis (DGGE), Community-level Physiological profiling (CLPP) and the standard plate count of colony forming unit (CFU), among many others. DGGE, an excellent tool for comparative investigations, is a method widely utilized to obtain profiles, and to describe microbial community structures and/or genetic diversity of complex microbial communities over time or in response to environmental changes (Hastings, 1999; Muyzer, 1999; Tourlomousis et al., 2010). DGGE is a molecular fingerprinting method based on the separation of polymerase chain reaction (PCR). Using denaturing gels and taking advantage of the electrophoretic mobility decrease of the partially melted DNA molecules DGGA allows the analysis of separated DNA fragments individually and hence allows for the identification of the main bacteria groups present in the biofilm (Muyzer, 1999; Tourlomousis et al., 2010).

CLPP, another excellent tool for comparative investigations, can be achieved using the BIOLOG® EcoPlates™ method (Weber and Legge, 2009). This method is used to identify microbial populations based on the type of carbon substrate the bacteria utilizes (Weber et al., 2007). In the BIOLOG® EcoPlates™ method aliquots are placed in EcoPlates™ which contain 31 different carbon substrates and their consumption is monitored by colorimetric means (optical

density measured at 590 nm) (Weber and Legge, 2009). A BIOLOG® EcoPlates™ analysis provides: the Average Well-Colour Development (AWCD) which is an indication of the amount of each carbon substrate that bacteria utilized; the metabolic richness which is simply the number of carbon sources utilized by bacteria with measured absorbance greater than 0.25; and with the previous two measures the Shannon-Wiener diversity index (H') or metabolic diversity (maximum value 3.46) can be determined (Salomo et al., 2009; Weber and Legge, 2011).

The CFU method is probably the oldest and most used method utilized in microbiology to estimate the number of live bacteria (Lazarova and Manem, 1995). The Heterotrophic Plate Count (HPC) is a CFU method used to estimate the number of live bacteria using organic carbon for growth (heterotrophic) that are present in any given environment without differentiating between pathogens and non-pathogens (Allen et al., 2004). HPC can be performed by spreading a diluted sample into a growing media (usually R2A for water samples) or by filtering the sample and then placing the filter on the media (Allen et al., 2004; Lazarova and Manem, 1995; Reasoner, 2004). After an incubation period at a determined temperature, the number of colonies is counted which yields the amount of bacteria present in each millilitre of sample (Allen et al., 2004; Reasoner, 2004). Because of the conditions of the method (medium, temperature, etc.) the HPC method enumerates only a fraction of the heterotrophic bacteria (Allen et al., 2004). HPC results are generally reported as CFU/mL, and it is assumed that each CFU represents an initial single, live bacterium that was capable of multiplying (Lazarova and Manem, 1995).

2.5 Biofilm growth control and removal

The literature indicates that biological deterioration during storage and distribution is the major problem faced by water suppliers (Momba et al., 2000). In drinking water treatment systems biofilm growth has been controlled by manipulating the factors affecting biofilm formation (pH, nutrients, temperature and planktonic bacteria (seed)). Some drinking water treatment plants have used different primary disinfectants and residuals, while others have implemented the removal of organic matter available during water distribution using GAC and/or sand filters (USEPA, 1992). Considering that biofilms have been found in ultra-pure systems the removal of organic matter seems to only slow down biofilm formation (Meyer, 2003).

New approaches such as catalyst modified surfaces, ultrasound, and electric fields have been used to increase the efficacy of a disinfectant on biofilm removal (Blenkinsopp et al., 1992; Meyer, 2003). Suspecting that the biofilm matrix is charged, therefore bonding with the antimicrobials before they reach the biofilm bacteria, Blenkinsopp et al. (1992) conducted some experiments disrupting this charge using electric current. In these experiments it was found that the biofilm removal efficacies from stainless steel by three industrial disinfectants were enhanced when the biofilm was exposed to a low-strength electric field with a low current density. Scientific knowledge also suggests that mechanical forces applied to a surface when removing biofilms plays an important role (Meyer, 2003).

The disinfection technique most used to remove biofilm is chlorination; probably because, in addition to killing bacteria, chlorine is able to remove extracellular polymeric material (EPS) from the surface making the attachment of new bacteria more difficult (Meyer, 2003). In the drinking water industry there are two types of chlorination used in distribution systems, pipes and reservoirs; continuous and shock chlorination. Continuous chlorination is the disinfection used in most drinking water distribution systems and consists of the constant addition of a low amount of chlorine so that a residual is maintain throughout the system (0.04 to 2.0 mg/L). Shock chlorination is usually used to disinfect storage tanks and wells. Shock chlorination consists of the addition of usually 4 to 5 times the standing well volume of a solution of chlorine at 50 to 200 mg/L allowing long enough contact time to guarantee proper disinfection (Schnieders, 2003).

After comparing three different disinfectants used in the water supply industry (monochloramine, chlorine and chlorine dioxide) LeChevallier et al. (1988) found that mono-chloramine was the most effective at controlling the proliferation of biofilm bacteria. LeChevallier et al. (1988) also concluded that the potential to be transported into the biofilm plays a more important role than the oxidation capacity of the disinfectant. This corroborates the fact that biofilm shrinks when it contacts oxidants becoming denser and therefore harder to penetrate (Schnieders, 2001).

2.6 Biofilm growth in water wells

Literature suggests that water wells are inevitably subject to biofilm formation considering that biofilm inhibition due to low organic matter content in groundwater is not possible (Meyer,

2003). Scientific knowledge indicates that problems caused by biofilm growth in water distribution systems and water wells include corrosion of metals, deterioration of concrete, deterioration of water quality, and decrease of well efficiency (clogging of pipelines and filters) (Nivens et al., 1995). Even though all the problems caused by bacteria are important the biological deterioration, which is measured with biological indicators (i.e. coliforms), is the problem of most concern. This particular concern arises because of the serious health implications biological indicators imply considering that their presence suggests that pathogens with similar routes of exposure may also be present (Conboy and Goss, 1999; Momba et al., 2000). Even more, typically opportunistic pathogenic biofilm bacteria (i.e. Helicobacter, Aeromonas, Mycobacterium, Legionella, etc.) can cause diseases in persons with skin lesions, pulmonary or immune dysfunctions, and chronic diseases (Vaerewijck et al., 2005). At the time this thesis was written no studies on health concerns due to biofilm bacteria from water wells were found.

3.0 Methods and Materials

3.1 Water extraction system

The water extraction system was the same system utilized by Javor (2010) (Figure 3.1). This system consisted of a water delivery line (1.91 cm (¾”) PVC pipe) sloping away from the test wells at 0.5 % extending from the wells to a sample collection facility. Four 170 L plastic barrels with screw top lids were conditioned to work as collection facilities allowing water samples to be collected and providing a convenient location to place a cumulative flow gauge (Omega FTB-4000, turbine meter). The water extraction system also included a check valve installed on the outlet of the water line. The water was delivered to the barrel followed by gravity discharge to a drainage ditch that runs along the northern boundary of the field site (Javor, 2010).

The water extraction system had been operating since November 25, 2008 using solar powered submergible impeller pumps (24 V, 16 Amp, Rule 3700) which were capable of pumping 20 L/minute at about 4.0 m of hydraulic head and were connected to an automated control system (Javor, 2010). The pumps were powered by 2-12 V deep cycle batteries in series which were charged by two 1.22 m x 0.61 m (48” x 24”), 24 Watt solar panels in series. An Allen-Bradley Pico programmable controller (model 1760-L12DWD) operated 30 Amp relays that turn the pumps on and off one at a time to avoid overloading the system since the pumps required high amperage (Javor, 2010).

3.2 Monitoring instrumentation

To monitor the water level fluctuations in the four test wells and two monitoring wells (MW2 and MW3) pressure transducers (Solinst Levelogger Junior in MW2, MW3, CTH1, ETH1, and ETH3, and a Solinst Levelogger Gold LTC in ETH2) were submerged in each well (Javor, 2010). All the pressure transducers provided an accuracy of ± 0.5 cm and a resolution of 0.028 % of the full scale of the measurement except for the pressure transducer in ETH2 which provided an accuracy of ± 0.3 cm and a resolution of 0.001 % of the full scale of the measurement (Javor, 2010).

Javor (2010) installed a thermocouple nest between 30 cm and 135 cm below ground surface (bgs) to obtain a soil temperature profile. Eight (8) thermocouples (Onset L-TMA-M006) with a

range of -40˚C to 100˚C and an accuracy of ± 0.7˚C were vertically placed every 15 cm. Two 4-channel data loggers (HOBO U12-008) were used to log and store the data and had been operating since December 2007 (Javor, 2010).

3.3 Water quality

Monthly sampling began in May 2009. Water samples were collected using 500 mL high-density polyethylene (HDPE) bottles and analyzed at the Centre for Alternative Wastewater Treatment (CAWT) Laboratory at Fleming College. Metals (Fe, Pb, Mn and Zinc) and cations (Ca^{+2}, Mg^{+2}, K^{+}, Na^{+}) concentrations are determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). Anions (Cl^{-}, NO_2^{-}, NO_3^{-}, SO_4^{-2}) were determined using ion chromatography (IC). Total coliforms and *E. coli* were determined using the ColiPlate 400 test. During sample collection quality control blanks were brought to the wells and were opened during sampling and were sealed once sampling was concluded. The ColiPlate 400 method has an accuracy restriction and a negative result was reported as <3 CFU indicating that the actual value lies between zero and three (3) CFU. Despite this restriction, this method was utilized because of its ease and practicality. For each sample and blank, 200 µL aliquots were dispensed to all ColiPlate wells. Plates were then covered and incubated at 35°C for 24 h. The number of wells that turned blue was used to obtain TC enumerations while *E. coli* enumerations were obtained using the number of wells that turned blue and fluoresced under UV light.

In addition to the above mentioned analysis, bi-weekly samples were collected and delivered to the Victoria County Health Unit (VHU) located in Lindsay, ON for microbial analysis using a membrane filtration method since April 2010. Table 3.1 includes the most relevant parameters analysed for water quality purposes, the method used and corresponding method detection limit (MDL).

3.4 Smoke tracer tests

As part of the performance monitoring of the test wells, smoke tracer tests were performed in December 2008, May 2009, March 2010, October 2010 and February 2011. The smoke test was developed by Javor (2010) to assess potential pathways between the atmosphere and the interior of the test wells. These pathways may allow airborne contaminants or insects to contact the water stored in the wells. These tests consisted of generating smoke using a chemical smoke

generator (Superior No. 1A) placed above the static water level. Using an adaptor attached to the lid (Figure 3.2) the interior pressure of the well was increased to between 68.9 and 103.4 kPa (10 and 15 psi) with the help of a Dewalt 1.6 HP, 56.8 L air compressor. Once the tests were completed the test wells were purged of smoke by removing the smoke generators and access lids. The flow rate and flow volume coming from the visually identified smoke pathways provide qualitative information on the degree of atmospheric interaction (Javor, 2010).

3.5 Geophysical methods

Also, as part of the ongoing performance monitoring of the test wells, the integrity of the different annular sealants were evaluated using a series of non-destructive geophysical tests consisting of ground penetrating radar (GPR) surveys. These geophysical measurements were conducted using the access tubes (10.2 cm (4 inch) diameter PVC) that were installed on each side of the annular sealant at each test well. One of the access tubes was placed in a vertical hole on the geologic formation side of the annular sealant and the other access tube was fixed to the nearest location on the interior casing wall for test wells CTH1, ETH1 and ETH2. The tubes were affixed to the interior wall of the casing with metal strapping and sealed with Portland cement. The installed access tubes protruded from the cover and lid assembly where they were sealed with Portland cement. Since the performance of GPR is reduced when the signal has to pass through steel, the interior access tube at test well ETH3 was installed on the outside of the corrugated galvanized steel casing. These access tubes extended from below the annular seal to the top of the well casing.

Borehole GPR has become a useful method for measuring the distribution of subsurface physical properties between boreholes (Gilson et al., 1996; Huisman et al., 2003; Parkin et al., 2000). Electromagnetic (EM) wave propagation is controlled by the velocity and attenuation of the material. These two properties are directly determined by measuring the time and root mean square (RMS) amplitude of the radar waves travelling from one borehole to another. The wave velocity is used to estimate the water content of the medium, and the wave attenuation or RMS can be used to estimate the electrical conductivity of the medium. The electrical conductivity of the medium is controlled by porosity, clay content, water content, dissolved electrolytic concentration, temperature and phase state of the pore water. The soil type for all wells consists of sandy clay which has a porosity of ~0.4; therefore, the volumetric water content of native soil

at saturation would be approximately 40%. In normal practice, the average value of the EM wave velocity is computed by dividing the distance between the transmitter in one borehole and a receiver in another borehole by the travel time of the EM wave between the two boreholes.

The water content is obtained from the measured velocity (*V*) by first calculating the relative dielectric permittivity (also known as the dielectric constant) *K* given by:

$$K = \frac{c^2}{V^2} \tag{1}$$

where *c* is the velocity of the EM wave in free space. The volumetric water content (θ) is calculated from the relative permittivity (*K*) using the following empirical relationship (Topp et al., 1980):

$$\theta = -5.3\times10^{-2} + 2.92\times10^{-2}K - 5.5\times10^{-4}K^2 + 4.3\times10^{-6}K^3 \tag{2}$$

GPR surveys were performed in each pair of access tubes installed at each test well using the pulseEKKO Pro GPR system (Sensors & Software, Mississauga, Ontario) with 200 MHz antennas. Due to the close spacing between access tubes, data was only gathered in the zero offset profile (ZOP) (Figure 3.3) mode which is used to obtain a one-dimensional profile between corresponding points from one access tube to the other. After calibration in air, a series of radar traces was recorded while both antennas were lowered down the pair of boreholes at equal 0.05 m intervals.

3.6 Laboratory biofilm experiments

To assess several techniques used to remove biofilm from large diameter drinking water well materials, it was necessary to produce biofilm both on a given coupon and between coupons with the number of bacteria statistically similar. All the coupons were cut 7 cm by 7 cm and two 2.5 cm by 5 cm areas were carved on each coupon as shown in Figure 1.2. Concrete coupons where obtained from a concrete tile which was cut into the desired size. Galvanized steel coupons were cut out from a galvanized steel sheet from the University of Waterloo’s machine shop. Fibreglass coupons were cut out to the desired size from a piece of fibreglass casing obtained from G.P. Fiberglass Ltd., Melfort, Saskatchewan.

The coupons were washed in a dishwasher and then autoclaved for 15 min (at 121 ^oC) before the experiments were conducted. To grow the biofilm on coupons several reactor designs, nutrient alternatives and incubation periods were evaluated in preliminary tests. Initially, abundant nutrients and bacteria seed were added to a reactor but it was noticed that frequently the reactor became anaerobic, or biofilm development was not observed. To avoid anaerobic conditions (known to be absent at the field site) it was decided to maintain aerobic conditions.

To guarantee that the bacterial composition of this laboratory-grown biofilm was similar to those potentially observed in the test wells, biofilm was produced using nutrients and water from the test wells as the bacteria seed. Preliminary tests were used to determine which nutrient and incubation duration were the most suitable for biofilm growth. Once the biofilm was grown an enumeration technique using ATP analysis was then evaluated.

After having mastered biofilm growth and biofilm enumeration some disinfection methods for the removal of biofilm were evaluated.

3.6.1 Reactor design

Laboratory-grown biofilm production was very challenging since it was difficult to mimic *in situ* conditions while encouraging biofilm formation. Various reactors designs and configurations were examined until biofilm growth was successful.

The biofilm reactors utilized for the production of biofilm consisted of unmodified 20 L fish tanks (Figure 3.4). For every experiment two reactors were used and identified as Reactor 1 (R1) and Reactor 2 (R2). Since the volume of well water added to each reactor was less than 5 L, the 15 L head space kept the solution (nutrients + test well water) aerobic. No gas or smell, characteristic of anaerobic conditions, was produced during the experiments. Each reactor was covered with a HEPA filter and placed inside a cardboard enclosure to prevent light from entering and to keep it at room temperature (20 ±2 ^oC).

Literature suggested that a solution of sodium acetate (BDH, VWR, Mississauga, ON), dibasic potassium phosphate (BDH, VWR, Mississauga, ON) and sodium nitrate (EMD, VWR, Mississauga, ON) with a concentration of acetate of about 50 mg/L and with a C:N:P molar ratio of 50:20:5 was a suitable feeding solution (Gagnon and Slawson, 1999). The carbon source was

later changed from sodium acetate to sucrose (160 mg/L) (Schnieders, 2010; Slawson, 2010). Preliminary tests suggested that an incubation period of 13 days was adequate.

3.6.2 Biofilm growth

Four (4) litres of water from CTH1, and 4 mL per litre of well water of a matrix feeding solution (40 g sucrose, 4 g $NaNO_3$ and 2 g K_2HPO_4 per litre) were added to each reactor to produce a concentration of 160 mg/L of sucrose. The solution in both reactors was homogenized for at least 24 hours with the help of a sterilized stir bar and magnetic plate. After the homogenization period 10 sterilized coupons (Figure 1.2) were placed in the bottom of each reactor (Figure 3.4). The coupons were left for an incubation period of 13 days and then the biofilm was quantified using the ATP method described below. It was expected that with the right pH, nutrient concentration, temperature and DO conditions the reactor should be able to generate the same amount of biofilm bacteria within the 13 day incubation period.

3.6.3 Biofilm enumeration

In the study by Javor (2010) biofilm removal was performed using one (1) sterilized swab which was then immersed in a buffer solution to resuspend the bacteria. Once the bacteria were resuspended an ATP extracting solution was added followed by a Luciferase/Luciferin (rL/L) solution. Immediately after the rL/L was added the RLU was read.

In this research, biofilm removal was performed using three (3) sterile swabs for each coupon area (denoted as Area A and B) (Figure 1.2). The bacteria densities measured on Area A were used as initial populations and the bacteria densities measured on Area B were used to determine the amount of bacteria removed by the different biofilm removal methods.

Each area was swabbed consecutively with 1 swab at a time to maximize the amount of biofilm removed (Figure 3.5). The tips of the used swabs were immediately cut off and inserted in a 15 mL sterile disposable test tube with 3 mL of filter-sterilized water (Figure 3.5 (b)). Once each area was swabbed with the third swab, the test tube with 3 swab tips was swirled for 2 minutes in a vortex to re-suspend the bacteria. One hundred (100) µL of suspended bacteria solution was added to a 1.7 mL micro centrifuge tube and mixed with 100 µL of ATP reagent. The RLU was

read immediately after the sample and ATP reagent were mixed using a luminometer (Turner Biosystems, Modulus 9988-9203 Fluorometer with luminometer module).

In this study the extracting and rL/L solutions, employed by Javor (2010), were replaced by a more precise ATP method (able to detect at least 10 bacteria cells) (Promega, Fisher Scientific, Whitby, ON). An ATP standard was used to generate a standard curve (10^{-1} to 10^{-3} μM ATP) which made it possible to obtain the initial ATP concentration from the RLU readings. The ATP samples were analyzed in duplicate and the standards were analyzed in triplicate. All bacteria counts are reported as the base 10 logarithm of cells per square centimeter.

Preliminary biofilm growth tests (Trial 1, 2 and 3) were used to determine whether the bacteria densities in Area A and Area B were the same (difference < 1 log unit).

3.6.4 Biofilm removal

Literature suggests that biofilm removal using disinfectants is not sufficient and that it is recommended to use some mechanical removal in addition to chemical disinfection. In this research the efficiency of some chemical disinfectants and of one mechanical method were evaluated.

3.6.4.1 Chlorination

As a baseline, chlorination (disinfection method recommended by the Ministry of the Environment to disinfect wells) was used to remove biofilm (Ontario Ministry of the Environment, 1990). A free chlorine solution of 120 to 150 mg/L was prepared using water at pH 7 (DI water pH 4.0 adjusted with NaOH 0.1N) and unscented household bleach (6% ClO^-) (Clorox). The Ontario Ministry of the Environment suggests that in order to achieve a proper disinfection the concentration of free chlorine residual in the well water should be at least 50 mg/L and not more than 200 mg/L; and that a contact time of at least 12 hours and not more than 24 hours after the water is dosed should be allowed (Ontario Ministry of the Environment, 1990).

After enumeration the coupons were placed in the free chlorine solution (130 – 150 mg/L) for at least 12 hours and not more than 24 hours. After the 24 hours contact time, the coupons were carefully removed from the free chlorine solution and placed in water (adjusted pH 7) to rinse

chlorine excess. After three rinses when most of the chlorine was removed (free chlorine <0.01 mg/L) biofilm enumeration was performed on Area B.

3.6.4.2 Pressurized water

A set of experiments were conducted to determine the efficiency of a mechanical method (i.e., pressurized water) to remove biofilm (Figure 3.6). After enumeration the coupons were power washed with tap water at 8960 kPa (1300 psi) working pressure with the nozzle set to a wide fan spray (25˚). The nozzle was moved from left to right 3 times to pressure wash the coupons maintaining a distance to the coupons between 10 and 15 cm. Once the coupons were washed a post-treatment biofilm enumeration was conducted on Area B.

3.6.4.3 Chlorination/Pressured washing Combination

A set of experiments were conducted to evaluate the efficiency of a mechanical method (i.e. pressurized water) followed by chemical disinfection (i.e. chlorination) to remove biofilm. After enumeration, the coupons were washed with pressurized water and then placed in a free chlorine solution of 120 to 150 mg/L for 24 hours. After the contact period, the coupons were removed and rinsed with water three times (see Section 3.6.4.1) and then a post-treatment biofilm enumeration was performed on Area B.

3.6.4.4 Chloramination

Chloramines (mostly monochloramine) were used to remove biofilm grown on coupons. Chloramines were prepared using unscented household bleach (6% ClO^-) (Clorox) and ammonium chloride (BDH, VWR, Mississauga, ON). Water at pH 7.5 (DI water (pH 4.0) adjusted with NaOH 0.1N) was mixed with enough bleach to produce a solution of free chlorine of 60 mg/L (final pH 8.5). Once the concentration of the free chlorine solution was analysed using a Hach DR 2800 portable spectrophotometer (Method 8021; Detection range 0.02 to 2.00 mg/L), 50 mL of ammonium chloride (4.8 g/L) was added to produce chloramines (final pH 7.5). Determination of chloramines was performed by measuring total chlorine and free chlorine after ammonium chloride addition. Considering that the pH was at all times > 7 it was assumed that most of the chlorine reacted with the ammonium to form monochloramine. After pre-treatment enumeration, the coupons were placed in the chloramine solution (60 to 70 mg/L) for 24 hours. Higher concentrations of chloramines were avoided because their high stability and persistence

make them unfeasible for field applications. After the contact period, the coupons were removed and rinsed with DI water (adjusted pH 7) as described for chlorination. After three rinses when most of the chloramine was removed (total chlorine in rinsing water <0.01 mg/L) biofilm enumeration was performed on Area B.

3.6.4.5 Hydrogen Peroxide

Huwa-San Peroxide® is a disinfectant consisting of a mixture of hydrogen peroxide and silver; and is recommended by its manufacturer to remove biofilm from drinking water distribution systems (SanEcoTec Ltd., 2011). The recommended concentration of active hydrogen peroxide is about 500 mg/L and the concentration of silver is unknown. In order to explore the effectiveness of this unusual disinfection method a solution of hydrogen peroxide without silver was employed to remove biofilm grown on coupons. Hydrogen peroxide at 500 mg/L was prepared from 35% hydrogen peroxide (BDH, VWR, Mississauga, ON) stock solution. Five (5) mL of the concentrated hydrogen peroxide were added to 4 L of water at pH 7 (DI water (pH 4.0) adjusted with NaOH 0.1N) to produce a H_2O_2 solution of 500 mg/L.

To confirm the desired concentration, an aliquot of the 500 mg/L solution acidified with sulphuric acid (Fisherbrand®, Fisher Scientific, Whitby, ON) and titrated with potassium permanganate (EM, VWR, Mississauga, ON) (US Peroxide, 2011). After enumeration, the coupons were placed in the hydrogen peroxide solution for 24 hours. After the contact period, the coupons were removed and rinsed with water at pH 7 (DI water adjusted to pH 7) as described above for chlorination. After three rinses biofilm enumeration was performed on Area B.

3.7 Biofilm field investigation

The test wells under investigation have been operating for about two years. Microbiological analysis found in Appendix B show that the wells have continuously tested positive for Total Coliforms (TC) (at least 35 positive samples out of 52 sampling events per test well). The test well that has tested positive for *E. coli* more frequently is CTH1 with 20 positive samples followed by ETH1, ETH2 and ETH3 with 8, 5, 2 positive samples respectively. This data indicates that the test wells were under the influence of surface water.

The lessons learned from the laboratory studies were used to determine the most efficient disinfection alternative(s) that will be used in the disinfection of the test wells. As part of the biofilm study the test wells were disinfected using this disinfection alternative to corroborate if it is as effective in the field as in the laboratory. It was decided that this biofilm removal field effort should be conducted from a known baseline or initial condition preferably without biofilm present. Given that there is likely biofilm presently on the test well casings it provided an opportunity to determine the biofilm composition prior to establishing a baseline condition. It was assumed that the types of bacteria within the biofilms may change with the casing material and thus having this knowledge will help in the interpretation of results.

The baseline biofilm characterization was conducted in three test wells (CTH1, ETH1 and ETH3). CTH1 (concrete cased) was selected because, as data show, it is the test well that shows frequent microbial contamination (TC and *E. coli*). ETH1 (concrete cased) and ETH3 (galvanized cased) were selected because they are representative of two of the three different casing materials under investigation. Considering that an extension of this research is likely to involve an extra well with fibreglass casing, fibreglass coupons were used to emulate the absent fibreglass cased test well.

To standardize the three (3) methods employed to analyse biofilm (denaturing gradient gel electrophoresis (DGGE), heterotrophic plate count (HPC) and Biolog® Ecoplates™, two (2) sets of background biofilm characterization samples were collected (spaced 8 days apart) before chlorine disinfection of all the test wells was performed.

Biofilm characterization performed by the Wilfrid Laurier University's biology department consisted of collecting and analysing samples from both the test well water and the casing walls. Swab samples were collected and preserved following the Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998). To collect samples from the test well water, two sterilized swabs (Figure 3.5 (c)) were submerging attached to fishing line (for easy recovery) for three (3) days. After the three (3) days suspension period, each swab was removed and placed into a 500 mL wide-mouth sterile jar filled with corresponding well water.

To collect biofilm samples from the casing walls, a sterilized swab was attached to the end of a telescopic pole and was used to swab a portion (about 200 cm^2) of the well casing below the

static water level. Two casing biofilm swabs were collected per well. After swabbing the casing wall the swab was placed into a 500 mL wide-mouth sterile jar filled with corresponding well water. The lids on the sampling jars were closed tightly and the jars were put into an ice-packer cooler and shipped to WLU for analysis.

Once in the laboratory the biofilm characterization samples were processed before analysis in order to resuspend bacteria. Bacteria resuspension consisted of shaking the samples at 130 to 180 rpm at 22 °C for 24 hours. Aliquots from the samples were analysed using CLPP, DGGE and HPC methods. Previous to the CLPP analysis the suspensions were analyzed at a wavelength of 420 nm to assess background carbon levels. Dilution of the suspensions was not required since all the values were <0.2. After the background carbon was assessed Biolog® Ecoplates™ were inoculated with 150 µL of sample and incubated in the dark at 22 °C and analyzed using a SpectaMax 190 (Molecular Devices, Sunnyvale, CA) spectrophotometer and data were collected using SoftMax Pro ver. 3.1.2 (Molecular Devices) every 24 hr.

DNA extraction for the DGGE analysis was performed on the residue resulted from filtering 250 mL of swab suspension using a sterilized 47 mm, 0.22 µm polycarbonate filter (Millipore), previously soaked in un-buffered PCR-grade Milli-Q (Millipore) water. After filtration, each filter was placed into a PowerSoil (Mo Bio Laboratories Inc., Solana Beach, CA) bead tube using sterile forceps, ensuring the filtrand was facing the middle of the tube and accessible to the tube contents. With the help of a new, sterile No. 11 blade (Feather, Fischer Scientific, Whitby, ON) on a sterilize No. 3 handled scalpel the filter was cut into small pieces in the bead tube. DNA was subsequently extracted following the protocol supplied by the manufacturer. Posteriorly, PCR was performed using 5 µL of the extracted template DNA and primers obtained from Sigma-Aldrich and confirmation of the presence of only a 233 base-pair band in sample wells and absence of any bands in the blank was performed using BioRad™ GelDock™ XR (Bio-Rad Laboratories) with amber filter. DGGE conditions, ladder creation and image acquisition are described in Appendix C.

For the HPC analysis the suspensions were diluted using 10-fold series from 10^0 to 10^6 using 9 mL dilution blanks containing sodium-free dilution buffer (APHA et al., 1998). 100 µL from each dilution was spread, in duplicate, onto R2A agar (BD Difco, Fisher Scientific, Whitby, ON)

plates. HPCs were counted after 5 to 7 days of incubation at 25 °C (APHA et al., 1998). The information obtained from the different characterization methods was used to determine if there was a difference between the bacteria grown in the different cased wells.

Following initial biofilm characterization sampling of sessile and suspended bacteria, all tests wells were reset to initial conditions using chorine disinfection following the procedure outlined by Javor (2010). It was believed that this will allow us to investigate the optimal biofilm removal method(s) determined from the laboratory experiments.

It was considered that future frequent biofilm sampling events from the well walls could increase contamination and be problematic. To avoid this concern four (4) sets of clean and sterilized coupons of the different casing materials (concrete, fibreglass and galvanized steel) were placed inside ETH1 (concrete cased) and ETH3 (galvanized cased) (12 coupons in each well). Interest was concentrated on ETH1 and ETH3 because of their enhanced designs; CTH1 was excluded because it was believed that due to its poor design bacteria contamination will be always present.

The coupons were suspended within the water fluctuation zone to expose them to what is considered the most critical conditions. After an incubation period of 74 days two sets of coupons were removed and shipped to WLU for biofilm characterization; the other two sets were intended to undergo ATP analysis but due logistic problems these coupons were not analysed.

