CHARACTERIZING AND MODELING IMPACTS OF CLIMATE AND LANDUSE VARIABILITY ON WATER QUALITY

Shawnigan Lake Community Water System and Watershed

Report on Results from Sept. 2010 to August 2011

Research Project Conducted by

Water and Aquatic Sciences Research Program

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INTRODUCTION

Shawnigan Lake is the second largest lake on southern Vancouver Island, providing a number of uses including drinking water, sport fisheries and recreation to a large number of people. Development within the watershed has increased steadily over the last several decades and the population continues to grow as more people make Shawnigan Lake their permanent residence rather than seasonal cottages. Shawnigan Lake is one of the four partner communities chosen for the overall research study initiated by the Water and Aquatic Sciences Research Program at the University. This community-based research project is a part of the NSERC Industry Research Chair (NSERC-IRC) Program on Environmental Management of Drinking Water at the University of Victoria. The NSERC-IRC program has studied water quality trends in Shawnigan Lake over a 7-year period (2001-2007) and presented the results to the community showing how the watershed activities and residential septic discharge have been deteriorating the water quality of this important water source. We showed that nutrients (phosphorus and nitrogen), that regulate algal biomass, turbidity and potential for toxic algae, as well as formation of disinfection byproduct following chlorination, have been increasing gradually. We also found that fecal bacteria and health-care products have been seeping into the lake causing potential health risks. We further collected sediment core and reconstructed the last 100-year history of the changes in water quality as a function of settlement, land-use and natural events like fire and flood in the watershed.

During a five-year program (started in 2010), the NSERC-IRC program at UVic proposed to characterize, quantify and model the impacts of climate variability and land-use on chemical, biological and microbial water quality, with a particular emphasis on the types and sources of

waterborne pathogens and associated health risks from source and drinking water. The importance of this project lies in the notion that it still not clear whether it is the climate change in terms of increasing air temperature or it is the climate variability in terms of episodic shifts temperature and rainfall that are more critical for outbreaks of waterborne pathogens. Current knowledge linking waterborne pathogens or disease with climate change or variability is at a very early stage, although there are a couple of existing waterborne disease outbreaks that were directly caused by the extreme climate events such as heavy precipitation as well as existing statistical relationship between infectious disease cases and weather conditions (Patz et al., 2005; Schuster et al., 2005; Stanwell-smith, 2001). We will evaluate whether the impacts of climate variability on waterborne pathogens would depend upon local or regional climate because it is the local or regional hydrology that leads to loading of pathogens into water. Climate change has multiple potential effects on pathogens in source water, and risks of waterborne diseases in Canada by 1) increased survival of fecal pathogens on land mediated by temperature and precipitation, 2) transport of pathogens over land and loading to water sources, 3) different types of land use and associated changes in density and distribution of animals will likely change the impacts of climate change on waterborne pathogens, and 4) increased risks from failure of water treatment and disinfections arising from flooding, and storm-water and sewage/septic overflows.

Waterborne diseases are caused by pathogenic microorganisms, which are directly transmitted, when contaminated fresh water is consumed. Contaminated fresh water, used in the preparation of food, can be the source of foodborne disease through consumption of the same microorganisms. According to the WHO, diarrheal disease accounts for an estimated 4.1% of the total daily global burden of disease and is responsible for the deaths of 1.8 million people every year (WHO, 2003; Ezzati et al., 2004). It was estimated that 88% of that burden is attributable to

unsafe water supply, sanitation and hygiene, and is mostly concentrated in children in developing countries. Pathogenic microorganism causing waterborne diseases is classified into three groups of microbial agents, bacterial protozoa, and virus, causing waterborne illnesses. Many of these pathogens are intestinal parasites. Some of these pathogens may be found naturally in the water, and the others are introduced into the water through human and animal waste contamination. Climate change directly and indirectly affects the survival and transmissions of these pathogenic microorganisms in ecosystems and environments (Figure 1).

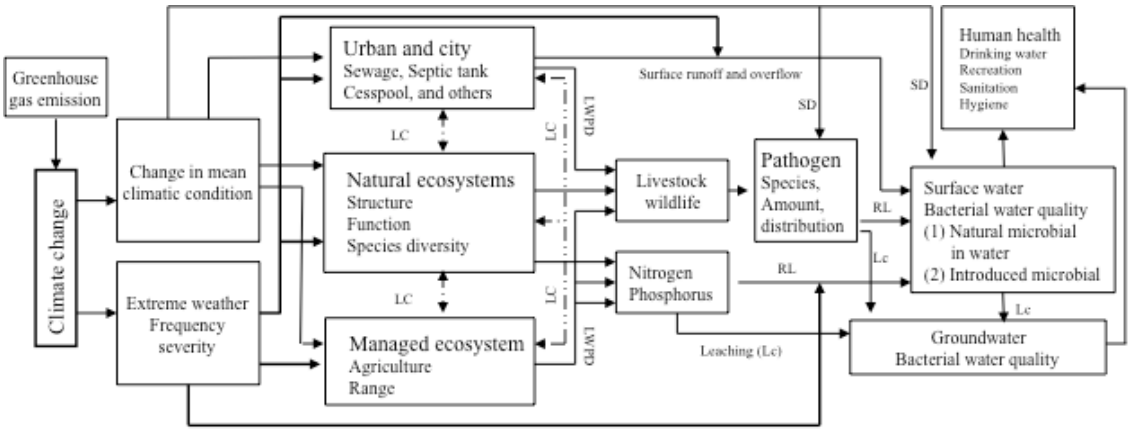


Figure 1. Diagram showing how climate change and along with ecosystem and landuse change affect waterborne pathogens and their transfer to surface and ground water systems. LC = Landuse Change, LWPD = Livestock Wildlife Population and Density, RL= Runoff and Lateral flow, and SD= Survival and Die off.

From the view of ecosystems and environment, direct impacts include the impact of temperature on the survival of pathogenic microorganisms and the impacts of precipitation on the transfers of pathogenic microorganisms to freshwater, which is typically caused by (1) the runoff of animal wastes to surface water from pastures and rangelands and (2) infiltration to groundwater from high concentrations of animal waste from confined animal feedlots. In city and urban areas, human and animal waste mostly contaminates freshwater or a water supply by the following ways: (1) failure of an on-site sewage disposal system (e.g., septic system) that causes direct infiltration to groundwater and/or provides runoff to surface water and (2) discharge of untreated

or improperly treated sewage to rivers and reservoirs, such as during operational malfunctioning. An extreme climate event such as a heavy storm with excessive storm water runoff often worsens the failures and cause waterborne disease outbreaks.

Indirect impacts of climate change on waterborne diseases is through its impacts on ecosystem structure, function, and land use, which determine the habitats for domestic and wild animals and further shift the origin and distribution of pathogenic microorganisms. The severity of waterborne diseases are affected by the climate factors because (i) survival and persistence of disease-causing organisms are directly affected by temperature, (ii) water is the media transporting disease-causing organism downstream to environment and ecosystems used for public use, thus precipitation and watershed hydrology can affect the transfer of waterborne disease organisms from animal sources to potable or recreational water, (iii) extreme climate events may accelerate the outbreak of waterborne disease, for example, old city sewer systems designed to convey sanitary sewage to waste treatment plants often leak or overflow untreated sewage with storm water into receiving waters after heavy rainfall events (Patz, et al. 2005), and (iv) land use change interacting with climate change may affect the bacteria sources and then affects the dynamics of the waterborne diseases. For example, fecal bacteria concentration in streams in agriculture and livestock dominated watersheds is higher than forest dominated watersheds (Crowther, 2002).

Existing evidences of the impacts of climate on waterborne diseases

The climate impacts on waterborne diseases are often demonstrated by the relationships between infectious disease cases and climate variables, i.e., temperature and precipitation. The evidences of temperature impacts on waterborne diseases includes: (1) increased temperature results in the increase of the reported cases of cholera caused by the blooms of cyanobacteria, dinoflagellates,

and pfiiesteria piscida (Colwell, 1996; Islam et al. 1009), (2) seasonal variation of waterborne disease is in correspondence with the change of seasonal temperature (Stanwell-Smith, 2001), and (3) close statistical relationship between cholera cases and sea surface temperature in El Nino years (Figure 2, Colwell, 1996).

