Burnt Bridge Creek Microbial Source Tracking

Identification of Sources of Microbial Pollution in Burnt Bridge Creek Watershed

DRAFT

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ABSTRACT

Burnt Bridge Creek (BBC) Vancouver, Washington, has been classified by the Washington State Department of Ecology as a Class A Stream. Violations of the fecal coliform standard and other standards for Class A Streams have placed Burnt Bridge Creek on the EPA 303d List of Impaired or Threatened Water Bodies.

To determine the source or sources of fecal contamination in BBC as measured by the fecal coliform level, Microbial Source Tracking (MST) was employed in a two-phase study, Phase One from July 1996 to February 1997 and Phase Two from May 1998 to March 1999. The MST method matches strains of *Escherichia coli* (*E. coli*) a fecal coliform, found in a stream with known *E coli* strains from suspected sources. The most frequently identified source of *E. coli* bacteria in Burnt Bridge Creek is of human origin. As BBC flows westward, the identified *E. coli* from humans increases from N.E. 137th Street, near the headwaters, at 4.4 percent to 20 percent at N.E. 2nd Avenue. The data strongly indicates that the source of human *E. coli* in Burnt Bridge Creek is from septic tank systems and not sanitary sewer lines. Other major sources of microbial pollution in BBC include pets, dogs and cats, migratory birds, urban wildlife, and livestock.

Recommendations for the control of microbiological pollution in BBC watershed include:

- Reducing the number of septic tank systems in the watershed, especially those nearest to BBC.
- Education of pet owners on the proper disposal of wastes from their pets.
- Discourage formation of resident populations of migratory birds.
- Control the population of urban wildlife, opossums, raccoons, and rodents.
- Encourage livestock owners to observe best management practices for pastures and animal waste handling.
- Continue tree planting along the streambeds to reduce the elevated water temperatures in BBC, which in turn will reduce fecal coliform regrowth.

Another recommendation is to increase the summer Burnt Bridge Creek water flow by the additional of high quality groundwater to the stream. If feasible, this action could improve BBC water quality in regards to fecal coliform, temperature, and dissolved oxygen levels, all currently in violation of Class A stream standards. The potential of infection from pathogenic bacteria resulting from stream contact would also be reduced. The addition of groundwater to BBC could be an interim measure to improve stream water quality if the other recommended activities are successful.

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1.0 INTRODUCTION

1.1 *Overview*

In June of 1996 this study was initiated to determine the sources of fecal contamination in Burnt Bridge Creek (BBC) which is located in Vancouver, Washington. Burnt Bridge Creek has been classified by the Washington Department of Ecology as a Class A stream, although it historically has had exceedences in temperature, pH, turbidity, dissolved oxygen, and fecal coliforms.

The present study is a part of a larger effort by the City of Vancouver to develop a comprehensive Water Systems Master Plan, which aims at protecting and managing the water resources within the Burnt Bridge Creek Watershed.

The methodology used to identify the sources of fecal coliforms in the BBC watershed is referred to as Microbial Source Tracking (MST). It is based on the ability to identify and match microorganisms found at different locations in the environment with the point or non-point sources (human, animals, etc.) of those organisms on the basis of their genetic fingerprint patterns. This is done by matching DNA fingerprint patterns of *Escherichia coli* strains isolated from the impacted site with the genetic fingerprint patterns of *E. coli* isolated from the suspected sources of microbial pollution in the watershed.

1.2 *Background*

Burnt Bridge Creek runs from 162nd Avenue into Vancouver Lake and is the main outlet for stormwater runoff in the BBC watershed.

The Burnt Bridge Creek Watershed is approximately 26 square miles. It is made up of 16 subbasins (drainage basins) that extend approximately 12 miles. Its boundaries are Vancouver Lake (east), 162nd Avenue (west), Mill Plain Boulevard (south), and 99th Street (north). Aquifers in the Burnt Bridge Creek Watershed are one of the main sources of the water for the City of Vancouver.

Several studies have shown water quality deterioration in the BBC and its tributaries. A study and management plan developed for BBC in 1977 identified septic tank effluent as a major source of pollution to the creek and called for elimination of septic systems in the basin. Agricultural activities, surface runoffs, and wildlife also impact the watershed.

From the standpoint of the watershed management policy it is important to identify the contributing sources of microbial pollution in the BBC Watershed, the BBC, and the underlying aquifers.

1.3 *Burnt Bridge Creek Watershed*

Location

The Burnt Bridge Creek Watershed (BBCW) extends approximately 12 miles from Vancouver Lake eastward to 162nd Avenue and north from Mill Plain Boulevard to 99th Street (Figure 1) and is made up of 16 subbasins. The watershed covers about 26 square miles (16,900 acres).

Groundwater hydrogeology and Geology

Most of the BBC watershed is relatively flat, 200 to 300 feet above the Columbia River. The water table lays 10-100 feet underground. The BBCW aquifers are the main source of water for the city of Vancouver and many industrial users in the area. A study by the US geological survey in 1988 showed that about one third of ground water used was from the water table aquifer and the other two third were from the deeper aquifers. It is estimated that of all the water entering the BBCW about 58 percent is from precipitation and 32 percent is groundwater flow from outside of the watershed. Approximately 10% of the BBCW water goes to the BBC base flow, 38% is extracted from wells, and 52% flows out of the watershed.

In the BBC basin rock materials at land surface consist of gravel and sand. The surface deposits are underlain by a series of older river-laid sediments including consolidated gravel and sand and silt.

Wetlands

Prior to about mid 19th century there was approximately 1800 acres of wetland in the area. Most of the wetlands were drained and converted to farmland. There are approximately 280 acres of wetland left in the BBC watershed.

Land Use

The BBC Watershed has undergone heavy urbanization (residential, commercial, industrial) during the past 50 years. Residual agricultural activities continue in the watershed. It has been estimated that there are 6,900 septic systems in the BBC basin.

Water Quality

Burnt Bridge Creek has been classified by the Washington Department of Ecology as a Class A stream. This classification is defined as excellent water quality with characteristic uses of water supply, stock watering, fisheries habitat, wildlife habitat, recreation, commerce and navigation (WAC 173-201A-030). There are two water quality criteria for fecal coliform bacteria levels in Class A freshwater. The concentration of organisms is not to exceed a geometric mean value of 100 colonies per 100 milliliters of water sampled, and not more than 10 percent of all samples obtained for calculating this mean can exceed 200 colonies per 100 milliliters.

Limited water quality testing in BBC was conducted by the Washington Department of Ecology in 1972-73, and in 1974 by the Southwest Washington Health District. The first extensive study of the BBC water quality was performed in 1976 by the consulting firm

of Kramer Chin Mayo (KCM). The KCM study showed violations of surface water standards in terms of temperature, dissolved oxygen, sediment, and bacteria, as well as problems with high levels of nutrients and metals. The KCM study concluded that the BBC showed progressive degradation from headwater to Vancouver Lake at its downstream end. The study attributed the degradation in the water quality of the BBC to agricultural and construction-related runoff, street runoff, lack of streamside shading, and failing septic systems.

A 1990 report on the water quality of BBC by the Southwest Washington Health District states that none of the sampling stations met all the water quality standards for a class A stream.

The Clark County Water Quality Division report for 1991-1993, showed that 61.6% of all the samples exceeded microbiological quality standards for a class A stream.

1.4 Study Goal and Objectives

The goal of the Microbial Source Tracking survey of Burnt Bridge Creek was to help determine the contribution to contamination by two primary potential sources, animals (livestock, pets, and wildlife) and septic systems. This was performed by sampling both the water from the stream and potential source fecal material from the watershed. The samples were then processed to establish collections of bacterial cultures representative of the *Escherichia coli (E.coli)* population in each sample. Genetic fingerprinting (using ribosomal RNA typing) was performed on each *E. coli* isolate. These patterns or DNA types, referred to below as ribotypes, were then used to match specific bacteria from a contaminated site in the creek to its source.

2.0 APPROACH AND METHODS

2.1 *Conventional Analysis*

Numerous human pathogens are spread by fecal contamination of water. Examples are *Vibrio cholera, Salmonella typhi, Giardia lamblia, Cryptosporidium parvum, E. coli* O157:H7 and Hepatitis A. These pathogens can be a risk to human health even at very low concentrations. Due to difficulties in the detection, identification, and enumeration of specific human pathogens in environmental and food samples, the concept of indicator organisms and related methodologies were developed and implemented in the late 1800's. Indicator organisms are used to assess the potential for the presence of pathogens. These organisms must be prevalent in feces, found in higher concentrations than pathogens, be more resistant to disinfectants (more persistent in the environment), and easy to quantify. The group of bacteria referred to as fecal coliforms meet these criteria. A formal definition of this group is that they are facultative anaerobic bacilli that ferment lactose with the production of gas within 48 hours at a temperature of 44.5°C. A prevalent and well-studied member of this group is the species *Escherichia coli.*

The concept of indicator organisms is the principal component of regulatory microbiology. The major limitation of this concept is that it is an oversimplification of the complex dynamics of microbial ecology, physiology, and genetics. It is true that often the presence of indicators can be associated with fecal contamination. However, it is also true that in many instances there may be little or no correlation between the presence of indicator organisms and the presence of fecal contamination and human pathogens.