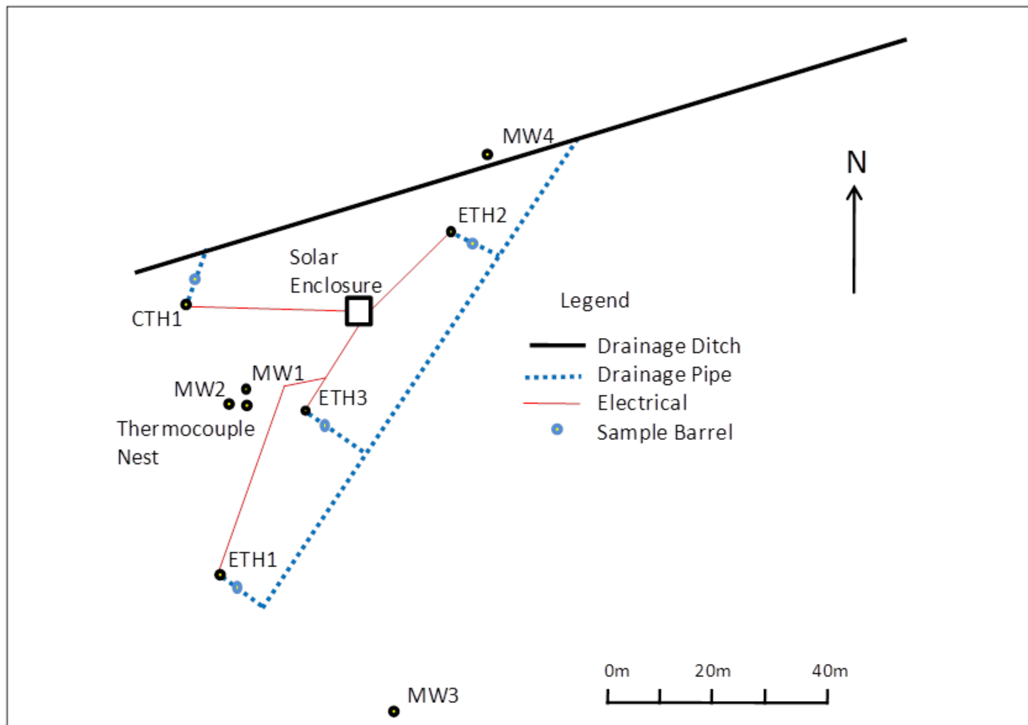

Figure 3.1. Site map (Javor, 2010).

Figure 3.2. Smoke tracer test (Javor, 2010).

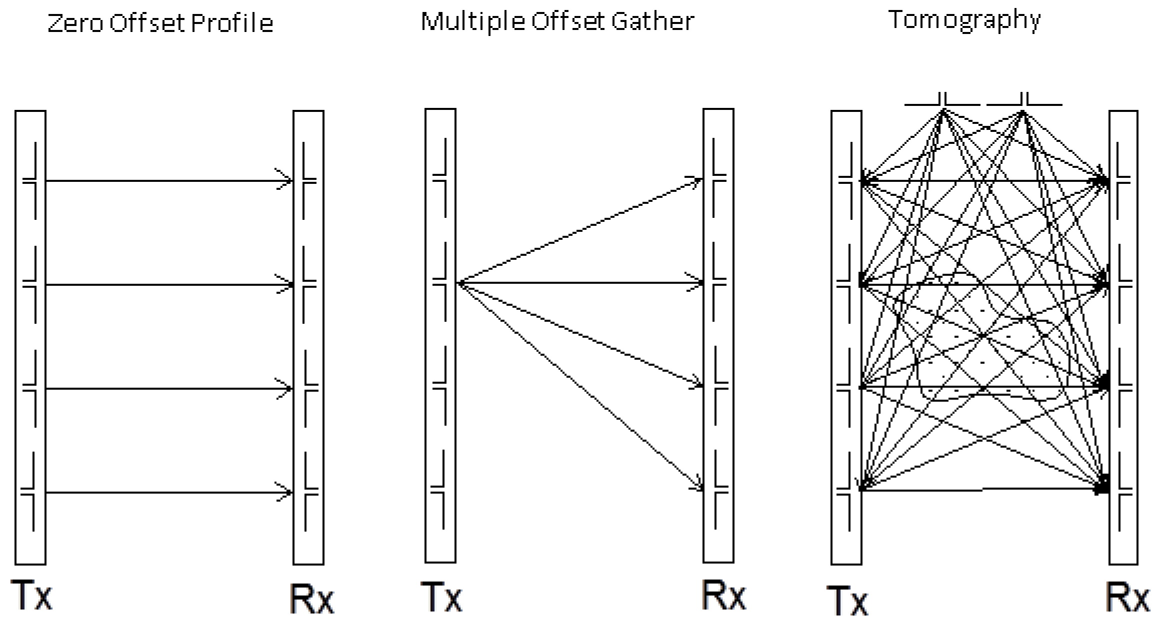

Figure 3.3. Borehole radar data acquisition modes showing typical ray path patterns between transmitter (Tx) and receiver (Rx) positions (Parkin et al., 2000).

Figure 3.4. Picture of biofilm reactor with coupons undergoing biofilm incubation.

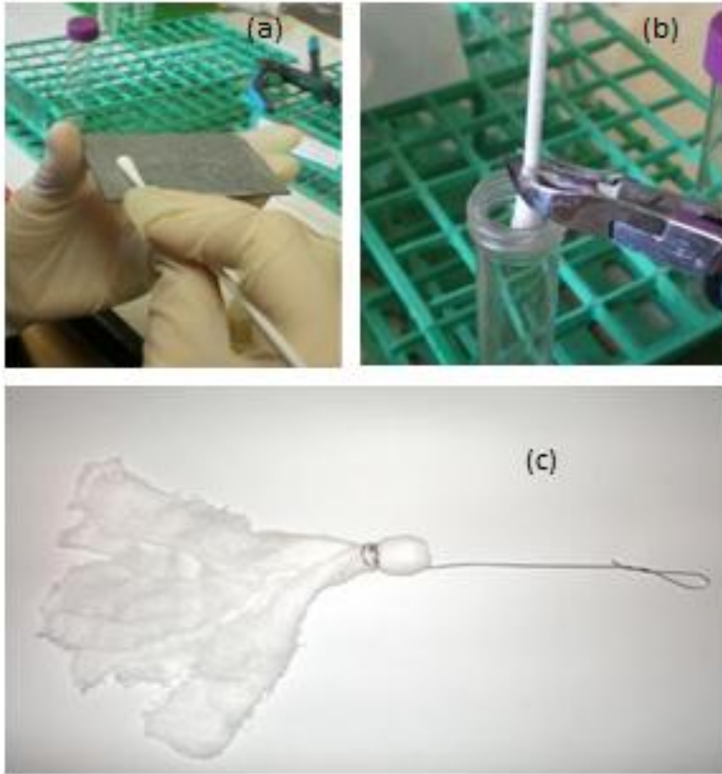

Figure 3.5. Photos of biofilm sampling showing (a) swabbing from coupon, (b) tip collection, and (c) swab used for biofilm and suspended bacteria collection from test wells.

Figure 3.6. Photo of fibre glass coupon being power washed.

Table 3.1. Methods and method detection limits (MDLs) used on water quality analysis.

Parameter	**Units**	**Method**	**MDL**
Alkalinity	mg/L $CaCO_3$	Titration	2 mg/L
Hardness	mg/L $CaCO_3$	Titration	2 mg/L
Calcium	mg/L	ICP-OES	0.03 mg/L
Magnesium	mg/L	ICP-OES	0.006 mg/L
Manganese	mg/L	ICP-OES	<0.004 mg/L
Potassium	mg/L	ICP-OES	0.01 mg/L
Sodium	mg/L	ICP-OES	0.25 mg/L
Chloride	mg/L	IC	<0.07 mg/L
Nitrite	mg/L as N	HACH method IC	<0.035 mg/L <0.03 mg/L
Nitrate	mg/L as N	HACH method IC	<0.05 mg/L <0.03 mg/L
Sulphate	mg/L as SO_4^{-2}	HACH method IC	<2 mg/L <0.28 mg/L
Iron	mg/L	ICP-OES	<0.01 mg/L
Lead	mg/L	ICP-OES	<0.05 mg/L
Zinc	mg/L	ICP-OES	<0.01 mg/L
Total coliforms	CFU/100mL	Coliplate -400 test kit	<3 CFU/100mL
E. coli	CFU/100mL	Coliplate -400 test kit	<3 CFU/100mL
Total coliforms (Health unit)	CFU/100mL	Membrane Filtration	<0 CFU/100mL
E. coli (Health unit)	CFU/100mL	Membrane Filtration	<0 CFU/100mL

4.0 Results and Discussion

4.1 Water extraction

The pumping schedule developed by Javor (2010) is still in place and no changes were made. This schedule was developed to remove about 1 m^3 every day (the average daily household water demand) from each well.

The pumps had been operational for 874 days at the time of the last reading (April 18, 2011) and the target amount of water removed from each test well was 874 m^3. Approximately 444.6 m^3 has been removed from CTH1, 671.8 m^3 from ETH1, 587.8 m^3 from ETH2 and 608.2 m^3 from ETH3. These water extractions indicate that the daily average pumping rates of CTH1, ETH1, ETH2 and ETH3 are 0.509 m^3/day, 0.769 m^3/day, 0.673 m^3/day and 0.696 m^3/day respectively. These pumping rates are less than the desired pumping rate (1 m^3/day). The cumulative volume pumped in all the test wells is mostly linear, however two plateaus (Figure 4.1) are observed during Winter 2010 (December 2009 to March 2010) and Winter 2011 (January 2011 to February 2011). The first plateau is believed to be a result of insufficient power/sunlight supply. This plateau is more prominent for CTH1 due to the freezing problems previously observed with the drainage system for this test well. A second plateau is observed during Winter 2011 but considerably less prominent than the one observed during the previous year. This time the plateau observed in CTH1 is not as different as the other wells suggesting that the freezing problem with the drainage system has been overcome.

4.2 Water level measurements and precipitation

Water levels have been monitored since May 2008 and fluctuations of as much as 2 meters have been observed (Figure 4.2 and Figure 4.3). While the prominent fluctuations can be attributed to seasonal changes as precipitation data suggests, the daily fluctuations are due to the pumping which causes drawdown and recharge. Water table elevation fluctuations and local precipitation seem to follow a similar pattern suggesting that the water in the test wells is groundwater under direct influence of surface water (GUDI) (Figure 4.2). However, a correlation analysis suggests that there is no direct relationship between a local precipitation event and the well water level measured the same day. Additionally, a similar correlation analysis between a given precipitation event and the corresponding following days' water level (considering infiltration

rate) suggests that a local precipitation event does not affect the water level measured the following four days (correlation 0.08 for all test wells). It is observed that the lowest water levels have occurred in September 2009 and September 2010. This could be attributed to the fact that the precipitation from July to September in 2009 and 2010 was about half the precipitation of 2008.

4.3 Soil temperature profile

The thermocouple nest was used to obtain the soil temperature profile which helps determine freeze – thaw cycles occurrences and the depth to which frost extends below the ground surface. Figure 4.4 shows the soil temperature profile from December 2007 to March 2011 and the average daily temperature recorded by Environment Canada at Trent University in Peterborough, ON. Over this period three complete freeze-thaw cycles have occurred. The soil temperature profile and the air temperature display a normal trend (temperature increasing with depth during winter and temperature decreasing with depth during summer). Soil temperatures below freezing were observed during the winters of 2009, 2010 and 2011 to the 30 cm depth. The temperature was >0 ˚C at the other depths. The information obtained from the soil temperature profile could support the theory on the damage suspected in some of the annular sealants from frost heave (see section 4.6). This alteration to the annular sealants is important because in the future they could become possible preferential contaminant pathways. As for the last GPR test there is no solid evidence that this damage due to frost have become contaminant pathways.

4.4 Water quality

Routine monitoring water samples have been collected every month (beginning in May 2009) from the test wells and an upgradient monitoring well thought to be representative of background water quality (MW2). Since May 2010 and due to microbial contamination, collection of water samples from MW2 was terminated and sample collection from MW1 and MW3 was initiated expecting that these monitoring wells were free of microbial contamination (Appendix B).

The water quality data obtained from the collected samples show that four parameters exceeded the Ontario Drinking Water Standards, Objectives and Guidelines' (2006) criteria in most of the test wells. Two of the parameters (TC, and *E. coli*) are bacteriological parameters and have non-detectable maximum acceptable concentrations (MAC). TC was used as an indicator of the

overall quality of water while *E. coli* was used as an indicator of fecal contamination (Health Canada, 2006). TC and *E. coli* data from well water samples analysed by the VHU and CAWT laboratory are shown in Figure 4.5 to Figure 4.6 (only integer values were plotted). A complete set of microbial data can be found in Appendix B. Being conservative, values reported as <3 CFU were highlighted as exceeding the Ontario Drinking Water Standards even though it was known the value lies between zero and three (3) CFU.

One parameter (chloride) has aesthetic objectives (AO) (Figure 4.7 to Figure 4.8) and one more (hardness) has operational guidelines (OG) (Figure 4.9 to Figure 4.10). Zinc (Figure 4.11) was found above the AO criteria in ETH3 (galvanized steel casing) during 2008 but in 2009 the concentration dropped to an acceptable level. Zinc concentration was determined again in November 2010, an even when the concentration on ETH3 was much higher (2 mg/L) than the other wells (0.1 mg/L) it did not exceed the 5 mg/L acceptable limit (Ontario Drinking Water Standards, Objectives and Guidelines, 2006). During the last sampling event (April 2011) zinc concentration dropped to 0.7 mg/L.

Javor (2010) found that TC counts were detected in all test wells and MW2 since sample collection started in March 2008 and February 2009, respectively. Considerable high counts of TC with values of 938, 137 and 219 CFU were found in CTH1, ETH1 and MW2 respectively. This trend did not change during the current research and counts as high as 1696 CFU using the ColiPlate 400 method and >80 CFU using the membrane filtration have been found in CTH1. In ETH1 the TC counts found are as high as 403 and >80 using the ColiPlate 400 and the membrane filtration methods respectively. In ETH2 the TC counts were observed to be as high as 11 and 41 using the ColiPlate 400 and the membrane filtration methods respectively. In ETH3 the TC counts observed were as high as 33 and 53 using the ColiPlate 400 and the membrane filtration methods respectively. The monitoring wells (MW1 and MW3) which have been sampled since May 2010 have constantly shown TC presence with counts as high as >80 CFU. The higher and frequent TC counts observed in CTH1 (Figure 4.5 (a)) since May 2010 suggests that this test well, as expected, is more vulnerable to airborne and surface contaminants than the enhanced test wells. The fact that all four (4) test wells present TC peaks in the same period of time suggests that the source of contamination could be infiltrating from the surface

directly into the groundwater, therefore the observed microbial contamination could be independent of the annular sealants.

E. coli counts have exceeded 20, 8, 5 and 2 times the criteria in CTH1, ETH1, ETH2 and ETH3 respectively since May 2009 (Table B.12 and Table B.13). The highest *E. coli* count was found in CTH1 with a value of 17 CFU determined using the membrane filtration method. Since April 2010 samples from MW1 have not shown *E. coli*, and samples from MW3 have exceeded the criteria five (5) times with one (1) CFU as the highest count. TC were found in the three (3) monitoring wells and *E. coli* has been found only in MW3. MW1 and MW2 are PVC cased wells located upgradient of the test wells, and MW3 is also a PVC cased well located downgradient of the test wells. TC and *E. Coli* in the monitoring wells is surprising considering that these wells are supposed to be PVC cased with no joints in the casing therefore their performance with regards to microbial contamination is expected to be better than that of large diameter wells. These observations also suggest that the source of microbial contamination is independent of the casing material and therefore contamination must be infiltrating directly into the groundwater. Furthermore, a detailed correlation analyses between available precipitation data and microbial contamination showed that there is no relationship between precipitation events and microbial contamination (Appendix D).

In all test wells and the monitoring wells the values for chloride and hardness are consistently above the AO of 250 mg/L and OG of 80 to 100 mg/L, respectively. Chloride and hardness concentrations showed two peaks in every well between August and September of 2009 and 2010 suggesting that the chloride and hardness impacts are independent of the annular sealant. These results indicate that there could be a source of chloride upgradient of the test wells. Also these peaks seem to be preceded by thaw periods (peaks start in April) suggesting that the chloride is being infiltrated into the groundwater after spring thaw. Hardness (Ca^{2+}) also presents two peaks in the same period of time as chloride. The peaks of hardness (~650 mg/L) and chloride (~500 mg/L) in ETH1 and ETH3 are the most prominent suggesting that if there is groundwater contamination, the center of this plume is closer to these test wells (south west of the study site). On the other hand ETH2 presents the least prominent peaks for both parameters suggesting that this test well is located farther from the center of the plume.

The concentrations of chloride and hardness found in MW1 and MW3 show behaviours similar to those found in the test wells. MW3 present concentration peaks for both chloride and hardness in September 2010. On the other hand, chloride concentration on MW1 increased (~300 mg/L) in September 2010 and stayed high for the following eight (8) months; while hardness seems to stay at a steady concentration since sampling started (~350 mg/L). Considering that groundwater flows south east (Javor, 2010) it is suspected that calcium chloride is being used for de-icing northwest of the site (on Fleming Campus) (Figure 1.1) and once the temperature raises the remains of calcium chloride infiltrates into the groundwater.

4.5 Smoke tracer test

A fourth smoke tracer test was conducted on the enhanced test wells in October 2010. The average temperature was 12 ^{o}C which is similar to the temperature conditions during smoke tracer Test #2 and #3. Hence the results were almost the same as smoke tracer Test #2 and #3. It was noticed though that new atmospheric pathways have appeared. The Poly-Lok lid and concrete cover joint, and the annular sealant were leaking on ETH3. The leaks noticed between the annular sealant and the casing material, and the exterior geophysical access tube at ETH3 could be generated by the already known casing joints leaks. On the other hand the leaking joint could be due to expansion/compression of the materials due to temperature changes.

A fifth smoke test was conducted in February 2011 to determine if there was a different behaviour under freezing conditions. During this fifth smoke test it was planned to remove some annular sealant material to confirm or disprove the possible explanation for the fourth smoke test findings on ETH3 but unfortunately the ground was frozen and the previously observed leaks were not observed. ETH2 has constantly shown throughout the different smoke tests leaks in the Poly-Lok lid seam and the interior geophysical access tube; however, the leak through the electrical conduit was not observed during this test (Table 4.2).

4.6 Geophysical measurements

Geophysical measurements were completed to assess non-destructively the integrity of the *in situ* annular sealant materials of the test wells. The baseline ground penetrating radar (GPR) survey was completed in March 2008 (M-08), a secondary GPR survey was completed in February 2010 (F-10) and a final GPR survey was completed in October 2010 (O-10). Each ZOP survey took

approximately 5 minutes to complete and was performed from two to four times to evaluate the repeatability of the results.

Processing of all the borehole GPR data was performed with data picking software that allows the selection of the wave arrival time and the duration of the first pulse on each GPR trace (Sensors and Software, Mississauga, Ontario). The first pick is used to compute the travel time of the EM wave between the two boreholes. The second pick is used to specify the end of the first pulse for the calculation of the RMS amplitude. The travel time is used to calculate the wave velocity; whereas, the RMS amplitude is proportional to the wave attenuation.

ETH1: The computed variation of water content and RMS amplitude with depth for this well is shown in Figure 4.12. For the measurements of M-08 and F-10, the water content increases with depth up to 0.9 m consistent with the location of the water table and then remains constant (water tables at 0.85 m and 1.36 m respectively). The measurements of O-10 show a deeper location of the water table (below 1.5m); which is consistent with a measured water table at 2.14m. The estimated water content at the surface (< 0.25 m) was higher in O-10 than in M-08 and F-10; likely because of infiltration events. The relatively high RMS values and low water content values measured in O-10 suggest that during the other two measurements (M-08 and F10) the medium conductivity was not controlled by the water content but by other variables such as an increase in dissolved ions and bentonite content. In general, the RMS amplitudes (M-08 and F-10) show a decreasing trend with depth consistent with the increase in water content (higher conductivity). However, the O-10 RMS values are similar to the previous measurements when the water table was higher; therefore, the RMS readings were governed by the electrical conductivity of the bentonite content and not by the water content. The uniform decay of the RMS data from M-08 and F-10 suggests that the annular sealant produced by the bentonite slurry with sand was homogeneous with depth. The RMS data from O-10 shows a peak at 1.25 m; which could represent a gap or a crack in the bentonite sealant likely induced by the seasonal change in water content. The linear increase in the RMS data at 1.5m could be related to the increase in water hardness detected in September, 2010 (Section 4.4).

ETH2: Figure 4.13 shows the variation of water content and RMS amplitude with depth for this well. Water content increases with depth until there is a sharp change at a depth of 0.75 m for

the data collected in 2008 suggestive of the location of the water table (measured water table at 0.35m). The calculated water contents of this well in M-08 are higher than the corresponding values for ETH1; this result could indicate the presence of water pockets in ETH2. The relative flat trend of the computed water content for F-10 and O-10 could suggest that the bentonite pellets underwent consolidation which generated a more uniform material than in M-08 (measured water tables at 0.6m for F-10 and 1.14m for O-10).

The RMS amplitude profile of M-08 clearly shows a valley starting at a depth of 1.3 m and extending over a length of approximately 1.0 m. This valley may have been generated by expansion of the clay after hydration; which caused a deformation of the external borehole and prevented the antenna to be advanced below the 1.5 m depth in F-10. The reduction in the RMS amplitudes from M-08 to F-10 and O-10 may also indicate that the bentonite chips after hydration and consolidation generated a more homogeneous material. The peak in the RMS amplitudes observed in O-10 at 0.5 m could also suggest a crack in the sealant. The linear increase after 1.4 m is consistent with the possible effect of harder water observed in ETH1.

ETH3: Figure 4.14 illustrates the variation of water content and RMS amplitude with depth for this well. Water content increases with depth (as in ETH2) until a sharp change at a depth of 1.0 m (M-08) suggestive of the location of the water table (measured water table at 0.6m). The calculated water contents of this well in M-08 are higher than the corresponding values for ETH1. As was the case for ETH2, indicating the possible presence of water pockets in ETH3. The relative flat trend of the computed water content for F-10 and O-10 could again suggest that the bentonite granules after hydration and consolidation become more uniform material. The measured water table of 0.9 m, in F-10 and 1.48 m, in O-10 are deeper than predicted from the water content evaluations; which could be caused by capillary rise in the bentonite. The variation in the RMS amplitude could be caused by the effect of pea-stones after the consolidation of the material; which is suggested by the increase in RMS with time because of a reduction in material conductivity (random distribution of pea stones in the sealant). The RMS variation in O-10 indicates a linear increase below approximately 1.0 m depth; likely generated by the increase in water hardness as mentioned above. On the other hand, the valley between 0.5 and 1.0 m could be generated by a deformation of the outer borehole; which prevented the penetration of the antenna below 1.4 m.

CTH1: The water content and RMS amplitude variation with depth for this well are shown in Figure 4.15. The measurements in F10 were not possible because a significant section of the borehole were frozen. Water content in M-08 increases more rapidly at 0.6 m depth; which is consistent with the measured water table (0.64 m). The volumetric water content in O-10 (between 50% and 60 %) suggests saturation of the sandy-clay with an assumed typical porosity of 0.4. The estimated location of the water table in O-10 was 1.5m; which confirms that capillary rise likely governed the water content computed from the GPR measurements. The linear increase of the calculated water content with depth in O-10 suggests that the drill cutting used as sealant produced a uniform sealant. The RMS amplitudes in M08 are almost constant (saturated instrument response) because of a low conductivity (e.g., low clay content). In O-10, the initial decrease in the RMS values is consistent with the increase in water content; however, the increase in RMS values after 1.0 m could suggest the effect of hard water as mentioned above.

4.7 Preliminary biofilm growth and enumeration

4.7.1 Trial 1

In this first trial 2 L of well water was added to each reactor with 4 mL of matrix nutrient solution producing a growing media of about 80 mg/L of sucrose. In each reactor 8 instead of 10 sterilized galvanized steel coupons were placed facing up in the bottom of each reactor. A stir bar sitting in the center of the reactor mixed the media for the entire period of incubation (7 days). Due to time constraints a short period of incubation was used even though it is known that these biofilms could have been different to those developed over a much longer time frame (months or years). After 7 days of incubation, the coupons were removed and the biofilm was measured in the two marked areas of each coupon (Figure 1.2). Figure 4.16(a) shows the bacteria enumeration of Area A and B for each coupon from both reactors as logs of cells per square centimeter; coupons R1-1 to R1-8 were taken from Reactor 1 and coupons R2-1 to R2-8 were taken from Reactor 2. The number of logs from this trial varies up to 2.9 units between areas on each coupon and each reactor.

4.7.2 Trial 2 and Trial 3

These trials followed the biofilm growth, enumeration and removal procedures described in Section 3. Figure 4.16(b) shows the bacterial enumeration of Area A and B for each coupon from Trail 2. The growth of biofilm in this experiment varied less than half a log between areas on each coupon which was the targeted value. In this trial increasing the amount of nutrients available and increasing the period of incubation seemed to have reduced the variation of the bacterial counts. These bacterial counts are higher than the ones obtained using the most effective resuspension method found by Gagnon and Slawson (1999) likely because this water was spiked with higher levels of nutrients. In Trial 3 (Figure 4.16 (c)) the number of logs varied less than half a unit between areas on each coupon and each reactor. This last trial confirmed reproducibility meaning that three out of the four goals of this laboratory effort had been achieved and different cleansing methods, the fourth goal, could be evaluated.

4.8 Biofilm removal

Unfortunately, at the time of these experiments concrete coupons were not available and fibreglass became available just after the fifth trial. The difference in the average bacterial enumerations determined on the coupons of both materials under the same conditions (same trial) before treatment were < 1 log unit in trial 5 to trial 10. These results suggest that bacteria do not have preference between these two casing materials and will attach indiscriminately to any of them (Table 4.3).

Even though the tests were conducted one at a time and using two (2) reactors, the tests and their corresponding reactors were enumerated to avoid confusion. The first test (chlorination) was labelled T1 and its duplicate T2; and the reactors for this test were labelled with the corresponding test number (i.e. R1 and R2). The second test (pressurized water) was labelled T3 and its duplicate T4; and the reactors used in this test were labelled R3 and R4; and so on. The following are the results of the different disinfection techniques:

4.8.1 Chlorination

In order to test the effectiveness of this disinfection method to remove biofilm, two reactors (R1 and R2) with 10 galvanized steel coupons each were used to produce lab-grown biofilm. Unfortunately fibreglass coupons were not available at the time of this test. Figure 4.17 shows

the average bacteria density obtained for the test (T1) and a duplicate (T2) before and after treatment. In T1 the average bacteria density measured on the coupons (from R1) was 8.06 ± 0.11 log units. After treating these coupons with 200 mg/L of chlorine for 24 hours the log reduction (LR) was determined to be 2.91 ± 0.09. In T2 the average bacteria density measured on the coupons (from R2) was determined to be 7.46 ± 0.28 log units. After treating these coupons with 200 ppm of chlorine for 24 hours the log reduction (LR) was determined to be 1.63 ± 0.14. Even though the difference in bacterial density between T1 and T2 was only half a log, the difference in log inactivation was over one (1) log. This difference in log inactivation was attributed to human error. Time constraints made it impossible to repeat these tests.

4.8.2 Pressurized water

To test the effectiveness of this mechanical biofilm removal recommended by Javor (2010), two reactors (R3 and R4) with 10 galvanized steel coupons each were used to produce lab-grown biofilm. Figure 4.18 shows the average bacteria density obtained for the test (T3) and a duplicate (T4) before and after treatment. For T3 the average bacteria density measured on the coupons (from R3) the average bacteria density was 8.03 ± 0.10 log units. After treating these coupons with pressurized water the LR was determined to be 2.60 ± 0.12. In T4 the average bacteria density measured on the coupons (from R4) was 7.26 ± 0.20 log units. After treating these coupons with pressurized water the LR was determined to be 2.22 ± 0.13.

4.8.3 Chlorination/Pressurized water combination

The effectiveness of a mechanical/chemical biofilm removal combination was tested. Two reactors (R5 and R6) were used to produce lab-grown biofilm on 10 galvanized steel coupons and 4 fibre glass. Average bacteria densities for the test (T5) and a duplicate (T6) can be found in Figure 4.19 and Figure 4.20. In T5 the average bacteria densities measured on coupons incubated in R5 for galvanized steel and fibre glass coupons were 8.04 ± 0.28 and 8.51 ± 0.08 log units respectively while in T6 the average bacteria densities from R6 were 7.90 ± 0.43 and 8.44 ± 0.11 log units. As stated in Section 3, after enumeration the coupons were pressure washed and then treated with chlorine for 24 hours. After chlorination the LRs in T5 were determined to be 4.25 ± 0.14 and 4.54 ± 0.12 for galvanized steel and fibre glass respectively. In T6 the LRs were determined to be 3.80 ± 0.22 and 4.35 ± 0.06 for galvanized steel and fibre glass respectively which are very close to the initial test. It was believed that pressurized water

followed by chlorination was more effective than in the reverse order since the effectiveness of a disinfectant is proportional to its penetration capacity. This option physically removed portions of biofilm allowing the chlorine to penetrate better the remaining biofilm. It was believed that if chlorination were used first, the biofilm would have hardened thus reducing the removal capacity of pressure washing.

4.8.4 Chloramination

The test of chloramines effectiveness to remove biofilm was conducted following a similar procedure as in chlorination. Two reactors (R7 and R8) with 6 galvanized steel coupons and 4 fibre glass each were used to produce lab-grown biofilm. Figure 4.21 and Figure 4.22 show the average bacteria density obtained for the test (T7) and a duplicate (T8) before and after treatment.

In T7 the average bacteria densities obtained from R7 were 7.87 ± 0.26 and 7.94 ± 0.31 log units for galvanized steel and fibre glass coupons respectively. In T8 the obtained average bacteria densities were 7.19 ± 0.69 and 7.24 ± 0.29 log units for galvanized steel and fibre glass coupons respectively. After treating the coupons with 70 ppm of chloramine for 24 hours the LR in T7 were determined to be 2.49 ± 0.15 and 2.66 ± 0.23 for galvanized steel and fibre glass respectively. In T8 the LR were determined to be 2.12 ± 0.35 and 2.49 ± 0.19 for galvanized steel and fibre glass respectively. These removal capacities showed that chloramine is as effective as chlorine removing biofilm.