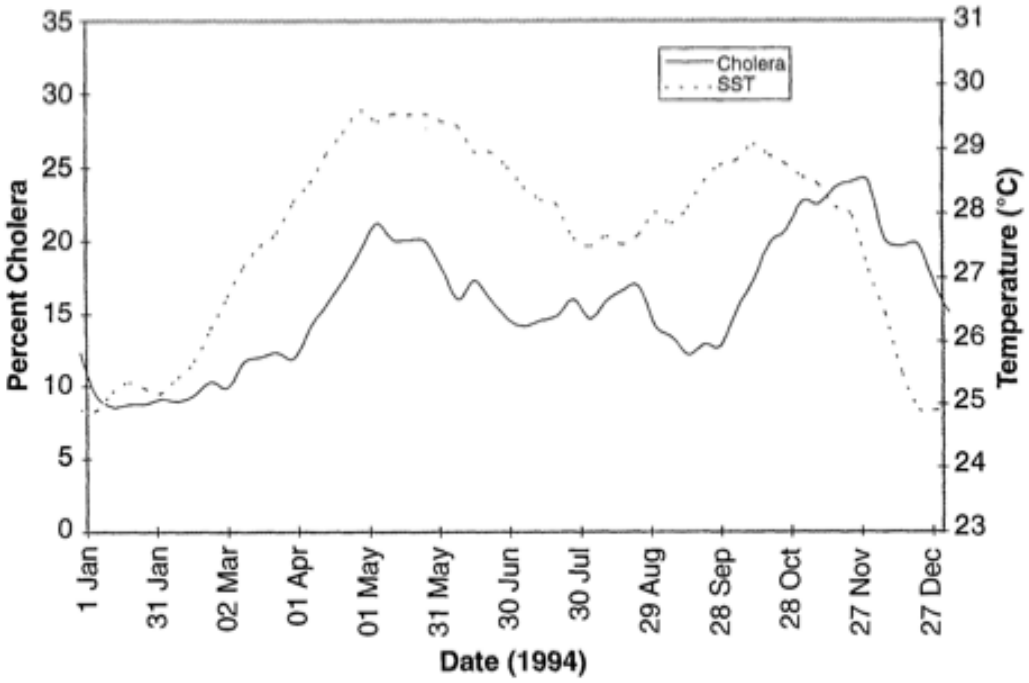


Figure 2. Relationship between sea surface temperature (SST, EI Niño events) and cholera cases in Bangladesh from January to December 1994 (Colwell,1996 Science 274, 2025-2031).

There are two well-known waterborne outbreaks caused by heavy rain in Canada. One is E. Coli Outbreak from May through December in 2000 in Walkerton, Ontario. As a result, seven people died from gastrointestinal illness. The city reported 160 confirmed cases of E. coli, more than 400 unconfirmed cases, and more than 2,300 people ill with gastrointestinal illness. This was caused by heavy rainfall, which washed cattle manure infected with *E. coli* O157:H7 and

Campylobacter jejuni into a shallow public-supply well for the community. Although the manure had been spread on agricultural land near the well in accordance with proper agricultural practices, the contaminants had passed from the soil down into the aquifer that fed the well. Officials found that the city's water-system operators had engaged in a series of improper monitoring practices (e.g., not collecting water samples from the appropriate sampling sites) and had falsified reports. Therefore, the contamination went undetected. An investigation published in 2002 offered recommendations for regulatory and policy reform in order to strengthen Ontario's protection of drinking water (O'Connor, 2002). The Walkerton E. coli O157:H7 outbreak is a chilling reminder that communities take high-quality drinking water for granted. Key factors in preventing the occurrence of similar outbreaks involve minimizing the chances of microbial contamination in water supplies: namely, protecting water sources; replacing aging portions of the water-supply infrastructure; properly maintaining existing structures; and improving training for water system operators (Auld et al., 2001; Hrudey, et al., 2003; and O'Çonnor, 2002). These could also be considered as adaptation strategies to mitigate climate impacts on waterborne outbreaks. The other waterborne disease is the outbreak of toxoplasmosis in Victoria, BC, which was associated with preceded high amount of precipitation and turbidity in water supply reservoirs (Bowie et al., 1997; Aramini et al., 1999). In total, there were 288 waterborne disease outbreaks in Canada from 1974 to 2001. Twenty-four of them have been linked to weather events (Schuster et al 2005; **Table 1**).

Table 1. Factors contributing to waterborne disease outbreaks in Canada (Schuster et al., 2005) *

Causative factor	Public	Semi-public	Private	Total
Weather Events				
Heavy rainfall	6	0	3	9
Drought	1	1	0	2
Flood	1	0	1	2
Spring runoff 8	1	1	1	0
Snow melt	1	0	0	1
Animals				
Wildlife	31	2	1	34
Livestock	6	2	0	8
Frozen wastes	2	0	0	2
Agriculture	4	0	1	5
People				
Septic tanks	1	13	7	21
Sewage	8	4	1	13
Cess pool	0	0	1	1
Non-specific Contamination†	6	45	14	65
Water Treatment Issues	34	11	2	47
Human				
Human source	1	0	0	1
Human error	4	2	0	6
Recommendations ignored	4	0	0	4
Sanitation	1	3	1	5
Communication	0	1	0	1
No community resistance to pathogen	1	0	0	1
Legislation/Enhanced Treatment Techniques‡	34	10	5	49
GWUDI§	3	5	1	9
Water Recycling	0	0	1	1

* Some outbreaks were associated with multiple causative factors

† Faecal coliforms were identified as being present, but the exact source was unknown

‡ If legislation had been in place or enhanced treatment technologies used (e.g., filtration), the outbreak would not have occurred

§ Groundwater under direct influence of surface water

In the U.S., detected *Escherichia (E.) coli* levels are as high as 2000-7000 colony forming units (CFU)/100ml have been observed following storm events around Milwaukee, Michigan. Storm event with 3 inches of rainfall within 24 hours can overwhelm the city combined sewer systems and lead to over flow. As a result, Lake Michigan *E. coli* concentration was 10 times higher than when there was no sewage overflows (Patz et al., 2005). Around 9 million annual cases of waterborne disease cases have been estimated in the United States (Patz et al., 2000). There were 525 waterborne disease outbreaks reported from 1948 to 1994. Of these outbreaks, 51% were preceded an extreme level of precipitation in the highest 10% (Curriero et al., 2001). The

majority of these outbreaks occurred in the summer and less in winter. Although researchers in UK did not find enough evidence linking drought or flooding events with outbreaks of waterborne diseases in UK (Hunter, 2003; Stanwell-Smith, 2001). According to the WHO data, climate change caused 1.458 million diarrhoeas per day in 2000 throughout the world. The situation is worse in undeveloped countries. These data do not classify how many cases directly belong to waterborne diseases (WHO, 2002; Ezzati, 2004). The WHO predicts that risk of diarrhoea will increase up to 9% in 2030. El-Niňo-years were associated with greater numbers of cholera incidences in Asia and South America when ocean surface temperature was higher than normal years (Colwell, 1996; Pascual et al., 2000; Lobitz et al., 2000; Speelmon et al., 2002).

The current research linking climate change and waterborne disease focuses on two approaches. One set of studies use the statistical relationship between infectious disease cases and climate variables. The other looks at the infectious disease outbreak and associated extreme climate conditions. Both of these approaches lack the mechanism explaining how climate change affects on waterborne disease dynamics and outbreaks. The identified research gaps, which lead to the objectives and activities of the overall program, are: (1) how climate variability affects the survival of pathogenic microorganisms and their transfers from sources to freshwater systems in the field condition? (2) what are the climate criteria and indices that could be used to forecast the potential outbreak of waterborne disease at community and regional levels; (3) how climate change affects waterborne disease through its impacts on landscape (watershed) structure and quality; (4) how the interactions between land use or landscape changes and climate change affect waterborne pathogens; (5) how to develop models and tools used as warning systems to predict the potential risk of waterborne diseases under changing climate conditions; (6) how do we develop robust adaptation strategies to cope with waterborne pathogens and disease risks

when we do not have much data or understanding of how climate and land use changes affect waterborne pathogens.

OBJECTIVES AND DELIVERABLES

The overall project is based on the existing data and newly collected data from the pilot communities where surface water is the principal source of drinking water, and where current/past land use, waste management, and water use for drinking, crop production, livestock farming and residential development have become critical impediments to sustainable clean and healthy water. Development of the project linking climate variability, waterborne pathogens and enteric illnesses took place at each community, but were tailored to the specific needs of each community. At each community, the research and knowledge transfer program engaged a collaborative team from provincial and local public health organizations, and involved expertise in hydrologic and land use modeling, Quantitative Microbial Risk Assessment (QMRA) modeling, microbiology of waterborne pathogens and down scaling of climate model.