The utility of the indicator concept is further limited by the lack of appropriate methodologies for tracking organisms associated with contamination to their potential sources. Sources of water pollution can be divided into two general groups, point and nonpoint sources. Point sources of pollution have defined discharge points such as pipes (municipal and industrial wastewater). Nonpoint sources of pollution do not have defined discharge points. Because of their diffuse nature, nonpoint sources are difficult to identify and control. Nonpoint sources of microbial pollution include wildlife, agricultural practices, on-site septic systems, commercial and recreational boating, aquaculture, and industrial practices. This impediment to the identification and control of sources of microbial pollution in water adversely affects the decision-making process of water quality and fisheries resources management.

Each year millions of dollars are spent on fecal and total coliform assays to determine the extent of bacterial pollution in water environments, and to satisfy increasingly rigid regulatory requirements concerning microbiological quality of water. Knowing the sources rather than just monitoring the level of pollution enables water quality management efforts to be more effective by directing source control measures where the greatest problem is. Although there are human pathogens associated with fecal pollution of animal origin, the risk to human health would presumably be greater if contamination

is caused primarily by human sources (mainly due to presence of human viruses). For these reasons, there is a need for the Microbial Source Tracking method described below to be used along with conventional analysis to more fully understand and address a bacterial pollution problem.

2.2 *Microbial Source Tracking*

Description

In response to limitations of conventional methods, we have developed the Microbial Source Tracking (MST) method (Samadpour, 1990). MST can be summarized in two steps. The first step is the molecular characterization of strains of the study organism, in this case *E. coli*, by DNA fingerprinting. Ribosomal RNA typing (ribotyping) is employed as the principle method for generating genetic fingerprints in MST studies. Secondly, ribotypes of *E. coli* strains isolated from potential sources are matched with the ribotypes of strains isolated from receiving water to determine the extent and distribution of each source's contribution to contamination.

The data resulting from a MST analysis can be used in:

- understanding the sources, distribution, and movement of microbial populations in the environment
- conducting risk and exposure assessment studies of the potential human effects associated with microbial pollution
- design and implementation of source controls
- studying the effects of control measures
- environmental litigation.

Definitions

An *isolate* is a pure culture of bacteria established from a source using sterile technique and appropriate growth media. The intent is that the culture originates from a single organism.

A *strain* is a classification of a group of organisms within a bacterial species based on relatedness resulting from *clonal descent*. A clone is defined as all the individuals (descendants) derived from a single individual (progenitor) by asexual reproduction (fission). The progenitor and descendants are genetically identical unless mutation occurs. A working definition of clone is: a group of bacterial cultures that have been isolated independently (from different sources, at different times, and in different places) and have so many genotypic and phenotypic characteristics in common that the most likely explanation for their relatedness is that they are of clonal origin.

A *ribotype* is a DNA pattern obtained from the DNA operon, or gene, that codes for ribosomal RNA (rRNA). This operon is highly conserved (not easily mutated) and can be used to distinguish between bacterial strains of the same species over many generations in the environment (Atlas et al., 1992) (Selander et al., 1987). Thus, within a

population of a given bacterial species there may be numerous isolates belonging to a single strain that can be distinguished from other strains of the same species by a unique ribotype.

MST makes use of the ability to classify organisms based on their genetic fingerprints into groups of clonal descent, or strains, as described above. The second concept forming the basis for the source tracking theory is that of *resident vs. transient strains* (Hartl and Dykhuizen, 1984). A bacterial strain that has adapted to a particular environment, or host (e.g. animal intestinal tract) is capable of colonizing that environment and competing favorably with members of the indigenous flora. These are called resident strains. Resident strains are usually shed over a long period of time from their host, thus providing a characteristic signature of their source. A transient strain is a bacterial strain that is introduced into a new environment, or host (e.g. into an animal by ingestion) but cannot colonize and persist in that environment. If the host is sampled over time for a given species of bacteria, a few resident strains are consistently observed in the system while a larger number of transient strains are seen passing through.

Rationale

Given that bacterial population structure is clonal and if within each species different clones have adapted to specialized environments, then it should be possible to:

- study a collection of bacterial isolates from a contaminated site (e.g. receiving water) and from possible sources of contamination
- divide the isolates into groups of clonal origin
- match the isolates from the contaminated site to the sources
- identify the contributing sources.

This requires the selection of an appropriate methodology for interstrain differentiation of bacteria. The method of choice needs to be sensitive enough to allow for dividing the species of interest into groups of clonal origin, and the results should be reproducible. The method should also be easy enough to perform, and the results should allow for comparing a large number of bacterial isolates. Ribosomal RNA typing with the use of appropriate restriction enzymes is the method of choice in MST studies of fecal coliforms. In special circumstances such as source tracking studies of *E. coli* O157:H7 (an *E. coli* strain associated with several food-related and recreational water outbreaks), it has been necessary to develop and use other methods of differentiation.

Other Studies

MST has been applied to other studies in addition to Burnt Bridge Creek. These include surveys of shellfish beds in Puget Sound, an urban watershed in northern Seattle, and a large primarily undeveloped watershed of a regional drinking water supply in King County. All of these studies demonstrate the usefulness of the methodology. Each one helps to develop a regional database of *E. coli* isolates and ribotypes. The ability of the method to track contamination is only as good as the information in the database. If

sources of contamination at a particular site have not been characterized, i.e. no source ribotype is available to match to an identified water ribotype, then a match cannot be made. As the database becomes more comprehensive and refined its effectiveness in helping to more fully characterize the nature of contamination at a site is improved.

2.3 *Sampling Procedures and Sampling Sites*

Burnt Bridge Creek Water

Phase one sampling of water from Burnt Bridge Creek was performed from June 1996 through July 1997. Sampling for the second phase of the study was performed from April 1998 to March 1999. The sampling stations for the BBC-MST study are indicated on Figure 1. Fecal coliforms were recovered from grab samples using the membrane filtration technique.

2.3.1 Burnt Bridge Creek (BBC) Sampling Stations

BBC1 (Burnt Bridge Creek at NE 2nd Avenue)

This is the flagship station on BBC. It was the major site for the original study and has been sampled for 25 years. Upstream influences on this site include I-5, two parks, and drainages from several sources including Cold Creek. This site is sampled upstream of a small bridge at NE 2nd Avenue. A short distance from this site BBC flows into wetlands, then Vancouver Lake.

BBC2 (Burnt Bridge Creek at NE18th Street)

This site is south, upstream, of NE 18th Street. A short distance upstream from BBC2 is an apartment complex with a resident population of ducks, approximately 60, several seagulls and aquatic mammals, six nutria and at least one beaver. Further upstream *are* agricultural fields (lettuce) and encroaching residential development.

BBC5 (Burnt Bridge Creek at 112th Avenue)

This site is directly upstream of NE 112th Avenue and south of the SR 500. BBC flows directly from east to west at this point. Immediately adjacent to this site new construction is taking place with the demolition of nearby houses and out buildings. From approximately one-half mile downstream of BBC5 to the creek's headwaters the stream is little more than a slow moving drainage ditch.

BBC 9B (Cold Creek at the confluence with BBC)

Cold Creek is a tributary to BBC. The sample site is upstream of the confluence and adjacent to the former Wildlife Department building. Cold Creek flows from east to west through a deep shaded canyon after meandering through fields with farm animals, light industrial sites, and residential areas. Cold Creek enters BBC 1/4 mile upstream of the most westerly site on Burnt Bridge Creek, BBC1.

BBCX (Burnt Bridge Creek at 137th Avenue)
This site is located downstream of the headwaters of BBC. At the headwaters, which are just west of NE 164th Avenue, BBC is a T-shaped ditch that flows slowly out of mint fields in a westerly direction. At NE 137th Avenue, the ditch is deep and slow moving. Upstream on the north side is a herd of 200 cattle and on the south side a few horses occupy a pasture. These animals do not have direct access to BBC, but they do have access to drainages to BBC. Samples *were* usually taken from the downstream side of NE 137th Avenue. due to the weeds and debris on the upstream side.

2.3.2 Pond Sample Sites

Grab samples were collected from two of a series of three ponds. The initial sample was collected from the inlet from the first pond and the outlet of the third pond. Samples were placed on ice in coolers and transported to the SWWHD. Within two hours the pond samples were filtered using membrane filtration for fecal coliform recovery. See Figure 1.

BBCMSTPO1 (Inlet to Upstream Pond off of Vancouver Mall Drive)
This site is found west of the Comfort Suites Motel and south of Vancouver Mall Drive. Samples were taken from the 36" inlet pipe flowing into the pond. This drainage is from the Vancouver Mall area and flows the year round.