4.8.5 Hydrogen peroxide

Effectiveness of hydrogen peroxide was tested as with the other chemicals. Two reactors (R9 and R10) with 6 galvanized steel coupons and 4 fibre glass each were used to produce lab-grown biofilm. Figure 4.23 and Figure 4.24 show the average bacteria densities obtained for the test (T9) and a duplicate (T10) before and after treatment.

In T9 the average bacteria densities were 8.11 ± 0.05 and 8.49 ± 0.07 log units for galvanized steel and fibre glass coupons respectively while in the duplicate the densities were 8.18 ± 0.11 and 8.68 ± 0.01 logs. After treating the coupons with 500 ppm of hydrogen peroxide for 24 hours in T9 the LR were determined to be 0.87 ± 0.15 and 3.35 ± 0.05 for galvanized steel and

fibre glass respectively; and in T10 0.95 ± 0.12 and 3.55 ± 0.05 for galvanized steel and fibre glass respectively.

4.8.6 Summary

Results showed that the oxidizing capacity of chlorine or chloramine with a contact period of 24 hours has a similar biofilm removal capacity (~2 Logs) compared to the physical removal exerted by pressure washing (Table 4.3). This observation is independent of the casing material. Oxidizing agents, chlorine and chloramine, appear to destroy the biofilm within the recommended period of contact. In contrast, hydrogen peroxide yielded better biofilm removal performance on fibreglass than galvanized steel casing material (> 3 LR and < 1 LR respectively). This observation suggests that bacteria on the galvanized steel casing material are able to protect themselves better compared to bacteria grown on fibreglass, or that hydrogen peroxide reacted with the galvanized steel (zinc and iron surface reactions) reducing the overall oxidation capacity in the system. The results from the biofilm removal experiments indicate, as literature suggests, that the use of a physical method for biofilm removal combined with chemical disinfection appears to be the best approach.

4.9 Biofilm field assessment

The results of the standardization (samples taken from well water and casing walls from CTH1, ETH1 and ETH3) of the Biolog® Ecoplates™ method employed to analyse biofilm assessment showed that the average well colour development (AWCD) values for suspended and sessile bacteria followed the same trend. AWCD values for suspended and sessile bacteria from the concrete cased wells (~0.25) (CTH1 and ETH1) were twice as high as the values for suspended and sessile bacteria (~0.1) from the galvanized steel cased well (ETH3). In the duplicate samples the AWCD values did not follow the same trend. The AWCD value from CTH1's suspended bacteria was high (~0.25); values from suspended bacteria from ETH1 and ETH3 were lower (~0.15). On the other hand, AWCD values for sessile bacteria from ETH1 and ETH3 (~0.32) were higher than the value obtained from CTH1 (~0.15) (Figure 4.25 (a)).

The results of metabolic richness from the standardization sampling events showed exactly the same trends as the ones observed for AWCD values (Figure 4.25 (b)). The Biolog® Ecoplates™ results show that the metabolic diversities obtained during both sampling events are usually

similar (first sample- duplicate <0.16); indicating that the results were replicated (Figure 4.25 (c)). Metabolic diversities of bacteria from ETH3 were the only values showing a considerable difference between sampling events (first sample – duplicate = 0.49); this difference could be attributed to the sensitivity to environmental changes of these bacteria. Overall, the standardization sampling results suggest that sessile bacteria from the three (3) wells, as literature suggests, are slightly more diverse than suspended bacteria; and that the bacteria from concrete casing wells have a higher metabolic diversity than bacteria from galvanized steel (Figure 4.25 (c)).

The results from the biofilm assessment performed on the coupons placed in ETH1 and ETH3 after disinfecting the wells indicate that there is a difference between AWCD values from the different coupons materials. AWCD values for bacteria from concrete are the highest, followed by fibreglass, and the bacteria with the lowest AWCD value is from galvanized steel (Figure 4.26). It was noticed that the AWCD value for bacteria from concrete incubated in ETH1 (concrete) is higher than the AWCD value for bacteria from concrete incubated in ETH3 (galvanized steel). Similarly, the AWCD value for bacteria from galvanized steel incubated in ETH3 are higher than the AWCD value for bacteria from galvanized steel incubated in ETH1. On the other hand, the AWCD values for bacteria from fiberglass seem to be independent of the incubating well. These data suggest that, as expected, native bacteria from a test well have a preference for the casing material.

It was noticed that bacteria grown on concrete utilized the most number of carbon substrates (average 19 carbon sources) followed by bacteria developed on fibreglass which utilized 14 carbon sources; on the other hand bacteria grown on galvanized steel utilized only an average of 2 carbon sources (Figure 4.27). The difference in metabolic diversity (Figure 4.28) on coupons incubated in ETH1 (concrete casing) is significant compared to the metabolic diversity difference observed on coupons incubated in ETH3 (galvanized steel casing). Bacteria from concrete and fiberglass incubated in ETH1 show a metabolic diversity of 3.10 and 2.84 respectively while bacteria grown on galvanized steel from the same well have a metabolic diversity of only 1.85. The metabolic diversity of bacteria from concrete and fiberglass incubated in ETH3 is very similar (2.94 and 2.89 respectively) while the metabolic diversity of bacteria grown on galvanized steel is 2.39.

Microbiological characterization suggests that the bacteria initially present in the wells did not change their metabolic diversity to a great extent with casing material. However, the microbial characterization performed on the coupons suggests that concrete, as the standardization results suggest, is the material of predilection for bacteria to establish biofilm indicating that this material lodges the most diverse microorganisms. These results also show that bacteria from ETH3 (galvanized steel) colonized more easily on the three casing materials while bacteria from ETH1 (concrete) seem to be more selective and appear to dislike galvanized steel. These data also suggest that bacteria from both test wells seem to have a similar preference for fibreglass as for concrete. More detail information about the biofilm field characterization can be found in Appendix C.

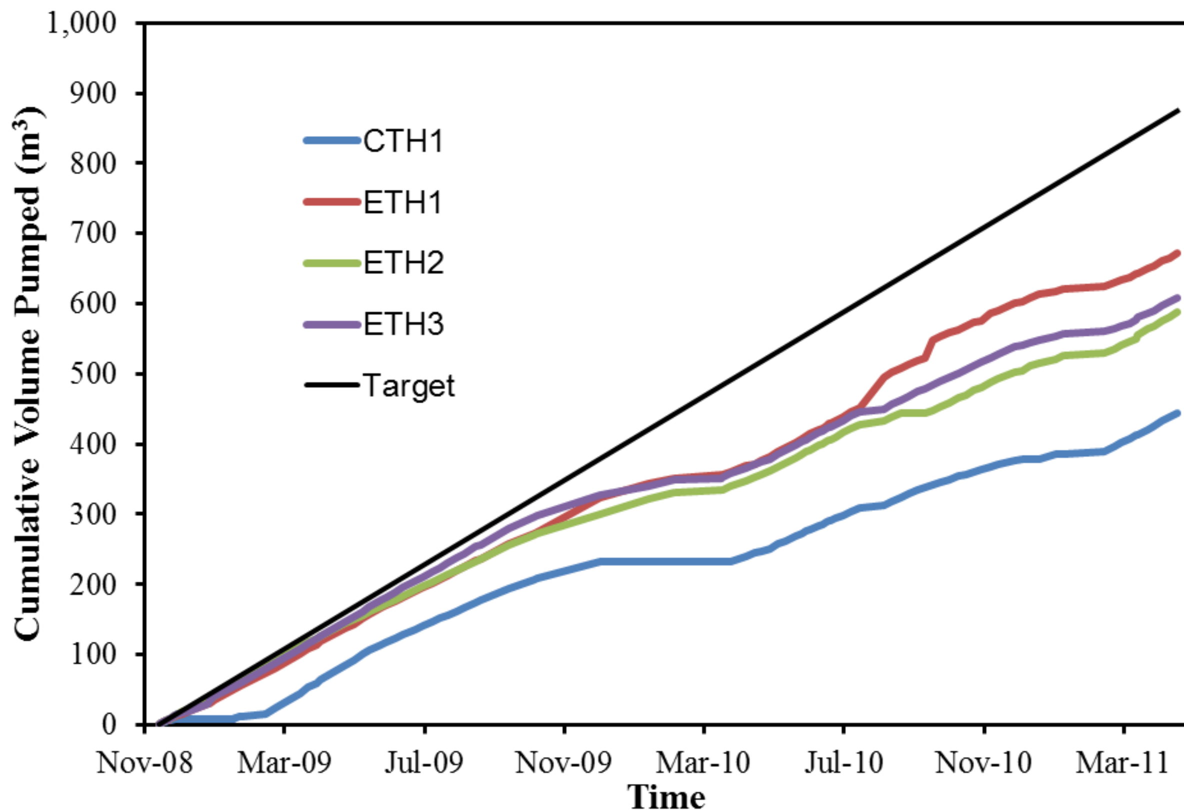

Figure 4.1. Cumulative volume of water pumped from test wells.

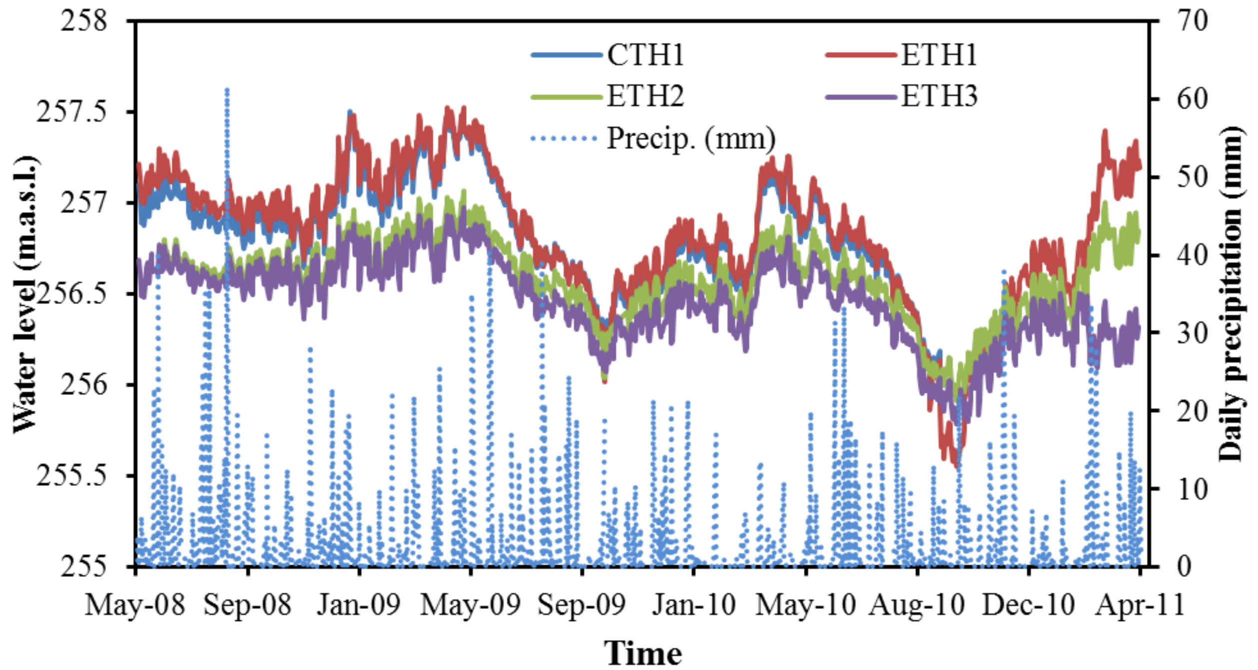

Figure 4.2. Test well water level measurements from pressure transducers.

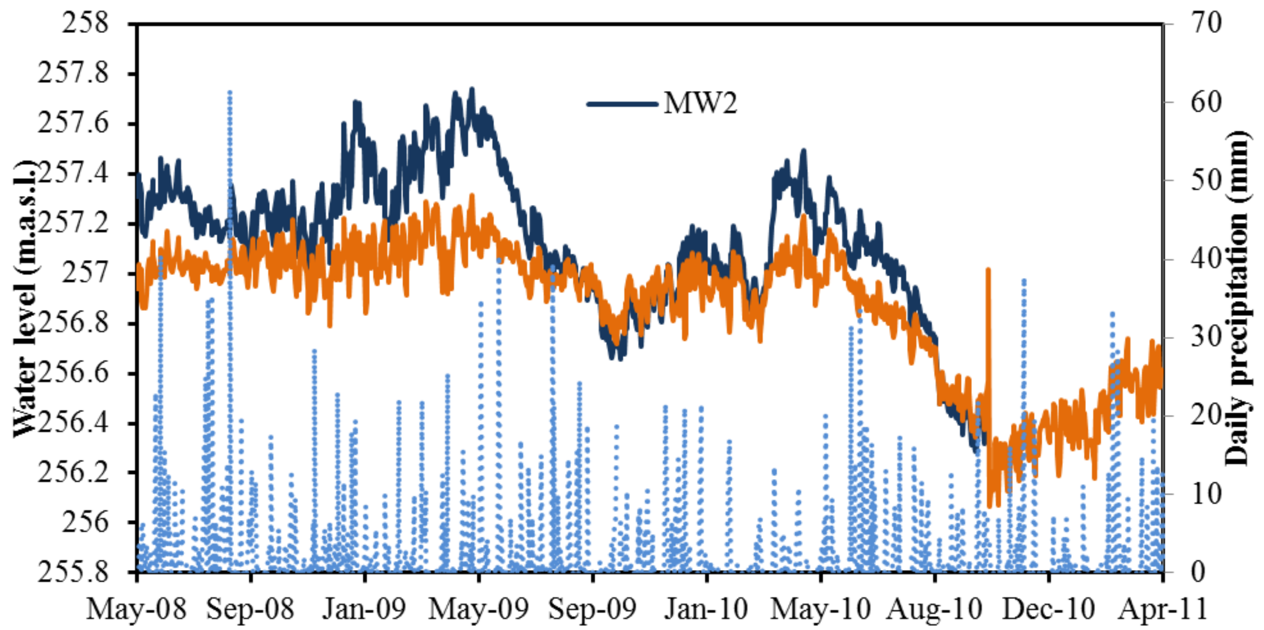

Figure 4.3. Monitoring well water level measurements from pressure transducers.

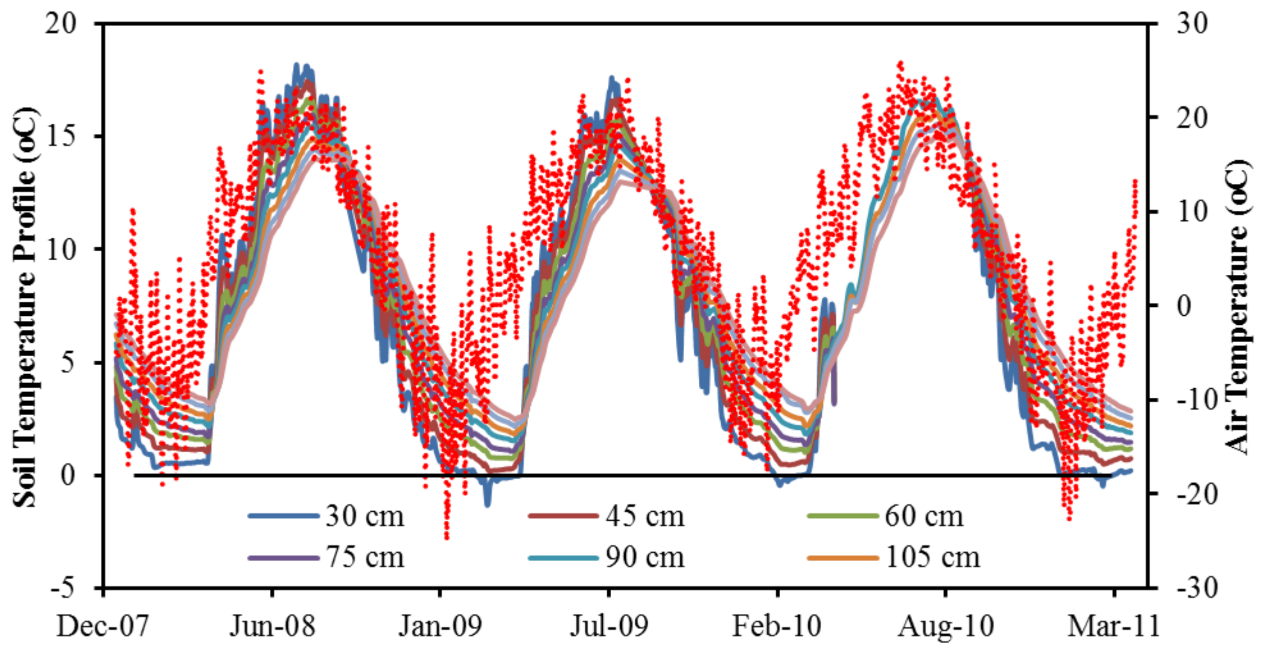

Figure 4.4. Soil temperature profile.

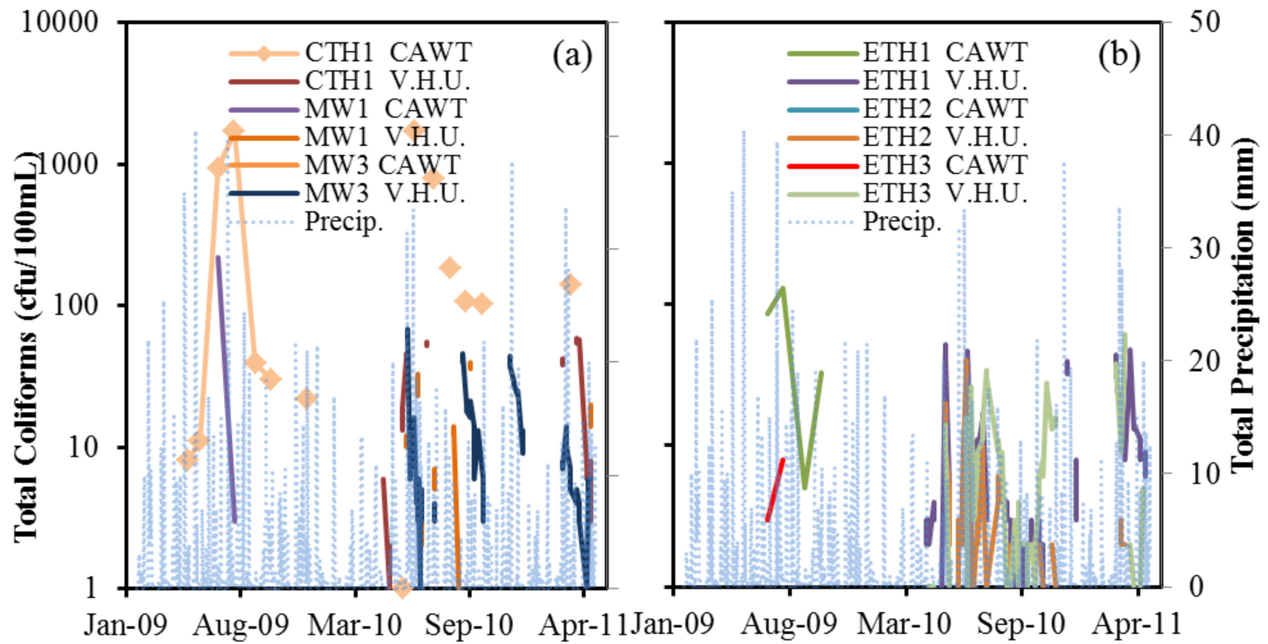

Figure 4.5. TC in (a) CTH1, MW1 and MW3; and in (b) ETH1, ETH2 and ETH3.

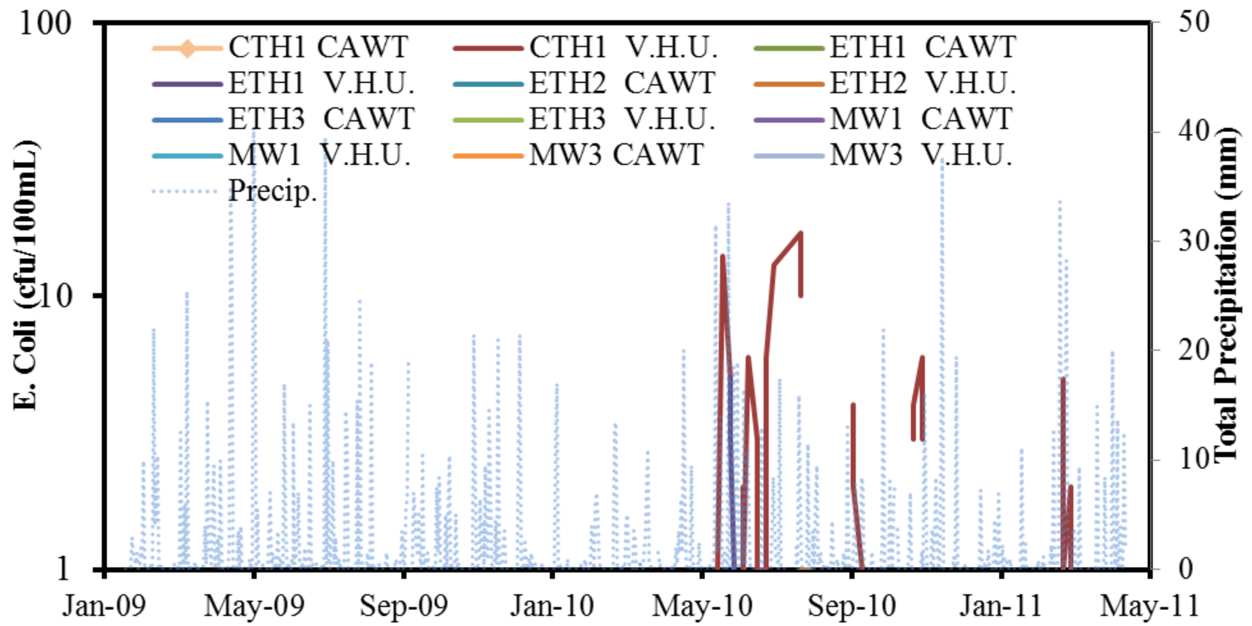

Figure 4.6. *E. coli* in CTH1, ETH1, ETH2, ETH3, MW1 and MW3.

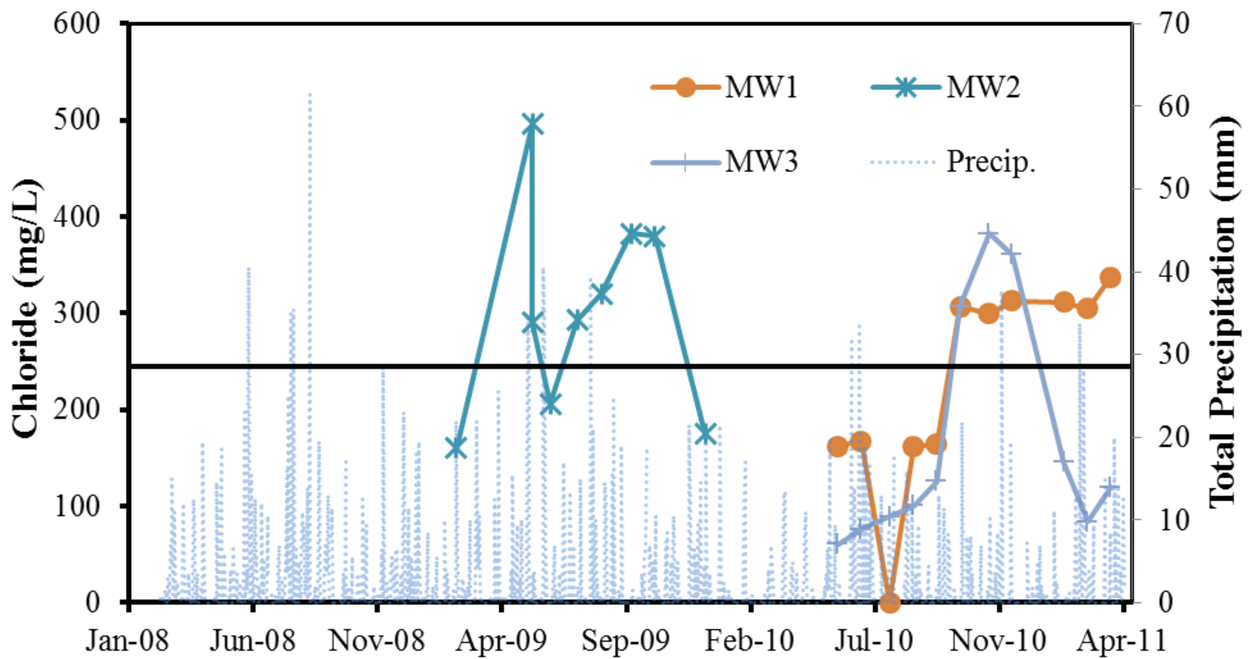

Figure 4.7. Chloride concentrations found in MW1, MW2 and MW3.

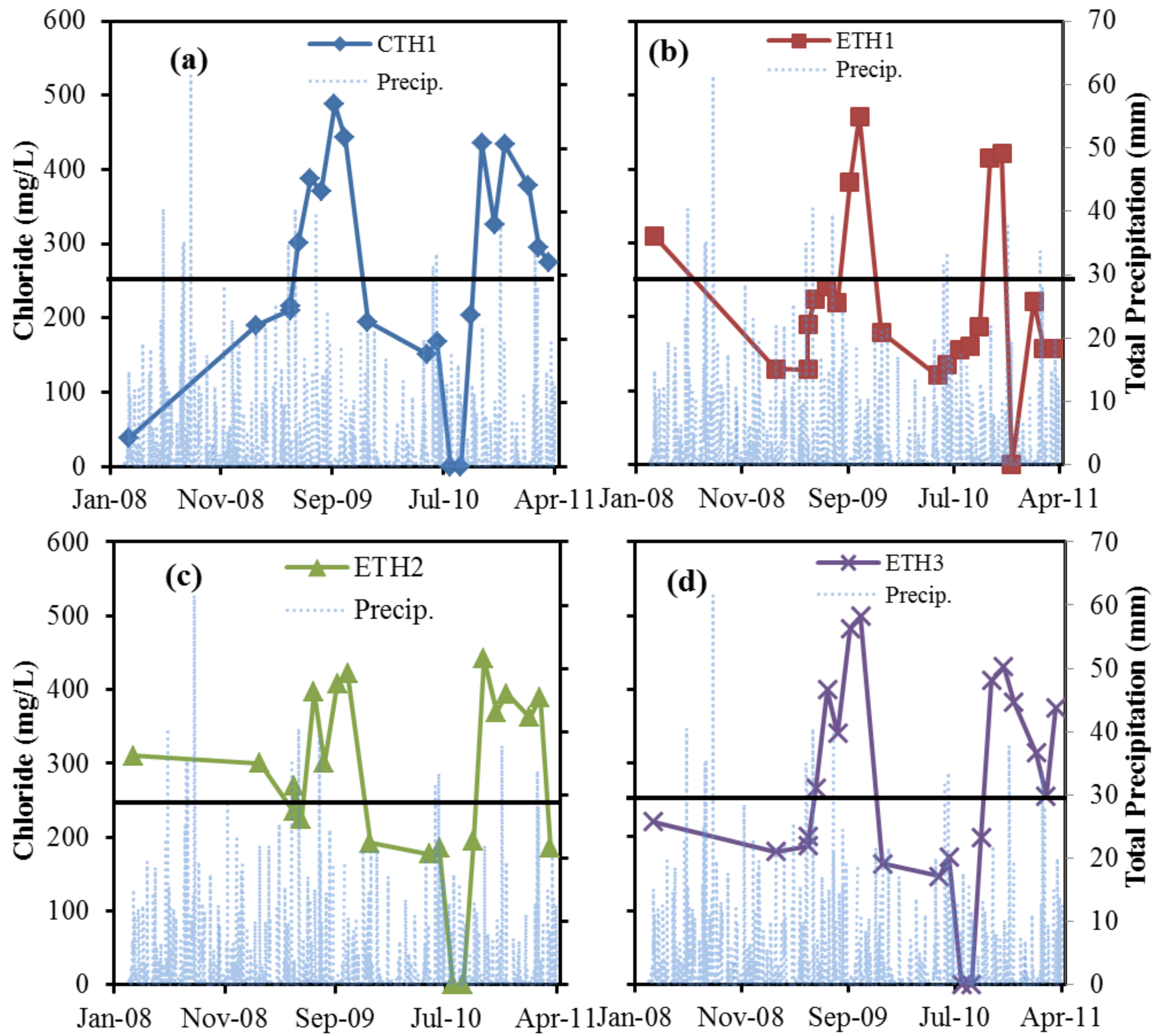

Figure 4.8. Chloride concentrations found in (a) CTH1, (b) ETH1, (c) ETH2, and (d) ETH3.