The proposed objectives are being addressed using two approaches, the first uses existing data on climatic events and their links to outbreaks of waterborne disease and boil-water advisories in Canadian communities (work with Health Canada, and provincial databases from BC), and the second is based on the extensive data collection on land use, hydrology, climate variability, types and concentrations of waterborne pathogens (WBP) in source water, archived information and community knowledge from pilot communities. GIS based hydrologic model and sources of waterborne pathogens are used to assess the risks of loading and transport of waterborne pathogens to water sources; and to model potential ways of climate change variables linked to intensities and occurrences of waterborne pathogens. The monitoring data from the pilot

communities are to be used to address the objectives of identifying risks from existing and emerging waterborne pathogens, of developing early warning systems, and of developing methods to optimize and adapt to waterborne disease risks.

Besides the overall objectives and deliverables of the project, which are due at the completion of the 5-year research project, the community of Shawnigan Lake has requested a delivery of the monthly results report and quarterly summary of the findings. From September 2010 to the current day, numerous raw and treated water samples have been analyzed for bacterial and chemical contents (turbidity, nutrients and DBPs). Samples of raw and treated water are collected by our partner at the lake and from the distribution system, placed in cooler and shipped to the laboratory of the University of Victoria for analysis.

Note that methods used at the University of Victoria may not be comparable to those used by the regulatory agencies like Vancouver Island Health Authority (VIHA) or by the CVRD Water Management Department. The results generated by the laboratory at UVic is for longer-term goals and objectives outlined above. Accordingly our results should not be compared with the data produced for compliance monitoring by regulatory departments and our results should not be used for regulatory purposes.

MATERIALS AND METHODS

Raw water samples are collected in 1 L sterile bottles in five replicates (5 L total). Treated water samples from water distribution system are collected at three locations (plant, midway and end of the distribution system) in 300mL and 40mL glass bottles for bacteriological and Disinfection By-Products (DBP) analysis, respectively. At the lake midpoint (location of a future intake), the water column is divided into epilimnion (integrated sample from the surface down to 5 meters), metalimnion (mid layer of the water column), and hypolimnion (deeper water column depth), from the surface to the bottom of the lake. Water sampling is carried out using 5m tubing for the surface water samples and the Beta Vertical Van Dorn water sampler (Wildlife Supply Co., Florida, USA) for the discrete grab samples at other depths. For Shawnigan Lake, raw water samples are taken from the intake from three lake layers (epi-, meta and hypo-limnion). *The reason for collecting samples from three depths is that the communities are interested in knowing how climate variability affects chemical and microbial water quality at various depths and they want to adjust intake depth as one of the adaptation strategies to mitigate or reduce waterborne pathogen risk at the intake.* The sampling is done biweekly. All samples are placed in coolers with ice packs and temperature monitoring device (Dickson, Illinois, USA; model SP125; setup sample interval: 10 min) and sent to the analytical laboratory of the University of Victoria, BC. On a microbiological point of view, a sample is considered as ‘valid’ for analysis if the temperature record is below 10°C from the sampling time until its arrival at the University of Victoria laboratory, based on the temperature monitoring device records within the cooler during transport.

Water filtration

After reception of the samples in the Water and Aquatic Sciences Research laboratory at the University of Victoria, all samples with raw water are mixed together in a 15 L Lowboy™ container and shaken to homogenize the samples (Figure 1a). A 40 mL of raw water are collected for turbidity measurement. All turbidity measurements are conducted at the laboratory of the University of Victoria using HACH Turbidimeter, model 2100A (Hach, CO, USA). Two duplicate samples of 100 mL each of raw water are filtered through 47 mm white gridded membrane filter papers (0.45µm pore size; Cole Parmer, QC, CA) under vacuum for *E. coli*, coliforms, and *Enterocccus* determination. Another 1 L of raw water is filtered through membrane filter papers (0.20 µm pore size; VWR, AB, CA) under vacuum and the filter is archived at -80 ^{o}C.

All 300 ml bottles of treated water are also shaken vigorously to homogenize the samples (Figure 1b). 100ml of each 300ml treated water samples is filtered through 47 mm white gridded membrane filter papers (0.45µm pore size; Cole Parmer, QC, CA) under vacuum for *E. coli and* coliforms, and *Enterocccus* determination. After filtration, the 0.45 µm membrane filters are incubated at 37 ^{o}C for 24 h on m-ColiBlue24 medium for *E. coli* and coliforms isolation, on MEI agar for *Enterococcus* isolation (for both raw and treated water). *E. coli* and coliforms colonies, (respectively blue and red on m-Coli Blue medium) and *Enterococcus* colonies (blue on MEI agar) are enumerated.

Figure 3a. ***Filtration process of the raw water samples***

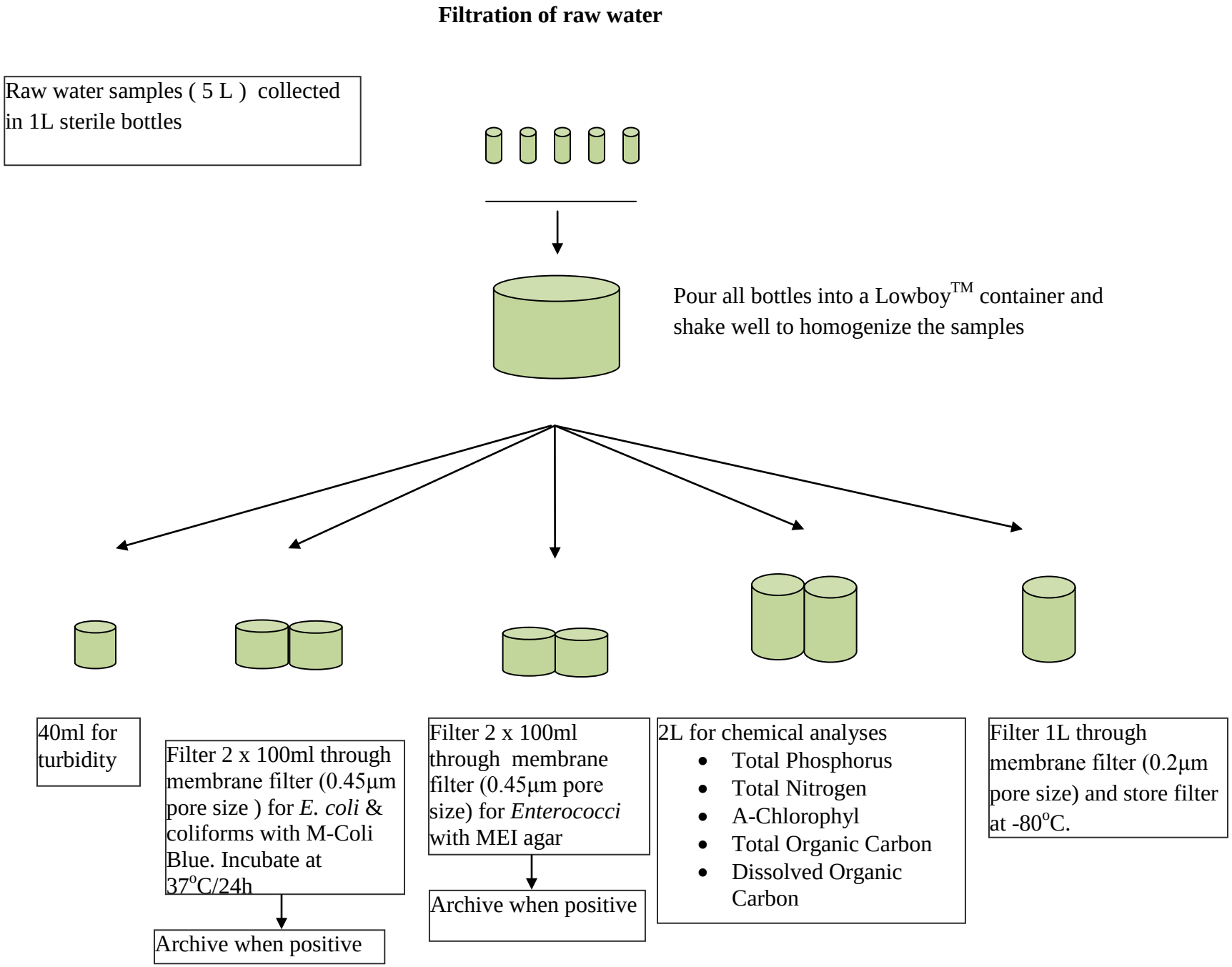
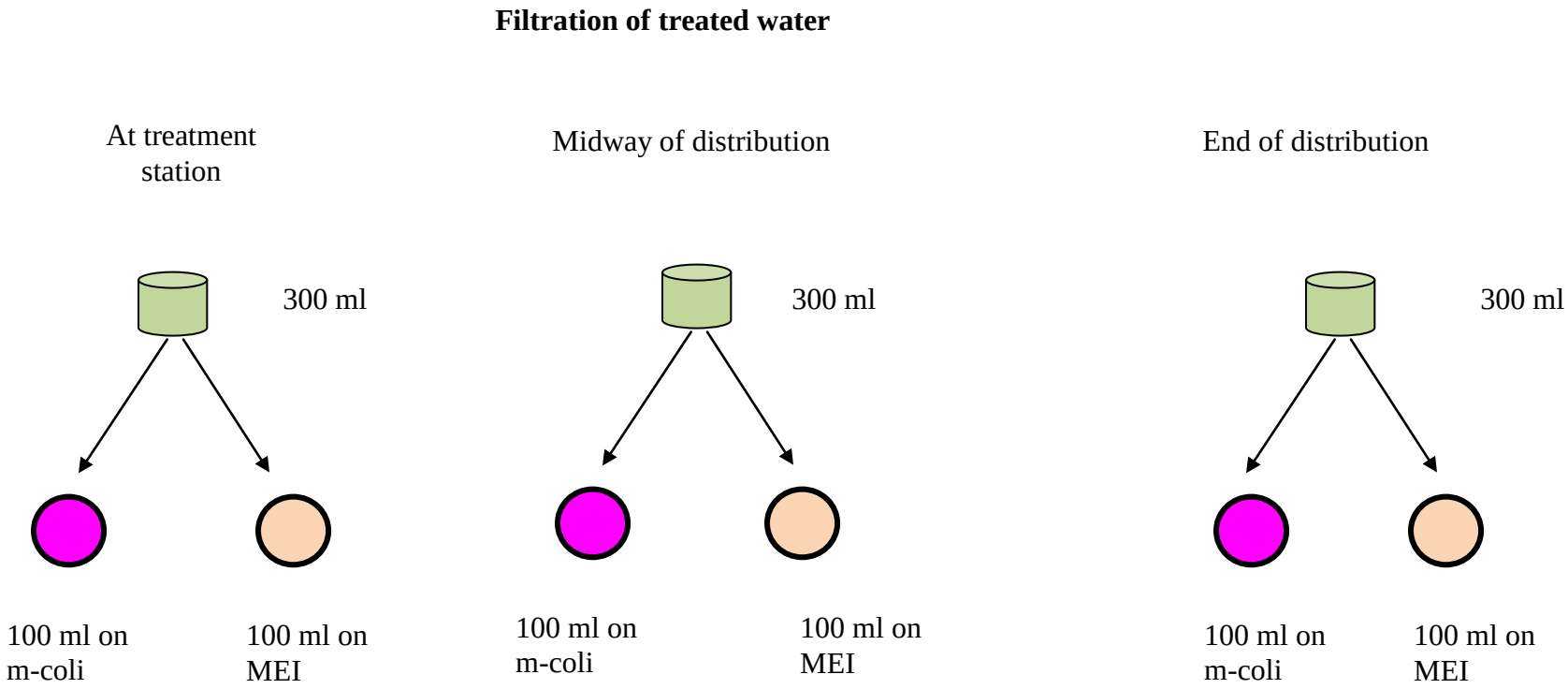