BBCMSTPO2 (Outlet from Pond at Heathman Lodge on Greenwood Drive)
This site is at the outlet from the pond next to the Heathman Lodge, approximately thirty feet from the southeast corner of the building. This pond is downstream of the two ponds along Vancouver Mall Drive. Ducks are often found on all three ponds. This water eventually empties into Burnt Bridge Creek at Andresen Road (BBCY).

2.3.3 Sanitary Sewer Line Sample Sites

These sample sites are sewer line manholes that were within 100 feet of Burnt Bridge Creek. See Figure 1. Samples were placed on ice, transported to the SWWHD, and streaked on MacConkey Agar plates within two hours of collection.

BBCMSTSS01 (Sewer line manhole number 1205, NE 121st Avenue)
This manhole is in the southbound lane approximately at the 5300 block of NE 121st Avenue, north of BBC.

BBCMSTSS02 (Sewer line manhole number 1154, East of the end of Murry Court)
This manhole is found east of Murry, also spelled Murray on some maps, Court in the yard of the Korean Church.

BBCMSTSS03 (Sewer line manhole west of Hazel Dell Avenue south of BBC)
This manhole is often obscured by the limbs of a large leafed Chinese Chestnut. The location is just west of Hazel Dell Avenue and south of BBC. No manhole number is given.

BBCMSTSS04 (Sewer line manhole number 1202 at NE 51st Street east of NE 112th Avenue)
Samples from this manhole were collected only when construction activities prevented sampling from BBCMSTSS01 on NE 121st Avenue.

2.3.4 Stormdrain Sample Sites

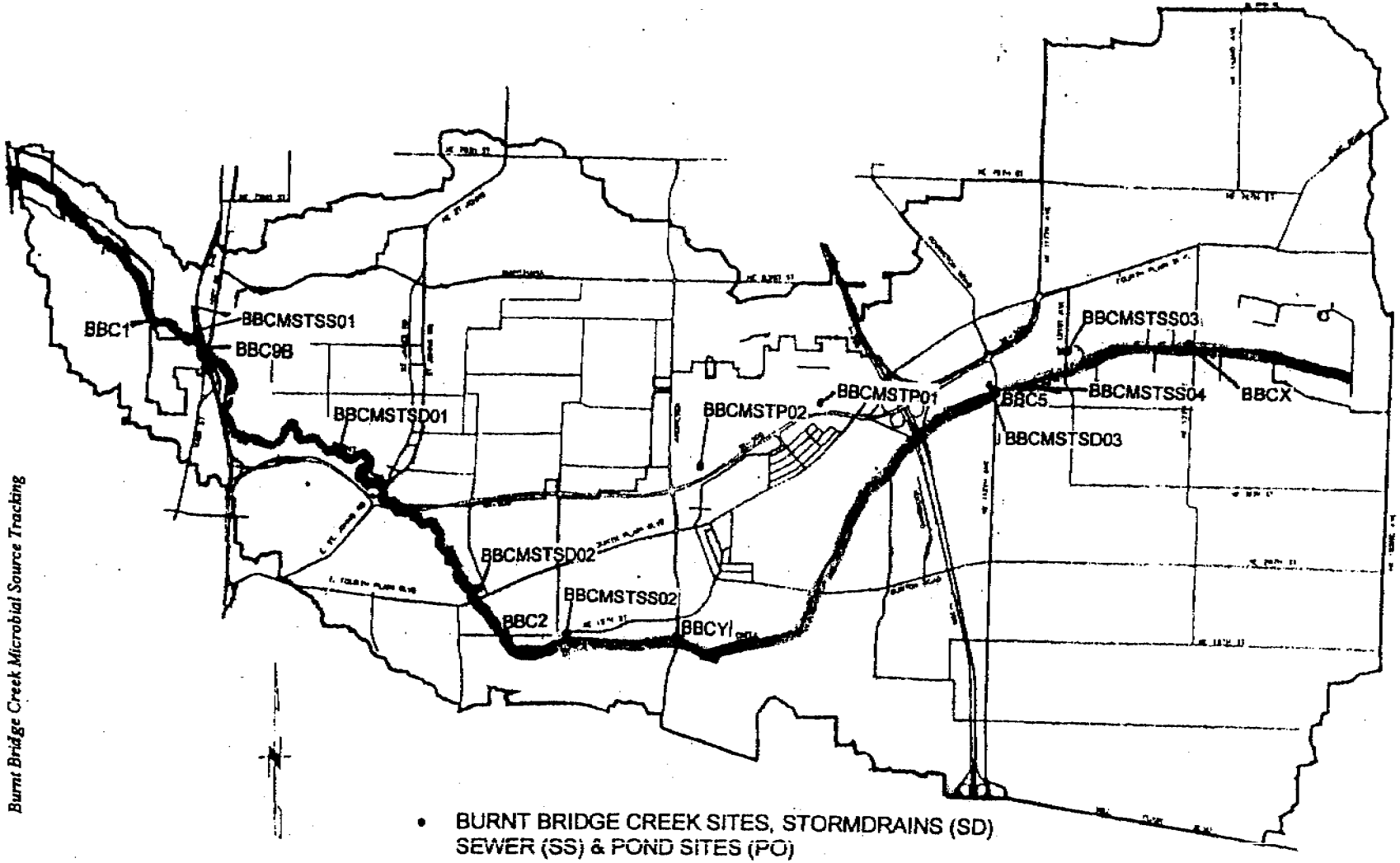
BBCY (Stormdrain at Andresen Road at BBC)
This site is on the west side of Andresen Road with samples taken from the 48" pipe flowing directly into BBC. The area drained includes the Vancouver Mall shopping center, typical strip development, motels, condominiums and apartments. Several upstream ponds have been constructed to control runoff. This storm drainage travels underground for over a mile before emptying into BBC at Andresen Road.

BBCMSTSD01 (Burnt Bridge Creek at Discovery Trail)
To reach this site one must park on NE 41st Street then travel east on Discovery Trail. Samples were collected from the drainage at the grate on the south side of the trail. This is found directly across from overflow structure in Oxbow Pond on the north side of the trail. Samples were collected from the grate on the creek bank located on the south side of the trail. This subbasin drainage area consists primarily of Rosemere residential neighborhood south of BBC and east of I-5.

BBCMSTSD02 (Burnt Bridge Creek at Linda Lane)
This storm drain to BBC is north of Fourth Plain Blvd. off of Linda Lane. The sample site is from the flow through a grated concrete structure. This drainage area consists largely of commercial establishments and residential neighborhoods. A long section of Fourth Plain Blvd., almost to Andresen Road, and a portion of SR 500 also drain to BBC at this site.

BBCMSTSD03 (East side of NE 112th at Burnt Bridge Creek)
This flow enters BBC from the northeast and drains an area roughly east of NE 137th Avenue and south of Fourth Plain. This drainage subbasin is rapidly developing into a mixed commercial, light-industrial, and residential area. Some livestock raising activities still remain. Residential neighborhoods with septic systems are found in the northeast section of this subbasin.

FIGURE 1
BURNT BRIDGE CREEK
MICROBIAL SOURCE TRACKING



2.3.5 Well Sample Sites

Well sample sites in the Burnt Bridge Creek Basin were selected from the Clark Public Utilities (CPU) 1990-91 Clark County study on private wells, health district personnel knowledge of well locations, and reports of private well water samples testing positive for fecal coliform. The wells selected from the CPU study had been reported to be positive for total coliform. See Figure 2.

Several liters to several gallons of water were purged from the well before collection of a sample. Samples were placed on ice and transported to the SWWHD water laboratory for fecal coliform recovery by membrane filtration.

BBCMSTB5 (NE 106th Avenue between NE 45th and NE 46th Street)
This is a monitoring well installed in 1984 for a study of septic tank effluent on shallow ground water in the Burtonwood and Serencourt developments. B5 is 10.4 feet in depth and is screened from 4 to 9 feet.

BBCMSTB8 (NE 47th Street at approximately NE 105th Avenue)
This monitoring well is within 20 feet of Burnt Bridge Creek and has a depth of 10.6 feet and is screened from 4 to 9 feet.