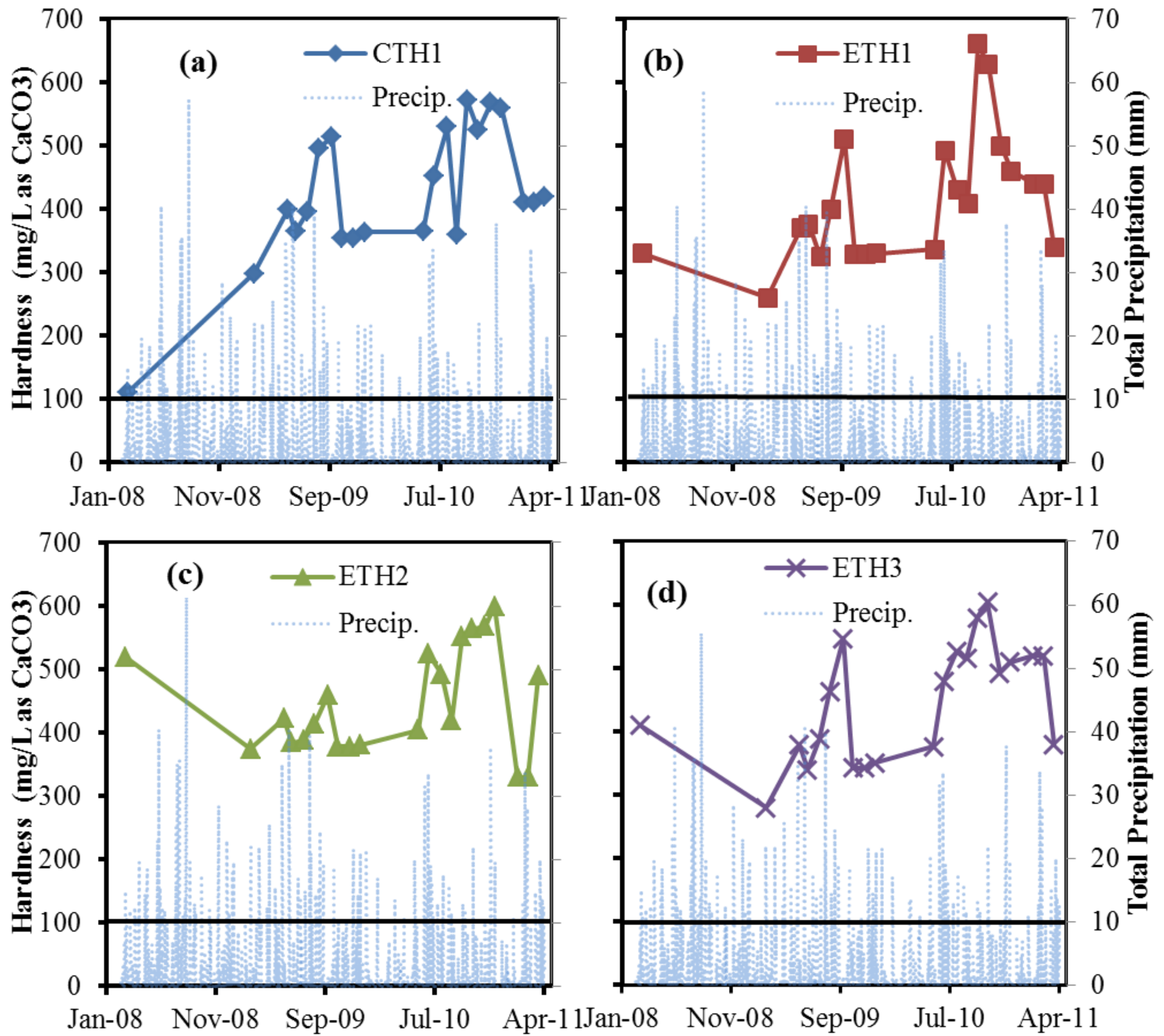

Figure 4.9. Hardness concentrations found in (a) CTH1, (b) ETH1, (c) ETH2, and (d) ETH3.

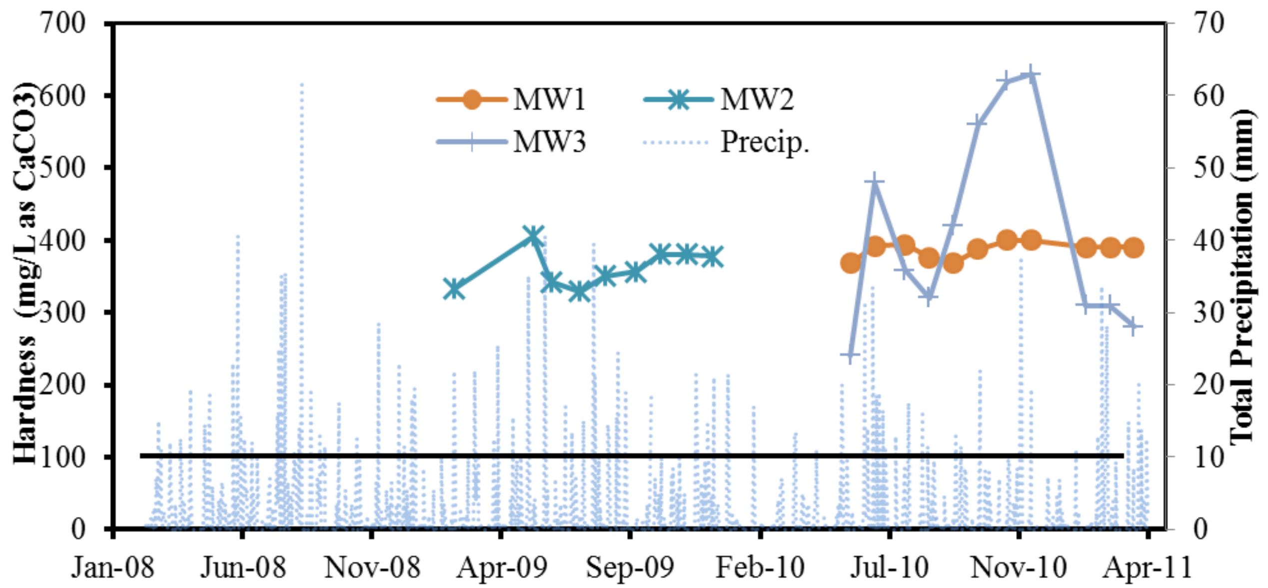

Figure 4.10. Hardness in MW1, MW2 and MW3.

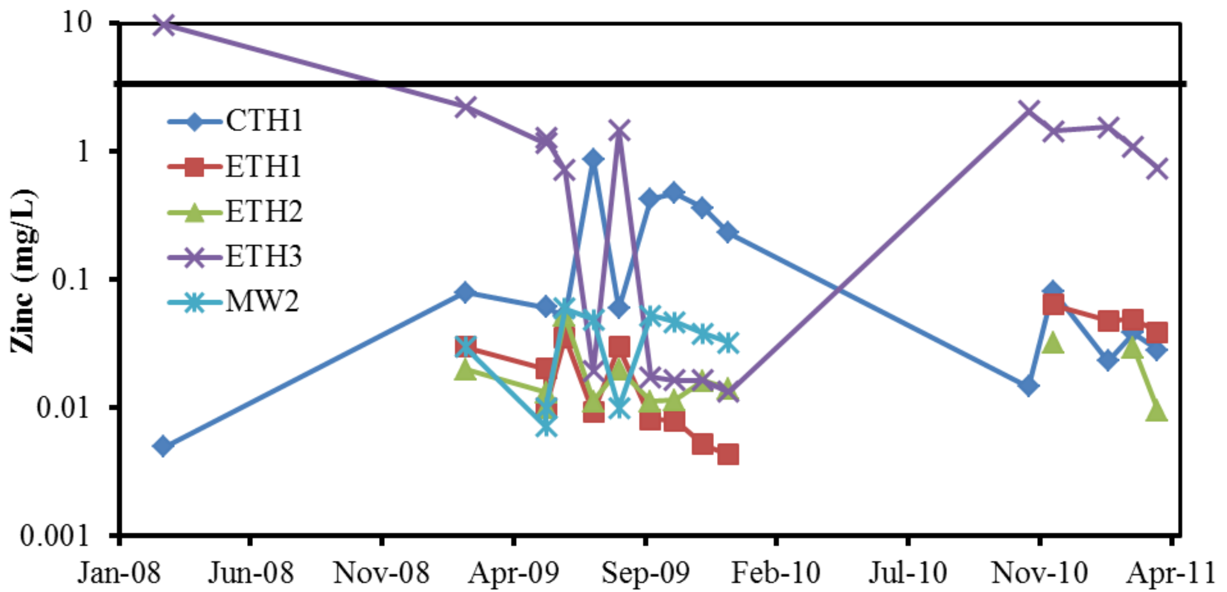

Figure 4.11. Zinc concentrations on the three test wells and MW2.

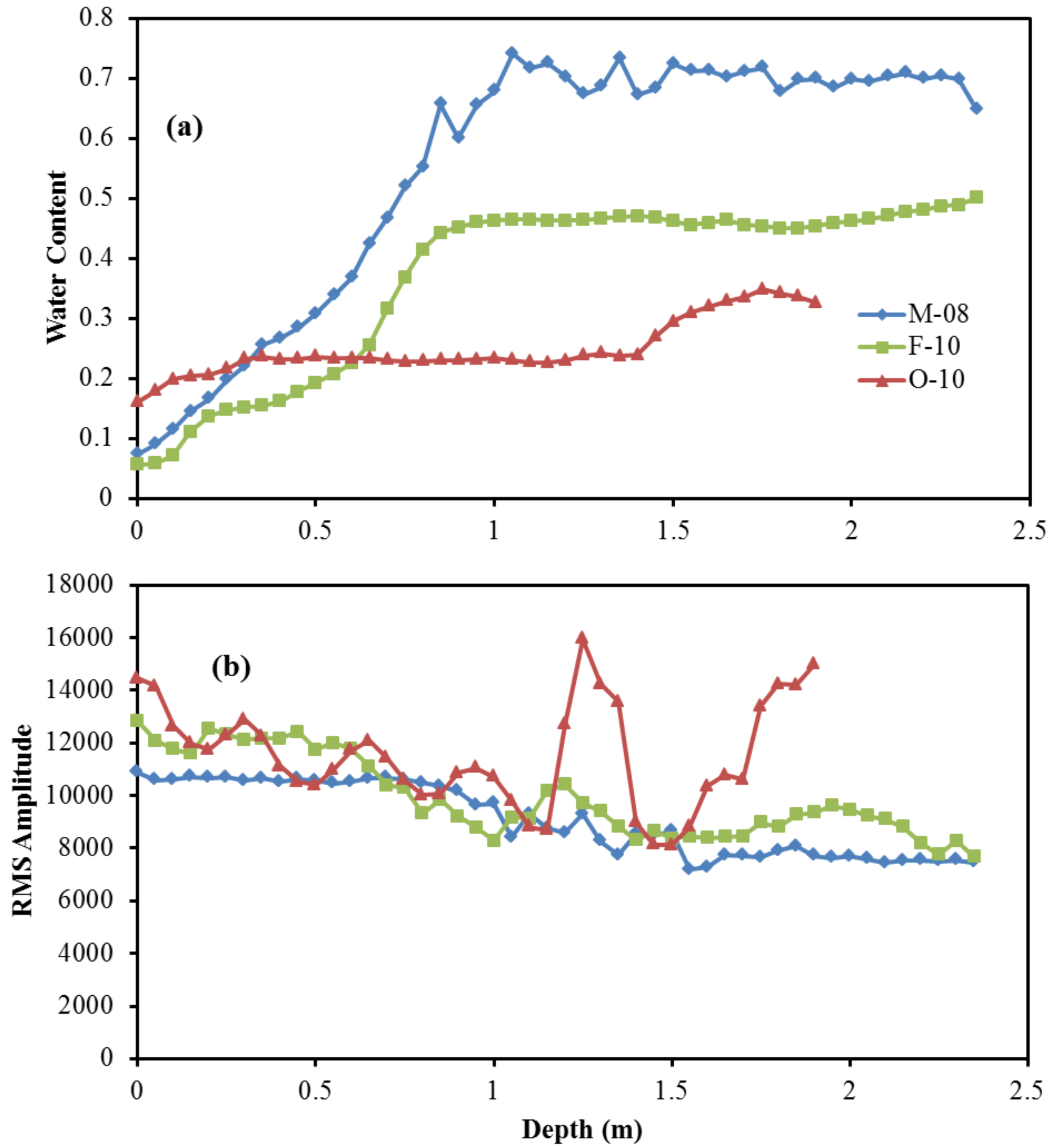

Figure 4.12. Variation of (a) water content and (b) RMS amplitude with depth for test well ETH1.

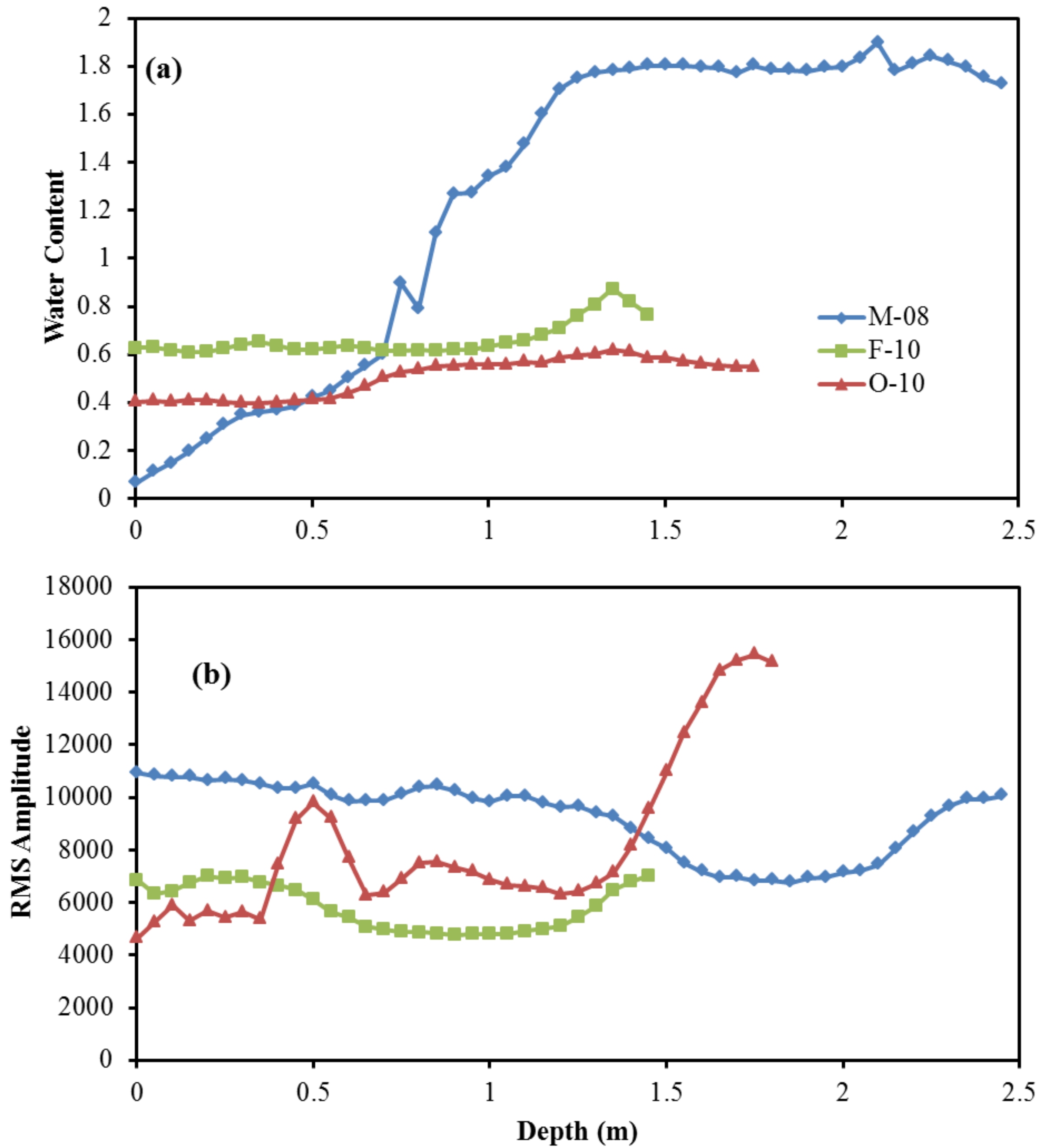

Figure 4.13. Variation of (a) water content and (b) RMS amplitude with depth for test well ETH2.

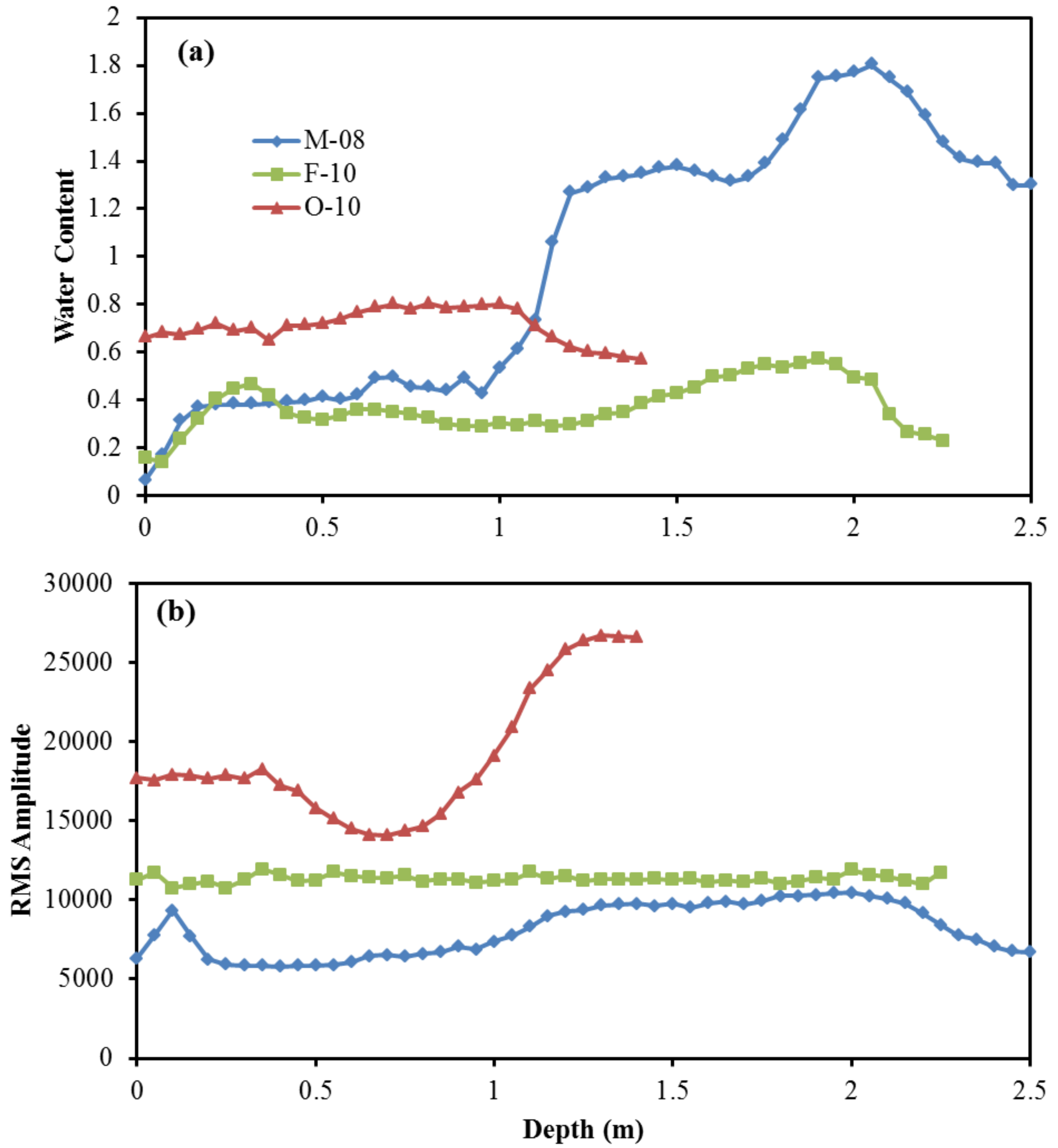

Figure 4.14. Variation of (a) water content and (b) RMS amplitude with depth for test well ETH3.

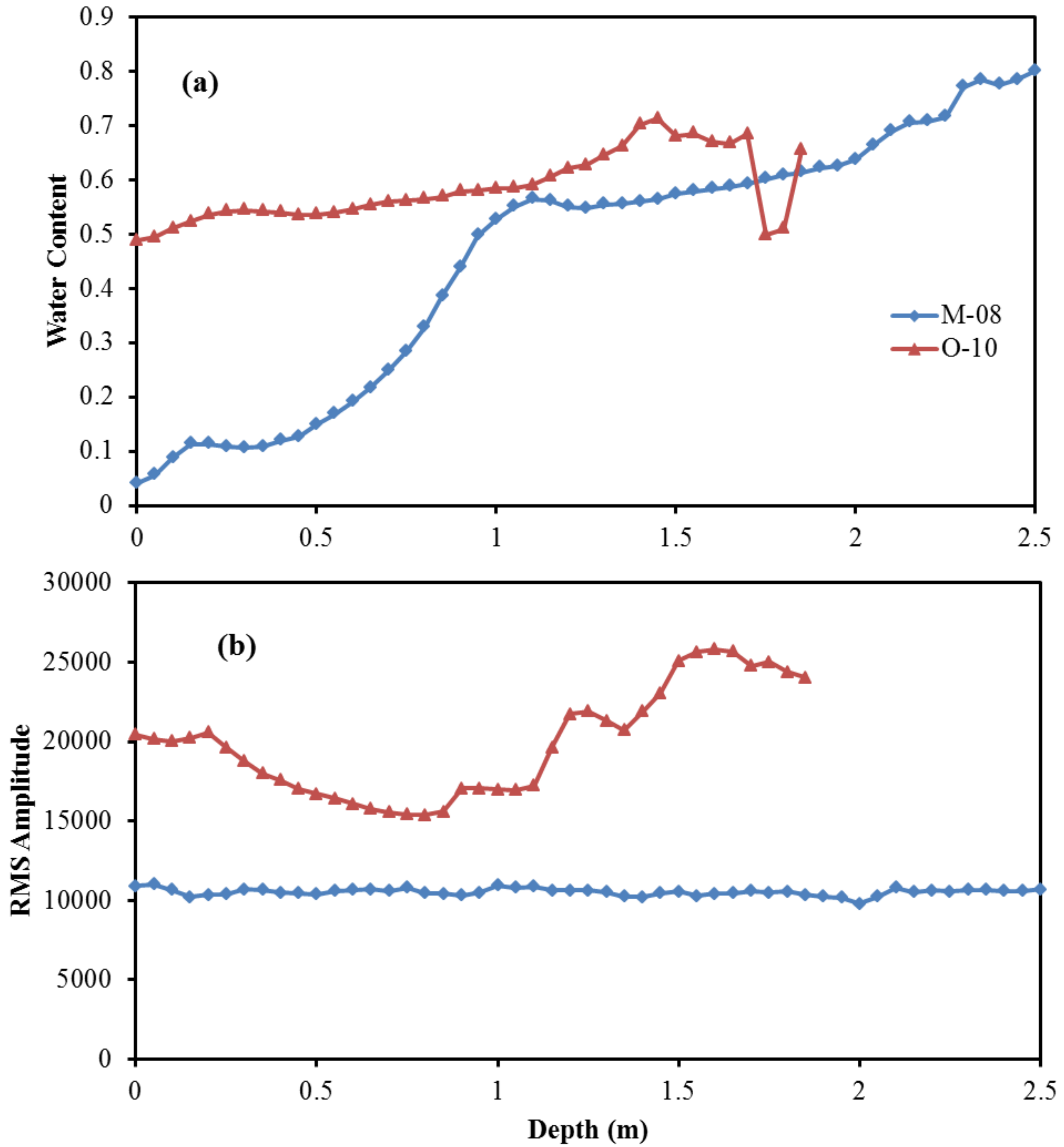

Figure 4.15. Variation of (a) water content and (b) RMS amplitude with depth for test well CTH1.

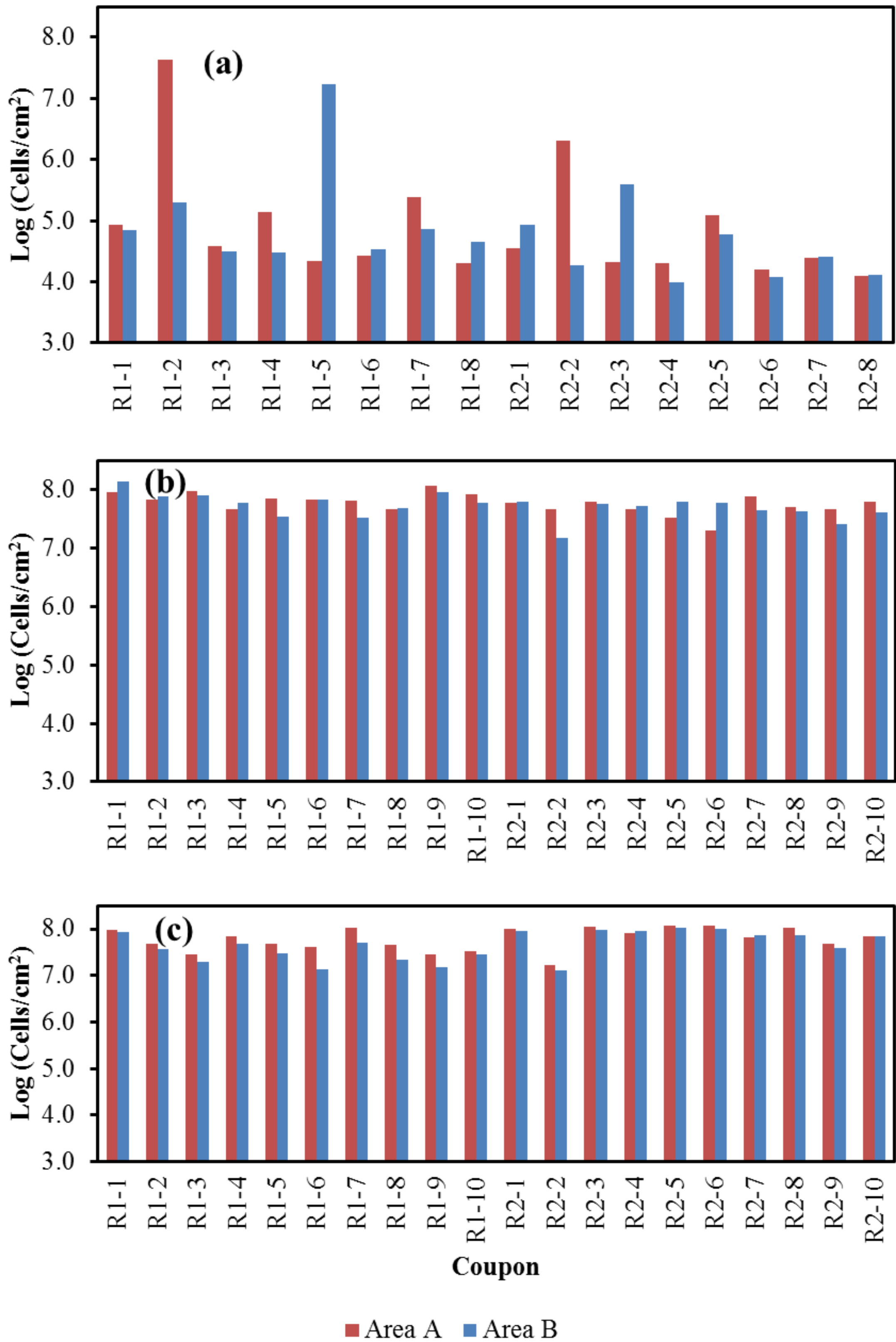

Figure 4.16. Preliminary biofilm growth - (a) Trial 1, (b) Trial 2, and (c) Trial 3.

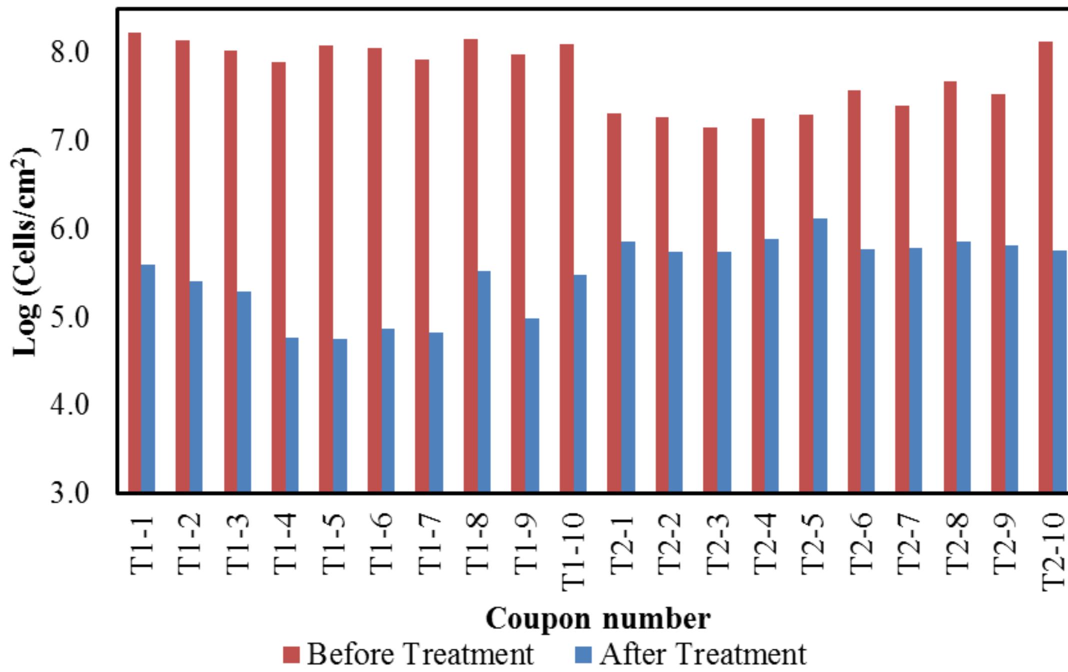

Figure 4.17. Biofilm removal by chlorination treatment from galvanized steel.