Figure 3b. Filtration process of the treated water samples



Chemical analysis of water samples

Raw water samples were analyzed for Total Organic Canbon (TOC), Dissolved Organic Carbon (DOC), total phosphorus, and total nitrogen concentrations. For the raw water, 2 L of water was taken from the 5 L of mixed sample for analyses as follows: 300 mL of raw water was analyzed for total phosphorus and total nitrogen (Lachat Autoanalyzer QuickChem 8000 and 8500 series, respectively, Lincolnshire, IL, USA). An additional 300 mL were filtered through a glass microfiber filter (GF/F, 0.7µm nominal rate) to determine a content of a-Chlorophyl in water column. The filtrate and the unfiltered water were analyzed for dissolved organic carbon (DOC) and total organic carbon (TOC) (TOC-V CPH Shimadzu, Kyoto, Japan), respectively according to the US EPA methods 415.1, Editorial Revision 1974 ("Organic Carbon, Total [Combustion or Oxidation]) and 9060A "Total Organic Carbon", November 2004, Revision 1.

From the treated water samples, disinfection by-products (DBP), such as Trihalomethanes (THM) and Haloacetic acids (HAA) were analyzed using USEPA methods 502.2, 551.1, and 552.2, respectively. THM compounds were extracted from treated water samples using solid phase micro extraction (SPME) procedure, followed by their analysis on gas chromatograph (Varian 3800, Palo Alto, USA) coupled with Ion Trap Mass Spectrometer (Varian Saturn 2200, Palo Alto, USA) as a detector. 40 ml of treated water was used in liquid-liquid extraction procedure for the extraction of HAAs followed by methylation procedure and their analysis on gas chromatograph equipped with VF-5ms 30m x 0.25mm column and an electron capture detector (ECD).

Microbiological analysis

Purification of presumptive E. coli and bacterial isolates archiving

Colonies of coliforms and *Enterococcus* were picked, inoculated in Luria-Bertani broth (LBB) and Muller Hinton broth, respectively, and incubated at 37^{o}C for 24h. After incubation, 200 µL of the broths was archived in 800 µL of 60% glycerol at -80^{o}C. Colonies of *E. coli* were picked and incubated in LBB at 37^{o}C for 24h. After incubation, aliquots of the broth were streak plated on McConkey agar for further purification (24h at 37 ^{o}C, twice).

Confirmation of presumptive E. coli by PCR

Whole cells from isolated presumptive *E. coli* colonies on McConkey agar were swirled in 200 µL nuclease free water and used as DNA templates. A PCR targeting *upsA* gene was used to confirm these isolated colonies as true *E. coli*. Forward primer sequence was 5'-CCGATACGCTGCCAATCAGT-3' and reverse primer sequence was 5'-ACGCAGACCGTAGGCCAGAT-3' (Chen *et al*., 1998). Final concentrations in the *uspA*-PCR reaction (25 µL) were 150 nM of each of the primers (Invitrogen), 3.75 mM of $MgCl_2$, 3 µM of dNTPs, 0.04 U *Taq* DNA Polymerase (Fermentas), PCR buffer and nuclease free water to complete the volume. Amplification was performed in a PxE0.2 thermal cycler (Thermo Electron Corporation, U.S.A.) with an initial denaturation at 95^{o}C for 5 min; 30 cycles consisting of 94 °C for 2 min, 70 °C for 1 min, and 72 °C for 1 min. *E. coli* K12 was used as the positive control, and nuclease free water as the negative control. The PCR products were separated by horizontal gel electrophoresis on a 1.8% agarose gel, stained in SYBR Safe DNA gel stain (Invitrogen Canada, Burlington, Canada) for 30 min, and the gel images were processed in a Gel Doc XR+ instrument (Bio-Rad, Canada). The expected amplicon size for a positive *uspA* sample was 884 bp. A BOX-

PCR was then performed on these 'true' *E. coli* in order to track the animal source of their presence in the raw water.

Statistical analysis of BOX-PCR DNA fingerprints

E. coli DNA fingerprints from water samples were compared against our existing *E. coli* DNA fingerprints library which is composed 535 of fingerprints from different known animal sources collected in Canada. Our library contains BOX-PCR DNA fragment sizes between 250-bp and 8000-bp (showing distinctive DNA bands) from human, horse, cow, dog, raccoon, mule deer, black bear, coyote, gull, marmot, elk, and wolf.

Discriminant function analysis (unstandardized function coefficients; unexplained variance method; all groups equal prior probabilities, within groups covariance matrix, within groups correlation matrix, leave-one-out classification) was performed on the library BOX-PCR DNA fingerprints to determine the number and percentage of isolates from each known source that were classified in the correct source categories (SPSS, ver. 19.0 for Windows). For each known source, the percentage of isolates that were classified in the correct source category is named rate of correct classification (RCC) while the weighted average of the percentages of all isolates correctly classified in their source categories corresponds to the average rate of correct classification (ARCC). The leave-one-out classification or cross-validation is generated when the library isolates are self-crossed as both the calibration data set and test data set. The characteristics of the Bacterial Source Tracking (BST) library used in this study are summarized in the following Table. This BST Library presents an original RCC of 93.1% and an overall cross-validation ARCC of 86.9% for the 12 source groups' distribution.