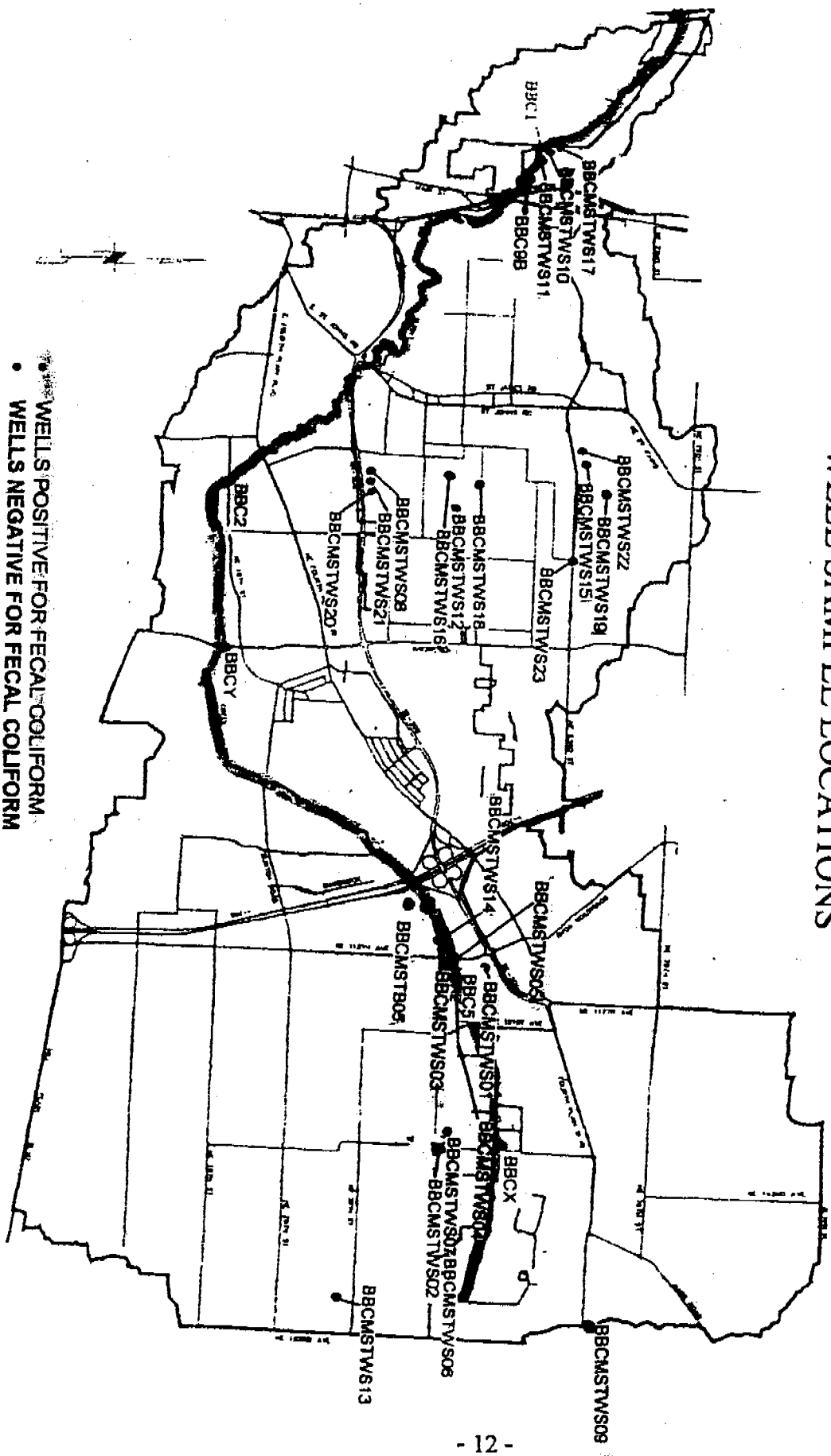
BBCMSTWS01-05, 07, 14, and 18
These are shallow hand dug wells with a water level from 6-10 feet. Several have been abandoned since sampled. BBCMSTWS14 and BBCMSTWS18 were negative for fecal coliform.

BBCMSTWS06, 10-12, 16-19, 22
These private wells are known to be drilled wells. Their selection was based on previously testing positive for total coliform. None of the wells were found positive for fecal coliform in this study.

BBCMSTWS02, 08, 09, 13, 15, and 23
Most of these private wells are believed to be drilled wells. However present residents are unsure of the well construction.

BBCMSTWS20 and 21
The well BBCMSTWS21 was reported to have been hand dug to a depth of 38 feet. The well water was also reported to require disinfection. The sample was collected before the UV disinfection unit. Well BBCMSTWS20 was dug to a depth of 25 feet.

FIGURE 2
BURNT BRIDGE CREEK MICROBIAL SOURCE TRACKING
WELL SAMPLE LOCATIONS



2.3.6 *Animal Fecal Samples*

First Phase Animal Fecal Samples

Fecal samples from various species of animals were collected from the watershed and vicinity from June 1996 through July 1997. The sampling focus was primarily on livestock, a suspected source for fecal coliform in BBC. Because of study limitations a more comprehensive sampling of domestic animals and wild animals was not done. The largest challenge to obtaining samples within the watershed was the lack of willingness, on the part of many private property owners, to participate in a study of this nature. Sample collection was also done in the vicinity of the watershed to obtain a greater number and diversity of samples. This also served the purpose of furthering the understanding of ribotype diversity within a population of animals of the same species living together and among members of the same species within a region.

All samples were collected with sterile implements and placed in sterile containers. The water samples were delivered to Coffey Labs Portland, Oregon, and the fecal and septage samples were delivered to the Southwest Washington Health District's microbiology laboratory for processing.

Second Phase Animal Fecal Samples

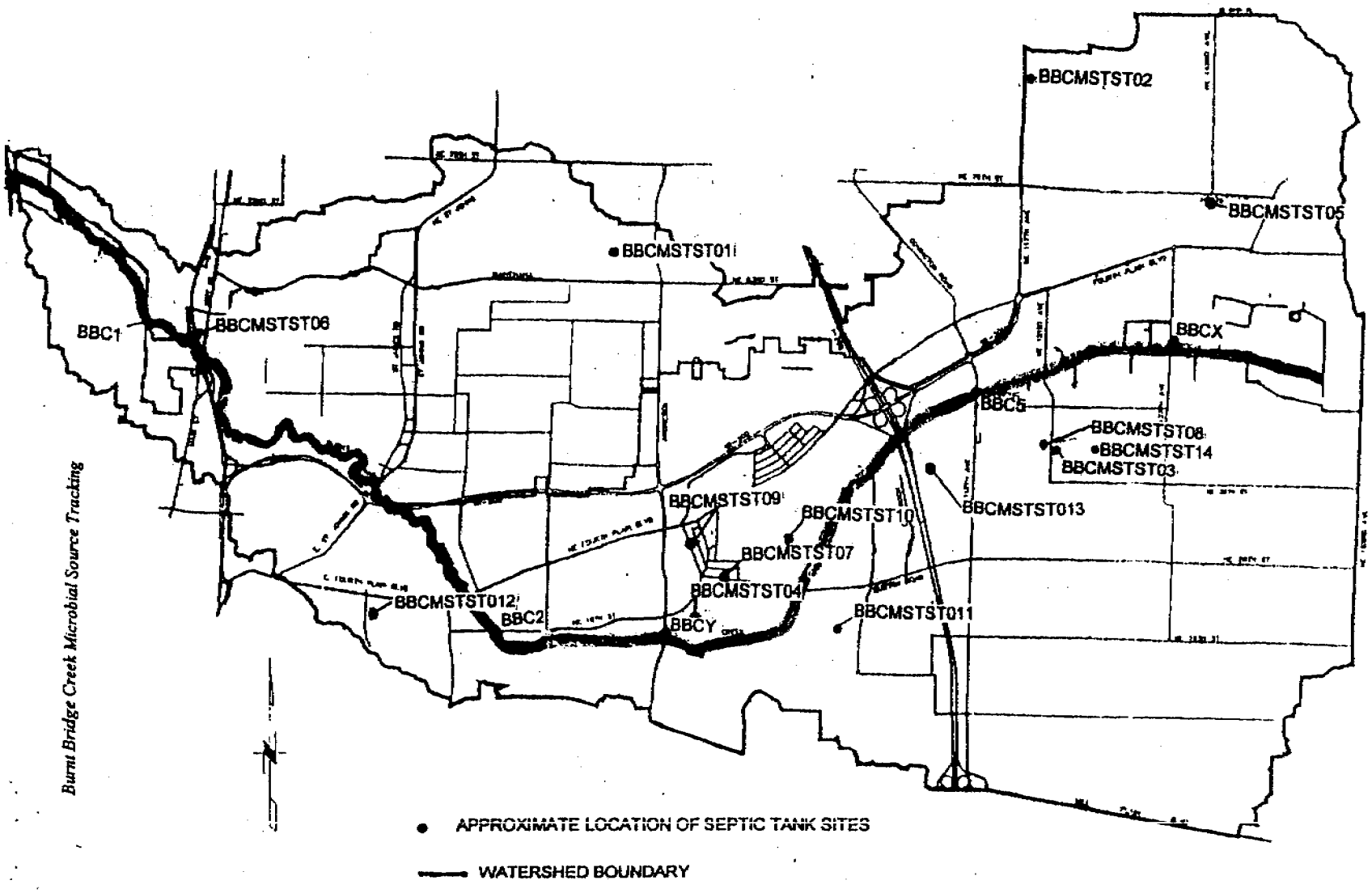
Additional fecal samples from livestock and pets were collected for this phase of the study. Unique in the second phase was fecal samples collected from mules living along Burnt Bridge Creek, a beaver, and a nutria. At least six nutrias live just upstream of BBC2. From the fecal sample collected from the nutria only two fecal coliform colonies were obtained from six heavily streaked MacConkey agar plates. Few fecal coliform colonies were recovered from the beaver fecal sample as well.