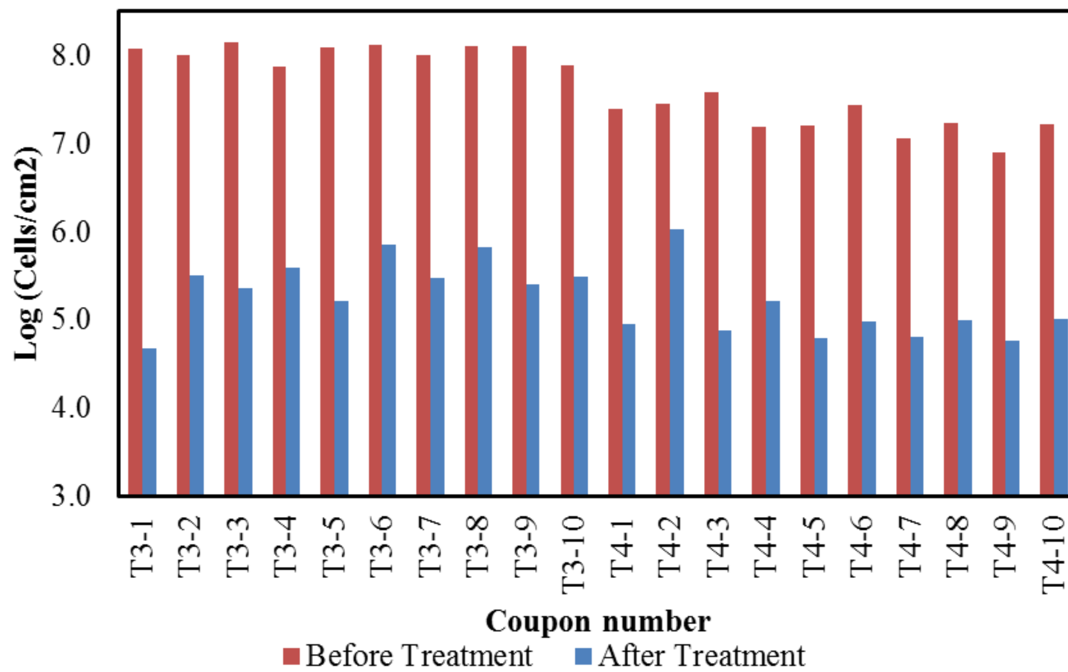

Figure 4.18. Biofilm removal by pressure washing treatment from galvanized steel.

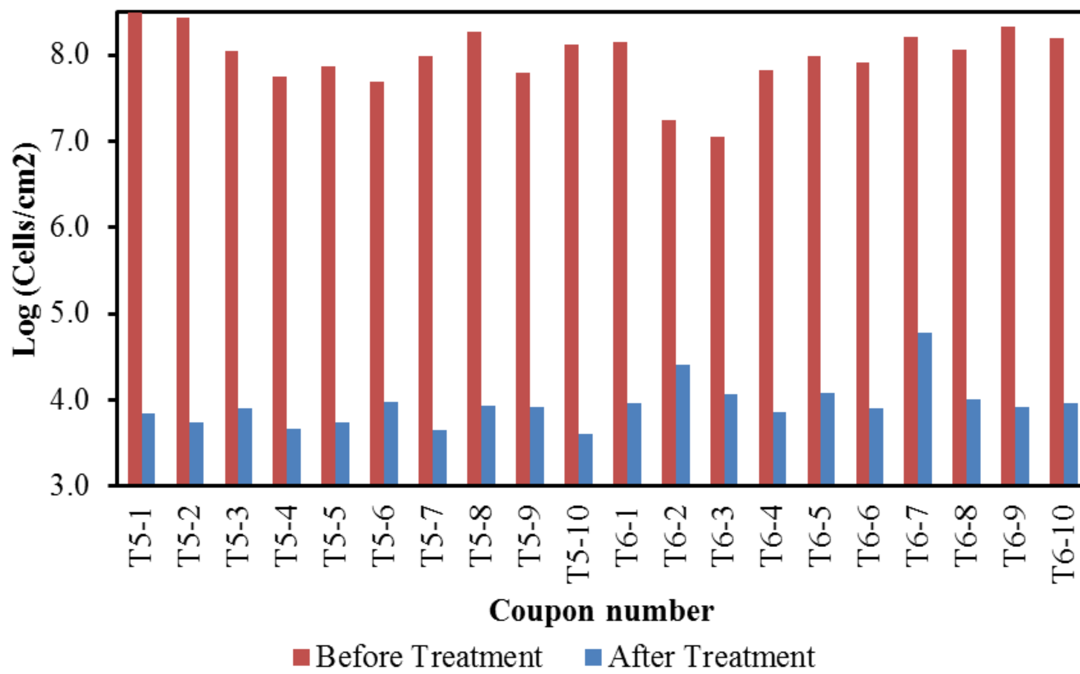

Figure 4.19. Biofilm removal by pressure washing/chlorination treatment from galvanized steel.

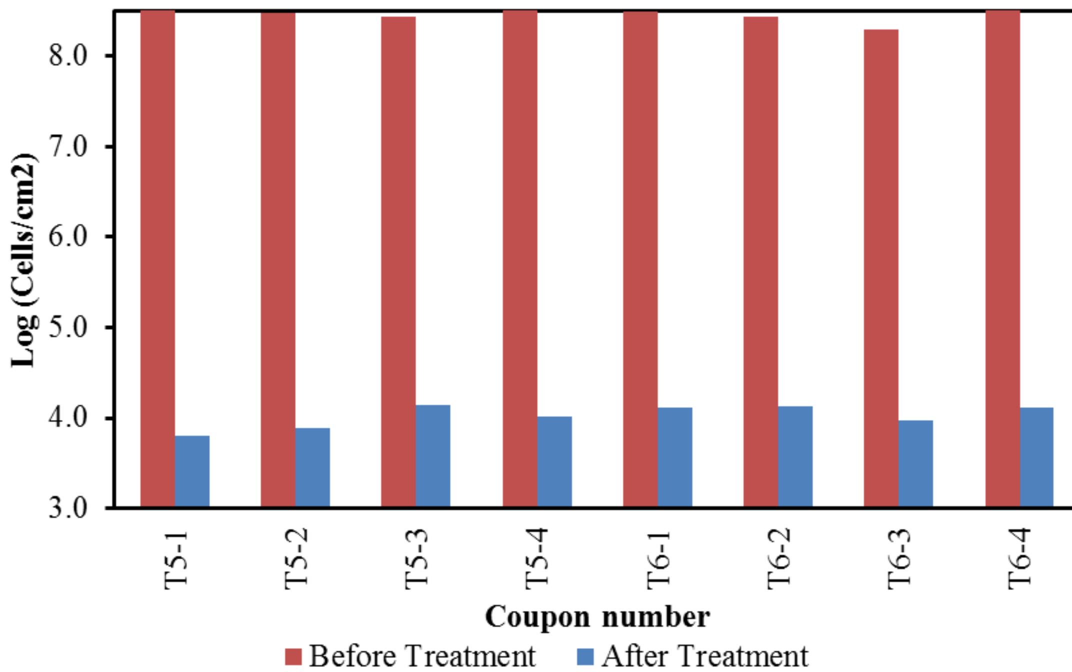

Figure 4.20. Biofilm removal by pressure washing/chlorination treatment from fibreglass.

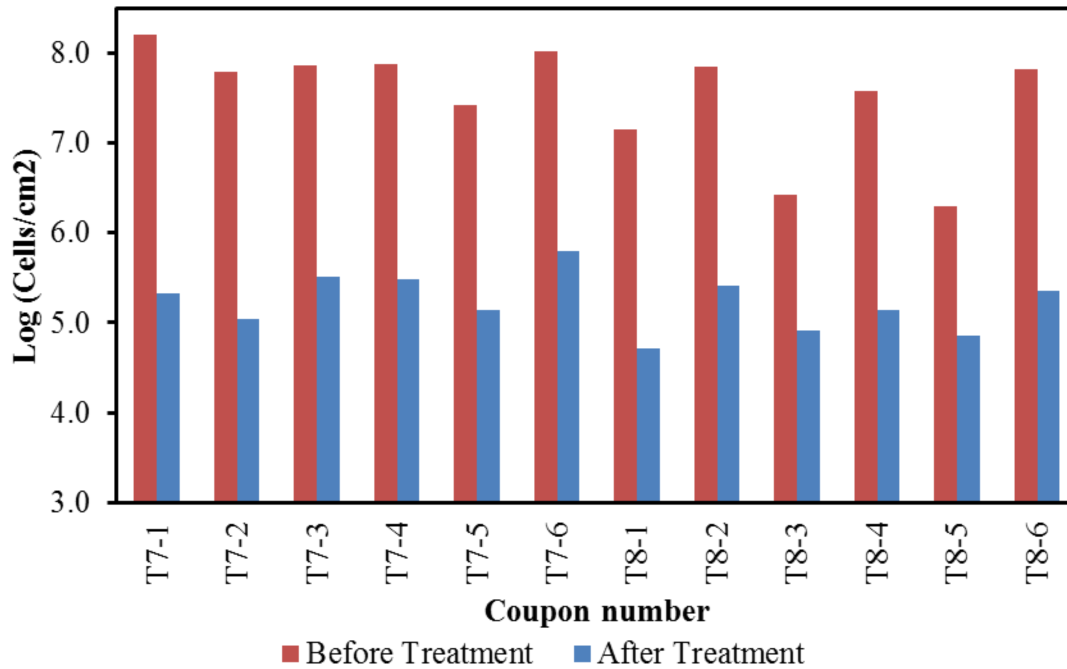

Figure 4.21. Biofilm removal by chloramination treatment from galvanized steel.

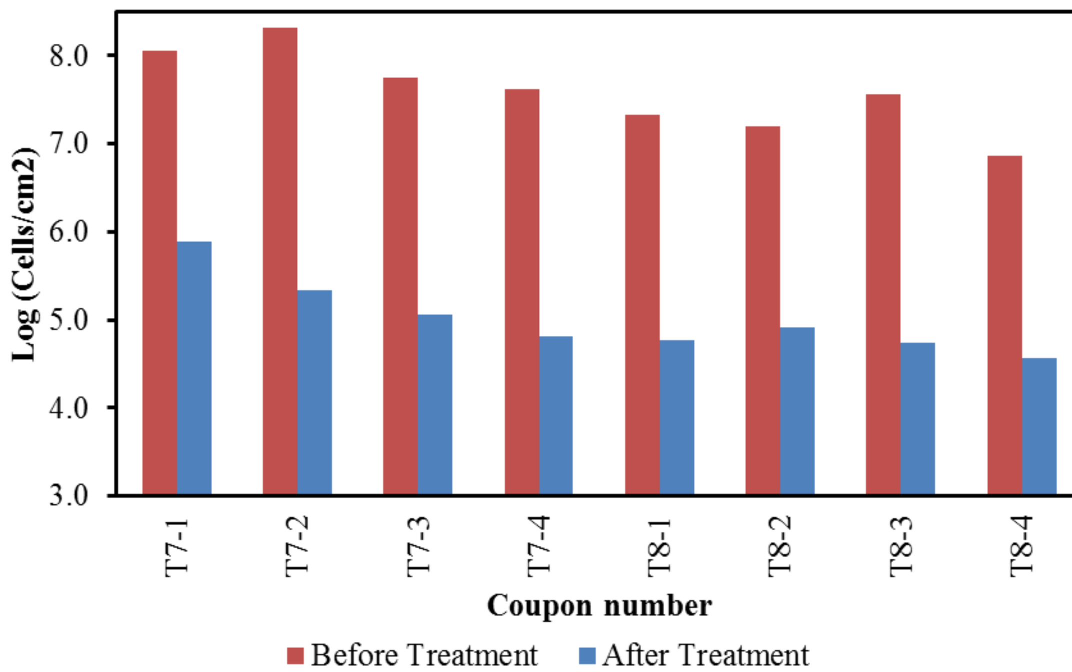

Figure 4.22. Biofilm removal by chloramination treatment from fibreglass.

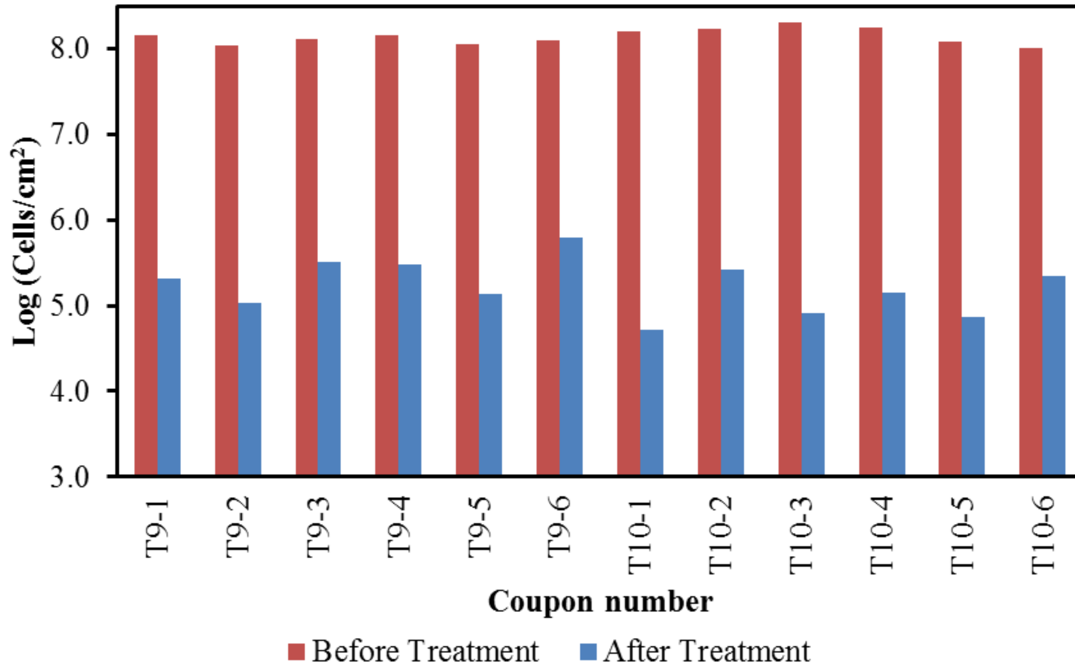

Figure 4.23. Biofilm removal by hydrogen peroxide treatment for galvanized steel.

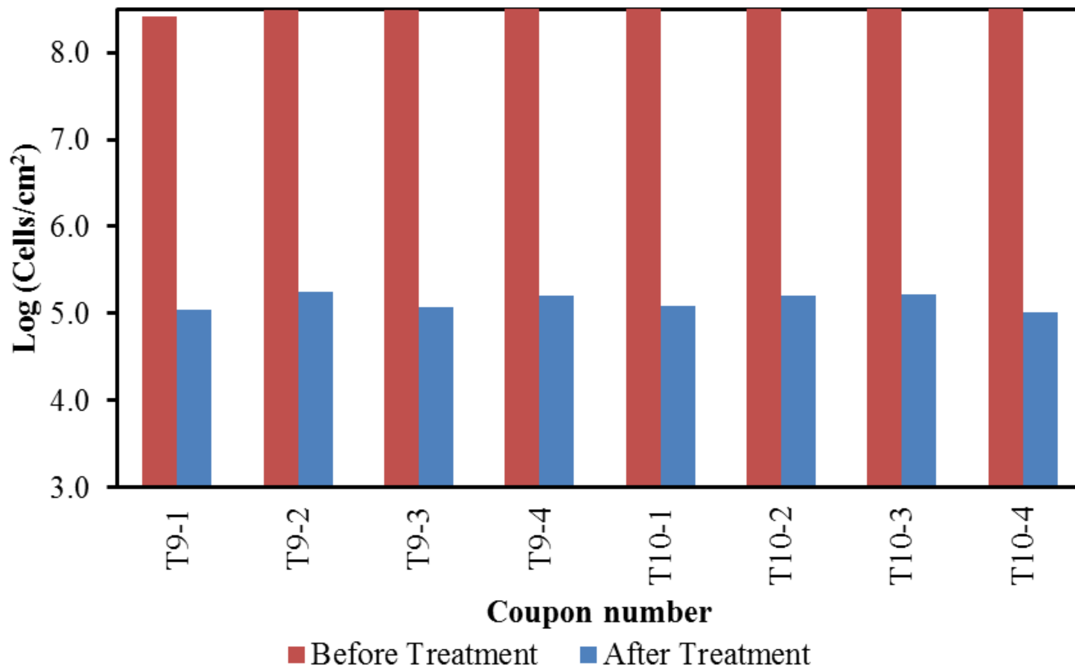

Figure 4.24. Biofilm removal by hydrogen peroxide treatment from fibreglass.

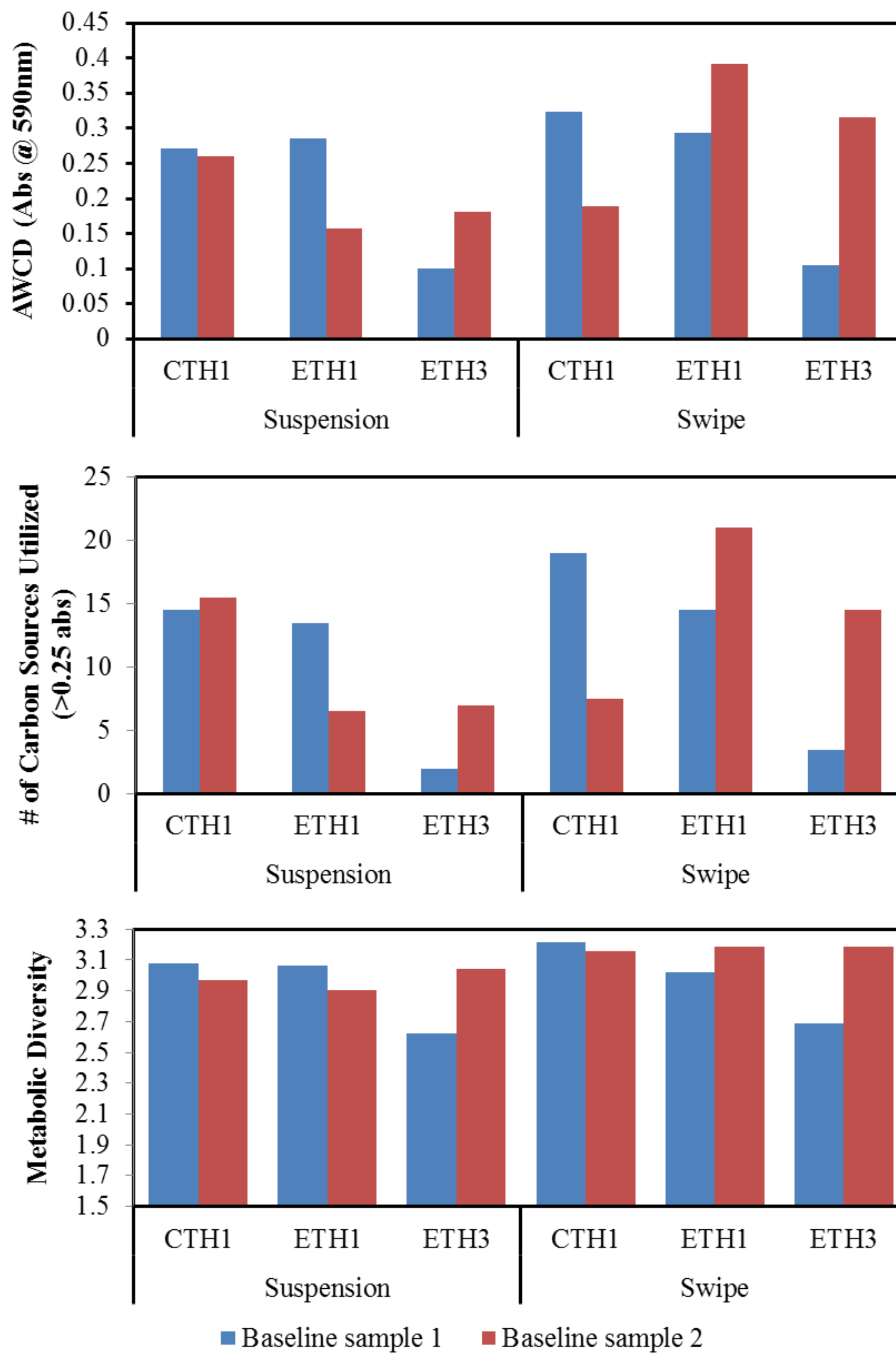

Figure 4.25. Wells biofilm characterization showing (a) AWCD, (b) metabolic richness and (c) metabolic diversity present in CTH1, ETH1 and ETH3.

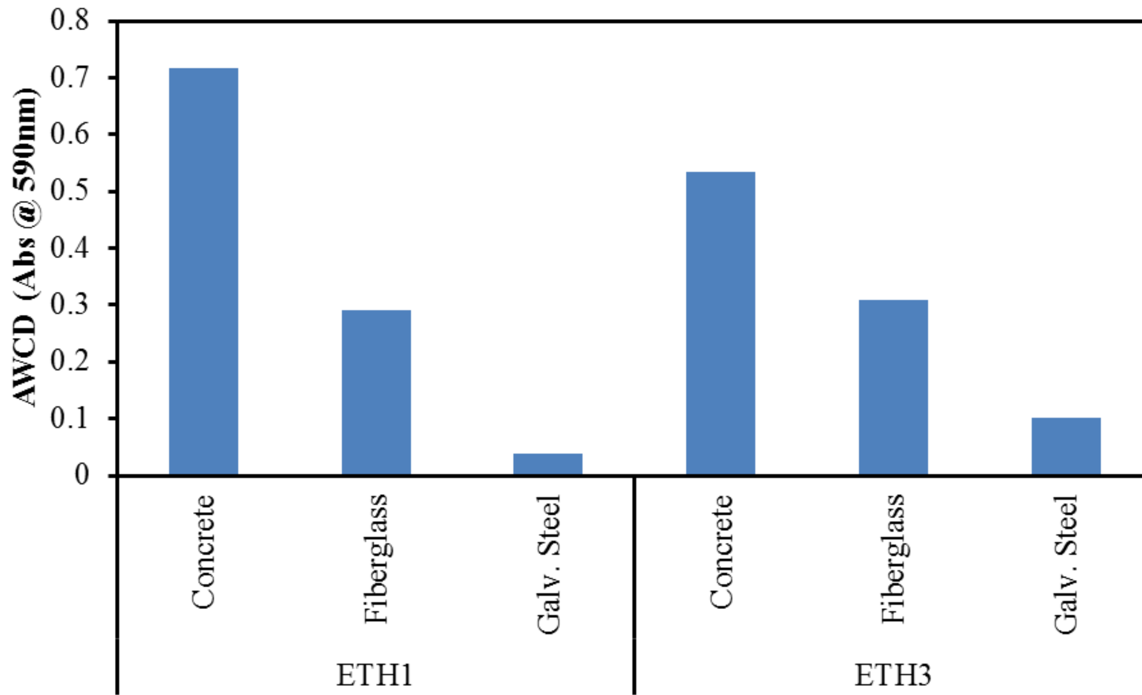

Figure 4.26. AWCD of biofilm on coupons incubated inside ETH1 and ETH3.

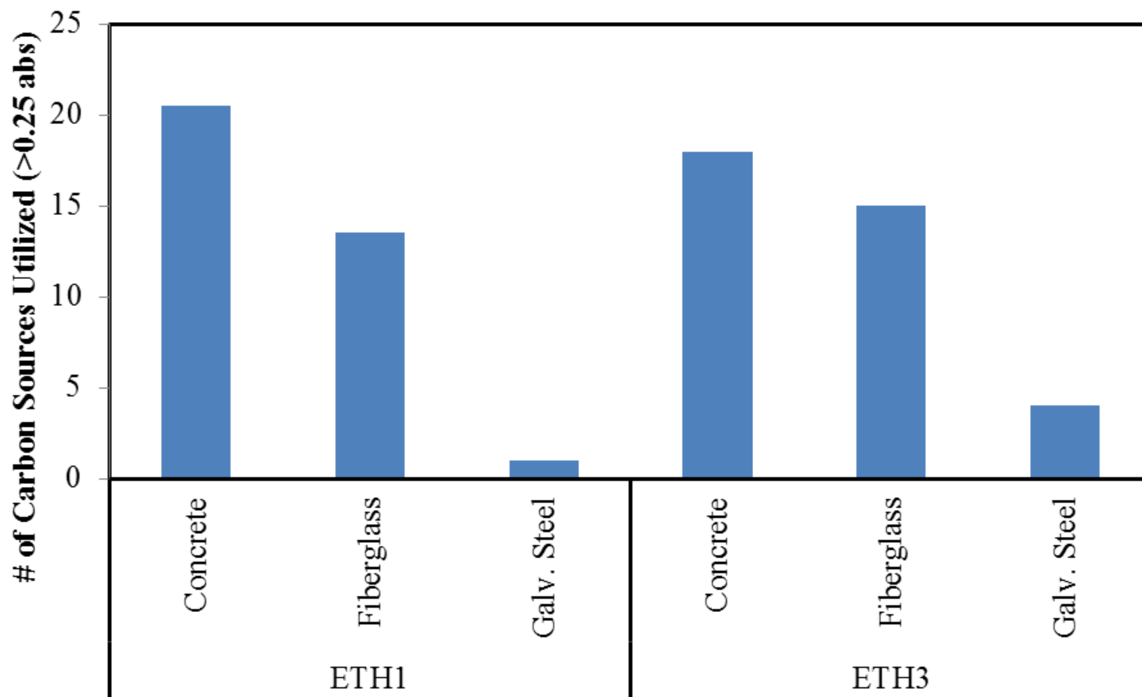

Figure 4.27. Metabolic Richness of biofilm on coupons incubated inside ETH1 and ETH3.

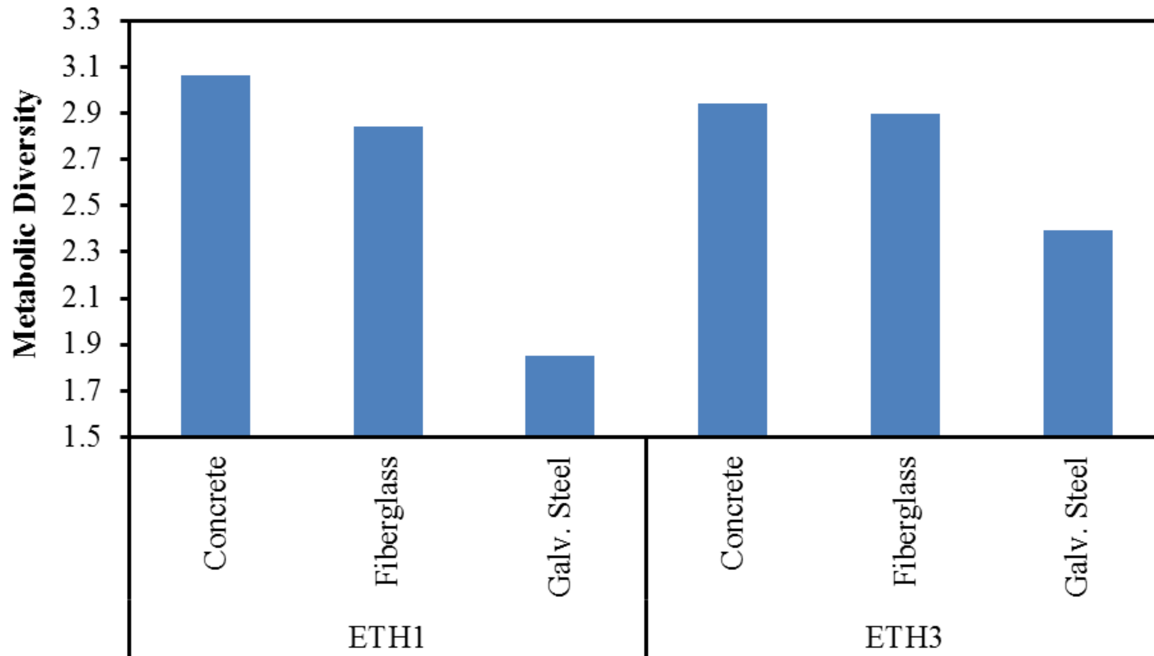

Figure 4.28. Metabolic diversity of biofilm on coupons placed inside ETH1 and ETH3.

Table 4.1. Daily test well pumping program (Javor, 2010).

Well		Daily Program Times			Total Pump Time
		A	B	C	
ETH2	On	11:40	13:20	14:45	40 min
	Off	12:00	13:30	14:55	
CTH1	On	12:01	13:31		40 min
	Off	12:21	13:51		
ETH3	On	12:22	13:52		40 min
	Off	12:42	14:12		
ETH1	On	12:50	14:20		40 min
	Off	13:10	14:40		

Table 4.2. Observations from five smoke tracer tests performed between December 2008 and February 2011.

Air Leak Location	CTH1	ETH1	ETH2	ETH3
Poly-Lok lid seam	NA	**Y/Y/Y/Y/Y**	**Y/Y/Y/Y/Y**	**Y/Y/Y/Y/Y**
Poly-Lok lid and concrete cover joint	NA	N/N/**Y**/**Y**/N	N/N/N/N/N	N/N/N/Y/N
Interior geophysical access tube	**Y/Y**	**Y/Y/Y/Y**/N	**Y/Y/Y/Y/Y**	NA
Electrical conduit	NA	**Y/Y/Y**/N/N	**Y/Y/Y/Y/Y**	**Y/Y/Y/Y**/N
Water line entry	N/N	N/N/N/N/N	N/N/N/N/N	N/N/N/N/N
Casing joints or seams	Y/Y	**Y**/N/N/N/N	**Y**/N/N/N/N	**Y/Y/Y/Y**/N
Annular sealant	N/N	N/N/N/N/N	N/N/N/N/N	N/N/N/**Y**/N
Exterior geophysical access tube	N/N	N/N/N/N/N	N/N/N/N/N	N/N/N/**Y**/N

Notes:
Test #1 performed December 2008.
Test #2 performed May 2009.
Test #3 performed March 2010.
Test #4 performed October 2010.
Test #5 performed February 2011.
NA – not applicable.
Y – Yes smoke leakage observed.
N – No smoke leakage observed.

Table 4.3. Pre-treatment logarithmic units and LR after treatment.