Animal Source	Number of library fingerprints	Original RCC %	Cross-Validation RCC %
Horse	38	100.0	97.4
Cow	55	100.0	100
Dog	45	100.0	100
Mule Deer	26	76.9	76.9
Elk	56	100.0	94.6
Wolf	60	90.0	85
Coyote	48	95.8	95.8
Black Bear	30	90.0	80
Marmot	53	100.0	98.1
Raccoon	37	100.0	100
Gull	28	85.7	64.3
Human	59	72.9	45.8
Total	535	93.1	86.9

RCC = Rate of Correct Classification

ARCC = Average Rate of Correct Classification

After analyzing unknown samples with Discriminant Analysis, the probabilities of group membership were analyzed manually in Excel (Microsoft Office 2007) and a threshold of group membership was set at 0.80. If a sample presented a group membership probability in one of the source groups superior or equal to 0.80, then it was classified in this group. If a sample presented a group membership probability inferior to 0.80, it was then assigned the Non-Identified (NI) tag.

DNA extraction from filtered water samples prior to real-time PCR assays

Genomic DNA of all bacteria from membrane filters were extracted using the Rapid Water DNA isolation kit (Mo-Bio, Canada) and according to the manufacturer's instructions. Several PCR-inhibitors are removed during this step. The concentration of isolated DNA was determined by measuring the optical density at 260 nm (Nano-Drop 2000, Thermo Fisher Scientific, Canada) and the purity was assessed by the examination of 260/280 nm optical density ratios. All DNA samples classified as pure, defined by having a 260/280 nm optical density ratio between 1.8 and 2, were adjusted to 20 $ng.\mu L^{-1}$ in TE buffer and stored at -20 °C until required for analysis.

Real-Time PCR analysis

Using the Standard mode of the7900 HT Fast Real-Time PCR System (Applied Biosystems, Canada), Power SYBR Green Master Mix (Applied Biosystems, Canada) was used for primers concentrations optimizations and for runs with primers sets only; TaqMan Universal Master Mix II with no AmpErase UNG (Applied Biosystems, Canada) was used for probes concentrations optimizations and for runs with primers and probes sets. When using primers sets only, amplification products were checked with melting curve analysis. The standard real-time PCR thermal cycling conditions are: 95°C for 10 min, followed by 35 cycles consisting of 95°C for 15s and 60°C for 1 min. For the fast mode, Fast SYBR Green PCR Master Mix or TaqMan Fast Universal PCR Mater Mix with no AmpErase UNG will be used (Applied Biosystems, Canada) for primers sets only, and primers and probes sets, respectively. All master mixes contain ROX as a passive reference dye.

All final PCR reaction volumes were 20 µL. Each sample was tested in triplicate, with 5 µL of sample DNA per reaction. Fluorescence of probes dyes was measured at their respective wavelengths during the annealing/elongation step of each cycle. If a sample presented no amplification in any of the primers and probes sets, then the assays were redone with 100 fold dilutions in order to circumvent possible PCR inhibitors and false negative results.

The analytical sensitivity of the PCR assay was determined using purified genomic DNA of cultures (standard curves) from reference strains. Genomic DNA of bacterial cultures was extracted using the InstaGene Matrix (Bio-Rad, Canada) and according to the manufacturer's instructions. The extracted DNA was serially diluted to construct standard curves. The reference bacterial strains used in this research project were *E. coli* W3110 (courtesy from Dr. Barbara Currie, University of Victoria, Canada) for the coliform bacteria and *E. coli* assays and *Enterococcus faecium* (ATCC 35667) for *Enterococcus* primers and probes sets. Others reference strains included *E. coli O157:H7* (ATCC 43895), *Salmonella enterica* (ATCC 14028), *Aeromonas hydrophila* (ATCC 35654), *Campylobacter jejuni*. (ATCC 33291). The amplification efficiency (E) of PCR was estimated by using the slope of the standard curve based on the following formula $E=10^{(-1/slope)}-1$. Data analysis was performed using SDS software (Applied Biosystems).

RESULTS AND DISCUSSION

From September 2010 to August 2011, numerous raw and treated water samples were analyzed for bacterial and chemical contents (turbidity, nutrients and DBPs). The majority of microorganisms detected in the raw water samples were non-*E. coli* coliforms followed by *Enterococcus* or *E. coli*. During this study period, non *E.coli* bacterial contamination was detected in the majority of raw water samples with the highest concentrations in the fall (September, October and November, see Excel file in the attachment). Organic matter, such as total organic carbon (TOC), was found in the range between 3 and 8 mg/L with a spike up to 23 mg/L in November due to probably a sample contamination. Total nitrogen (TN) was the second highest nutrient in concentration found in the raw water. Total phosphorus was consistently low in all samples. The level of DBP compounds in treated water samples varied ranging from 30 µg/L to the levels exceeding the GCDWQ regulation. Bacteria source tracking (BST) analyses were performed using our *E. coli* DNA fingerprint library containing 535 known sources from around British Columbia. In general, it showed very few bacterial contaminations from human source, but the sampling period started during late summer, after the swimming and recreational time slowed down.

Water Chemistry & Nutrients

Total phosphorous (TP) concentrations in Shawnigan Lake generally remained below 7 µg/L, except for 3 peaks of 12.75 µg/L seen in the lake in September 2010, and around 8 µg/L in February 2011. During 2010-2011, the mean epilimnetic TP has been 6.7 µg/L, which is higher long-term average TP for Shawnigan Lake of 5.9 µg/L.

Nitrogen and total organic carbon concentrations were also below guideline levels and showed no trend over the period of record. The nutrient results indicate that Shawnigan Lake continues to be oligotrophic, which is desirable from a recreational and drinking water supply water quality perspective (Figure 4). TN remains below 250 µg/L at all depths, and mean epilimnetic TN is at 213.22 µg/L. Note that TOC is the precursor for the formation of disinfection by-products following chlorination of water.

The nitrogen to phosphorus ratio (TN: TP) is a good indicator of which of these two nutrients is the limiting factor in lake productivity (algal growth); TN: TP > 20 indicates phosphorus (P) limitation, while TN: TP < 20 indicates nitrogen (N) limitation (Wetzel 2001). Shawnigan Lake has been P-limited during our study since September 2010 with the mean TN:TP ratio at 31.8. TN:TP ratio below 15 as it happens under high P loading leads to bloom of blue-green algae with potential for taste and odour and algal toxins in water.