All samples were grab samples taken in sterile containers. Standard quality assurance and quality control procedures were observed in the field including the collection of field and trip blanks. Samples were promptly placed on ice and delivered within one to two hours to the SWWHD laboratory to be analyzed within the specified holding time.

2.3.7 *Septic Tank System Samples*

Another suspected major source of fecal coliform in BBC is septic tanks. In phase one of the study 20 septic tank systems were sampled from businesses, churches, and schools. In phase two, 12 domestic septic tank systems were sampled. Effluent samples were collected from the pump chamber to the drainfield. One sample was collected directly from the septic tank. These samples were streaked on MacConkey agar plates. An effluent sample from a sand filter was collected, with fecal coliform recovered using membrane filtration. See Figure 3 for the locations for phase two septic samples.

FIGURE 3
BURNT BRIDGE CREEK
MICROBIAL SOURCE TRACKING



2.4 Bacterial Culture

The water samples were analyzed for fecal coliform enumeration by Coffey laboratory (Portland, OR) using the membrane filtration method (APHA, 1992) in phase one of the study and at the SW Washington Health District laboratory for phase two of the study. After analysis, the plates were transported to Dr. Samadpour's lab. Morphologically appropriate colonies (round, blue, and flat) were chosen from these plates and streaked for isolation onto MacConkey media and incubated at 37° C for 24 hours.

The fecal, septage, and sewer samples were transported directly to the Southwest Washington's Health District's microbiology laboratory. They were streaked onto MacConkey media plates and incubated at 35° C for 24 hours. The MacConkey plates were then sent to Dr. Samadpour's lab. Characteristic colonies (round, purplish red, typically flat) were chosen from these plates to be streaked for re-isolation on MacConkey media.

Isolated colonies that fermented lactose on MacConkey were then restreaked onto Tripticase Soy Agar (TSA). An average of five isolates was obtained from each water and fecal sample. Biochemical analysis was done to positively identify E. coli. This was done by inoculating each isolate into a tryptophane broth and onto a sodium citrate slant and incubating at 37° C for 24 hours. Isolates that were able to produce indol from tryptophane and not able to utilize sodium citrate as a sole source of carbon were positively identified as E. coli. These isolates were then assigned an isolate number and cultured again on TSA to obtain enough cells for storage in LB-15% glycerol freezing media at -70° C and for genomic (chromosomal) DNA isolation.

2.5 DNA Isolation and Digestion

Confluent growth of each isolate was scraped with a sterile flat-headed toothpick from TSA plates and suspended in Tris-EDTA buffer. The suspension was mixed well by pipetting up and down. To lyse the cells sodium dodecyl sulfate (SDS) and proteinase K (Pharmacia, Piscataway, N.J.) were added. These preparations were then incubated at 40° C for one hour. This was followed by phenol extraction to remove cellular material other than DNA. The preps were vortexed and then centrifuged for five minutes. The top aqueous layer containing DNA was removed and extracted with chloroform to further purify the DNA. DNA was precipitated out of solution by adding 2.5-3 times the prep volume of absolute ethanol. The DNA was spooled onto a glass capillary pipette, washed with absolute ethanol, dried, and resuspended in enough sterile distilled water (approximately 500 µL) to obtain a consistent DNA concentration among all preps.

Restriction endonuclease digestions of each DNA prep were done by using 10 units of appropriate restriction enzymes (Boehringer Mannheim, GmbH, Germany) as instructed by the manufacturer and 4 µL of DNA. Each 20 µL digestion prep was incubated at 37° C overnight. The preps were then centrifuged and 3 µL of stop dye were added to arrest the digestion reaction and prepare for loading into gels for electrophoresis.

2.6 Gel Electrophoresis and DNA Probing

The fragments of DNA produced by the enzyme digestion were resolved by agarose gel electrophoresis. The DNA fragments were then transferred from the gel by blotting onto a Nitran filter (Schleicher & Schuell, Keene, N.H.) in high salt solution (Maniatis et al., 1982) (Southern, 1975). These blots were baked at 80° C for one hour.

The blotted DNA was then hybridized with a radioactively labeled ribosomal RNA (rRNA) probe (Maniatis et al., 1982). The probe was labeled with [α-32P] dCTP, using random primers and incubated at 37° C for 30 minutes. The double stranded DNA molecules are denatured into single strands during the blotting process. During the hybridization reaction, the single stranded probe joins to single stranded DNA that contains segments of ribosomal RNA operon. Hybridization of the probe to the blotted DNA was done under stringent conditions.

After hybridization the blots were washed, dried, and exposed to X-ray film (Kodak, Rochester, N.Y.) with an intensifying screen at -70° C. Two to three different exposures were done to ensure all DNA bands that hybridized with the probe would be visible on film. The X-ray image of the DNA banding produced in this way for each isolate is termed an autoradiogram. The actual banding pattern is a ribotype.

2.7 Ribotyping and Analysis

Figure 4. illustrates the autoradiograms from one gel or blot. Each row, or lane, of bands represents DNA from one E. coli isolate and is headed by a number. The isolates represented in lanes 7 and 8 have identical banding patterns, the same ribotype and therefore belong to the same strain. This is also true for isolates in lanes 13, 14, 16, 17, and 19. The two groups belong to different strains determined by their unique ribotypes. Using an algorithm, which was developed for this purpose, the ribotypes were converted to an alphanumeric pattern.

The data for each isolate was entered into a computer database (using Microsoft Access 2.0). Isolates were sorted by ribotypes. Potential ribotype matches between isolates obtained from water and source samples, source samples of the same type, source samples of different types, and different water samples were confirmed by further inspection of the autoradiograms.

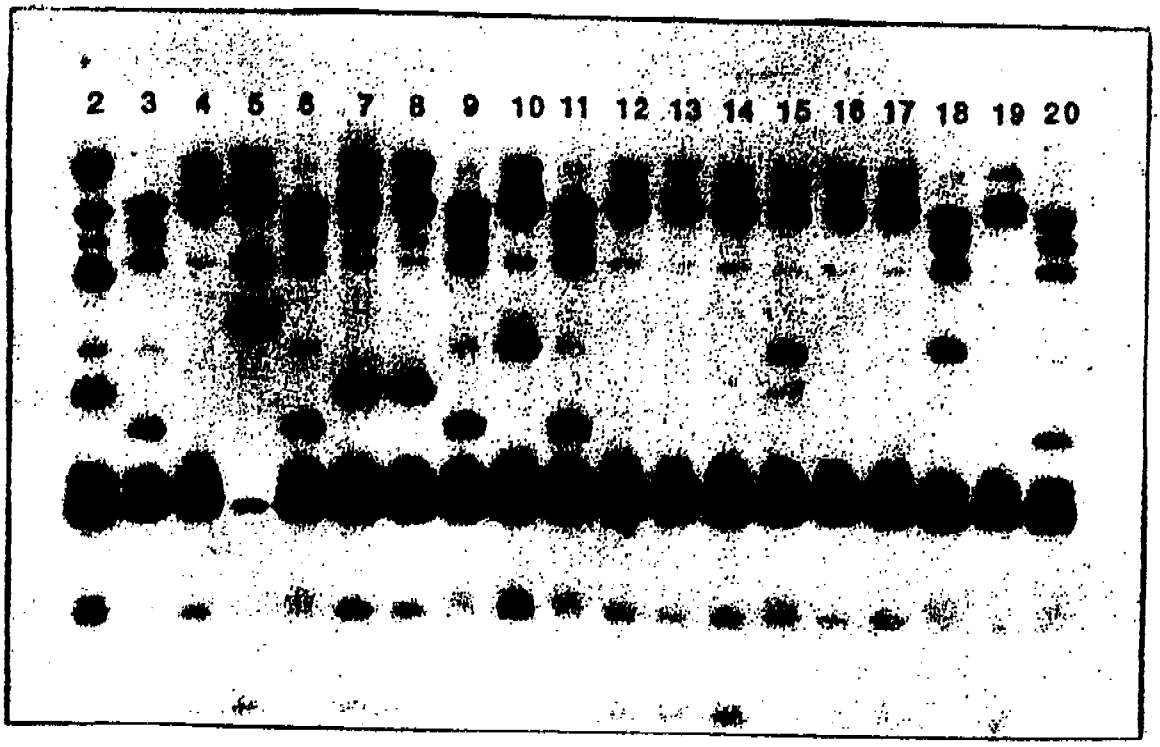


Figure 4. Examples of ribotype autoradiograms from a group of *E. coli* isolates.

3.0 RESULTS AND DISCUSSION

3.1 *Fecal Coliform Enumeration*

Table 1 gives the data from sampling, and indicates whether a base or storm flow was sampled for each stream location in phase one. The six primary sample stations locations are described above. Stations BBC9B and BBCX were not sampled in the second phase of the study.

For phase one the fecal coliform numbers found in the samples were generally high. Only 13 of the 42 samples have fecal coliform numbers below 100. Cold Creek appears to be as polluted as the BBC. As it is expected, storm events after periods of dry weather result in very elevated fecal coliform level numbers. This is clearly seen in all samples taken at 7/18/1996. Phase two fecal coliform results were similar to the levels in Phase One. Likewise, fecal coliform levels were elevated during a storm event.

Table 1. Fecal Coliform levels in samples taken at various BBC stations (per 100 ml).

Date	Flow Conditions	BBC 1	BBC9B	BBC2	BBCY	BBC5	BBCX
3/17/99	High flow, rain			15		9	
3/2/99	Base flow, rain	20		29	2	57	
2/16/99	High flow, rain	27		22	50	57	
2/10/99	Base flow, rain				7		
2/2/99	Base flow, rain			71	580	152	
1/12/99	Base flow, rain				350		
1/5/99	Base flow, dry	33		13	31	6	
12/16/98	High flow, rain				12		
12/7/98	High flow, rain			252			
12/3/98	High flow, dry	193				105	
11/17/98	Base flow, rain	290		110	6	48	
10/5/98	High flow, dry			150	60	120	
9/25/98	High flow, dry	265					
9/14/98	Base flow, dry	570			12		
9/3/98	Base flow, dry	250		350		155	
8/24/98	Base flow, dry				24		
8/18/98	Base flow, dry	190		140	45	165	
7/28/98	Base flow, dry				298		
7/21/98	Base flow, dry				85		
7/14/98	Base flow, dry	250		230	145	150	
6/10/98	Storm event	2,820		1,030	85	600	
5/26/98	Base flow, dry			400		360	

5/14/98	Base flow, dry				400		
5/7/98	Base flow, rain	250		160		150	
2/17/97	Base flow, dry	12	24	264	86	4	6
1/20/97	Base flow, rain	298	1110	312	284	102	118
11/18/96	Base flow, rain	180	680	80	490	100	52
10/21/96	Base flow, rain	72	54	134	90	52	50
9/9/96	Base flow	380	680	870	300	240	1140
8/12/96	Base flow	340	650	94	103	550	410
7/18/96	Storm Event	10,000	14,000	1000	26,000	1640	3000

Table 2. Samples taken and processed for the BBC microbial source tracking study, Phase One and Phase Two.

Sample Type	Sample Source	Number of Samples	Number of Isolates
Water	BBC1	23	98
Water	BBC2	23	95
Water	BBC5	23	104
Water	BBC9B	7	50
Water	BBCX	7	45
Water	Groundwater	10	42
Water	BBCY	29	137
Water	Storm Drain	12	39
Water	Pond	20	76
Septage	BBC Watershed	40	202
Sewer	Sanitary Sewer	29	174
Fecal	Cow	15	97
Fecal	Horse	10	25
Fecal	Dog	21	62
Fecal	Cat	15	45
Fecal	Chicken	5	27
Fecal	Duck	10	42
Fecal	Llama	1	5
Fecal	Sheep	1	5
Fecal	Mule	2	5
Fecal	Beaver	1	2
Total		304	1367

3.2 Water-to-source Ribotype Matches

BBCX

Forty-five *E. coli* strains were isolated from 7 water samples collected from BBC at this location during the first phase of the study. Of the 45 *E. coli* strains 11 (24.4%) were matched to cows, 4 (8.9%) to horses, 3 (6.7%) to humans, 2 (4.4%) to cats, 2 (4.4%) to raccoons, 1 (2.2%) to dogs, 1 (2.2%) to sea gull, 2 (4.4%) to rodents, and 1(2.2%) to opossum.

BBC5

Table 3 shows the results of source tracking at BBC5 site. Human and bovine sources at 15 percent each are the major sources of fecal coliforms at this site, they are followed by the avian sources (13%), dogs and cats (13% combined), and urban wildlife (10%).

Table 3. Summary of the Phase One and Phase Two results for the BBC5 sampling station.

Matched to Source	Phase One, 44 isolates	Phase Two, 60 isolates	Total 104
Human	6 (14%)	10 (17%)	16 (15%)
Cow	9 (20%)	7 (12%)	16 (15%)
Avian	1 (2%)	2 (3%)	3 (3%)
Duck	2 (4%)	4 (7%)	6 (6%)
Geese	0	2 (3%)	2 (2%)
Sea Gull	1 (2%)	1 (2%)	2 (2%)
Cat	1 (2%)	4 (7%)	5 (5%)
Dog	3 (7%)	5 (8%)	8 (8%)
Raccoon	1 (2%)	3 (5%)	4 (4%)
Opossum	2 (4%)	2 (3%)	4 (4%)
Rodent	0	2 (3%)	2 (2%)
Unknown	18 (41%)	18 (30%)	36 (34%)

BBCY

The results of the MST study of the BBCY site are shown in Table 4. Avian sources account for the majority of impact (26%) at this site, followed by dogs and cats (16%), urban wildlife (raccoon, opossum and rodents) at 19%, human at 4% and bovine at 2%.

Table 4. Summary of the Phase One and Phase Two results for the BBCY sampling station.

Matched to Source	Phase One, 46 isolates	Phase Two, 90 isolates	Total 136 isolates
Human	4 (9%)	2 (2%)	6 (4%)
Cow	3 (7%)	0	3 (2%)
Avian	2 (4%)	5 (5%)	7 (5%)
Duck	1 (2%)	3 (4%)	4 (3%)
Geese	2 (4%)	7 (8%)	9 (7%)
Sea Gull	3 (7%)	6 (7%)	9 (7%)
Crow	2 (4%)	3 (3%)	5 (4%)
Cat	3 (7%)	5 (5%)	8 (6%)
Dog	5 (11%)	8 (9%)	13 (10%)
Raccoon	3 (7%)	4 (4%)	7 (5%)
Opossum	4 (9%)	5 (5%)	9 (7%)
Rodent	3 (7%)	6 (7%)	9 (7%)
Unknown	11 (24%)	36 (40%)	47 (34%)

Storm Drainages

Three other stormdrains besides BBCY were studied. The MST results are shown in Table 5. Pets, avian and urban wildlife appear to be the major impactors for the three stormdrains. Only a single match (3%) was found to *E. coli* of human origin in one of the stormdrains. This low level match to human sources implies that the drains are free of cross connection to sewer lines. The match could be due to groundwater entrainment, or the human clone may be a transient clone.

Table 5. Summary of the results for the three stormdrain sites.

Matched to Source	Storm Drain 1 19 Isolates	Storm Drain 2 14 Isolates	Storm Drain 3 6 isolates	Total 39 isolates
Human	0	1	0	1 (3%)
Dog	2	1	1	4 (10%)
Cat	1	0	1	2 (5%)
Duck	1	0	0	1 (3%)
Avian	1	1	0	2 (5%)
Sea gull	2	1	0	3 (8%)
Raccoon	2	2	1	5 (13%)
Opossum	1	2	0	3 (8%)
Rodent	2	1	1	4 (10%)
Unknown	7	5	2	14 (36%)

BBC2

Human related sources (human, dog, and cat) appear to be the major impactor at this station, accounting for 27% of the source matches (Table 6). Avian sources at 23% are the second major impactor, followed by wildlife at 11%.

Table 6. Summary of the Phase One and Phase Two results for the BBC2 sampling station.

Matched to Source	Phase One 43 Isolates	Phase Two, 52 Isolates	Total 95 Isolates
Human	6 (14%)	6 (11%)	12 (13%)
Beaver	1 (2%)	0	1 (1%)
Avian	1 (2%)	3 (6%)	4 (4%)
Duck	4 (9%)	9 (17%)	13 (14%)
Geese	1 (2%)	2 (4%)	3 (3%)
Sea Gull	0	1 (2%)	1 (1%)
Crow	0	1 (2%)	1 (1%)
Cat	3 (7%)	3 (6%)	6 (6%)
Dog	4 (9%)	3 (6%)	7 (8%)
Raccoon	1 (2%)	2 (4%)	3 (3%)
Opossum	1 (2%)	3 (6%)	4 (4%)
Rodent	1 (2%)	2 (4%)	3 (3%)
Unknown	20 (46%)	17 (33%)	37 (39%)

Unknown	20	(46%)	17	(33%)	37	(39%)

BBC9B

Fifty *E. coli* strains were isolated from the confluence of cold Creek with BBC during the first phase of the study. *E. coli* of human origin accounts for 11 (42%) of the matches at this station, together with dogs 5 (19%) and cats 3 (12%) the human related sources are the major contributors at this site. The second largest contributor is urban wildlife at 5 (19%) and is followed by avian sources at 3 (12%).

BBC1

E. coli of human origin accounts for 20% of the matches at this station, together with dogs (5%), and cats (3%), the human sources are the major impactor at this site. The second major impactors are the avian sources at 21%, followed by urban wildlife at 11% and agriculture (cows and horses) at 8%. (Table 7)

Table 7. Summary of the Phase One and Phase Two results for the BBC1 sampling station.

Matched to Source	Phase One 44 Isolates		Phase Two 54 Isolates		Total 98 Isolates	
Human	8	(18%)	11	(20%)	19	(20%)
Cow	4	(9%)	2	(4%)	6	(6%)
Horse	1	(2%)	1	(2%)	2	(2%)
Avian	2	(4%)	3	(6%)	5	(5%)
Duck	2	(4%)	4	(7%)	6	(6%)
Geese	2	(4%)	2	(3%)	4	(4%)
Sea Gull	1	(2%)	3	(6%)	4	(4%)
Crow	0		2	(3%)	2	(2%)
Cat	2	(4%)	1	(2%)	3	(3%)
Dog	2	(4%)	3	(6%)	5	(5%)
Raccoon	1	(2%)	3	(6%)	4	(4%)
Opossum	1	(2%)	2	(3%)	3	(3%)
Rodent	2	(4%)	2	(3%)	4	(4%)
Unknown	16	(14%)	15	(28%)	31	(32%)

Ponds

The ponds are mostly impacted by the avian sources at 28%, wildlife at 15%, and pets at 12% (Table 8).

Table 8. Summary of the results for Ponds 1 and 2.

Matched to Source	Pond 1 29 Isolates	Pond 2 47 Isolates	Total 76 Isolates	
Dog	3	3	6	(8%)
Cat	1	2	3	(4%)
Duck	3	5	8	(10%)
Goose	1	2	3	(4%)
Avian	2	3	5	(7%)
Sea gull	2	3	5	(7%)

Raccoon	2	3	5 (7%)
Opossum	1	2	3 (4%)
Rodent	2	1	3 (4%)
Unknown	12	23	35 (46%)

A summary of the results for BBC MST study is shown in Table 9 total of 528 *E. coli* strains were isolated from water samples taken at six creek sampling stations in the BBCW. Sources were identified for 336 (63.6%) of the *E. coli* isolates.

The results indicate that the sources of microbial pollution in BBC and Cold Creek in the order of their significance are: humans (12.7%), cows (6.8%), dogs (7.4%), ducks (5.7%), cats (5.1%), opossums (4.2%), raccoons (4.2%), rodents (4.2%), avian (5.7%), geese (3.4%), sea gull (3.2%), crows (1.5%), horses (1.1%), and beavers (0.2%).

If we divide the sources into four categories; human related (humans, dogs and cats) agricultural (cows, horses) avian (ducks, geese, crows, sea gulls) and urban wildlife (raccoons, opossums, rodents) human related sources will become the major contributor in the BBCW, accounting for 25.2% of the microbial load identified at the BBC. The next major source of microbial pollution in the BBC is the avian source (17.8%), followed by urban wild life (12.6%). Agricultural sources account for 7.9% of *E. coli* pollution load.

It appears that the agricultural impact on the BBC is on the decline while both the urban and wildlife impacts are increasing. The percent of matches to bovine sources in BBC-5 decreased from 20% in the first phase of the study to 12% during the second phase. The percentage for BBC1 decreased from 9% for the first phase to 4% during for the second phase of the study.

Table 9. Summary of the Phase One and Phase Two results for all the BBCW creek sampling stations.

Matched to Source	BBC X	BBC 5	BBCY	BBC2	BBC9B	BBC 1	Total
Human	3 (6.7%)	16 (15%)	6 (4%)	12 (13%)	11 (22%)	19 (20%)	67 (12.7%)
Cow	11 (24.4%)	16 (15%)	3 (2%)	0	0	6 (6%)	36 (6.8%)
Avian	0	3 (3%)	7 (5%)	4 (4%)	2 (4%)	5 (5%)	21 (4.0%)
Duck	0	6 (6%)	4 (3%)	13 (14%)	1 (2%)	6 (6%)	30 (5.7%)
Geese	0	2 (2%)	9 (7%)	3 (3%)	0	4 (4%)	18 (3.4%)
Sea Gull	1 (2.2%)	2 (2%)	9 (7%)	1 (1%)	0	4 (4%)	17 (3.2%)
Cat	2 (4.4%)	5 (5%)	8 (6%)	6 (6%)	3 (6%)	3 (3%)	27 (5.1%)
Dog	1 (2.2%)	8 (8%)	13 (9%)	7 (8%)	5 (10%)	5 (5%)	39 (7.4 %)
Raccoon	2 (4.4%)	4 (4%)	7 (5%)	3 (3%)	2 (4%)	4 (4%)	22 (4.2%)
Opossum	1 (2.2%)	4 (4%)	9 (7%)	4 (4%)	1 (2%)	3	22 (4.2%)

Rodent	2 (4.4%)	2 (2%)	9 (7%)	3 (3%)	2 (4%)	4 (4%)	22 (4.2%)
Horse	4 (8.9%)	0		0	0	2 (2%)	6 (1.1%)
Crow			5 (4%)	1 (1%)	0	2 (2%)	8 (1.5%)
Beaver				1 (1%)	0		1 (0.2 %)
Total Matched	27 (60%)	68 (66%)	89 (66%)	58 (61%)	27 (54%)	67 (68%)	336 (63.6%)
Unknown	18 (40%)	36 (34%)	47 (34%)	37 (39%)	23 (46%)	31 (32%)	192 (36.4%)
Total	45	104	136	95	50	98	528

Unknown Sources

Of the 528 *E. coli* strains isolated from water samples taken at BBC and Cold Creek sampling stations, 336 (63.6%) were matched to known sources. The water isolates that were not matched to sources may be attributable to unsampled source types, or source types that are underrepresented in our database

3.3 Septic Versus Sewer

In the course of the study we analyzed a total of 202 E. coli isolates from 40 septic tank samples and 174 *E. coli* isolates from 29 wastewater samples taken from the sewer lines. There were 48 ribotypes among the 202 *E. coli* strains isolated from the 40 septic tank samples. The 174 E. coli strains from the sewer lines were divided into 118 groups on the basis of their ribotypes. Three of the ribotypes seen in the septic tank samples matched three of the ribotypes seen in the sewer line samples. These results strongly indicate that a limited number of *E. coli* clones have adapted to the septic tank environment. This allows for assessing the impact of septic tanks versus sanitary sewer contribution to the levels of microbial pollution. All but four of the human clones seen in the water samples were matches to septic tank samples. The data strongly suggests that sewer lines are not contributing to the levels of microbial pollution seen in the BBC.

4.0 RECOMMENDATIONS

4.1 Control of Microbiological Pollution

The results of this study provide some directions for the control of microbial pollution in the BBC watershed.

- The human sources of microbial pollution appear to be the major contributor of Burnt Bridge Creek *E.coli*. Furthermore the majority of the human matches are to *E.coli* clones found in septic tanks. Reducing the number of septic tanks in the watershed, especially in areas close to the BBC, may result in improving the water quality of BBC. In addition, the threat of infection by human pathogens from contact with water in BBC will be reduced.

- Dogs and cats are another major contributor of fecal coliform contamination of the BBC. Pets in urban environment are becoming a significant source of pollution in storm runoff, urban lakes, and creeks. Attempts should be made to inform pet owners of this growing problem and educate them in proper waste handling for their pets.

- The data points at avian and urban wildlife as two other major contributors of fecal coliforms to the BBC. Although the use of the creek by migratory birds is inevitable, attempts should be made to discourage the formation of resident populations. It is also prudent to control the population of opossum, raccoons, and rodents in the BBC basin. Aside from their contributions of fecal coliforms to the creek, they can also act as reservoirs for human pathogens in the watershed.

- Encourage livestock owners to observe best management practices for pastures in general and particularly those with direct access to the BBC, its tributaries, and drainages to the creek. This involves fencing to restrict access, streamside vegetation effective at filtering pollutants, avoidance of over pasturing resulting in bare and/or compacted earth, collection and proper storage/disposal of animal wastes, and alternatives to direct stream watering of animals.

- Continue planting trees along the banks of BBC to provide more shading and reduce stream temperatures. Reducing stream temperatures during the summer months reduce the potential for regrowth of fecal coliform in Burnt Bridge Creek.

4.2 Increasing BBC Flow

The feasibility of increasing Burnt Bridge Creek stream flow, especially during the summer months, through pumping groundwater into the creek should be evaluated. Burnt Bridge Creek has been classified by the Washington State Department of Ecology as a Class A stream, however BBC water quality is in violation of Class A standards for

fecal coliform, temperature, dissolved oxygen, and pH. As such BBC has been place on the EPA 303d List of Impaired or Threatened Water Bodies. The addition of the high quality groundwater to BBC should be upstream of N.E. 112th Street, BBC5. This action could improved the stream's water quality in the following ways:

- As BBC fecal coliform levels exhibit seasonal highs from June through September (Woodward, 1998) increasing the stream flow will decrease the density of fecal coliform as well as the potential for infection from pathogenic organisms from contact with the stream's water.

- Like fecal coliform, the BBC stream temperature shows seasonal highs in the summer months. The temperature standard for Class A streams in Washington State, 18.0 °C, was exceeded 20 percent of time at BBC2 in 1999. The addition of groundwater to BBC will decrease the stream temperature, increasing the likelihood of BBC meeting the Class A standard. Decreased stream temperature will also reduce the regrowth of fecal coliform in BBC.

- The dissolved oxygen concentration in Burnt Bridge Creek violated the Class A standard, 8 mg/L, for all but two months in 1999 at BBC5, and for five months in 1999 at BBC2. The addition of groundwater to the stream would reduce the temperature increasing the water's capacity to contain oxygen. The groundwater itself could be aerated or spilled into the creek to increase the dissolved oxygen.

5.0 REFERENCES

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APPENDIX

BBC MST Phase Two Site Addresses and GPS Locations

BBCMST PHASE TWO SITE ADDRESSES & GPS LOCATIONS					
Site Number	Address	Type of Sample	Longitude	Lattitude	Zip Code
BBC1	BBC @ NE 2nd Avenue	Stream	122 40 09.752 -	45 39 41.279 +	98663
BBC2	BBC @ NE 18th Street	Stream	122 37 26.669 -	45 38 04.782 +	98661
BBC5	BBC @ NE 112th Avenue	Stream	122 33 27.767 -	45 39 30.524 +	98682
BBCY	BBC @ Andresen Road	Storm Drainage	122 36 01.361 -	45 38 06.109 +	98661
BBCMSTSD01	BBC @ Discovery Trail	Storm Drainage	122 38 57.456 -	45 39 05.466 +	98663
BBCMSTSD02	BBC @ Linda Lane Drainage	Storm Drainage	122 37 40.512 -	45 38 19.875 +	98661
BBCMSTSD03	BBC @ NE 112th Ave. Drainage	Storm Drainage	122 33 29.86 -	45 39 30.334 +	98662
BBCMSTB05	NE 106th Ave. S of 46th Street	Shallow Well	122 33 50.221 -	45 39 17.787 +	98682
BBCMSTB08	NE 47th St.E of NE 106th Ave.	Shallow Well	122 33 53.222 -	45 39 21.543 +	98682
BBCMSTWS01	NE 112th Ave. & SR 500	Shallow Well	122 33 28.326 -	45 39 40.339 +	98682
BBCMSTWS02	5113 NE 137th Ave.	Drilled Well?	122 31 52.934 -	45 39 36.387 +	98682
BBCMSTWS03	North of NE 49th St. & West of 112th	Shallow Well	122 33 30.036 -	45 39 36.588 +	98682
BBCMSTWS04	Approx. NE 52 St. & 113th Ave.	Shallow Well	122 33 22.807 -	45 39 36.408 +	98682
BBCMSTWS05	NE 51 St. & 112th Ave. (NE Corner)	Shallow Well	122 33 26.591 -	45 39 33.251+	98682
BBCMSTWS06	13408 NE 52nd Street	Drilled Well	122 32 06.015 -	45 39 36.22 +	98682
BBCMSTWS07	13408 NE 52nd Street	Shallow Well	122 32 05.344 -	45 39 37.012 +	98682
BBCMSTWS08	4509 NE 38th Street	Drilled Well?	122 37 28.901 -	45 38 55.125 +	98661
BBCMSTWS09	16807 NE 4th Plain Rd.	Drilled Well?	122 29 57.985 -	45 40 18.406 +	98682
BBCMSTWS10	6106 Alki Road	Drilled Well	122 40 32.707 -	45 40 04.413 +	98663
BBCMSTWS11	6212 Alki Road	Drilled Well	122 40 28.525 -	45 40 00.003 +	98663
BBCMSTWS12	5000 NE 51st Street	Drilled Well	122 37 13.37 -	45 39 37.89 +	98661
BBCMSTWS13	3208 NE 148th Street	Drilled Well?	122 31 16.854 -	45 38 43.491 +	98682
BBCMSTWS14	North of NE 49th St. & West of 112th	Shallow Well	122 33 33.438 -	45 39 26.646 +	98662
BBCMSTWS15	4305 NE 66th Ave	Drilled Well?	122 36 15.504 -	45 39 12.092 +	98661
BBCMSTWS16	4504 NE 48th Street	Drilled Well	122 37 31.088 -	45 39 23.173 +	98661

BBCMST PHASE TWO SITE ADDRESSES & GPS LOCATIONS

Site Number	Address	Type of Sample	Longitude	Lattitude	Zip Code
BBCMSTWS17	5808 Alki Rd.	Drilled Well	122 40 22.408 -	45 39 53.34 +	98661
BBCMSTWS18	4615 NE 54th Street	Shallow Well	122 37 24.303 -	45 39 39.512 +	98663
BBCMSTWS19	6912 NE 47th Avenue	Drilled Well	122 37 24.274 -	45 40 21.006 +	98661
BBCMSTWS20	4607 NE 38th Street	25' Dug Well	122 37 27.184 -	45 38 54.808 +	98661
BBCMSTWS21	4510 NE 38th Street	38' Dug Well	122 37 29.414 -	45 38 56.197 +	98661
BBCMSTWS22	4112 NE 66th Avenue	Drilled Well	122 36 17.208 -	45 39 08.298 +	98661
BBCMSTWS23	5501 NE 66th Avenue	Drilled Well?	122 36 13.793 -	45 39 43.921 +	98662
BBCMSTP01	South of Vancouver Mall Drive	Pond	122 34 46.782 -	45 39 24.792 +	98662
BBCMSTP02	Heathman Lodge, 7801	Pond	122 35 27.94 -	45 39 11.078 +	
	NE Greenwood Drive				98682
BBCMSTSS01	NE 121st Ave. N of BBC	Sewer	122 40 06.099 -	45 39 40.902 +	98661
BBCMSTSS02	Murry Ct. off NE 18th Street	Sewer	122 37 04.902 -	45 38 02.959 +	98663
BBCMSTSS03	Hazel Dell Ave. near BBC	Sewer	122 32 56.301 -	45 39 37.708 +	98682
BBCMSTSS04	NE 112th Ave.& 51st St.	Sewer	122 33 14.409 -	45 39 34.219 +	98661
BBCMSTST01	6314 NE 66th Ave.	Septic Tank Effluent	122 36 25.594 -	45 39 52.345 +	98661
BBCMSTSF01	6314 NE 66th Ave.	Sand Filter Effluent	122 36 25.594 -	45 39 52.345 +	98682
BBCMSTST02	8705 NE 117th Ave.	Septic Tank Effluent	122 33 04.229 -	45 41 07.145 +	98682
BBCMSTST03	4400 NE 122nd Ave.	Septic Tank Effluent	122 32 58.325 -	45 39 08.058 +	98684
BBCMSTST04	7525 NE 18th Street	Septic Tank Effluent	122 35 44.151 -	45 38 13.029 +	98682
BBCMSTST05	7713 NE 142nd Ave.	Septic Tank Effluent	122 31 31.612 -	45 40 45.836 +	98663
BBCMSTST06	5405 NE Hazel Dell Ave.	Septic Tank Effluent	122 40 04.284 -	45 39 41.901 +	98662
BBCMSTST07	8104 NE Burton Rd.	Septic Tank Effluent	122 35 22.915 -	45 38 25.415 +	98682
BBCMSTST08	4508 NE 122nd Avenue	Septic Tank Effluent	122 32 53.148 -	45 39 17.652 +	98662
BBCMSTST09	2980 Littler Avenue	Septic Tank Effluent	122 35 46.888 -	45 38 35.706 +	98662
BBCMSTST10	3007 NE 93rd Avenue	Septic Tank Effluent	122 34 36.22 -	45 38 39.312 +	98664
BBCMSTST11	9400 NE Alpine Street	Septic Tank Effluent	122 34 35.224 -	45 37 48.026 +	

BBCMST PHASE TWO SITE ADDRESSES & GPS LOCATIONS					
Site Number	Address	Type of Sample	Longitude	Lattitude	Zip Code
BBCMSTST12	1903 Grand Blvd.	Septic Tank Effluent	122 38 29.919 -	45 38 07.588 +	98661
BBCMSTST13	3916 NE Burtonwood Ct.	Septic Tank Effluent	122 33 51.869 -	45 39 03.465 +	98682
BBCMSTST14	12715 NE 44th Street	Septic Tank	122 32 30.835 -	45 39 11.191 +	98682
BBCMSTDS01-04	4601 E 18th St.	Duck Fecal Samples	122 37 15.81 -	45 38 01.91+	98661
BBCMSTC01-03	S. of 147th Ave. & 4th Plain	Cattle Fecal Samples	122 31 16.652 -	45 40 16.413 +	98682
BBCMSTB01	Near BBC2 (NE 18th Ave.)	Beaver Fecal Sample	122 37 26.383 -	45 38 04.508 +	98661
BBCMSTN01	4601 E 18th St.	Nutria Fecal Sample	122 37 15.81 -	45 38 01.91+	98661
BBCMSTM01-M02	1806 41st Cir.	Mule Fecal Samples	122 39 06.812 -	45 39 05.786 +	98663