		Galvanized Steel		Fibreglass	
		Initial Logs	Logs Removed	Initial Logs	Logs Removed
Chlorination					
Trial 1	n	10	10		
	Average	**8.06**	**2.91**		
	Standard Deviation	0.10	0.27		
	Variance	0.011	0.070		
Trial 2	n	10	10		
	Average	**7.46**	**1.63**		
	Standard Deviation	0.28	0.33		
	Variance	0.080	0.107		
Power Washing					
Trial 3	n	10	10		
	Average	**8.03**	**2.60**		
	Standard Deviation	0.10	0.36		
	Variance	0.010	0.126		
Trial 4	n	10	10		
	Average	**7.26**	**2.22**		
	Standard Deviation	0.20	0.35		
	Variance	0.041	0.119		
Power washing/Chlorination					
Trial 5	n	10	10	4	4
	Average	**8.04**	**4.25**	**8.51**	**4.54**
	Standard Deviation	0.28	0.32	0.08	0.22
	Variance	0.081	0.104	0.007	0.049
Trial 6	n	10	10	4	4
	Average	**7.90**	**3.80**	**8.44**	**4.35**
	Standard Deviation	0.43	0.54	0.10	0.06
	Variance	0.182	0.292	0.011	0.003
Chloramine					
Trial 7	n	6	6	4	4
	Average	**7.87**	**2.49**	**7.94**	**2.66**
	Standard Deviation	0.26	0.27	0.31	0.35
	Variance	0.070	0.074	0.095	0.125
Trial 8	n	6	6	4	4
	Average	**7.19**	**2.12**	**7.24**	**2.49**
	Standard Deviation	0.69	0.50	0.29	0.26
	Variance	0.476	0.252	0.084	0.067
Hydrogen Peroxide					
Trial 9	n	6	6	4	4
	Average	**8.11**	**0.87**	**8.49**	**3.35**
	Standard Deviation	0.05	0.37	0.07	0.08
	Variance	0.003	0.133	0.005	0.006
Trial 10	n	6	6	4	4
	Average	**8.18**	**0.95**	**8.68**	**3.55**
	Standard Deviation	0.11	0.26	0.01	0.10
	Variance	0.013	0.068	0.000	0.010

5.0 Conclusions and Recommendations

5.1 Conclusions

The performance of the different large diameter residential drinking water wells were studied over two freeze/thaw cycles. The pumping system was able to emulate a daily water demand. The average daily pumping volume (0.7 m^3) was lower than the desired (1.0 m^3) in all wells due to insufficient power generated by the solar system during cloudy days. The lowest daily pumped volume was observed in CTH1 (0.5 m^3) which in addition to the lack of enough power experienced freezing problems with the drainage system during the first year of operation.

Water quality data indicated that the monitored wells (four (4) test and two (2) monitoring wells) were affected by total coliforms with values as high as 938 CFU. In addition to the bacterial presence, the data showed that following the onset of spring (approximately April) the operational guideline for hardness and the aesthetic objective for chloride were exceeded in all of the monitored wells. The highest concentration of these two parameters reached a peak (usually by September) with values between 400 and 500 mg/L for chloride, and between 400 and 650 mg/L for hardness. The presence of bacteria and elevated concentrations of some ions in all the monitored wells indicates that the source is independent of the annular sealant and beyond the boundaries of the study site. It is suspected that the source of dissolved ions is road salt applied upgradient on the Fleming College Campus, but the microbial source is unknown. Collectively, these observations indicate that these wells are under the direct influence of surface water.

Smoke tracer tests conducted under normal and freezing conditions on the enhanced test wells did not show significant pathway differences between the atmosphere and the interior of the test wells. The only variations in pathways observed over the monitoring period are related to various seals (casing seams, electrical conduit). Some of the geophysical access tubes (under non-freezing conditions) show additional pathways due to expansion/compression of the materials as a result of temperature changes.

Geophysical measurements were completed to assess non-destructively the integrity of the *in situ* annular sealant materials of the test wells. The non-destructive assessment of the annular sealant materials showed that bentonite slurry with sand (annular sealant for ETH1) was homogeneous

with depth since this well was installed. This observation is in contrast to the annular sealants using bentonite pellets (ETH2), bentonite granules (ETH3) and drill cuttings (CTH1) which required hydration and/or consolidation to generate a homogenous response in water content and electrical conductivity (RMS).

Initial laboratory experiments allowed for the development and standardization of a procedure to grow biofilm under similar *in situ* conditions as those observed in the large diameter test wells at the study site. These experiments confirmed that the bacteria density grown on both areas of the coupons were within an acceptable range (< 1 log unit). The laboratory experiments also led to the development of biofilm sampling and enumeration methods.

Biofilm cleaning experiments were conducted using galvanized steel and fibreglass casing materials; concrete coupons were not available at the time of the experiments. It was found that pressure washing had similar biofilm removal capabilities to chlorine. However when this mechanical method to remove biofilm was combined with chlorination the combined removal potential was almost equal to the sum of their individual capabilities. This combination had a slightly better performance on fibreglass casing material. The potential use of some unconventional methods to remove biofilm was also explored. The disinfection capabilities of chloramines and hydrogen peroxide were tested. It was found that chloramines were able to remove a similar number of log units as chlorination and pressure washing. Hydrogen peroxide only removed 1 log unit from galvanized steel coupons but surprisingly was able to remove 3.5 log units from fibreglass.

Biofilm assessment in three (3) of the test wells indicated that sessile bacteria are slightly more diverse than suspended bacteria, and that the bacteria from the concrete cased wells have a higher metabolic diversity than bacteria from the galvanized steel cased well. The microbial characterization performed on the coupons indicated that concrete supports the most diversity of microorganisms. The bacteria in ETH3 (galvanized steel) grew more easily on all the casing materials while the bacteria in ETH1 (concrete) did not colonize on galvanized steel. Bacteria from test wells ETH1 and ETH3 seem to have a similar preference for fibreglass as for concrete casing materials.

The results from this study indicated that large diameter wells constructed using an enhanced design reduce airborne and surface water pathways making them less prone to these sources of contamination compared to conventional wells (i.e., CTH1). Even though complete sterilization of large diameter wells is not possible, this laboratory study determined that the use of pressure washing combined with chlorination was able to remove more biofilm than was possible with a single disinfection method. It was also observed that a greater degree of biofilm was able to be removed from fibreglass casing material than from galvanized steel casing material.

5.2 Recommendations

This research focused on assessing various biofilm removal methods, and the performance monitoring of large diameter drinking water wells over two freeze/thaw cycles. The following recommendations are provided to extend the value of this research:

- Continue with routine monitoring of the existing test wells to determine the effect of well age on performance. Monitoring activities should include monthly water quality sample collection and analysis, water elevation, soil temperature, and cumulative volume purged.

- Construct and monitor the performance of another large diameter well using fibreglass casing material. This material may provide a better alternative to the continuous galvanized casing with riveted seams and the concrete casing with sealed joints.

- Conduct an additional smoke tracer test once the Poly-Lok lid and seal is replaced to determine whether this is the cause of the observed air pathways.

- Perform microbial characterization before and after biofilm removal using the best alternative found in this study to determine its efficacy in the field.

- Collect cores from the annular sealants of the test wells to verify the findings from the GPR surveys.

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Appendix A. Biofilm experimental method.

Appendix A contains the detailed methods used to create biofilm in the laboratory, the cleaning methods used in this study and the analytical methods used in the analysis.

A1 Biofilm preparation

A.1.1 Materials

- large diameter well casing material coupons (7 cm x 7 cm) of galvanized steel, and fibreglass (5 of each)
- an etching tool to carve galvanized steel and fibreglass coupons
- autoclave
- marker
- biofilm reactor

A.1.2 Procedure

1. Etch 2, 5 cm by 2.5 cm, rectangles (Figure 1.2) into the galvanized steel coupons and carve 2, 5 cm by 2.5 cm, rectangles into the fibreglass. Mark the areas as A and B.
2. Sterilize all coupons in an autoclave at 120 degrees Celsius for 20 minutes (Forster C.J. et al, 2001).
3. Prepare biofilm reactor (Figure 3.4) by filling with raw water (as seed), DI water (pH adjusted to 7) and nutrients.
4. Homogenize the solution for at least 24 hours.
5. After the mixing period place the coupons with the markings facing up in the bottom of the reactor.
6. Leave in biofilm reactor incubating for 13 to 15 days for biofilm formation.
7. After the incubation period remove the samples from the biofilm reactor.

A2 Cleaning methods

Disinfection using chlorination was used as baseline. Pressure washing was the only mechanical cleaning method tested to remove biofilm from the coupons of the different casing materials. Once removed from the biofilm reactor the cleaning methods were tested. To ensure repeatable results standard methods for cleaning have been developed.

A.2.1 Materials

- Bleach - (6%)

- Ammonium chloride (4.8 g/L)
- Hydrogen peroxide (35%)
- Clean biofilm reactors
- Pressure washer (25˚ nozzle tip, 1300 psi working pressure)
- Clamps
- Coupons with biofilm growth

A.2.2 Procedures

A.2.2.1 Chlorination

1. Prepare a bleach solution 50 to 200 ppm in a clean reactor (1.25 to 3.25 mL bleach/L DI water).
2. Place the coupons in the bottom of the reactor with the marked areas facing up.
3. Allow 24 hours contact time before removing the coupons.
4. Rinse the coupons submerging them in clean DI water (pH 7). Remove the coupons and submerge them in another batch of DI water (pH 7). Repeat one more time.
5. Refer to the sample collection section for the next steps.

A.2.2.2 Pressure washing

1. Clamp the coupons to a surface in a drainable area.
2. Pressure wash each coupon individually, using a wide fan setting (25˚ nozzle tip). Keeping the nozzle 10 to 15 cm above the coupons sweep the coupons with the water jet 3 times back and for.
3. Refer to the sample collection section for the next steps.

A.2.2.3 Pressure washing followed by chlorination

1. Clamp the coupons to a surface in a drainable area.
2. Pressure wash each coupon individually, using a wide fan setting (25˚ nozzle tip). Keeping the nozzle 10 to 15 cm above the coupons sweep the coupons with the water jet 3 times back and for.

3. Prepare a bleach solution 50 to 200 ppm in a clean reactor (1.25 to 3.25 mL bleach/L DI water).
4. Place the coupons in the bottom of the reactor with the marked areas facing up.
5. Allow 24 hours contact time before removing the coupons.
6. Rinse the coupons submerging them in clean DI water (pH 7). Remove the coupons and submerge them in another batch of DI water (pH 7). Repeat one more time.
7. Refer to the sample collection section for the next steps.

A.2.2.4 Chloramination

1. Prepare a chloramine solution 50 to 200 ppm in a clean reactor (1.25 to 3.25 mL bleach/L DI water followed by 10 to 40 mL of ammonium chloride. Make sure the pH is at all times over 7 to guarantee monochloramine formation.
2. Place the coupons in the bottom of the reactor with the marked areas facing up.
3. Allow 24 hours contact time before removing the coupons.
4. Rinse the coupons submerging them in clean DI water (pH 7). Remove the coupons and submerge them in another batch of DI water (pH 7). Repeat one more time.
5. Refer to the sample collection section for the next steps.

A.2.2.5 Hydrogen peroxide

1. Prepare a hydrogen peroxide solution 400 to 500 ppm in a clean reactor (1.25 to 1.5 mL hydrogen peroxide/L DI water.
2. Place the coupons in the bottom of the reactor with the marked areas facing up.
3. Allow 24 hours contact time before removing the coupons.
4. Rinse the coupons submerging them in clean DI water (pH 7). Remove the coupons and submerge them in another batch of DI water (pH 7). Repeat one more time.
5. Refer to the sample collection section for the next steps.

A3 Sample collection

To retrieve biofilm samples from the coupons, this will be swabbed and analyzed for residual biofilm on the sterile swabs. To ensure repeatable results, standard methods for collecting samples of the biofilm have been developed.

A.3.1 Materials

1. nitrile/latex gloves
2. sterile swabs
3. biofilm grown coupons
4. 15 mL sterile disposable test tubes
5. filter-sterilized water
6. VWR® SuperClear™ Microcentrifuge Tubes
7. micropipette 200 μL

A.3.2 Procedure

1. Remove the coupons from the reactor.
2. Retrieve a sample from each Area A by swabbing with 1 swab.
3. Immediately cut off the tip of the used swab and place it in a 15 mL sterile disposable test tube with 3 mL of filter-sterilized water.
4. Repeat steps 2 and 3 two times collecting the tips in the same 15 mL sterile disposable test tube.
5. Swirled for 2 minutes in a vortex the test tubes with the 3 swab tips.
6. Collect two 100 μL aliquots in two VWR® SuperClear™ microcentrifuge tubes immediately after swirling.
7. Refer to the ATP analysis section for the next steps

A4 ATP Analysis

A.4.1 Materials

Chemicals should be kept frozen at -20 degrees Celsius.

- Promega BacTiter-Glo Microbial Cell Viability Assay (BacTiter-Glo™ Buffer and BacTiter-Glo™ Substrate (lyophilized).

- Adenosine 5′-triphosphate disodium salt solution 100 mM.
- 1 protocol
- luminometer
- micropipette 200 μL
- cuvette rack
- nitrile/latex gloves

Chemicals should be kept at -20 ˚C.

A.4.2 Reading procedure

Method as outlined by Promega's Enliten Total ATP Bio-contamination Detection Kit.

1. Tap the BacTiter-Glo™ substrate to ensure that all dried material is at the bottom. Wear gloves to prevent contamination of the rubber stopper.
2. Thaw the BacTiter-Glo™ substrate and the BacTiter-Glo™ buffer and let reach room temperature.
3. Transfer the BacTiter-Glo™ substrate and the BacTiter-Glo™ buffer in the substrate container to form the BacTiter-Glo™ Reagent. Optional: a syringe with a needle can be used to perform this step.
4. Mix the reagent by gently vortexing, swirling or by inverting the bottle until a homogeneous solution is obtained.
5. Store left overs at -20 ˚C for up to 5 days and at -70 ˚C for a few weeks.
6. Transfer 100 μl of BacTiter-Glo™ reagent to each VWR® SuperClear™ microcentrifuge tube with samples on them.
7. Mix and read the luminescence immediately and record the RLU reading.

A.4.3 ATP standard curve

1. Prepare 1μM ATP from the concentrated.
2. Prepare serial dilutions of ATP (10^{-1}μM, $5x10^{-2}$μM, 10^{-2}μM and 10^{-3}μM).
8. Place 100 μl of each standard in a labeled VWR® SuperClear™ microcentrifuge tube.

9. Add a volume of BacTiter-Glo™ Reagent (100 µl) equal to the volume of ATP standard present in each microcentrifuge tube (1:1 ratio).
10. Mix contents and read the luminescence immediately and record the RLU reading.

Appendix B. Experimental Data.

Appendix B contains water quality data and biofilm growth data.

Table B.1. CTH1 water quality data.

CTH1													
	Units	4-Mar-08	11-Feb-09	14-May-09	14-May-09	4-Jun-09	7-Jul-09	5-Aug-09	10-Sep-09	8-Oct-09	9-Nov-09	8-Dec-09	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		Lab	Lab	Fleming	Lab	Fleming	Fleming	Lab	Fleming	Fleming	Fleming	Fleming	Limits
Alkalinity ($CaCO_3$)	mg/L	NA	318	312	308	304	279	291		282	312.5	274	30-500*
Hardness ($CaCO_3$)	mg/L	110	298	NA	400	364	396	495	514	355	364	360	80-100*
Calcium (Ca^{+2})	mg/L	38	108	135	130	138	145	181	194	129	132	131	
Magnesium (Mg^{+2})	mg/L	3.50	6.57	8.23	7.82	7.07	7.90	10.80	7.17	7.65	8.25	8.18	
Manganese (Mn^{+2})	mg/L	NA	0.008	0.013	0.011	0.015	0.009	0.003	0.010	0.009	0.004	0.004	0.05
Potassium (K)	mg/L	59.0	6.16	5.84	6.01	0.16	0.16	5.30	0.17	0.18	0.16	0.15	
Sodium (Na)	mg/L	29	116	105	67	84	85	120	114	112	110	110	200
Anions													
Chloride (Cl)	mg/L	38	190	210	216	301	388	370	488	443		195	250
Nitrite (N)	mg/L	0.020	<0.060	<0.060	<0.015	<0.015	<0.015	<0.060	non detect	non detect		non detect	1
Nitrate (N)	mg/L	0.90	2.21	2.03	0.20	2.05	2.44	2.59	2.68	2.87		2.77	10
Sulphate (SO_4)	mg/L	34	24	21	21	21	23	24	25	26		28	500
Metals													
Iron (Fe)	mg/L	<0.01	0.29	0.04	0.04	0.08	0.03	0.04	0.02	0.01	0.01	0.01	0.3
Lead (Pb)	mg/L	<0.0005	<0.005	<0.003	<0.003	0.005	0.016	<0.005	0.015	0.016	0.015	0.013	0.01
Zinc (Zn)	mg/L	<0.005	0.08	0.06	0.06	0.05	0.87	0.06	0.43	0.48	0.37	0.23	5
Bacteria													
Total Coliform	cfu/100mL	NA	0	23	8	11	938	320	39	30	<3**	22	Not Detectable
E. Coli	cfu/100mL	NA	0	0	<3**	<3**	<3**	19	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL	NA	0	0	NA	NA	NA	25					Not Detectable
Other Parameters													
pH		NA	8.08	7.69	7.08	6.99	6.99	7.71	7.09	7.44	6.98	7.11	6.5-8.5*
Conductivity	µS/cm	NA	1110	1120	1137	1275	1401	1660	1833	1628	1644	1292	
Total Dissolved Solids	mg/L	NA	623	706	NA	NA	NA	1070					500

* operational guideline range

(highlighted) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.2. CTH1 water quality data (cont.).

		CTH1											
	Units	17-May-10	14-Jun-10	19-Jul-10	16-Aug-10	13-Sep-10	12-Oct-10	15-Nov-10	13-Dec-10	14-Feb-11	14-Mar-11	11-Apr-11	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Limits**
Alkalinity ($CaCO_3$)	mg/L	302	268	302.5	300	292.5	310	318	368	318	293	313	30-500*
Hardness ($CaCO_3$)	mg/L	364	452	530	360	572	524	500	460	410	410	420	80-100*
Calcium (Ca^{+2})	mg/L	170	180		214	199	202	159	173	180	157	148	
Magnesium (Mg^{+2})	mg/L	11.62	12.29		15.77	14.26	12.71	12.46	10.82	9.05	8.64	8.33	
Manganese (Mn^{+2})	mg/L	<0.004	<0.004	<0.004	<0.004	<0.004	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.05
Potassium (K)	mg/L	8.20	7.00		7.49	6.24	6.07	not done	7.50	5.21	4.96	4.60	
Sodium (Na)	mg/L	131	129		126	160	152	149	252	153	141	137	200
Anions													
Chloride (Cl)	mg/L	151	168	over range	over range	203	435	421		378	294	274	250
Nitrite (N)	mg/L	non detect	non detect	non detect	non detect	<0.03	<0.03	<0.03		<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	2.03	2.03	2.39	2.32	2.61	2.99	2.97		2.48	2.32	1.34	10
Sulphate (SO_4)	mg/L	21	24	26	26	27	25	30		29	26	23	500
Metals													
Iron (Fe)	mg/L	0.02	0.01	<0.010	0.02	0.02	0.01	0.02	0.01	<0.010	<0.010	<0.010	0.3
Lead (Pb)	mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.01
Zinc (Zn)	mg/L	0.02	0.03	0.01	<0.010	0.03	0.02	0.01	0.03	0.02	0.04	0.03	5
Bacteria													
Total Coliform	cfu/100mL	not done	1696	794	182	106	102	not done	<3**	<3**	141	<3**	Not Detectable
E. Coli	cfu/100mL	not done	<3**	<3**	<3**	<3**	<3**	not done	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL												Not Detectable
Other Parameters													
pH		6.96	6.96	7.2	7.08	6.97	7.24	7.09	7.69	7.29	7.23	7.19	6.5-8.5*
Conductivity	μS/cm	1497	1629	1875	1897	1972	1923	1808	2260	1815	1555	1493	
Total Dissolved Solids	mg/L												500

* operational guideline range

(yellow) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.3. ETH1 water quality data.

ETH1													
	Units	4-Mar-08	11-Feb-09	14-May-09	14-May-09	4-Jun-09	7-Jul-09	5-Aug-09	10-Sep-09	8-Oct-09	9-Nov-09	8-Dec-09	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		**Lab**	**Lab**	**Fleming**	**Lab**	**Fleming**	**Fleming**	**Lab**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Limits**
Alkalinity ($CaCO_3$)	mg/L	NA	335	305	307	306	286	284		290	297.5	312	30-500*
Hardness ($CaCO_3$)	mg/L	330	260	NA	370	376	324	400	510	328	330	336	80-100*
Calcium (Ca^{+2})	mg/L	120	94.2	120	155	122	123	145	193	119	119	121	
Magnesium (Mg^{+2})	mg/L	10.00	5.95	7.61	7.93	6.64	6.82	9.07	7.17	7.45	7.83	7.93	
Manganese (Mn^{+2})	mg/L	NA	<0.001	0.0003	<0.001	0.002	0.002	<0.001	0.002	0.005	0.005	0.006	0.05
Potassium (K)	mg/L	69.0	3.9	3.7	3.7	0.2	0.4	4.5	0.2	0.2	0.2	0.3	
Sodium (Na)	mg/L	150	107	65	102	72	65	75	72	74	66	65	200
Anions													
Chloride (Cl)	mg/L	310	130	129	190	224	240	220	383	471	over range	180	250
Nitrite (N)	mg/L	<0.010	<0.060	<0.015	<0.060	<0.015	<0.015	<0.060	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	2.80	2.15	0.10	2.11	2.13	2.03	1.98	2.42	2.68	2.49	3.33	10
Sulphate (SO_4)	mg/L	31	23	18	20	20	19	18	21	24	26	27	500
Metals													
Iron (Fe)	mg/L	<0.01	0.02	0.12	<0.01	0.07	0.09	<0.01	0.092	0.063	0.083	0.076	0.3
Lead (Pb)	mg/L	NA	<0.005	<0.003	<0.005	<0.003	<0.003	<0.005	<0.003	<0.003	<0.003	<0.003	0.01
Zinc (Zn)	mg/L	NA	0.03	0.02	0.01	0.04	0.01	0.03	0.01	0.01	0.01	0.00	5
Bacteria													
Total Coliform	cfu/100mL	NA	0	<3**	0	<3**	87	137	5	33	<3	<3	Not Detectable
E. Coli	cfu/100mL	NA	0	<3**	0	<3**	<3**	66	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL	NA	0	NA	0	NA	NA	99					Not Detectable
Other Parameters													
pH		NA	8.02	7.14	7.70	7.01	7.04	7.74	7.12	7.16	6.88	7.27	6.5-8.5*
Conductivity	μS/cm	NA	995	1202	1070	1119	1081	1190	1562	1653	1732	1383	
Total Dissolved Solids	mg/L	NA	591	NA	669	NA	NA	709					500

* operational guideline range

(highlighted) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.4. ETH1 water quality data (cont.).

		ETH1											
	Units	17-May-10	14-Jun-10	19-Jul-10	16-Aug-10	13-Sep-10	12-Oct-10	15-Nov-10	13-Dec-10	14-Feb-11	14-Mar-11	11-Apr-11	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Limits
Alkalinity ($CaCO_3$)	mg/L	302	284	295	287.5	292.5	297.5	295	315	332.5	312.5	312.5	30-500*
Hardness ($CaCO_3$)	mg/L	336	492	430	408	660	628	568	600	440	440	340	80-100*
Calcium (Ca^{+2})	mg/L	141	147	216	226	156	153	186	202	150	119	122	
Magnesium (Mg^{+2})	mg/L	10.25	10.58	15.13	16.56	11.48	11.32	14.69	9.74	7.29	6.78	6.97	
Manganese (Mn^{+2})	mg/L	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.05
Potassium (K)	mg/L	7.6	8.2	5.9	6.6	6.8	7.8	not done	6.3	4.9	4.1	4.0	
Sodium (Na)	mg/L	106	102	130	133	61	62	147	149	111	102	109	200
Anions													
Chloride (Cl)	mg/L	121	135	156	160	187	414	369	394	221	157	158	250
Nitrite (N)	mg/L	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	1.82	1.75	1.98	1.93	2.24	2.30	4.10	2.73	2.16	1.86	1.32	10
Sulphate (SO_4)	mg/L	19	20	22	22	25	25	31	33	26	23	19	500
Metals													
Iron (Fe)	mg/L	0.011	0.010	<0.010	<0.010	0.011	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.3
Lead (Pb)	mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.01
Zinc (Zn)	mg/L	0.01	<0.010	<0.010	<0.010	0.01	<0.010	<0.010	0.06	0.05	0.05	0.04	5
Bacteria													
Total Coliform	cfu/100mL	not done	403	3	<3**	<3**	<3**	not done	3	3	<3**	<3**	Not Detectable
E. Coli	cfu/100mL	not done	<3**	<3**	<3**	<3**	<3**	not done	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL												Not Detectable
Other Parameters													
pH		7.03	7.01	7.23	7.08	7.00	7.18	7.09	7.37	7.5	7.3	7.22	6.5-8.5*
Conductivity	µS/cm	1237	1326	1415	1518	1789	1983	1969	1883	1373	1142	1183	
Total Dissolved Solids	mg/L												500

* operational guideline range; [yellow highlight] Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.5. ETH2 water quality data.

ETH2													
	Units	4-Mar-08	11-Feb-09	14-May-09	14-May-09	4-Jun-09	7-Jul-09	5-Aug-09	10-Sep-09	8-Oct-09	9-Nov-09	8-Dec-09	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		**Lab**	**Lab**	**Fleming**	**Lab**	**Fleming**	**Fleming**	**Lab**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Limits**
Alkalinity ($CaCO_3$)	mg/L	NA	327	296	300	292	282	299		290	305	310	30-500*
Hardness ($CaCO_3$)	mg/L	520	374	NA	422	384	388	414	547	378	380	370	80-100*
Calcium (Ca^{+2})	mg/L	190	137	137	137	120	188	152	199	139	139	136	
Magnesium (Mg^{+2})	mg/L	11.00	7.81	7.93	8.18	6.63	7.58	8.49	12.02	7.75	7.89	7.63	
Manganese (Mn^{+2})	mg/L	NA	<0.001	0	<0.001	0.001	0.001	<0.001	0.002	0.006	0.004	0.004	0.05
Potassium (K)	mg/L	2.8	2.9	2.3	2.3	0.4	0.4	2.6	0.1	0.5	0.5	0.5	
Sodium (Na)	mg/L	130	150	82	134	71	103	109	103	105	108	109	200
Anions													
Chloride (Cl)	mg/L	310	300	235	270	225	397	300	482	422	over range	192	250
Nitrite (N)	mg/L	<0.010	<0.060	<0.015	<0.060	<0.015	<0.015	<0.060	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	2.30	1.94	0.10	2.23	2.12	1.92	1.86	2.61	2.15	2.69	2.85	10
Sulphate (SO_4)	mg/L	31	27	26	25	20	28	27	24	26	30	27	500
Metals													
Iron (Fe)	mg/L	<0.01	<0.01	0.03	<0.01	0.05	0.04	0.01	0.05	0.04	0.06	0.04	0.3
Lead (Pb)	mg/L	NA	<0.005	<0.003	<0.005	<0.003	<0.003	<0.005	0.01125	<0.003	<0.003	<0.003	0.01
Zinc (Zn)	mg/L	NA	0.02	0.01	0.01	0.05	0.01	0.02	0.02	0.01	0.02	0.01	5
Bacteria													
Total Coliform	cfu/100mL	NA	0	<3**	1	<3**	<3**	3	<3**	<3**	13	<3**	Not Detectable
E. Coli	cfu/100mL	NA	0	<3**	0	<3**	<3**	0	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL	NA	0	NA	0	NA	NA	0					Not Detectable
Other Parameters													
pH		NA	8.00	7.05	7.71	7.05	7.06	7.72	7.09	7.19	6.95	7.05	6.5-8.5*
Conductivity	μS/cm	NA	1390	1430	1350	1120	1428	1470	1790	1576	1504	1175	
Total Dissolved Solids	mg/L	NA	834	NA	843	NA	NA	826					500

* operational guideline range

(highlighted) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.6. ETH2 water quality data (cont.).

ETH2													
	Units	17-May-10	14-Jun-10	19-Jul-10	16-Aug-10	13-Sep-10	12-Oct-10	15-Nov-10	13-Dec-10	14-Feb-11	14-Mar-11	11-Apr-11	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Fleming	Limits
Alkalinity ($CaCO_3$)	mg/L	286	289	295	297.5	297.5	302.5	310	482.5	317.5	305	295	30-500*
Hardness ($CaCO_3$)	mg/L	404	524	492	420	552	564	492	510	330	330	490	80-100*
Calcium (Ca^{+2})	mg/L	188.9	195.2	203.7	196.2	199.2	190.2	156	183.0	192.6	186.0	179.0	
Magnesium (Mg^{+2})	mg/L	12.04	12.09	13.11	12.59	14.32	12.23	11.14	8.02	8.67	9.51	9.07	
Manganese (Mn^{+2})	mg/L	<0.004	<0.004	<0.004	0.004	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.05
Potassium (K)	mg/L	4.885	5.005	5.104	7.763	7.945	6.026	not done	3.672	3.371	3.163	2.900	
Sodium (Na)	mg/L	141.5	144.8	154.4	149.1	161.2	153.3	139	235.5	140.3	161.8	162.3	200
Anions													
Chloride (Cl)	mg/L	178	185	over range	408	195	442	431	383	363	389	185	250
Nitrite (N)	mg/L	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	1.46	1.63	1.76	1.76	1.88	2.58	2.90	3.29	2.29	2.23	1.31	10
Sulphate (SO_4)	mg/L	24	27	28	26	27	25	30	42	30	29	21	500
Metals													
Iron (Fe)	mg/L	<0.010	<0.010	<0.010	<0.010	0.02	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.3
Lead (Pb)	mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.01
Zinc (Zn)	mg/L	0.01	<0.010	0.03	0.03	0.05	<0.010	<0.010	0.03	<0.010	0.03	0.01	5
Bacteria													
Total Coliform	cfu/100mL	not done	11	5	<3**	<3**	<3**	not done	<3**	<3**	<3**	<3**	Not Detectable
E. Coli	cfu/100mL	not done	<3**	<3**	<3**	<3**	<3**	not done	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL												Not Detectable
Other Parameters													
pH		7.02	6.90	7.27	6.99	6.98	7.11	7.05	7.54	7.32	7.23	7.19	6.5-8.5*
Conductivity	µS/cm	1716	1768	1812	1836	1883	1911	1695	1935	1796	1865	1793	
Total Dissolved Solids	mg/L												500

* operational guideline range; [yellow highlight] Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.7. ETH3 water quality data.