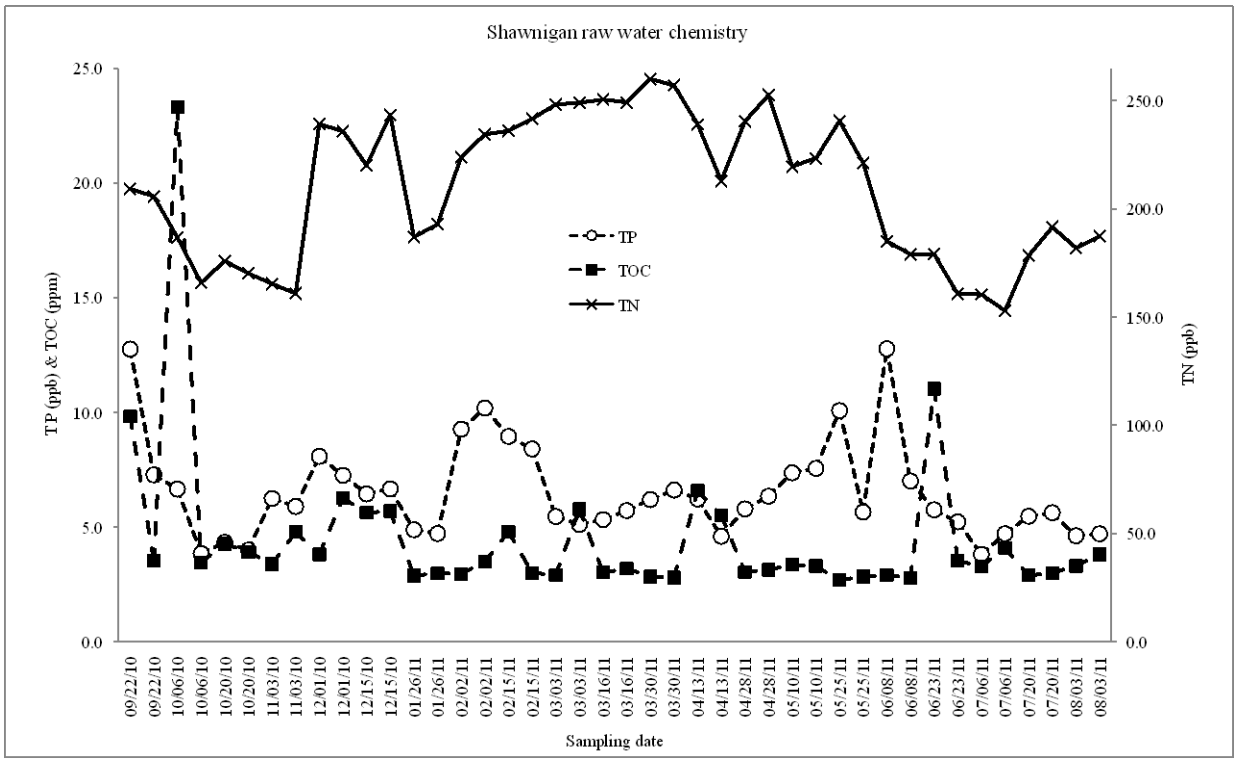


Figure 4. ***Water chemistry of the samples of raw water from Shawnigan Lake.***

Total organic carbon (TOC) concentrations are also, typical of oligotrophic lakes (Figure 2). The majority of carbon in the reservoir is in a dissolved state. In Shawnigan Lake Reservoir, TOC ranged from about 3 to 6 mg/L, with one spike up to 23.5 mg/L range in Sep-Oct. due to probably a sample contamination. The turbidity of the untreated water is low (below 1 NTU), Table 2. The level of *chlorophyll A* extracted from untreated water samples is in the range between 0.009 and 0.045 ug/L rising in October, November and March, Table 2.

Field ID	Sampling date	Turbidity (NTU)	Chloro.A(ug/L)
SHAW-01 (EPI)	09/22/10	na	0.018
SHAW-01(HYPO)	09/22/10	na	0.022
SHAW-01 (EPI)	10/06/10	na	0.019
SHAW-01(HYPO)	10/06/10	na	0.017
SHAW-01 (EPI)	10/20/10	na	0.029
SHAW-01(HYPO)	10/20/10	na	0.026
SHAW-01 (EPI)	11/03/10	na	0.033
SHAW-01(HYPO)	11/03/10	na	0.036
SHAW-01 (EPI)	12/01/10	na	0.018
SHAW-01(HYPO)	12/01/10	na	0.018
SHAW-01 (EPI)	12/15/10	na	0.019
SHAW-01(HYPO)	12/15/10	na	0.019
SHAW-01 (EPI)	01/26/11	0.5	0.019
SHAW-01(HYPO)	01/26/11	0.4	0.024
SHAW-01 (EPI)	02/02/11	0.5	0.010
SHAW-01(HYPO)	02/02/11	0.5	0.007
SHAW-01 (EPI)	02/15/11	0.4	0.009
SHAW-01(HYPO)	02/15/11	0.4	0.011
SHAW-01 (EPI)	03/03/11	0.6	0.016
SHAW-01(HYPO)	03/03/11	0.7	0.016
SHAW-01 (EPI)	03/16/11	0.8	na
SHAW-01(HYPO)	03/16/11	0.8	na
SHAW-01 (EPI)	03/30/11	0.5	0.045
SHAW-01(HYPO)	03/30/11	0.8	0.039
SHAW-01 (EPI)	04/13/11	0.55	0.029
SHAW-01(HYPO)	04/13/11	0.85	0.031
SHAW-01 (EPI)	04/28/11	0.5	tba
SHAW-01(HYPO)	04/28/11	0.47	tba
SHAW-01 (EPI)	05/10/11	0.51	tba
SHAW-01(HYPO)	05/10/11	0.42	tba
SHAW-01 (EPI)	05/25/11	0.34	tba
SHAW-01(HYPO)	05/25/11	0.35	tba
SHAW-01 (EPI)	06/08/11	0.45	tba
SHAW-01(HYPO)	06/08/11	0.65	tba
SHAW-01 (EPI)	06/23/11	0.45	tba
SHAW-01(HYPO)	06/23/11	0.4	tba
SHAW-01 (EPI)	07/06/11	0.41	0.014
SHAW-01(HYPO)	07/06/11	0.38	0.013
SHAW-01 (EPI)	07/20/11	0.45	tba
SHAW-01(HYPO)	07/20/11	0.51	tba
SHAW-01 (EPI)	08/03/11	0.36	0.009
SHAW-01(HYPO)	08/03/11	0.37	0.009
SHAW-01 (EPI)	08/17/11	tba	tba
SHAW-01(HYPO)	08/17/11	tba	tba

Table 2. **Chlorophyll A** ***(ug/L) and Turbidity (NTU) data of the samples of untreated water from Shawnigan lake in the period from September 2010 to August 2011.***

Disinfection By-Products (DBPs)

During the study period, from September 2010 to August 2011, the Cowichan Valley Regional District in partnership with the Laboratory Water and Aquatic Sciences Research at the University of Victoria provided biweekly samples of treated drinking water for analysis for the presence of DBPs. The samples were taken at three locations of the distribution system, at the chlorination station, the middle point and the end of the distribution system. Results show that total Halo-Acetic Acids (THAAs) range from 29.8 to 146.98 μg/L at the chlorination station, from 40.5 to 187.13 μg/L at the middle, and from 30.4 to 175.81 μg/L at the end, with a yearly mean of 83.6 μg/L (Figure 3) and an annual standard deviation of 40.0 μg/L. Total Trihalomethanes (TTHMs) were also variable, with the ranges of 25.5 to 142.1, 34.2 to 194.6 and 59 to 217.0 μg/L at the start, middle and the end of the distribution system, respectively. A yearly mean for TTHMs is 98.2 μg/L (Figure 3) with an annual standard deviation of 50.5. Note that regulatory guidelines for THM and HAA are 100 μg/L and 80 μg/L, and the concentrations are frequently above guidelines.

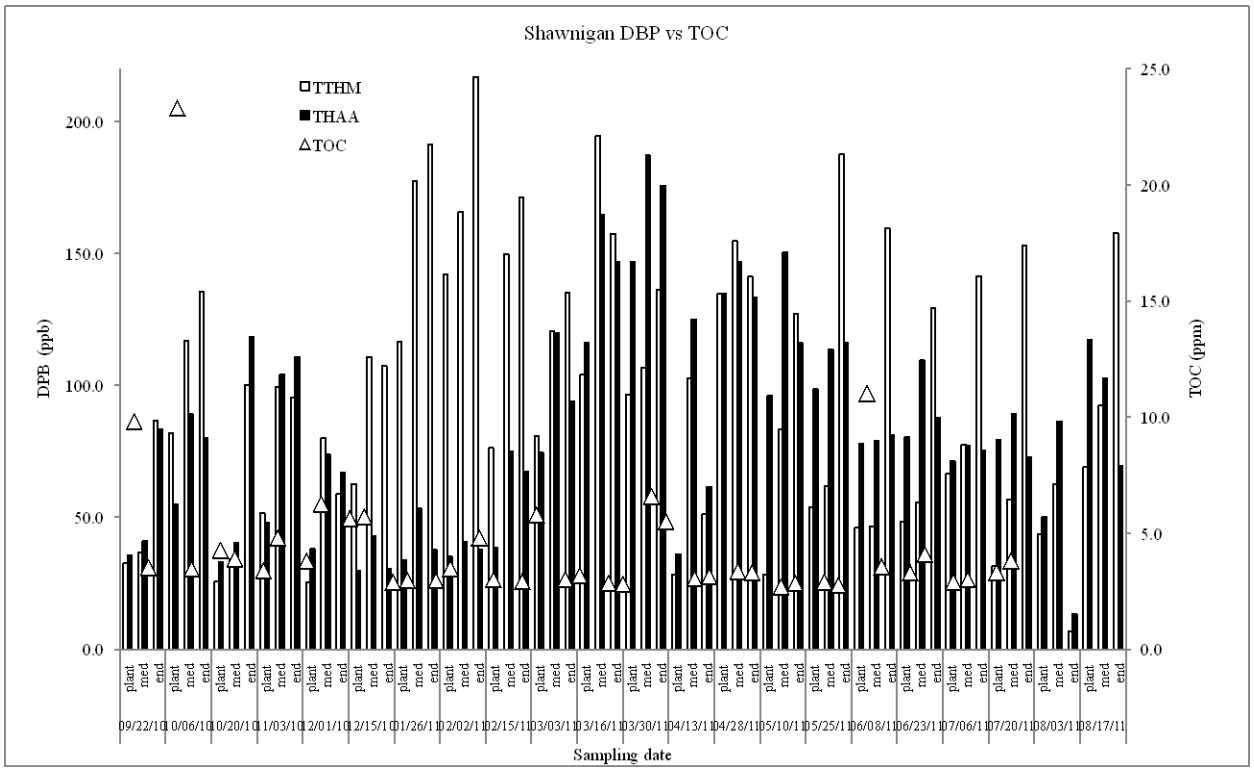


Figure 5. ***Results of DBP and TOC analysis obtained on treated water samples from Shawnigan Lake.***

Bacteriological Analysis of Raw Water

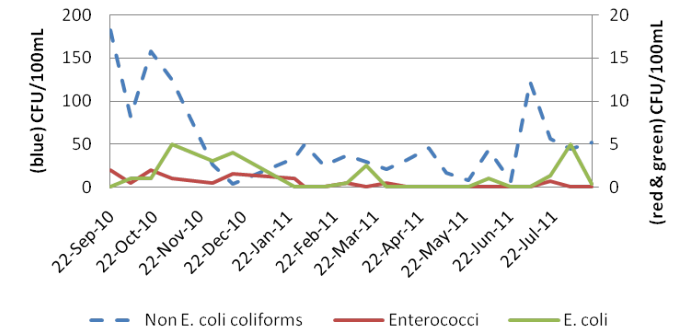
Raw and treated water samples (n=110) were collected and analyzed biweekly.

Bacterial concentrations of non-*E. coli* coliforms shown to be higher through September to November, and in July and August, and relatively low and constant from November to June, in both the epilimnion (Figure 3 a, b) and the hypolimnion (Figure 3 c, d) layers of the lake. Relatively very low *E. coli* have been isolated from the raw water samples. Overall bacterial source distribution in raw water samples is shown in Figure 4 a, b, while the ones corresponding to the epilimnion and hypolimnion layers are shown in Figure 3 b, d respectively.

The BST (Bacterial Source Tracking) analysis (Figure 4 a, b) of *E. coli* isolates in raw water

samples showed that almost one third of the contamination came from horse, while another third of the contamination came from the following three sources: gull, black bear or dog.

A

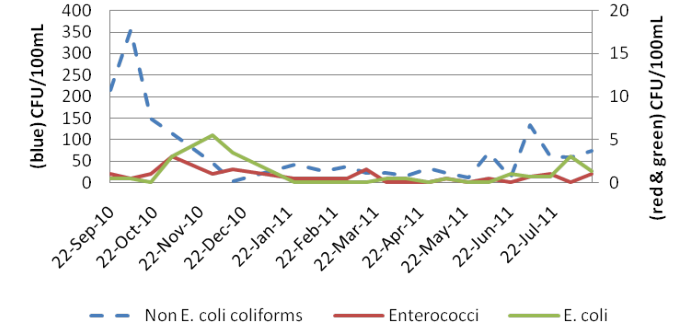


B

Shawnigan - Epi BST

Source	Number of isolates	Percentage (%)
Horse	4	19.0
Cow		0.0
Dog	2	9.5
Mule Deer		0.0
Elk	1	4.8
Wolf	1	4.8
Coyote		0.0
Black Bear	2	9.5
Marmot		0.0
Raccoon	1	4.8
Gull	3	14.3
Human	3	14.3
NI	4	19.0
Total	21	100.0

C



D

Shawnigan - Hypo BST

Source	Number of isolates	Percentage (%)
Horse	7	36.8
Cow	1	5.3
Dog	2	10.5
Mule Deer	1	5.3
Elk	1	5.3
Wolf	1	5.3
Coyote		0.0
Black Bear	2	10.5
Marmot		0.0
Raccoon		0.0
Gull	3	15.8
Human		0.0
NI	1	5.3
Total	19	100.0

Figure 6a,b,c,d. ***Bacterial counts and sources of fecal bacteria in raw water from Shawnigan Lake.***

Concerning the treated water, samples were taken at plant, in the middle, and at the end of the distribution system. Our analysis showed the presence of non-*E. coli* coliforms in the following treated samples:

Figure 7. ***Overall bacterial source distribution in raw water collected at Shawnigan lake. A: results' table; B: pie chart.***

A

Shawnigan - Global BST

Source	Number of isolates	Percentage (%)
Horse	11	27.5
Cow	1	2.5
Dog	4	10.0
Mule Deer	1	2.5
Elk	2	5.0
Wolf	2	5.0
Coyote	0	0.0
Black Bear	4	10.0
Marmot	0	0.0
Raccoon	1	2.5
Gull	6	15.0
Human	3	7.5
NI	5	12.5
Total	40	100.0

B

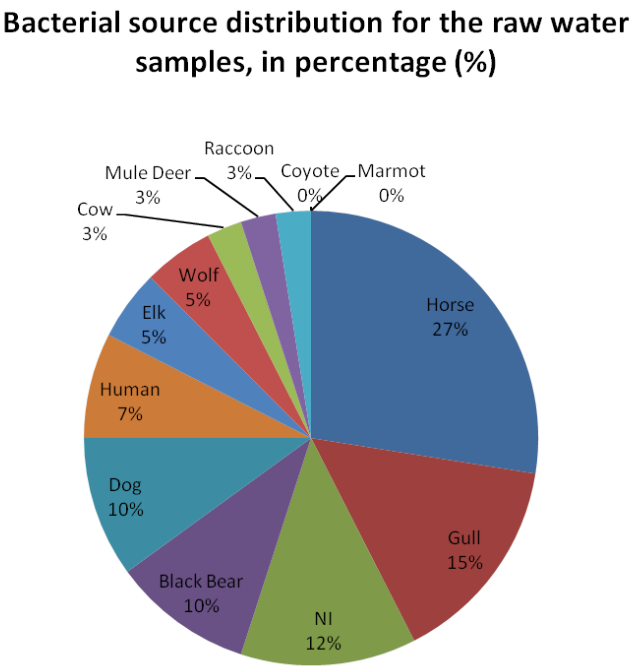


Figure 8. ***Non*-E. coli *coliform counts in treated water from Shawnigan distribution system.***

	Distribution system:		
Date	Plant	Middle	End
22-Sep-10	5	7	0
20-Oct-10	2	1	0
1-Dec-10	31	0	4
2-Feb-11	4	0	0
20-Jul-11	1	0	2
3-Aug-11	3	0	0
17-Aug-11	1	0	0

No Enterococci or *E. coli* bacteria have been found in treated water.

REFERENCES

Aramini, J. J., Stephen, C., Duby, J. P., Engelstoft, C., Schwantje, H., and Ribble, C. S. 1999. Potential contamination of drinking water with *Toxoplasma gondii* oocysts. *Epidemiol. Infect.* 122, 305–315.

Auld, H., Klaassen, J., and Geast, M. 2001. Report on an assessment of the historical significance of rainfalls in the Walkerton area during May, 2000. Background for testimony to the Walkerton Inquiry in January 2001. Atmospheric Science Division, Meteorological Service of Canada, Environment Canada, Toronto, ON.

Bowie, W. R., King, A. S., Werker, D. H., Isaac-Renton, J. L., Bell, A., Eng, S. B., and Marion, S. A. 1997. Outbreak of toxoplasmosis associated with municipal drinking water. BC Toxoplasma Investigation Team. *Lancet* 350, 173–177.

Carson, C. A., Shear, B.L., Ellersieck, M.R., and Schnell, J.D. 2003. Comparison of ribotyping and repetitive extragenic palindromic-PCR for identification of fecal *Escherichia coli* from humans and animals. *Appl. Environ. Microbiol.* **69:** 1836-1839.

Center for disease control and prevention. 1993. Surveillance for Waterborne Disease Outbreaks -- United States, 1991-1993. *Morbidity and Mortality 2eekly Report*. 43:1-33.

Clesceri LS, Greenberg AE & Eaton AD 1998. Standard methods for the examination of 2ater and wastewater, 30th edition, American Public Health Association, washington, D.C.

Colwell, R.R., 1996. Global change and infectious disease: the cholera paradigm. Science 274, 2025-2031.

Crowther, J. Kay, D., and Wyer, MD., 2002. faecal-indicator concentrations in waters draining lowland pastoral catchments in the UK: relationships with land use asn farming practice. Water Resources 36, 1725-1734.

Curriero, F.C., Patz, J.A., Rose, J.B., and Lele, S., 2001. The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948-1994. America Journal of Public Health 91, 1194-1199.

Davies, J.M., Mazumder, A. 2003. Health and environmental policy issues in Canada: the role of 2atershed management in sustaining clean drinking 2ater quality at surface sources. *J. Environ. Manage.* **68**: 373-386.

Dombek, P.E., Johnson, L.K., Zimmerley, S.T., Sadowsky, M.J. 2000 Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. *Appl. Environ. Microbiol.* **66:** 3573-3577.

Ezzati, M., Lopez., A.D., Rodgers, A., and Murray, C.J.L., 2004. Comparative Quantification of Health Risks: Global and Regional Burden of Diseases due to Selected Major Risk Factors. World Health Organization, Geneva.

Golfinopoulos, S. K. and Nikolaou, A. D. 2005. Formation of DBPs in the drinking water of Athens, Greece: a ten-tear study. Global NEST Journal. 7: 106-118.

Hunter, P.R., 2003. Climate change and waterborne and vector-borne disease. Journal of Applied Microbiology 94, 37S-46S.

Johnson, L.K., Brown, M.B., Carruthers, E.A., Ferguson, J.A., Dombek, P.E., Sadowsky, M.J. Sample size, library composition, and genotypic diversity among natural populations of *Escherichia coli* from different animals influence accuracy of determining sources of fecal pollution. *Appl. Environ. Microbiol.* 3004; **70:** 4478-4485.

Islam, M.S., Draser, B.S., and Bradley, D.J., 1990. Long-term persistence of toxigenic *Vibrio cholerae* O1 in the mucilaginous sheath of a blue-green alga, *Anabaena variabilis*. Amer. J. Trop. Med. Hyg. 53, 133–139.

Hrudey, S. E., Payment, P., Huck, P. M., Gillham, R. W., and Hrudey, E. J. 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: Comparison with other waterborne outbreaks in the developed world. *Water Sci. Technol.* 47:7–14.

Levy, D.A., M.S. Bens, G.F. Craun, R.L. Calderon, Herwaldt, B.L. 1998. Surveillance for waterborne-Disease Outbreaks—United States, 1995-1996. Center for Disease Control Morbidity and Mortality 2eekly Report **47 (SS-5)**:1-34.

Lobitz, B., Beck, L., Hug, A., Wood, B., Fuchs, G., Faruque, A.S.G., and Colwell, R., 2000. Climate and infectious disease: use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. Proceedings of the National Academy of Science 97, 1438-McLellan, S.L., Daniels, A.D., Salmore, A.K. 2003. Genetic characterization of *Escherichia coli* populations from host sources of fecal pollution by using DNA fingerprinting. *Appl. Environ. Microbiol.* **69:** 3587-3594.

Meays, C.L., Broersma, K., Nordin, R., Mazumder, A. 2003. Source tracking fecal bacteria in 2ater: a critical revie2 of current methods. *J Environ. Manage.* 3004; **73:** 71-79.

Mohapatra, B.R., Broersma, K., Nordin, R., Mazumder, 2007a. A. Evaluation of repetitive extragenic palindromic-PCR for discrimination of fecal *Escherichia coli* from humans, and different domestic- and 2ild-animals. *Microbiol. Immunol.* **51**: 733-740.

Mohapatra, B.R., Broersma, K., Mazumder, A. 2007b. Comparison of five rep-PCR genomic finger printing methods for differentiation of fecal *Escherichia coli* from humans, poultry and wild birds. FEMS *Microbiol. Lett.* **377**: 98-106.

O'Connor, D. R. 2002. *Report of the Walkerton Inquiry. Part one: The events of May 2000 and related issues.* Toronto: Queen's Printer for Ontario. Available at www.walkertoninquiry.com/report1/index.html#full.

Pascual, M., Rodó, X., Ellner, S.P., Colwell, R., and Bruma, M.J., 2000. Cholera dynamics and El-Nino-Southern Oscillation. Science 289, 1766-1769.

Patz, J.A., Vavrus, S.J., Uejio, C.K., McLellan, S.L., 2008. Climate change and waterborne disease risk in the Great Lakes Region of the U.S. Am. J. Pre. Med. 35, 451-458.

Patz, J.A., McGeehin, M.A., Bernard, S.M., Ebi, K.L., Epstein, P.R., Grambsch, A., Gubler, D.J., Reiter, P., Romieu, I., Rose, J.B., Samet, J.M., and Trtanj, J., 2000. The potential health

impacts of climate variability and change for the United States: Executive summary of the report of the health sector of the U.S. national assessment. Environmental Health Perspectives 108, 367-376.

Patz, J.A., Campbell-Lendrum, D., Hollowat, T., and Foley, J.A., 2005. Impacts of regional climate change on human health. Nature 488, 310-317.

Schuster, C.J., Aramini, J.J., Ellis, A.G., Marshall, B.J., Robertson, W.J., Medeiros, D.T., Charron, D.F., 2005. Infectious disease outbreaks related drinking water in Canada, 1974-2001. Canadian Journal of Public Health 96, 254-258.

Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R., Lukasik, J. 2003. Microbial source tracking: current methodology and future directions. *Appl. Environtl. Microbiol.* **68:** 5796-5803. Simpson, J.M., Domingo, J.2.S., Reasoner, D.J. 2003. Microbial source tracking: state of the science. *Environ Sci. Technol.* **36:** 5379-5388.

Serodes, J.-B., Rodrigues M.J., Li, H., and Bouchard, C. 2003. Occurrence of THMs and HAAs in experimental chlorinated waters of the Quebec City area (Canada). *Chemosphere.* 51: 253-263.

Seurinck, S., Verstraete, 2., and Siciliano, S.D. 2003. Use of 16S-33S rRNA intergenic spacer region PCR and repetitive extragenic palindromic PCR analyses of *Escherichia coli* isolates to identify nonpoint fecal sources. *Appl. Environ. Microbiol.* **69:** 4943-4950.

Speelmon, E.C., Checkley, W., Gilman, R.H., Patz, J., Caleron, M. and Manga, S., 2000. Cholera incidence and El-Nino-related higher ambient temperature. Journal of the American Medical Association 283, 3072-3074.

Stanwell-smith, R., 2001. Waterborne disease and climate change. C in Health effects of climate change in the UK. Website: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4007935.

Upton, S.J. 1999. Waterborne/foodborne outbreaks of *Cryptosporidium parvum.* http://222.ksu.edu/parasitology/2ater. Accessed on 19 November, 2007.

USEPA. 2002a. Wastewater technology fact sheet, bacterial source tracking. Office of water, United States Environmental Protection Agency, Washington, D. C., EPA 832-F-02-010. pp. 1-12.

USEPA. 2005 Microbial source tracking guide. Office of Research and Development, United States Environmental Protection Agency, Cincinnati, Ohio, EPA/600-R-05-064. pp. 150.

Versalovic, J., Koeuth, T., Lupski, J.R. Distribution of repetitive DNA-sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991; **19:** 6833-6831.

Versalovic, J., Schneider, M., de Bruijn, F.J., Lupski, J.R. 1994. Genomic fingerprinting of bacteria 2ith repetitive sequence-based polymerase chain reaction. *Methods Mol. Cell Biol.* **5:** 35-40.

Vitousek, P.M., Mooney, H.A., Lubchenko, J., Mellilo, J.M. 1997. Human domination of Earth's ecosystem. *Science* **377:** 494-499.

WHO 1993. Waterborne infections, gudelines for drinking water quality. World Health Organization, Geneva (online), http://www.studiengang-wasser.de/VorlHygieneTeil5.pdf, accessed 13 December 2006.

WHO 2003 Emerging issues in water and infectious diseases. World Health Organization, Geneva (online), http://www.who.int/water_sanitation_health/emerging/en/emerging.pdf, accessed 13 December 2006.

Wooa, D.M., Vicente, K.J. 2003. Sociotechnical systems, risk management, and public health: comparing the North Battleford and 2alkerton outbreaks. *Reliability Engineering and System Safety*. 80: 353-36.