		ETH3											
	Units	4-Mar-08	11-Feb-09	14-May-09	14-May-09	4-Jun-09	7-Jul-09	5-Aug-09	10-Sep-09	8-Oct-09	9-Nov-09	8-Dec-09	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		Lab	Lab	Fleming	Lab	Fleming	Fleming	Lab	Fleming	Fleming	Fleming	Fleming	Limits
Alkalinity ($CaCO_3$)	mg/L	NA	331	310	309	310	283	293		290	298	314	30-500*
Hardness ($CaCO_3$)	mg/L	410	280	NA	379	340	388	463	547	342	330	353	80-100*
Calcium (Ca^{+2})	mg/L	150	101	124	86	129	142	169	199	124	119	129	
Magnesium (Mg^{+2})	mg/L	9.40	6.39	7.86	8.11	6.96	8.07	10.20	12.02	7.68	7.83	7.20	
Manganese (Mn^{+2})	mg/L	NA	0.005	0.002	0.001	0.002	0.001	0.001	0.002	0.002	0.005	0.002	0.05
Potassium (K)	mg/L	4.0	3.6	3.3	3.3	0.1	0.1	4.3	0.1	0.1	0.2	0.1	
Sodium (Na)	mg/L	120	120	69	106	80	85	104	103	103	66	105	200
Anions													
Chloride (Cl)	mg/L	220	180	188	200	265	399	340	482	499	over range	163	250
Nitrite (N)	mg/L	0.030	<0.060	<0.015	<0.060	<0.015	<0.015	<0.060	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	2.50	2.34	0.70	2.09	2.17	2.48	2.41	2.61	2.77	2.49	2.81	10
Sulphate (SO_4)	mg/L	33	24	18	21	20	22	23	24	26	26	32	500
Metals													
Iron (Fe)	mg/L	<0.01	0.17	0.04	0.03	0.10	0.04	0.01	0.05	0.03	0.08	0.03	0.3
Lead (Pb)	mg/L	<0.0005	<0.005	<0.003	<0.005	<0.003	0.016	<0.005	0.011	<0.003	<0.003	0.006	0.01
Zinc (Zn)	mg/L	9.80	2.23	1.16	1.28	0.72	0.02	1.46	0.02	0.02	0.01	0.01	5
Bacteria													
Total Coliform	cfu/100mL	NA	0	<3**	1	<3**	3	0	<3**	<3**	<3**	<3**	Not Detectable
E. Coli	cfu/100mL	NA	0	<3**	0	<3**	<3**	0	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL	NA	0	NA	0	NA	NA	0					Not Detectable
Other Parameters													
pH		NA	8.04	7.05	7.76	7.07	7.07	7.63	7.09	7.32	6.88	7.06	6.5-8.5*
Conductivity	µS/cm	NA	1080	1185	1140	1219	1375	1540	1790	1722	1732	1360	
Total Dissolved Solids	mg/L	NA	637	NA	671	NA	NA	969					500

* operational guideline range

(yellow highlight) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.8. ETH3 water quality data (cont.).

		ETH3											
	Units	17-May-10	14-Jun-10	19-Jul-10	16-Aug-10	13-Sep-10	12-Oct-10	15-Nov-10	13-Dec-10	14-Feb-11	14-Mar-11	11-Apr-11	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Limits**
Alkalinity ($CaCO_3$)	mg/L	310	262	302.5	300	302.5	305	307.5	337.5	323	295	318	30-500*
Hardness ($CaCO_3$)	mg/L	376	480	526	516	580	604	568	560	520	520	380	80-100*
Calcium (Ca^{+2})	mg/L	157	188	182	211	204	197	208	191	170	145	128	
Magnesium (Mg^{+2})	mg/L	11.35	13.24	13.19	13.79	14.22	13.53	12.73	9.28	8.68	8.08	7.17	
Manganese (Mn^{+2})	mg/L	<0.004	<0.004	<0.004	0.004	<0.004	0.004	0.006	<0.004	0.017	0.005	<0.004	0.05
Potassium (K)	mg/L	7.2	7.2	8.0	6.7	6.2	6.5	not done	5.7	4.9	4.3	3.5	
Sodium (Na)	mg/L	127	138	138	157	121	153	156	195	132	136	125	200
Anions													
Chloride (Cl)	mg/L	146	173	over range	over range	199	412	326	433	314	255	374	250
Nitrite (N)	mg/L	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	2.03	2.09	2.25	2.17	2.36	2.42	3.92	2.68	2.24	1.97	1.28	10
Sulphate (SO_4)	mg/L	21	23	25	26	27	25	34	33	29	27	25	500
Metals													
Iron (Fe)	mg/L	0.01	<0.010	<0.010	0.04	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.3
Lead (Pb)	mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.01
Zinc (Zn)	mg/L	0.93	1.07	0.70	1.42	1.35	1.22	2.07	1.44	1.55	1.08	0.74	5
Bacteria													
Total Coliform	cfu/100mL	not done	<3**	5	<3**	<3**	<3**	not done	<3**	<3**	33	<3**	Not Detectable
E. Coli	cfu/100mL	not done	<3**	<3**	<3**	<3**	<3**	not done	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL												Not Detectable
Other Parameters													
pH		7.02	6.9	7.09	6.81	6.88	7.84	7.06	7.39	7.35	7.17	7.36	6.5-8.5*
Conductivity	µS/cm	1464	1665	1768	1813	1930	1940	1950	2037	1675	1473	1310	
Total Dissolved Solids	mg/L												500

* operational guideline range

[highlighted] Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.9. MW2 water quality data.

MW2													
	Units	**4-Mar-08**	**11-Feb-09**	**14-May-09**	**14-May-09**	**4-Jun-09**	**7-Jul-09**	**5-Aug-09**	**10-Sep-09**	**8-Oct-09**	**9-Nov-09**	**8-Dec-09**	**Ontario Drinking Water Standards, Objectives and Guidelines**
Cations		**Lab**	**Lab**	**Fleming**	**Lab**	**Fleming**	**Fleming**	**Lab**					**Limits**
Alkalinity ($CaCO_3$)	mg/L	-	313	266	272	266	250	289		264	272.5	252	30-500*
Hardness ($CaCO_3$)	mg/L	-	333	NA	405	342	329	350	357	380	378	347	80-100*
Calcium (Ca^{+2})	mg/L	-	122	79	147	67	67	69	71	80	78	70	
Magnesium (Mg^{+2})	mg/L	-	6.85	44.54	46.90	41.55	43.39	43.50	43.94	43.65	44.26	42.13	
Manganese (Mn^{+2})	mg/L	-	0.021	0.020	0.022	0.010	0.010	0.011	0.010	0.009	0.009	0.005	0.05
Potassium (K)	mg/L	-	3.5	9.8	9.7	0.2	0.2	9.6	0.3	0.3	0.3	0.4	
Sodium (Na)	mg/L	-	100	83	132	98	101	119	107	106	106	105	200
Anions													
Chloride (Cl)	mg/L	-	160	496	290	206	293	320	382	380		175	250
Nitrite (N)	mg/L	-	<0.060	<0.015	<0.060	<0.015	<0.015	<0.060	<0.03	<0.03		<0.03	1
Nitrate (N)	mg/L	-	2.28	<0.10	<0.05	0.36	0.27	<0.05	non detect	non detect		non detect	10
Sulphate (SO_4)	mg/L	-	25	3	1	1	1	1	0	0		0	500
Metals													
Iron (Fe)	mg/L	-	1.31	0.88	1.12	0.05	0.12	0.73	0.16	0.01	0.01	0.01	0.3
Lead (Pb)	mg/L	-	<0.005	<0.003	<0.005	0.010	0.019	<0.005	0.019	0.018	0.015	0.012	0.01
Zinc (Zn)	mg/L	-	0.03	0.01	<0.01	0.06	0.05	<0.01	0.05	0.05	0.04	0.03	5
Bacteria													
Total Coliform	cfu/100mL	-	10	<3**	0	<3**	219	0	<3	<3	<3	<3	Not Detectable
E. Coli	cfu/100mL	-	0	<3**	0	<3**	<3**	0	<3	<3	<3	<3	Not Detectable
Fecal Coliforms	cfu/100mL	-	0	NA	0	NA	NA	0					Not Detectable
Other Parameters													
pH		-	7.89	7.43	7.95	7.44	7.54	NA	7.51	7.78	7.49	7.54	6.5-8.5*
Conductivity	µS/cm	-	994	1368	1330	1447	1407	1380	1448	1356	1367	1135	
Total Dissolved Solids	mg/L	-	566	NA	823	NA	NA	723					500

* operational guideline range

[Yellow highlight] Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.10. MW1 water quality data.

		MW1											
	Units	17-May-10	14-Jun-10	19-Jul-10	16-Aug-10	13-Sep-10	12-Oct-10	15-Nov-10	13-Dec-10	14-Feb-11	14-Mar-11	11-Apr-11	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Limits**
Alkalinity ($CaCO_3$)	mg/L	266	230	272.5	252.5	257.5	265	250	255	257.5	257.5	255	30-500*
Hardness ($CaCO_3$)	mg/L	368	392	394	376	368	388	400	400	390	390	390	80-100*
Calcium (Ca^{+2})	mg/L	78.978	79	51	78	79	75	73	79	72	75	73	
Magnesium (Mg^{+2})	mg/L	55.111	56.77	55.90	50.07	52.92	55.11	51.44	43.50	44.22	44.62	44.52	
Manganese (Mn^{+2})	mg/L	0.007836	0.007	<0.004	0.007	0.006	0.007	<0.004	0.008	0.009	0.009	0.009	0.05
Potassium (K)	mg/L	11.1	11.2	11.3	11.7	11.4	11.2		11.2	10.9	10.7	10.3	
Sodium (Na)	mg/L	129	133	126	129	131	126	118	141	121	130	130	200
Anions													
Chloride (Cl)	mg/L	162	167	over range	162	164	307	300	313	312	305	337	250
Nitrite (N)	mg/L	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	10
Sulphate (SO_4)	mg/L	0.69	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	0.29	<0.28	0.74	1.05	500
Metals													
Iron (Fe)	mg/L	0.25	0.07	<0.010	0.02	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.3
Lead (Pb)	mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.01
Zinc (Zn)	mg/L	0.022	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.042	<0.010	0.024	<0.010	5
Bacteria													
Total Coliform	cfu/100mL	not done	<3**	19	5	65	65	not done	19	<3**	<3**	<3**	Not Detectable
E. Coli	cfu/100mL	not done	<3**	<3**	<3**	<3**	3	not done	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL												Not Detectable
Other Parameters													
pH		7.47	7.32	7.59	7.12	7.43	7.69	7.47	7.66	7.73	7.54	7.52	6.5-8.5*
Conductivity	µS/cm	1450	1460	1457	1432	1444	1439	1419	1442	1448	1455	1470	
Total Dissolved Solids	mg/L												500

* operational guideline range; (yellow highlight) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.11. MW3 water quality data.

MW3													
	Units	17-May-10	14-Jun-10	19-Jul-10	16-Aug-10	13-Sep-10	12-Oct-10	15-Nov-10	13-Dec-10	14-Feb-11	14-Mar-11	11-Apr-11	Ontario Drinking Water Standards, Objectives and Guidelines
Cations		**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Fleming**	**Limits**
Alkalinity ($CaCO_3$)	mg/L	304	268	300	290	285	285	280	303	338	368	253	30-500*
Hardness ($CaCO_3$)	mg/L	240	480	358	320	420	560	620	630	310	310	280	80-100*
Calcium (Ca^{+2})	mg/L	110.03	114	65	156	201	198	232	213	132	109	99	
Magnesium (Mg^{+2})	mg/L	8.7664	9.03	9.75	10.46	14.09	9.03	16.36	11.12	6.92	6.30	5.94	
Manganese (Mn^{+2})	mg/L	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.05
Potassium (K)	mg/L	5.6	5.5	6.2	7.5	7.6	5.4	not done	6.0	4.2	3.7	3.3	
Sodium (Na)	mg/L	58	53	52	62	151	74	90	106	78	68	61	200
Anions													
Chloride (Cl)	mg/L	61	76	89	101	126	307	383	361	146	84	119	250
Nitrite (N)	mg/L	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	1
Nitrate (N)	mg/L	1.45	1.51	1.73	1.73	1.98	2.00	2.38	2.39	2.12	2.36	1.05	10
Sulphate (SO_4)	mg/L	15.32	16.20	17.15	17.18	18.92	19.01	25.42	29.06	23.72	21.67	16.12	500
Metals													
Iron (Fe)	mg/L	0.01	0.01	0.02	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.3
Lead (Pb)	mg/L	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.01
Zinc (Zn)	mg/L	0.030	<0.010	0.020	<0.010	<0.010	<0.010	<0.010	0.026	<0.010	0.028	<0.010	5
Bacteria													
Total Coliform	cfu/100mL	not done	8	49	8	<3**	<3**	not done	3	<3**	<3**	3	Not Detectable
E. Coli	cfu/100mL	not done	<3**	<3**	3	<3**	<3**	not done	<3**	<3**	<3**	<3**	Not Detectable
Fecal Coliforms	cfu/100mL												Not Detectable
Other Parameters													
pH		7.15	7	7.24	6.72	7.07	7.28	6.96	7.22	7.35	7.27	7.22	6.5-8.5*
Conductivity	μS/cm	854	913	952	1048	1215	1540	1781	1755	1119	910	824	
Total Dissolved Solids	mg/L												500

* operational guideline range

(yellow highlight) Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

** method used for analysis requires 0 counts to be reported as <3

Table B.12. TC and *E. coli* (Victoria County Health Unit and CAWT Labs).

	CTH1				ETH1				ETH2				ETH3				MW1				MW3			
	CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni	
Date	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*
14-May-09	8	<3			<3	<3			<3	<3			<3	<3			<3	<3						
4-Jun-09	11	<3			<3	<3			<3	<3			<3	<3			<3	<3						
7-Jul-09	938	<3			87	<3			<3	<3			3	<3			219	<3						
4-Aug-09	1696	<3			132	<3			11	<3			8	<3			3	<3						
10-Sep-09	39	<3			5	<3			<3	<3			<3	<3			<3	<3						
8-Oct-09	30	<3			33	<3			<3	<3			<3	<3			<3	<3						
9-Nov-09	<3	<3			<3	<3			13	<3			<3	<3			<3	<3						
8-Dec-09	22	<3			<3	<3			<3	<3			<3	<3			<3	<3						
6-Apr-10			O/G	O/G			2.0	0			0.0	0			0.0	0			1.0	0			O/G	O/G
6-Apr-10			O/G	O/G			3.0	0			0.0	0			0.0	0			0.0	0			O/G	O/G
12-Apr-10			>80	0			3.0	0			0.0	0			0.0	0			0.0	0			O/G	O/G
12-Apr-10			>80	0			2.0	0			0.0	0			1.0	0			0.0	0			O/G	O/G
20-Apr-10			6.0	0			4.0	0			0.0	0			1.0	0			0.0	0			O/G	O/G
20-Apr-10			6.0	0			3.0	0			0.0	0			1.0	0			0.0	0			O/G	O/G
3-May-10			1.0	0			0.0	0			0.0	0			0.0	0			0.0	0			O/G	O/G
3-May-10			2.0	0			1.0	0			0.0	0			0.0	0			0.0	0			O/G	O/G
10-May-10			>80	0			52.0	0			20.0	0			14.0	0			0.0	0			0.0	0
10-May-10			>80	0			41.0	1			9.0	0			10.0	0			0.0	0			3.0	0
17-May-10			>80	0			5.0	0			5.0	0			1.0	0			0.0	0			0.0	0
17-May-10			>80	0			2.0	0			2.0	0			0.0	0			0.0	0			2.0	0
25-May-10	1.0	0	13.0	0			0.0	0			0.0	0			0.0	0			0.0	0			O/G	O/G
25-May-10	0.0	0	19.0	0			0.0	0			0.0	0			0.0	0			0.0	0			O/G	O/G
31-May-10			42.0	0			2.0	0			1.0	0			1.0	0			10.0	0	0.0	0	O/G	O/G
31-May-10			46.0	0			3.0	1			3.0	0			1.0	0			12.0	0	0.0	0	O/G	O/G
3-Jun-10			>80	0			2.0	0			0.0	0			0.0	0			0.0	0			57.0	0
3-Jun-10			>80	1			0.0	0			0.0	0			0.0	0			0.0	0			68.0	0
7-Jun-10			O/G	13			9.0	0			2.0	0			1.0	0			2.0	0			6.0	0
7-Jun-10			O/G	14			9.0	0			5.0	0			0.0	0			0.0	0			7.0	0
14-Jun-10	1696.0	<3	>80	5	403.0	<3	>80	5	11.0	<3	32.0	1	<3	<3	11.0	0	<3	<3	0.0	0	8.0	<3	15.0	0
14-Jun-10			>80	0			>80	3			41.0	0			16.0	0			0.0	0			16.0	0
17-Jun-10			O/G	O/G			46.0	1			10.0	0			2.0	0			0.0	0			4.0	0
17-Jun-10			O/G	O/G			47.0	0			12.0	0			1.0	0			0.0	0			5.0	0
21-Jun-10			>80	0			13.0	0			4.0	0			0.0	0			33.0	0			6.0	0
21-Jun-10			>80	0			15.0	0			6.0	0			0.0	0			23.0	0			3.0	0
23-Jun-10			>80	0			22.0	0			3.0	0			12.0	0			0.0	0			6.0	0
23-Jun-10			>80	0			25.0	0			3.0	0			10.0	0			0.0	0			3.0	0
24-Jun-10			>80	2			12.0	0			1.0	0			26.0	0			0.0	0			1.0	0
24-Jun-10			>80	1			9.0	0			2.0	0			21.0	0			0.0	0			3.0	0
28-Jun-10			>80	6			6.0	1			0.0	0			3.0	0			3.0	0			5.0	0
28-Jun-10			>80	6			10.0	1			1.0	0			3.0	0			2.0	0			3.0	0
5-Jul-10			55.0	3			11.0	0			3.0	0			7.0	0							O/G	O/G
5-Jul-10			52.0	1			9.0	0			2.0	0			9.0	0							O/G	O/G
12-Jul-10			>80	1			16.0	0			10.0	0			11.0	0			O/G	O/G			O/G	O/G
12-Jul-10			>80	6			17.0	0			6.0	0			15.0	0			O/G	O/G			O/G	O/G
19-Jul-10	794.0	<3	>80	13	3.0	<3	4.0	0	5.0	<3	1.0	0	5.0	<3	34.0	0	19.0	<3	7.0	O/G	49.0	<3	4.0	0
19-Jul-10			>80	13			3.0	0			1.0	0			30.0	0			5.0	O/G			3.0	0
9-Aug-10			>80	17			>80	0			4.0	0			9.0	0			O/G	O/G			O/G	O/G
9-Aug-10			>80	10			>80	0			6.0	1			11.0	0			O/G	O/G			O/G	O/G
16-Aug-10	182.0	<3	26.0	0	<3	<3	7.0	0	<3	<3	4.0	1	<3	<3	6.0	0	5.0	<3	O/G	O/G	8.0	3	O/G	1
16-Aug-10			26.0	0			4.0	0			4.0	0			9.0	0			O/G	O/G			O/G	O/G
23-Aug-10			O/G	O/G			4.0	0			0.0	0			1.0	0			12.0	0			O/G	O/G
23-Aug-10			O/G	O/G			3.0	0			1.0	0			1.0	0			14.0	0			O/G	O/G

*O/G = Overgrown, too many different kinds of bacteria present from contamination to determine if there are positive counts of Total Coliform and E.Coli

Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

Table B.13. TC and *E. coli* (Victoria County Health Unit and CAWT Labs) (cont.).

	CTH1				ETH1				ETH2				ETH3				MW1				MW3			
	CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni		CAWT		Victoria Co. Health Uni	
Date	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*	Tot. Coli	*E. Coli*
30-Aug-10			>80	0			3.0	0			2.0	0			1.0	0			1.0	0			O/G	O/G
30-Aug-10			>80	0			1.0	0			1.0	0			1.0	0			1.0	0			O/G	O/G
7-Sep-10			>80	0			1.0	0			0.0	0			3.0	0			>80	0			40.0	0
7-Sep-10			>80	0			2.0	0			0.0	0			2.0	0			>80	0			46.0	0
13-Sep-10	106.0	<3	>80	0	<3	<3	O/G	O/G	<3	<3	3.0	0	<3	<3	1.0	0	65.0	<3	>80	0	<3	<3	20.0	1
13-Sep-10			>80	0			O/G	O/G			0.0	0			4.0	0			>80	0			18.0	0
20-Sep-10			>80	4			2.0	0			0.0	0			0.0	0			36.0	0			16.0	0
20-Sep-10			>80	2			1.0	0			0.0	0			2.0	0			40.0	0			21.0	0
27-Sep-10			>80	1			0.0	0			1.0	0			2.0	0			O/G	O/G			14.0	0
27-Sep-10			>80	0			0.0	0			0.0	0			1.0	0			O/G	O/G			6.0	0
4-Oct-10			>80	0			2.0	0			1.0	0			0.0	0			O/G	O/G			12.0	1
4-Oct-10			>80	0			3.0	0			0.0	0			2.0	0			O/G	O/G			13.0	0
12-Oct-10	102.0	<3	>80	0	<3	<3	0.0	0	<3	<3	0.0	0	<3	<3	1.0	0	65.0	3	O/G	O/G	<3	<3	6.0	0
12-Oct-10			>80	0			0.0	0			0.0	0			2.0	0			O/G	O/G			3.0	0
18-Oct-10			>80	0			3.0	0			2.0	0			4.0	0			O/G	O/G			>80	0
18-Oct-10			>80	0			2.0	0			2.0	0			6.0	0			O/G	O/G			>80	1
25-Oct-10			>80	0			2.0	0			1.0	0			8.0	0			O/G	O/G			>80	0
25-Oct-10			>80	0			1.0	0			1.0	0			6.0	0			O/G	O/G			>80	0
1-Nov-10			>80	0			1.0	0			0.0	0			28.0	0			O/G	O/G			>80	0
1-Nov-10			>80	0			1.0	0			0.0	0			26.0	0			O/G	O/G			>80	0
8-Nov-10			O/G	3			O/G	O/G			0.0	0			13.0	0			O/G	O/G			>80	1
8-Nov-10			O/G	4			O/G	O/G			2.0	0			13.0	0			O/G	O/G			>80	1
15-Nov-10			O/G	6			O/G	O/G			1.0	0			16.0	0			O/G	O/G			>80	0
15-Nov-10			O/G	3			O/G	O/G			0.0	0			14.0	0			O/G	O/G			>80	0
29-Nov-10			0	0			0	0			0	0			0	0			O/G	O/G			44	0
29-Nov-10			0	0			0	0			0	0			0	0			O/G	O/G			37	0
6-Dec-10			0	0			40	0			0	0			O/G	O/G			O/G	O/G			29	0
6-Dec-10			0	0			32	0			0	0			O/G	O/G			O/G	O/G			28	0
13-Dec-10	<3	<3			3	<3	0	0	<3	<3	0	0	<3	<3	0	0	19	<3	O/G	O/G	3	<3	23	0
13-Dec-10							0	0			0	0			0	0			O/G	O/G			25	0
20-Dec-10			0	0			8	0			0	0			0	0			O/G	O/G			9	0
20-Dec-10			0	0			3	0			0	0			0	0			O/G	O/G			13	0
14-Feb-11	<3	<3			3	<3			<3	<3			<3	<3			<3	<3			<3	<3		
14-Feb-11																								
28-Feb-11			42	0			44	0			0	0			38	0			0	0			O/G	O/G
28-Feb-11			38	0			41	0			0	0			30	0			0	0			O/G	O/G
1-Mar-11			>80	0			26	0			0	0			34	0			O/G	O/G			8	0
1-Mar-11			>80	0			25	0			0	0			26	0			O/G	O/G			7	0
8-Mar-11			O/G	5			18	0			3	0			10	0			O/G	O/G			14	0
8-Mar-11			O/G	1			22	0			2	0			9	0			O/G	O/G			10	0
14-Mar-11	141	<3	>80	2	<3	<3	10	0	<3	<3	2	0	33	<3	53	1	<3	<3	0	0	<3	<3	7	0
14-Mar-11			>80	1			8	0			0	0			62	0			0	0			5	0
24-Mar-11			59	0			40	0			1	0			0	0			0	0			4	0
24-Mar-11			54	0			48	0			0	0			2	0			5	0			5	0
29-Mar-11			>80	0			13	0			1	0			1	0			O/G	O/G			4	0
29-Mar-11			58	0			14	0			0	0			0	0			O/G	O/G			3	0
11-Apr-11	<3	<3	7	0	<3	<3	11	0	<3	<3	1	0	<3	<3	0	0	<3	<3	0	0	3	<3	1	0
11-Apr-11			6	0			8	0			0	0			1	0			0	0			1	0
15-Apr-11			O/G	O/G			>80	0			0	0			4	0			O/G	O/G			5	0
15-Apr-11			O/G	O/G			>80	0			1	0			5	0			O/G	O/G			6	0
18-Apr-11			3	0			6	0			O/G	O/G			0	0			14	0			6	0
18-Apr-11			8	0			9	0			O/G	O/G			0	0			20	0			6	0

*O/G = Overgrown, too many different kinds of bacteria present from contamination to determine if there are positive counts of Total Coliform and E.Coli

Does not meet Ontario Drinking Water Standards, Objectives and Guidelines

Table B.14. Biofilm growth Trial 1, 2 and 3

	Trial 1			Trial 2			Trial 3		
Coupon #	**Area A**	**Area B**	**Δ Log**	**Area A**	**Area B**	**Δ Log**	**Area A**	**Area B**	**Δ Log**
R1-1	4.93	4.85	0.07	7.95	8.14	0.19	7.99	7.94	0.05
R1-2	7.63	5.30	2.33	7.84	7.89	0.05	7.69	7.57	0.12
R1-3	4.59	4.50	0.09	7.97	7.91	0.06	7.45	7.30	0.15
R1-4	5.15	4.48	0.67	7.67	7.78	0.11	7.84	7.67	0.17
R1-5	4.34	7.23	2.89	7.85	7.55	0.30	7.67	7.48	0.20
R1-6	4.42	4.53	0.11	7.83	7.83	0.00	7.61	7.14	0.47
R1-7	5.38	4.87	0.51	7.81	7.51	0.30	8.02	7.70	0.32
R1-8	4.30	4.66	0.35	7.67	7.68	0.01	7.66	7.34	0.32
R1-9				8.06	7.95	0.11	7.45	7.17	0.28
R1-10				7.92	7.78	0.14	7.51	7.45	0.06
	Average		0.88			0.13			0.21
	Standard Deviation		1.10			0.11			0.13
	Variance		1.21			0.01			0.02
	Min		0.075			0.001			0.049
	Max		2.89			0.30			0.47

	Trial 1			Trial 2			Trial 3		
Coupon #	**Area A**	**Area B**	**Δ Log**	**Area A**	**Area B**	**Δ Log**	**Area A**	**Area B**	**Δ Log**
R2-1	4.55	4.94	0.39	7.77	7.79	0.02	8.00	7.95	0.06
R2-2	6.30	4.27	2.03	7.67	7.18	0.49	7.22	7.11	0.11
R2-3	4.32	5.60	1.28	7.80	7.75	0.04	8.05	7.97	0.08
R2-4	4.30	4.00	0.30	7.66	7.72	0.06	7.91	7.97	0.06
R2-5	5.08	4.77	0.31	7.52	7.79	0.27	8.07	8.02	0.06
R2-6	4.20	4.07	0.13	7.30	7.77	0.47	8.08	8.01	0.07
R2-7	4.39	4.40	0.01	7.88	7.65	0.23	7.82	7.86	0.04
R2-8	4.09	4.12	0.03	7.70	7.62	0.08	8.02	7.86	0.17
R2-9				7.67	7.41	0.25	7.67	7.60	0.08
R2-10				7.79	7.62	0.17	7.84	7.85	0.01
	Average		0.56			0.21			0.07
	Standard Deviation		0.72			0.17			0.04
	Variance		0.52			0.03			0.00
	Min		0.012			0.022			0.014
	Max		2.03			0.49			0.17

Table B.15. Biofilm removal results

		Galvanized Steel			Fibreglass		
		Initial Logs	Final Logs	Logs Removed	Initial Logs	Final Logs	Logs Removed
		Chlorination					
Trial 1	n	10	10	10			
	Average	8.06	5.15	**2.91**			
	Standard Deviation	0.105	0.341	0.265			
	Variance	0.011	0.116	0.070			
	Min	7.90	4.75	2.62			
	Max	8.22	5.60	3.33			
	Standard Error			**0.09**			
Trial 2	n	10	10	10			
	Average	7.46	5.8	**1.63**			
	Standard Deviation	0.282	0.111	0.327			
	Variance	0.080	0.012	0.107			
	Min	7.2	5.7	1.19			
	Max	8.1	6.1	2.36			
	Standard Error			**0.14**			
		Power Washing					
Trial 3	n	10	10	10			
	Average	8.03	5.4	**2.60**			
	Standard Deviation	0.099	0.330	0.356			
	Variance	0.010	0.109	0.126			
	Min	7.9	4.7	2.25			
	Max	8.1	5.9	3.39			
	Standard Error			**0.12**			
Trial 4	n	10	10	10			
	Average	7.26	5.0	**2.22**			
	Standard Deviation	0.202	0.369	0.345			
	Variance	0.041	0.136	0.119			
	Min	6.9	4.8	1.42			
	Max	7.6	6.0	2.70			
	Standard Error			**0.13**			
		Power washing followed by Chlorination					
Trial 5	n	10	10	10	4	4	4
	Average	8.04	3.8	**4.25**	8.51	4.0	**4.54**
	Standard Deviation	0.284	0.133	0.323	0.083	0.147	0.220
	Variance	0.081	0.018	0.104	0.007	0.022	0.049
	Min	7.7	3.6	3.71	8.4	3.8	4.29
	Max	8.5	4.0	4.69	8.6	4.1	4.82
	Standard Error			**0.14**			**0.12**
Trial 6	n	10	10	10	4	4	4
	Average	7.90	4.1	**3.80**	8.44	4.1	**4.35**
	Standard Deviation	0.427	0.285	0.541	0.105	0.072	0.056
	Variance	0.182	0.081	0.292	0.011	0.005	0.003
	Min	7.0	3.9	2.83	8.3	4.0	4.30
	Max	8.3	4.8	4.42	8.5	4.1	4.42
	Standard Error			**0.22**			**0.06**
		Chloramine					
Trial 7	n	6	6	6	4	4	4
	Average	7.87	5.4	**2.49**	7.94	5.3	**2.66**
	Standard Deviation	0.264	0.275	0.272	0.309	0.461	0.353
	Variance	0.070	0.076	0.074	0.095	0.213	0.125
	Min	7.4	5.0	2.23	7.6	4.8	2.16
	Max	8.2	5.8	2.89	8.3	5.9	2.98
	Standard Error			**0.15**			**0.23**
Trial 8	n	6	6	6	4	4	4
	Average	7.19	5.1	**2.12**	7.24	4.7	**2.49**
	Standard Deviation	0.690	0.282	0.502	0.291	0.145	0.259
	Variance	0.476	0.080	0.252	0.084	0.021	0.067
	Min	6.3	4.7	1.43	6.9	4.6	2.28
	Max	7.9	5.4	2.47	7.6	4.9	2.83
	Standard Error			**0.35**			**0.19**
		Hydrogen Peroxide					
Trial 9	n	6	6	6	4	4	4
	Average	8.11	7.2	**0.87**	8.49	5.1	**3.35**
	Standard Deviation	0.054	0.394	0.365	0.069	0.099	0.079
	Variance	0.003	0.155	0.133	0.005	0.010	0.006
	Min	8.0	6.4	0.56	8.4	5.0	3.23
	Max	8.2	7.5	1.59	8.6	5.2	3.42
	Standard Error			**0.15**			**0.05**
Trial 10	n	6	6	6	4	4	4
	Average	8.18	7.2	**0.95**	8.68	5.1	**3.55**
	Standard Deviation	0.112	0.290	0.261	0.013	0.102	0.099
	Variance	0.013	0.084	0.068	0.000	0.010	0.010
	Min	8.0	6.8	0.53	8.7	5.0	3.46
	Max	8.3	7.6	1.21	8.7	5.2	3.66
	Standard Error			**0.12**			**0.05**

Appendix C. Denaturing gradient gel electrophoresis (DGGE), heterotrophic plate count (HPC) and Biolog® Ecoplates™S report by Mike Mitzel.

Appendix C contains the methods used on the microbial characterization performed on the wells and coupons.

Well Project - Summary of Experimental Procedures,
Data Analysis, Results and Work Performed

Experimental Summary

Sample Processing

Upon receipt of the first two round of samples, consisting of suspended swabs and side-well swipes suspended in well water and contained in 500 mL sample bottles, were incubated at 22°C overnight and set to shake at ~130 rpm for the first round and ~180 rpm for the second. HPC's, CLPP and DNA extraction were performed the next day, following overnight incubation/shaking. Upon receipt of the third round of samples, coupons were transferred aseptically to new double-bagged stomacher bags[1] and all bags we filled with sterile PBS to an estimated fill-line of 500 mL. The third round samples were then subjected to sonication at 55 kHz for 1 min and the suspension PBS used in CLPP and DNA extraction immediately following. This suspension was stored overnight at 4°C and HPC's were performed the following morning.

HPC's

Heterotrophic plate count (HPC) were done using R2A agar (BD Difco, Fisher Scientific, Whitby, ON) after creation of a dilution series from 10^0 to 10^6 using 9 mL dilution blanks containing sodium-free dilution buffer (APHA, 1998) of which 100 µL from each dilution was spread, in duplicate, onto R2A plates. HPCs were counted after 5 to 7 days of incubation at 25°C in accordance with the standard method (APHA, 1998).

CLPP

Community-level Physiological profiling (CLPP) was done using Biolog™ ECOplates™ (Biolog Inc., Hayward, CA), inoculated following considerations presented in Garland (1997), Garland *et al.* (2007), Calbrix *et al.* (2005), Weber *et al.*, (2007) and Weber and Legge (2010). Prior to inoculation, samples were spectrophotometrically analyzed at a wavelength of 420 nm as a means to assess background carbon levels (Weber & Legge, 2010). If the value was greater than 0.2, the sample was diluted one order of magnitude and analyzed again. Dilution to fulfill this guideline was not required for any samples. Biolog™ ECOplates™ were inoculated with 150 µL of sample per well, with care taken not to cross-contaminate any wells; incubated at 22°C in the dark; analyzed using a SpectaMax 190 (Molecular Devices, Sunnyvale, CA) spectrophotometer and data collected using SoftMax Pro ver. 3.1.2 (Molecular Devices) every 24 hr.

DNA Extraction

250 mL of swab, wipe or coupon suspension solution was filtered onto a sterilized 47 mm, 0.22 µm polycarbonate filter (Millipore), soaked in un-buffered PCR-grade Milli-Q (Millipore) water. Each filter was placed into a PowerSoil (Mo Bio Laboratories Inc., Solana Beech, CA) bead tube using sterile forceps, ensuring the surface containing the filtrand was facing the middle of the tube and accessible to the tube contents. The filter was then cut into small pieces using a new, sterile No. 11 blade (Feather, Fischer Scientific, Whitby, ON) on a sterilize No. 3 handled scalpel

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[1] Concrete coupons received on Jan. 18th had leaked original sample transport fluid (made by Hector?) out of sample transport bags. Fiber glass and Galvanized steel did not, and their sample transport solutions were conserved and added with the coupon the stomacher bags prior to sonication.

for approximately 5 minutes in the bead tube. DNA was subsequently extracted following the protocol supplied by the manufacturer.

PCR for DGGE

PCR was performed using 5 µL of template DNA with the primers 357f (5'-CCTACGGGAGGCAGCAG-3') with a GC-clamp (5'- CGCCCGCCGCGCCCCGCG CCCGTCCCGCCGCCCCCGCCCG-3') added to the 5' end, and 518r (5'-ATTACCGCGGCTGCTGG-3'), modeled after Ogino *et al.* (2001) and obtained from Sigma-Aldrich. The primer set used is considered a universal set and targets the hyper-variable V3 region of bacterial 16S rDNA (Muyzer *et al.*, 1993). PCR mastermix for this universal primer set was prepared such that each 50 µL reaction contained 1x Go-Taq™ Flexi (Promega) Green PCR Buffer, 0.5 µM of each primer, 1.5 µM MgCl, 1.5U Go-Taq™ Flexi (Promega), 200 µM dNTP (Promega) and 21.3 µL of Milli-Q (Millipore) water. PCR was performed using a BioRad™ I-cycler iQ PCR machine (Bio-Rad Laboratories). Touch-down PCR conditions were based on those described in Muyzer *et al.* (1993) and consisted of an initial denaturation step of 94°C for 5 min, followed by 20 cycles of 94°C, 65°C and 72°C for 1 min each, in which the annealing temperature of 65°C was decreased by 1°C every 2 cycles to a temperature of 56°C on the 20th cycle. Ten additional cycles of 94°C, 55°C and 72°C for 1 min each followed. PCR concluded with a 7 min, 72°C extension step and was held at 4°C until storage at -10°C. PCR reaction success was measured by loading 10 µL PCR product into 1.5% agarose gel in 1x TAE buffer. Gels were run for 60 min at 100 V, stained with ethidium bromide solution for 15 min and visualized using BioRad™ GelDock™ XR (Bio-Rad Laboratories) with amber filter to confirm the presence of only a 233 base-pair band in sample wells and absence of any bands in the blank, which consisted of the 5 µL of the same Milli-Q water used in creation of the mastermix instead of template DNA.

DGGE Conditions

For all of the samples collected from the three scales, DGGE was performed following the methods and rationale presented by Green *et al.* (2009) with slight modification. 8% (wt/vol) acrylamide gels containing a linear denaturant gradient ranging from 40 to 65%, with 100% denaturant defined as a solution of 7M urea and 40% formamide. Gels were run for 17 hrs at 70 V (1190 V·hr) using a CBS Scientific™ DGGE-2401 machine (CBS Scientific Inc., Del Mar, CA) set to a constant temperature of 60°C. 15 µL of sample PCR product was added to each lane, allowing free lanes for the DGGE ladder.

DGGE Ladder Creation

DGGE ladder was created using 8 cloned sequences from various environmental samples known to move distinctly and consistently through the DGGE gel. These cloned and purified sequences were obtained from Dr. Josh Neufeld (University of Waterloo) and amplified individually using the 357f-gc and 518r primer set and PCR reaction conditions described in the PCR for DGGE subsection (see pgs. 23 & 24), modified to include 3 µL of template instead of 5 µL. The remaining 2 µL volume was replaced by increasing Milli-Q water to 23.3 µL. The ten PCR reactions were pooled post-PCR to create 500 µL of DGGE ladder and this was used in all DGGE gels, which was diluted by adding 500 µL 10 mM Tris-HCl (pH 8.0; Sigma-Aldrich). 7 µL of the ladder was added to a central lane and both outside lanes.

DGGE Image Acquisition

Gels were stained with 1x SYBR Gold solution (10,000x stock diluted in 1x TAE; Invitrogen, Burlington, ON) for approximately 1 hr. Gels were then placed in a BioRad™ Gel Doc™ XR (Bio-Rad Laboratories) and flooded with deionized water before being photographed with

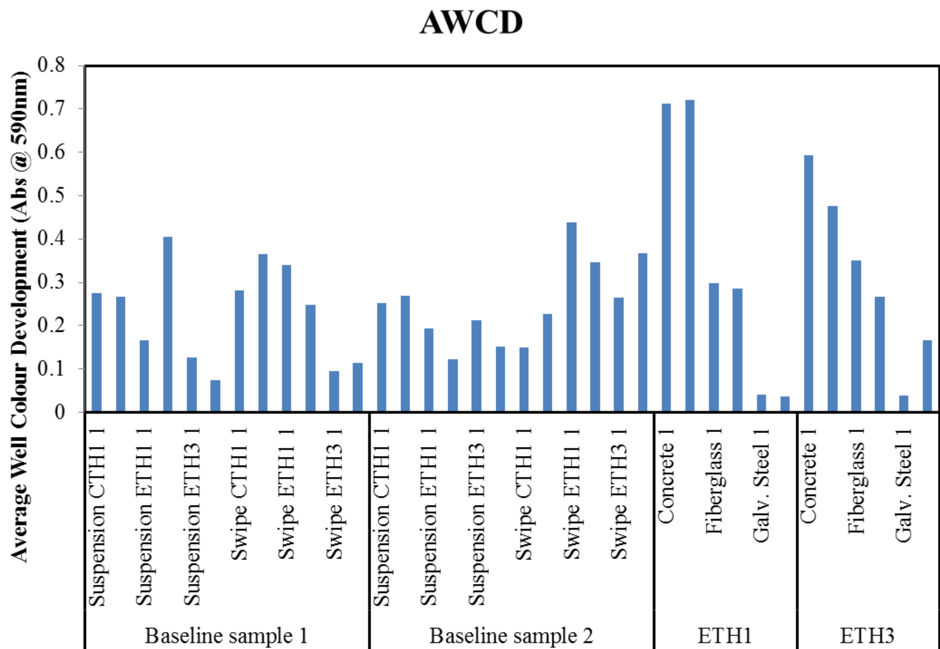
BioRad™ SYBR Gold filter (Bio-Rad Laboratories). The resulting image was captured using Quantity One® software (Bio-Rad Laboratories), ensuring the gel was exposed for less than the time required to produce saturated pixels. The image was then exported to an 8-bit .tif file, excluding overlays, and saved at the scan resolution (2879 dpi) and size of 1360 x 1024.

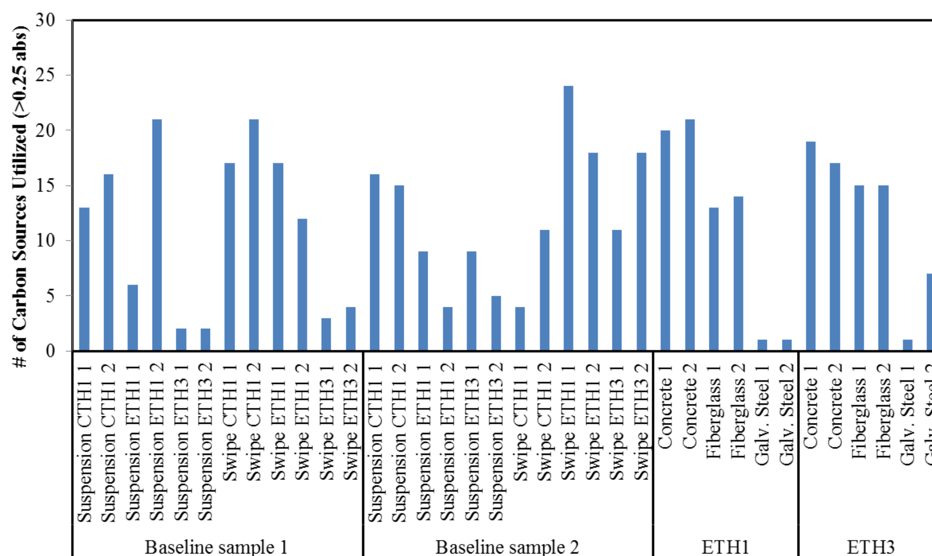
Data Analysis

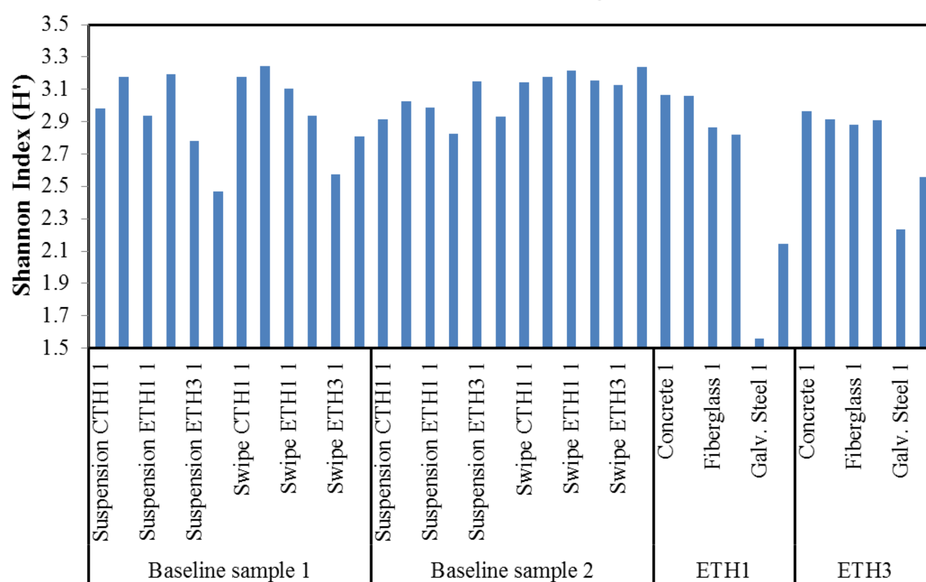
HPC's were counted after 5 days. No change was observed from any counts taken on the 5th day by the 7th day.

CLPP data from the 96 hr time point was analyzed for three sample events, based on assessment of standard deviations and number of observed absorbance values greater than 1.99.

DGGE images were loaded into GelComparII (Applied Maths, Austin, TX) software following the manufactures instructions. Bands were detected using the automated band detection algorithm provided in the program, set to the minimum cut-off that detected only the expected bands in the ladder-containing lanes. The resulting band detection output was examined using the original image to ensure only bands clearly visible to the unaided eye in the original picture were selected and that bands were not placed in areas which contained peaks due to dust or other image inconsistencies. Bands that appeared to the unaided eye but were over looked by the band search algorithm were excluded to ensure consistency regarding the treatment of background intensity. Densitometric peaks for each banding pattern in each lane were exported into text files conferring both band movement (i.e., number of pixels from the top of the gel; R_f) and relative band intensities (i.e., intensity of each band compared to total intensity of each lane, for each separate lane).







HPC's
-compiled into spreadsheet, not yet graphed.

DGGE
-Not yet analyzed

Work Performed
3 rounds of sampling consisted of the receipt of 36 samples (12 per round).
- First was received Oct. 26th 2010. HPC's, CLPP & DNA extraction performed by M.R.M. CLPP read by R.M.S. and M.R.M.

- Second received on Nov. 9th 2010. HPC's performed by R.C.; CLPP & DNA extraction performed by M.R.M. CLPP read by R.M.S., R.C. and M.R.M.
- Third samples received on Jan. 18th 2011. HPC's, CLPP & DNA extraction performed by M.R.M. CLPP read by R.M.S. and M.R.M.

Preparation/Clean-up
-All sample events required making of R2A plates, sterile buffers/tips/filters and filter apps.
-Pipettes were also sterilized prior to use.
-All waste was autoclaved and test-tubes/dishes washed following samples.

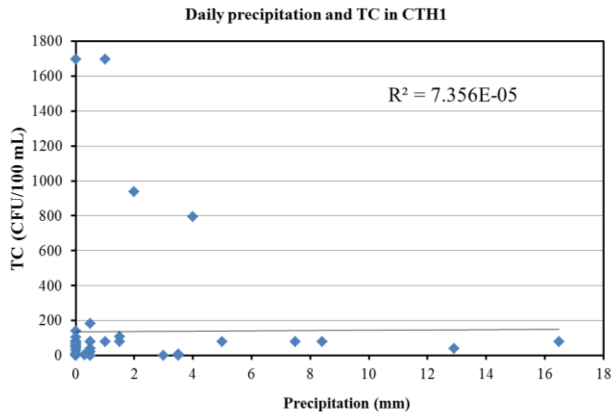
Data collection
-HPCs were counted by hand.
-CLPP-derived output was collected from the computer connected to the spectrophotometer and converted from .pda to .txt, and then to .xls and arranged in a summary sheet.
-DGGE images were normalized and inspected and the densitometric curves and number of bands extracted using GelComparII. This output is stored as a .txt.
PCR/DGGE
-This technique was run twice following the final sampling event, using aliquots of the same PCR reaction.

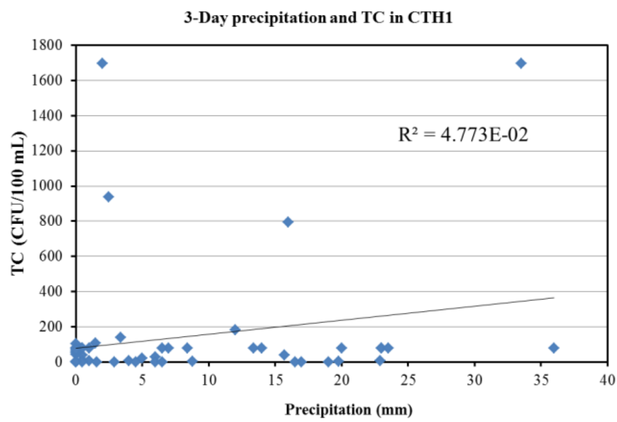
Data Analysis
-CLPP data was compiled into a summary spreadsheet. AWCD, Richness and Shannon Index (H') were extracted. Kurtosis and Skewness were assessed. Individual replicates, as well and the averaged replicates were transformed using a taylor transformation.

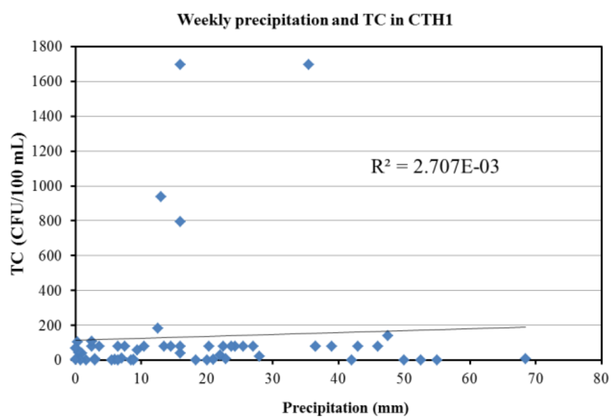
Appendix D. Correlations analysis between precipitation events and microbial contamination.

Appendix D contains the correlation analysis performed to determine if there was a relationship between a precipitation event and samples with positive microbial indicator.

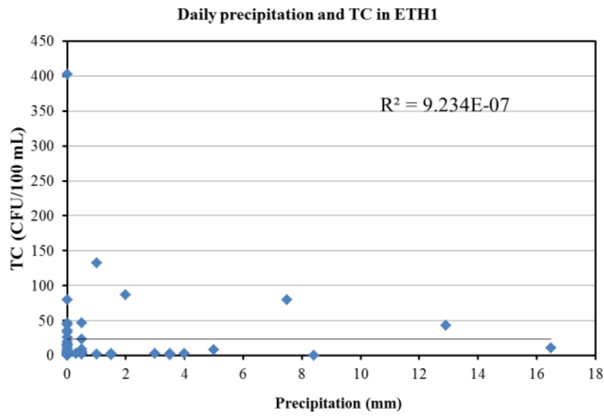
Correlation between daily, 3-day and weekly precipitation and TC presence in CTH1:

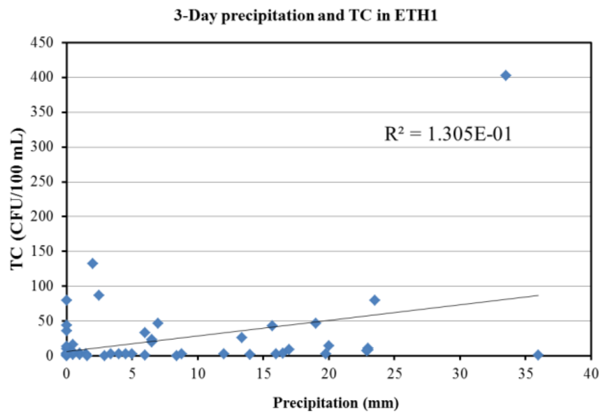


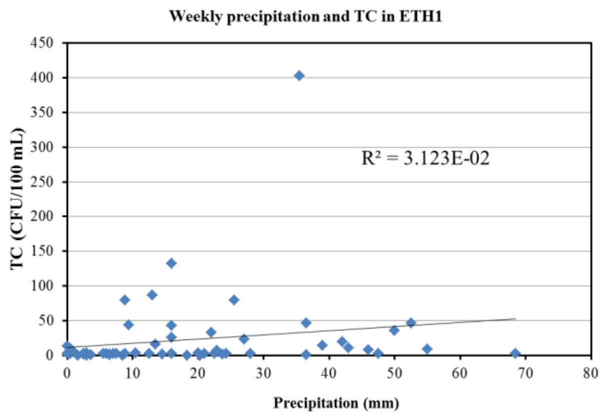




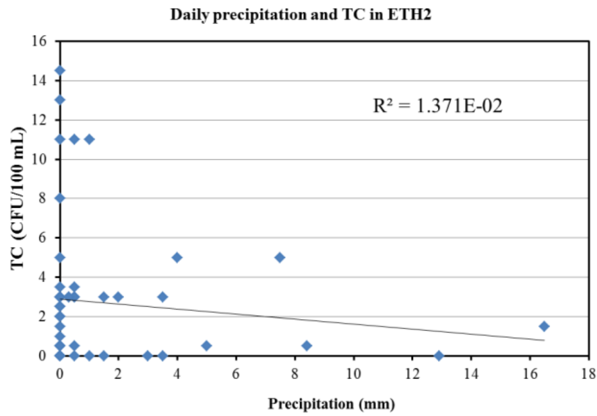
Correlation between daily, 3-day and weekly precipitation and TC presence in ETH1:

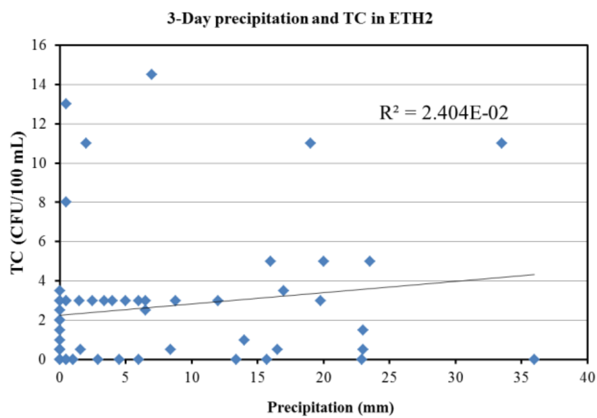


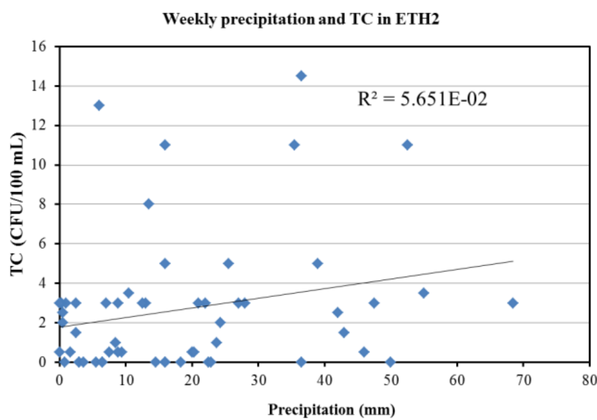




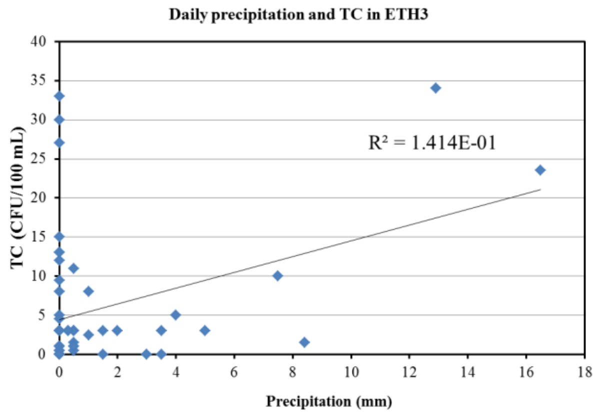
Correlation between daily, 3-day and weekly precipitation and TC presence in ETH2:

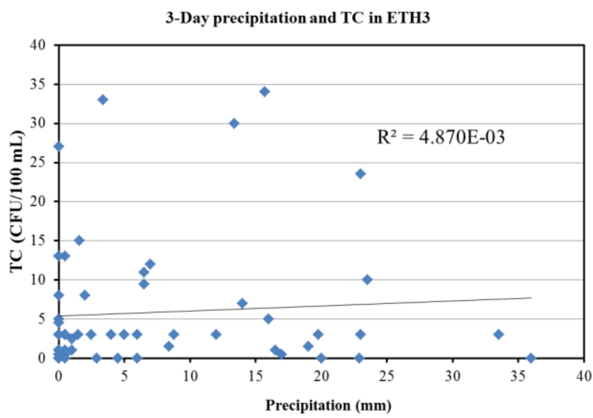


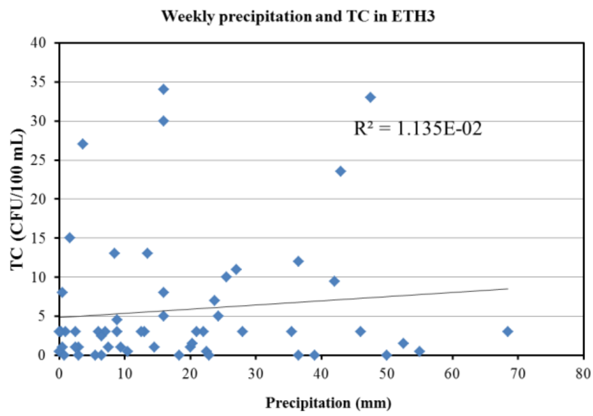




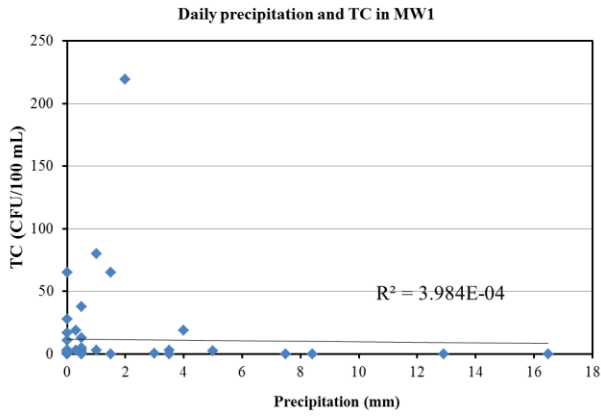
Correlation between daily, 3-day and weekly precipitation and TC presence in ETH3:

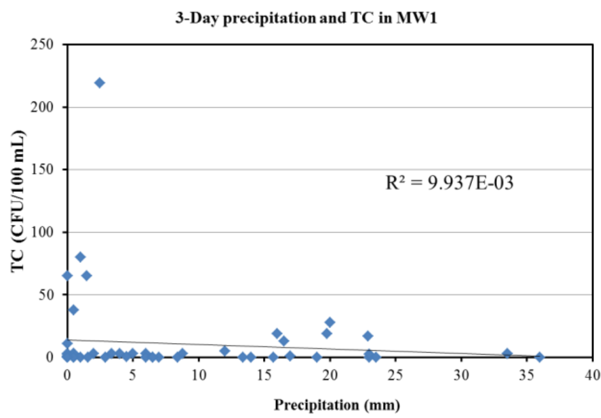


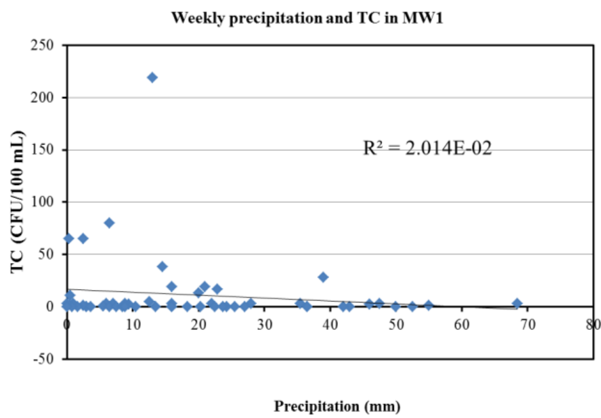




Correlation between daily, 3-day and weekly precipitations and TC presence in MW1:







Correlation between daily, 3-day and weekly precipitation and TC presence in MW3: