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An Ecotypic Differentiation of Human and Bovine Fecal Streptococci, with Application to Human Pollution in the Yakima River

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AN ECOTYPIC DIFFERENTIATION OF HUMAN AND BOVINE FECAL STREPTOCOCCI, WITH APPLICATION TO HUMAN POLLUTION IN THE YAKIMA RIVER

A Thesis

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of the Requirements for the Degree

Master of Science

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by

Harlen Harvey Johnson

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APPROVED FOR THE GRADUATE FACULTY

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PREFACE

"If you visit American city you will find it very pretty
Just two things of which you must beware: don't drink the water and don't breathe the air
Pollution - Pollution
They got smog and sewage and mud
Turn on the tap and you get hot and cold running crud"

This stanza from a song by Tom Lehrer expresses a growing concern in the United States over the contamination of the atmosphere and the nation's water supply. It is the aim of this thesis to show ecotypic dependence of fecal streptococci on certain species of mammals, and to make application of this ecotypic dependence in showing the presence of human contamination in the Yakima River.

Special appreciation and graditude to the following people, who so willing gave of their time: Mr. Curt Wiberg, Miss Janet Lowe, Dr. Virginia Harden, Dr. Dan Willson, and Dr. Jack Peary.

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CHAPTER I

INTRODUCTION AND DEFINITION OF TERMS

In the past decade, the nation has become keenly aware of the alarming increase in the contamination of the atmosphere, soil, and water. With a greater awareness on the part of the public, the demand for more and better measures to control and to detect the sources of contamination has increased. New methods must be developed and old ones must be revised to be able to combat the increase in contamination.

I. PURPOSE

Statement of purpose. It was the purpose of this study to report on the development of a technique for distinguishing human fecal streptococci from bovine fecal streptococci, and to make application of this technique in describing some of the sources of pollution in the Yakima River as contributed by the Wilson Creek drainage system, in the Ellensburg area of Washington.

Available sources of study. At the present time, the major indicator organisms of mammalian pollution are the coliform bacteria (*Escherichia coli* and *Aerobacter aerogenes* and their varieties). Not only are the coliform bacteria found in the intestines of homeothermic animals, but they are also normal constituents of the soil bacteria. Therefore,

they can enter lakes and streams with the run-off waters and give an indication of fecal pollution which is not valid. However, with the advent of the development of Escherichia coli media (BBL), the soil coliforms no longer present this problem, since only the coliforms from homeothermic vertebrates will grow on the media.

A less used, but often mentioned group of indicator organisms consist of the enterococci or fecal streptococci. These organisms are found only in the intestines of homeothermic vertebrates and are not found in the soil. Furthermore, with the development of M-Enterococcus media (Difco), their density in water was determined to be seven or eight times greater than that of the coliforms, making the detection of organic pollution highly accurate. (9:1052).

Selected source for this study. The fecal streptococci were chosen as test organisms because of this high density and the fact that they inhabit animal intestines exclusively. It was hoped, that even though the species are the same, an ecotypic dependence of the organisms on man and cattle would be present. A study by Cooper and Ramadan suggests that such a separation is possible. (3:180 - 190).

In their study, they have shown that fresh fecal samples from cows and sheep will not survive a treatment of 0.04% potassium tellurite medium, a modification of Harold's medium, and 30 minutes of exposure to a temperature

of 63° C. (3:180 -190). Fecal streptococci from a human source would survive under these conditions. Many of the species of fecal streptococci found in cattle and sheep are the same as those found in man. However, since the fecal streptococci in man survived treatment, it would show an ecotypic variation within the enterococcus group. Other investigators also indicate the presence of an ecotypic variation within the group. (1:1545 - 1552). (8:1553 - 1558). With the development of M-Enterococcus media (Difco), which enables one to enumerate a greater number of enterococci, it was necessary to try to duplicate Cooper and Ramadan's work with an improved media and note whether the same or similar results would occur.

II. ORGANIZATION OF REMAINDER OF THESIS

The remainder of this thesis is devoted to an explanation of the method which was used, the results obtained, and finally a description of the level of pollution in the Yakima River and Wilson Creek.

III. DEFINITION OF TERMS

Enterococcus or Fecal Streptococci. These two terms are synonymous and are used to define the members of the genus Streptococcus which inhabit the intestine of homeothermic vertebrates. They include the following species:

Streptococcus bovis, Streptococcus equinus, Streptococcus fecalis, Streptococcus fecalis var. liquefaciens, Streptococcus fecalis var. zymogenes, and Streptococcus durans. These organisms in themselves, are not pathogenic and only indicate a possible presence of pathogenic bacteria and viruses. (3:181).

Ecotype. An organism which, although morphologically identical to another, is physiologically dependent on a specific habitat. If removed and transplanted into a habitat of its morphologically identical relative, it would die or compete very poorly. (17:15).

Organic Pollution. Organic pollution is a condition in which fecal material is present in detectable amounts and is usually used in connection with the degree of pollution in still or running waters. (7:57).

CHAPTER II

LOCATION OF COLLECTION SITES

The primary objective of this study was to describe the existing contamination levels of Wilson Creek, and the Yakima River below its confluence with Wilson Creek.

I. STREAM ZONATION

A stream starts as an unpolluted body of moving water. The area through which the stream moves before coming in contact with pollutants is referred to as the Unaffected Zone. This is followed by the Degradation Zone or the area where organic pollution enters the stream. This is typified by such symptoms as an increase in the biochemical oxygen demand (B.O.D.) and the decreese in the dissolved oxygen (D.O.). Also, sludge begins to build up and the turbidity increases. In addition, sewage molds, fungi and filamentous bacteria may be found in abundance. In general, the bacteria population is increased and the intolerant or sensitive bottom dwelling organisms are eliminated. (6:24).

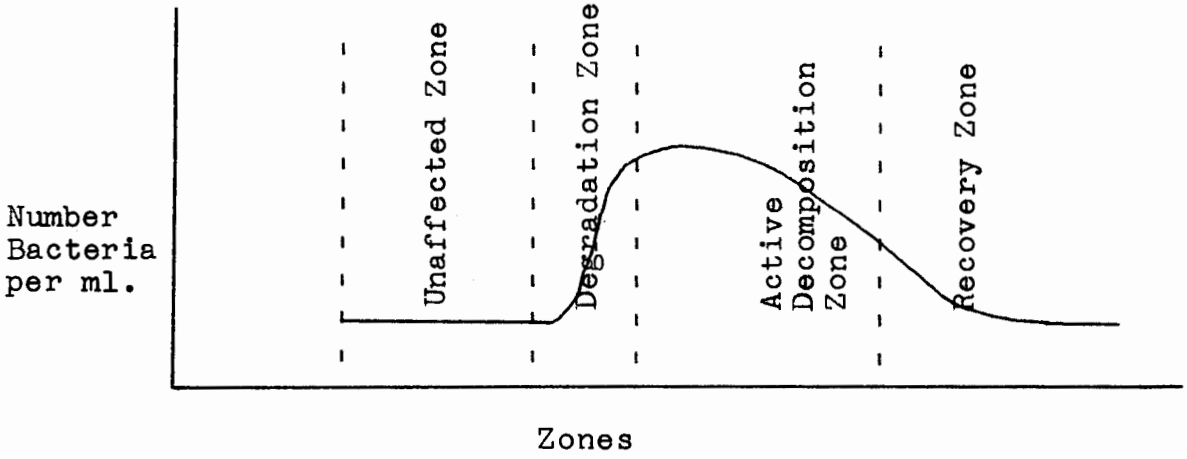
This often is followed by the Active Decomposition Zone, in which the B.O.D. undergoes partial satisfaction. The D.O. reaches its low point and may go completely to zero in the upper end of the zone. Sludge deposits are at their maximum depth at the upper limit of the zone and

turbidity gradually diminishes through the zone. The molds, fungi, and filamentous bacteria reach peaks in the upper limits of the zone and, also, gradually diminish. (6:17).

Following the Active Decomposition Zone is what is termed the Recovery Zone. In this zone, the B.O.D. decreases and the D.O. increases to the Unaffected level. The molds and fungi have been replaced by algae. The population abundance decreases and the numbers of bottom dwelling organisms increases. (6:38).

Under ideal conditions, the data gathered from such a stream, and plotted graphically would be a modified bell shaped curve. (See Figure 1).

Figure 1



With these ideas in mind, the Yakima River was sampled to locate these zones. It is known that Wilson Creek is one of the major sources of Yakima River pollution in the Ellensburg area.

II. FIRST TECHNIQUE

Sample techniques. The first technique used to locate the above mentioned zones and sampling stations involved total bacteria count by the plate count method on nutrient agar. The samples were collected on October 9, 1967. A total of 30 samples of water were collected and included the region just above Wilson Creek in the Yakima River, Wilson Creek, and thirteen locations in the Yakima River below its confluence with Wilson Creek.

Two samples were collected per site and four plate counts were made per site. The plate counts were in concentrations of one milliliter per plate and one tenth milliliter per plate. Two plates of each concentration were made. These were then incubated for 24 hours at 37° C and counted. The results of the counts indicated that either the concentrations were not dilute enough or that the bacteria were very motile. At any rate, the plates were so overgrown that no adequate counts could be made.

It was necessary to use an alternate method to obtain countable plates. For this, a selective medium was used. The medium was lactose fermentation broth for the enumeration of coliform bacteria by the Most Probable Number technique. By using this method, the samples were limited to only a portion of the bacteria in the water. However, the results should be proportionately the same. The collection of samples

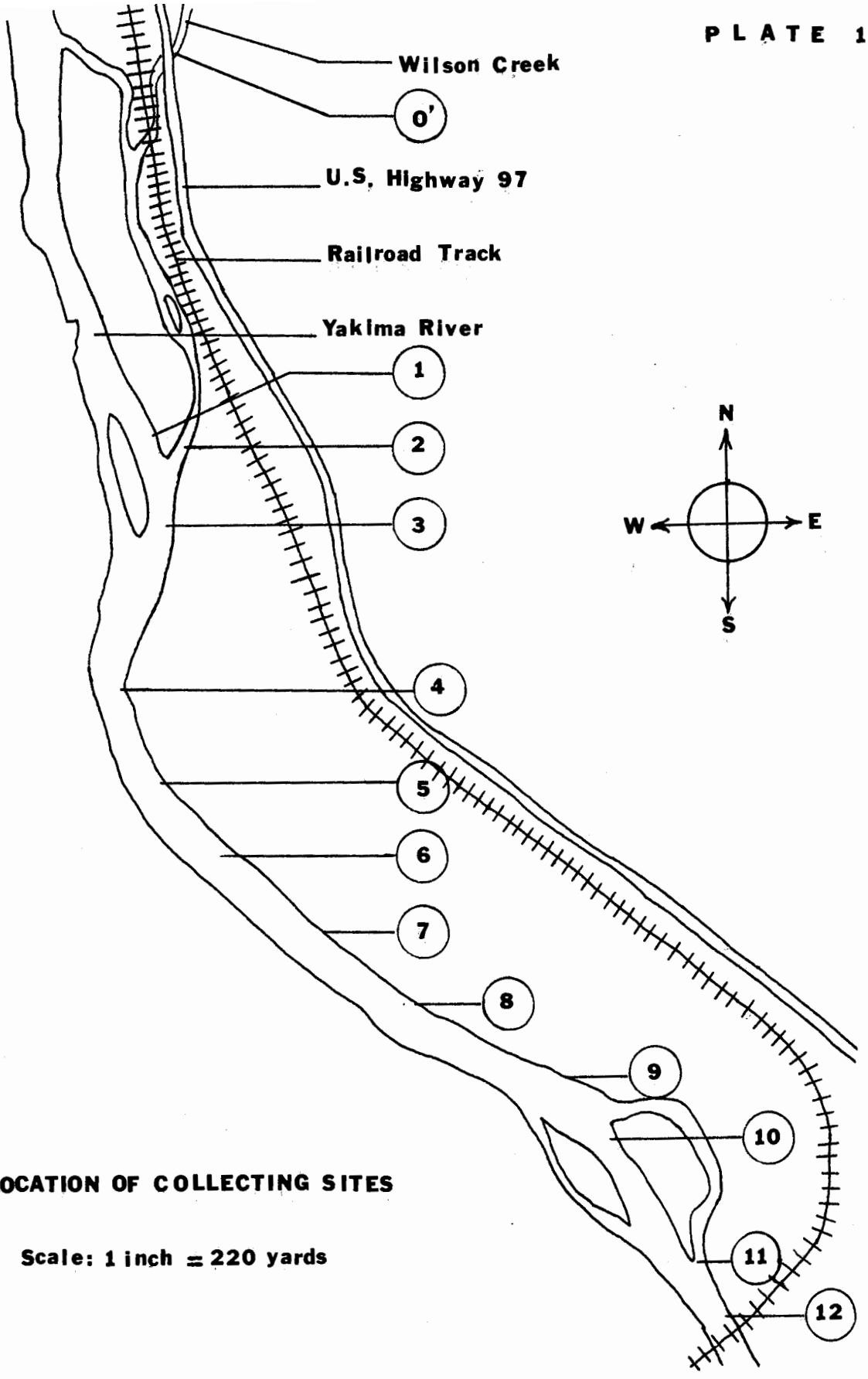
was made on October 21, 1967. All samples were collected by the method described in the Standard Methods for the Examination of Water and Wastewater, 12th edition.

A total of twelve locations, (See map, Plate 1), were sampled, (each at intervals of approximately 200 yards from the previous one), and only one bottle of water per station was collected. Dilutions were made as follows: three tubes of 10 milliliters of sample per 10 milliliters of double strength lactose broth, three tubes of 1 milliliter of sample per 10 milliliters of single strength lactose broth, and three tubes of one tenth milliliter of sample per 10 milliliters of single strength lactose broth.

One set of dilutions as described above was made for each sample collected and the samples were incubated at 37° C for 24 hours and 48 hours, and read according to the most probable number chart in the Standard Methods for the Examination of Water and Wastewater.

The coliform bacteria group are known to ferment lactose broth within 24 to 48 hours. If there is gas produced in the lactose broth, it can be presumed that the fermenting bacteria belong to the coliform group. If no gas develops in 24 hours, it is advised, by the Standard Methods, to continue incubating the cultures for another 24 hours. If, at the end of 48 hours, there was no gas present it can concluded that there were no lactose fermenting bacteria and therefore no coliform

PLATE 1



LOCATION OF COLLECTING SITES

Scale: 1 inch = 220 yards

bacteria. To confirm the presence of coliform bacteria, the IMViC Test, as described in the Standard Methods, was used. The results can be seen on Plate II.

Results. The results of this sampling shows that above site one the Yakima River is relatively unaffected by organic pollution. By using the results of the 48 hours incubation period, the area of the Yakima River below its confluence with Wilson Creek from site one to site four could be classified as a Degradation Zone.

The results from the sample collected at site three were not expected. This may be due to the way the sample was collected. According to field notes, the site was quite deep allowing materials to settle out.

From site four to site eight could be classified as an Active Decomposition Zone. Barring any new introduction of organic pollutants at site eight, from site nine on should be a Recovery Zone, however, it can be seen in Plate II that an apparently new Degradation Zone is present. At site eight there is a sudden change. This may be explained by the fact that a house situated along Highway 97 is directly in line with this site, and constitutes a possible source of additional organic pollution. The 48 hours reading tends to make even a smoother graph of this zonation as it occurs in the Yakima River. (Plate II).

LOCATION OF ZONES IN THE YAKIMA RIVER

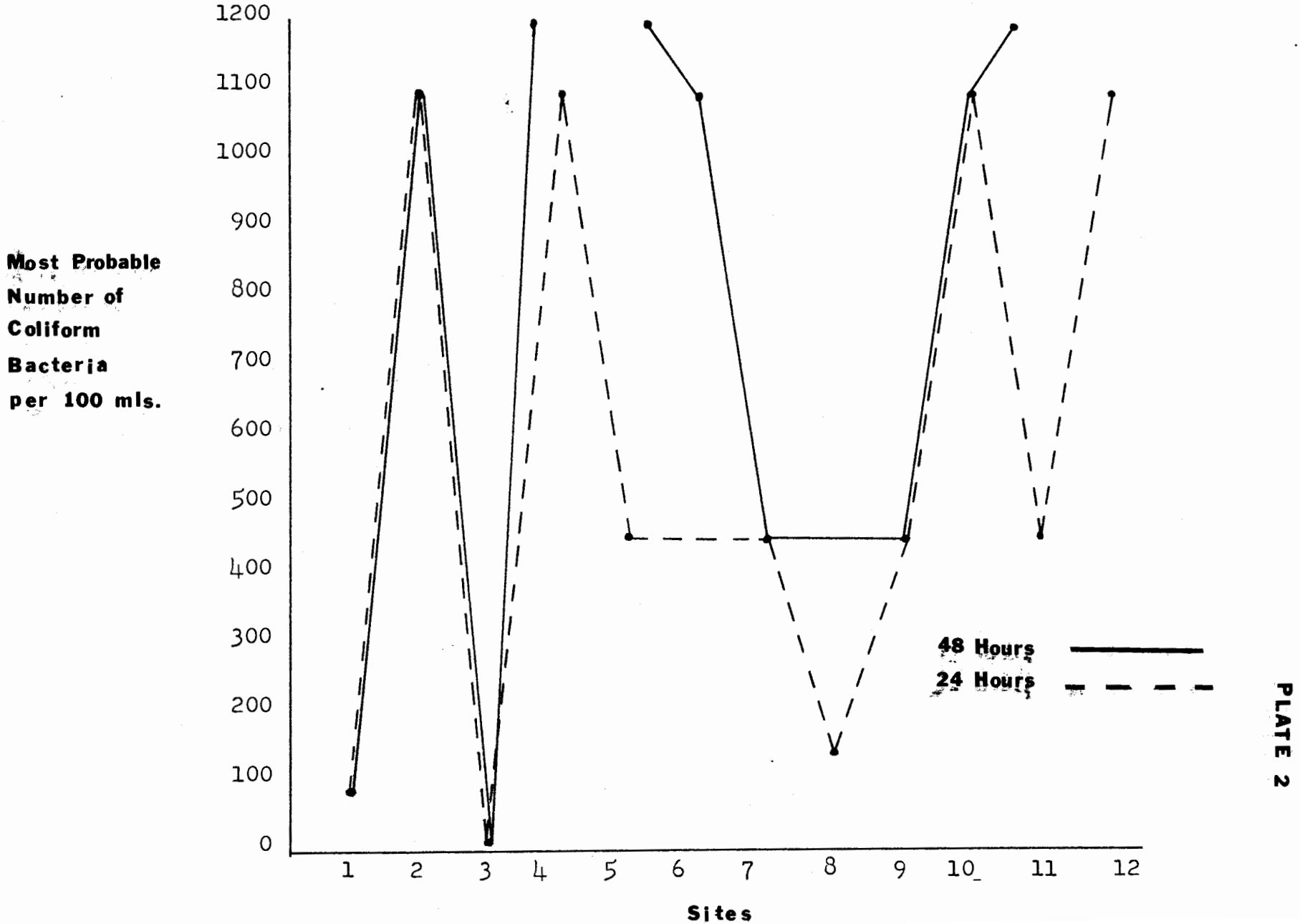


PLATE 2

III. FINAL SELECTION OF SITES

As a result of the data from the initial survey, the following sites were selected.

Site 0': Located under Highway 97 brdige on Wilson Creek.

Site 1: Located in the Yakima River about 50 yards upstream from its confluence with Wilson Creek.

Site 4: Located about 500 yards downstream from site 1.

Site 5: Located about 200 yards downstream from site 4.

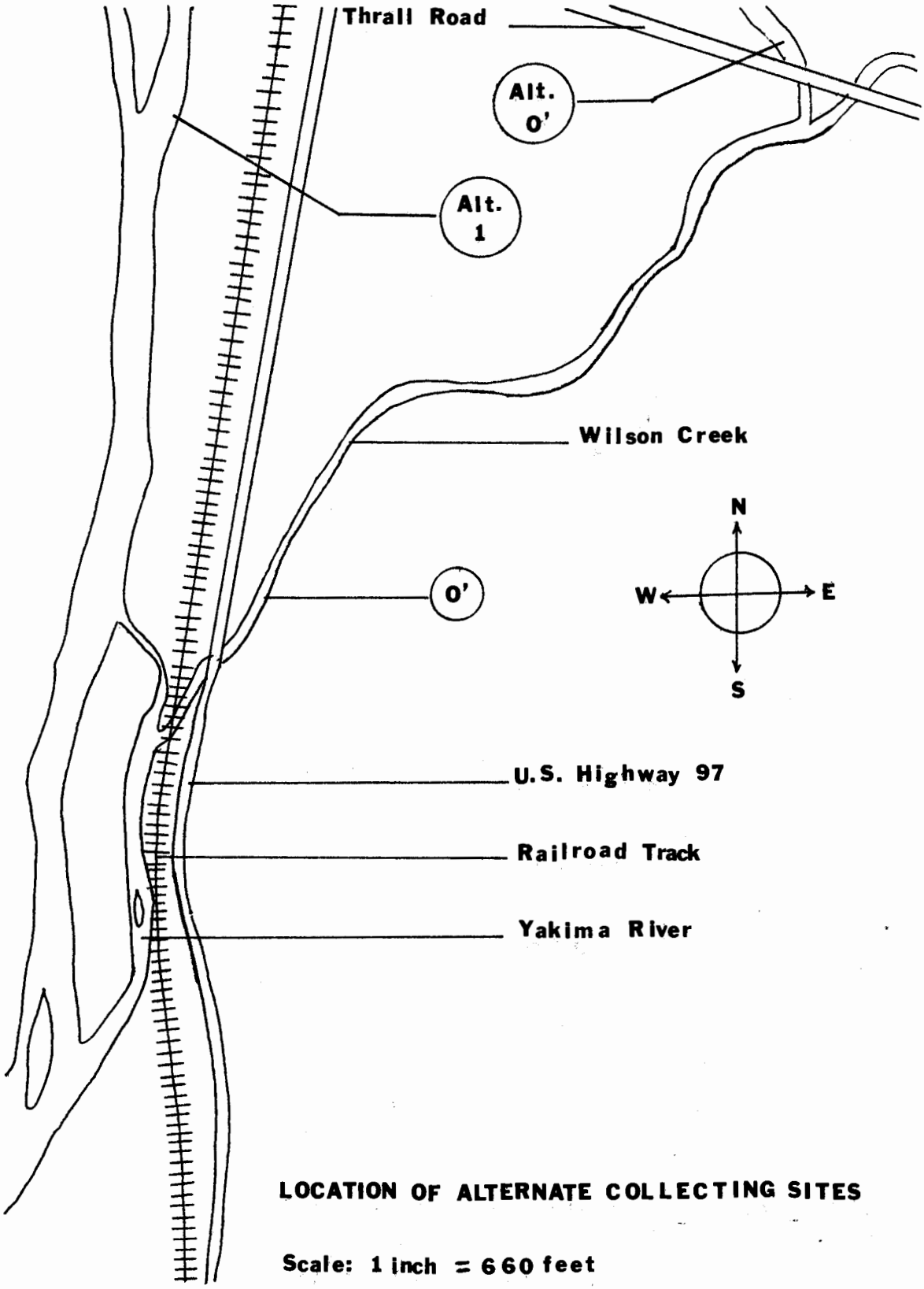
Site 7: Located about 400 yards downstream from site 5.

IV. STATION ALTERNATES

During periods of high water it became necessary to find alternate locations for certain collection stations since the increase in water level made it impossible to obtain specimens. Plate III shows the location of the alternate sites.

The location of site 0' in Wilson Creek was moved upstream from the Highway 97 bridge since the number of enterococci per milliliter was inconsistant from week to week. At 0' a greater consistancy was obtained. Later this site had to be moved again for the above reason.

PLATE 3



LOCATION OF ALTERNATE COLLECTING SITES

Scale: 1 inch = 660 feet

CHAPTER III

METHODS AND MATERIALS

I. MEMBRANE FILTER TECHNIQUE

The membrane filter technique as described by the Standard Methods for the Examination of Water and Wastewater, (12th edition), was the method used.

II. FIRST GOAL AND NECESSARY REVISIONS

Meidia. The first objective was to try the method of Cooper and Ramadan using M-Enterococcus agar in place of the potassium tellurite medium, since the M-Enterococcus media is more selective than the potassium tellurite. It was found that the temperature of 63° C for 30 minutes used by Cooper and Ramadan, was too great for growth and that the enterococci from water would not survive under these conditions. This would indicate that the bacteria had been attenuated by their exposure to water which was much below the body temperature of the host mammal. It was found after some trial and error sampling, that a temperature of 55° C for five minutes was the best temperature and time combination for the survival of the attenuated bacteria. The temperature and length of exposure were varied until a high degree of survival was obtained. Next, the strength of the tellurite solution was found to be too high. Cooper and Ramadan used a 0.04% solution,

which was found to kill the attenuated bacteria. Chapman tellurite solution, a salt of potassium and telurium, is a bactericidal agent which permits the isolation of enterococci from highly contaminated sources. (4:277). Again, through trial and error, this time by adding diminishing amounts of Chapman tellurite to the media, it was found that a $2 \times 10^{-5}\%$ solution of Chapman tellurite was best. (This is equal to 0.02 milliliters of a 1% Chapman tellurite solution for every 10 milliliters of media).

It was found, however, that a combination of a little more Chapman tellurite solution and a little less heat gave better results, since more bacteria survived. Therefore, a temperature of 50° C for five minutes with $3 \times 10^{-5}\%$ solution of Chapman tellurite was used throughout the experiment. (This is equal to 0.03 milliliters of a 1% Chapman tellurite solution for every 10 milliliters of media). The M-Enterococcus media contains: 2% tryptose, 0.5% yeast extract, 0.2% glucose, 0.04% dipotassium phosphate, 0.04% sodium azide, 0.01% 2,3,5, triphenyltetrazolium chloride, and 1% agar, with a pH of 7.2 and is commercially available from Difco. (16:591).

Media Preparation. The dehydrated media was prepared as directed. It was allowed to cool to 45° C before it was added to the Chapman tellurite solution. Since the tellurite is heat sensitive, and cannot be added to media which is over 55° C, it was pipetted by the use of a sterile micropipette

into clean test tubes in amounts of 0.03 milliliters per test tube. Next the media was added, using an automatic pipette which was set to deliver 10 milliliters at a time. In this way, it was felt that the tellurite solution would be thoroughly mixed throughout the media. The media was then poured into the petri dishes and allowed to solidify.

III. METHODS OF SAMPLE COLLECTION

Sterile screw capped test tubes were used for collecting the sewage specimens. They held approximately 20 milliliters of liquid and were uncapped under water at the Ellensburg Municipal Sewage Plant intake pipes. The tubes were three quarters filled, allowing for room to shake the samples and evenly distribute the sediment in them. The collection of water samples from the Yakima River and Wilson Creek were made in sterile sample bottles which had a capacity of approximately 125 milliliters and were filled to about 110 milliliters leaving just enough room to shake the contents and disperse the sediments evenly. Fecal samples from cattle were collected in sterile screw capped test tubes which contained a wooden applicator stick. These samples were collected at the holding pens at the Schaake Meat Packing Company Plant in Ellensburg, Washington, and were from the freshest material available. Enough fecal material was collected to make an emulsion of four loop fulls of feces per 100 milliliters of sterile buffered water, pH 7.

IV. CULTURES

All cultures were incubated at 37° C for 48 hours and then the colonies were counted under a dissecting microscope with 10X magnification. On M-Enterococcus medium, the enterococci appear as pink to red colonies and the following species of Streptococci are known to occur, according to Slanetz and Bartley: Streptococcus bovis, Streptococcus equinus, Streptococcus fecalis, Streptococcus fecalis var. liquefaciens, Streptococcus fecalis var. zymogenes, and Streptococcus durans. None of the following organisms are known to grow on this media: Escherichia coli, Aerobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Sarcena lutea, Bacillus subtiles, Bacillus cereus, all of which are common contaminants when working with fecal materials. (16:592).

V. WATER QUALITY

In order to ascertain the water quality, the state guide, Water Quality Standards for Interstate and Coastal Waters of the State of Washington, requires that more than one characteristic of the water be sampled. In addition to the bacteria level, the pH, temperature, and dissolved oxygens were measured. The actual methods and procedures are described below.

pH. The pH measurement of the Yakima River and Wilson Creek waters was determined either by pH paper or by a Beckman portable pH meter. The pH paper samplings ran from pH 4 to pH 6, while the range for the Beckman portable pH unit samplings ran from pH 7.5 to pH 8.2. Since the Beckman field pH unit was standardized before going out into the field, the pH measurements by this method were probably more accurate.

Temperature. The water temperatures of the Yakima River and Wilson Creek were taken by a specially constructed devise which held a reservoir of water after it was withdrawn from the river or creek. The thermometer bulb was resting in the reservoir and could be easily read.

Dissolved Oxygens. The dissolved oxygens were done using a powder pillow method which is commercially available from the Hach Chemical company. The method is a modification of the Standard Winkler Method Alsterberg Modification, which is described in the Standard Methods for the Examination of Water and Wastewater. The method involves the substitution of phenylarsene oxide (PAO) for sodium thiosulfide. The PAO solution is completely stable and performs identically with sodium thiosulfide. The manganous sulfite, alkaline iodine-azide, sulfamic acid were sealed in polyethylene tubes or powder pillows. One powder pillow of each reagent was added

to a 300 milliliter BOD (Biochemical Oxygen Demand) bottle, and then 200 milliliters of sample was titrated with PAO solution and the results read as parts per million dissolved oxygen.

CHAPTER IV

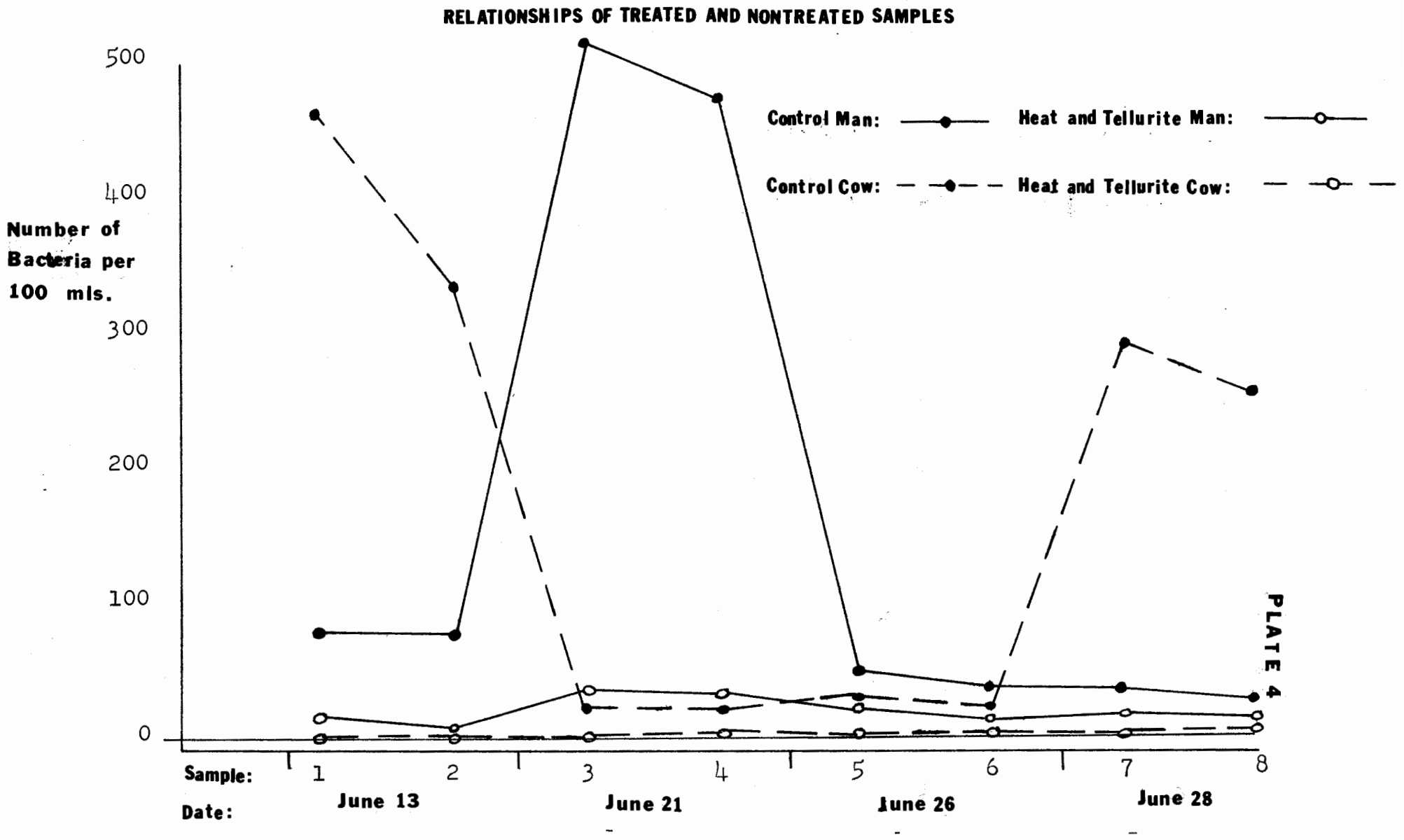
VALIDATION OF TECHNIQUE

I. RELATIONSHIPS OF TREATED AND NONTREATED SAMPLES

As previously mentioned in Chapter III, a great amount of time was spent in finding a method suitable to distinguish between human and bovine fecal streptococci. The aforementioned method was developed in late April of 1968 and needed to be repeated a number of times, obtaining satisfacotry results each time, to be validated. During the month of June, 1968 a total of eight successful trials were made. Plate IV shows graphically the results of these eight samplings.

A major problem in this sampling was obtaining a consistant bacterial density from one sampling to the next. Even though the same amount of sewage or fecal material was used as an inoculum each time, there was no way of being sure that the bacterial density of the inoculum was consistant. Therefore, wide ranges in number of bacterial colonies were obtained from the various samples.

In interpreting the graph, the treated human samples tend to parallel that of the nontreated human samples. On the other hand, there is no positive correlation between the treated bovine samples and the untreated samples. Comparing the median values of untreated human and bovine samples with the median values of treated human and bovine samples, the


RELATIONSHIPS OF TREATED AND NONTREATED SAMPLES
Control Man:
Heat and Tellurite Man:
Control Cow:
Heat and Tellurite Cow:
Number of Bacteria per 100 mls.
500
400
300
200
100
0
Sample:
1
2
3
4
5
6
7
8
Date:
June 13
June 21
June 26
June 28
PLATE 4

percentage of survival was computed. The median values for the human samples were as follows: 79.5 colonies per 100 milliliters of dilution for the untreated sample and 8 colonies per 100 milliliters of dilution for the treated samples. The median values for the bovine samples were as follows: 140 colonies per 100 milliliters of dilution for the untreated samples and no colonies per 100 milliliters of dilution for the treated samples. This gave a survival percentage for the human enterococci of 10.06% and a survival percentage of 0% for the bovine enterococci. In almost every case there is no growth of the treated bovine samples even when the density of the untreated sample reached 461 colonies per 100 milliliters of sample. In those instances where a colony did grow, it was always growing around a piece of straw or undigested hay which may have provided enough insulation to enable the bacteria to survive the heat treatment. In no case did more than two colonies survive treatment.

II. RELATIONSHIPS OF MIXED AND HUMAN TREATED SAMPLES

Furthermore, when comparing the graph (Plate V) of the treated human samples with that of a mixture of treated human and bovine samples, the results again are fairly parallel.

Considering the method used in preparing the samples from fresh sewage and cattle feces, this graph tends to show that the cattle enterococci are eliminated by the treatment, and that it is possible to detect human pollution

RELATIONSHIPS OF MIXED AND HUMAN TREATED SAMPLES

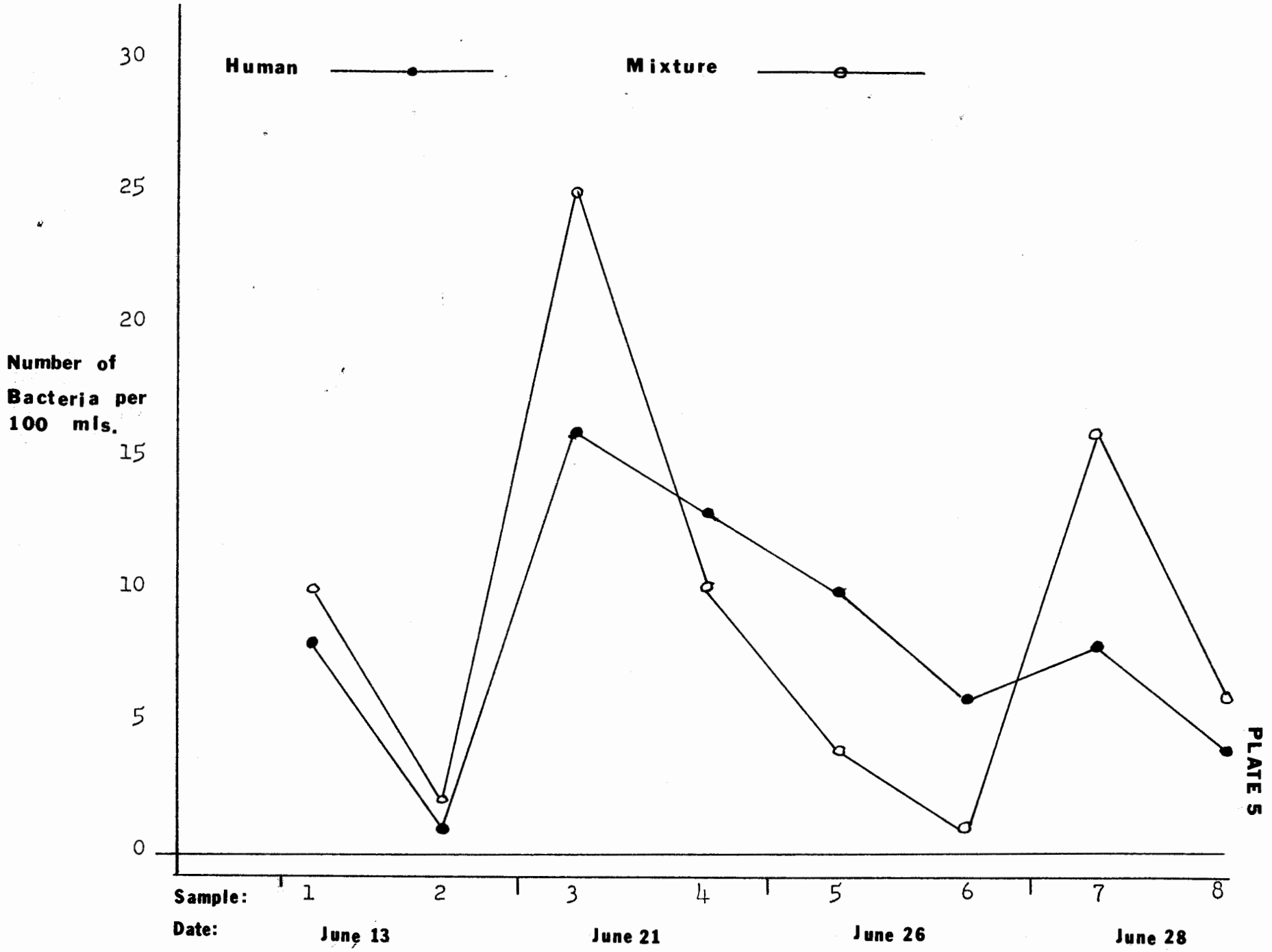


PLATE 5

in still and running waters which receive contamination from a number of different sources.

III. FURTHER VERIFICATION OF ECOTYPIC DEPENDENCE

In order to further verify the technique, a sample of water from the irrigation ditch which flows through the Central Washington State College campus was collected by the Chestnut Street bridge, and a sample of sewage were treated by the heat and tellurite method. After incubation, a well isolated colony was selected from both samples and inoculated on M-Enterococcus media slants. Tube "A" was a good colony from the sewage sample and tube "B" was a good colony from the irrigation ditch sample. These samples were sent to the State of Washington Department of Health Enivronment Laboratory Services Section, Smith Tower, Seattle, Washington for identification. The results of their identification showed that isolate "A" was a:

> "Gram positive coccus, catalase negative, non-hemolytic on rabbit blood agar surface growth, does not liquify gelatin (incubated one week), grows in 6.5% NaCl at 10° C and 45° C, grows in 0.1% methylene blue, reduces TTC, ferments mannitol and sorbitol. These characteristics indicate the organism is Streptococcus fecalis.
>
> Isolate "B" has the same characteristics except that gelatin is liquified by the organism. This would place it as Streptococcus fecalis var. liquifaciens."

Both these organisms are found in human and bovine feces, although the Streptococcus fecalis found in bovine

feces is usually an atypical form. (3:184). Therefore, these isolates are presumably human.

CHAPTER V

WATER QUALITY OF THE YAKIMA RIVER BELOW ITS CONFLUENCE WITH WILSON CREEK

The State Guide to Water Quality Standards lists four categories in which interstate and coastal waters are grouped. The first of these is Class AA, extraordinary. The water of this quality "markedly and uniformly exceeds the requirements for all or substantially all uses". (19:3).

Class A, excellent, is the second category. The water of this quality also "exceeds or meets the requirements for all or substantially all uses". (19:4).

Class B, good, is next. In general its waters "exceed or meet the requirements for most uses". (19:5). The last category is Class C, fair. Water of this quality "exceeds or meets the requirements of selected and essential uses". (19:6). These uses include commerce and navigation, cooling waters, boating, and fish passage.

The Yakima River, below Wilson Creek could be properly classified as a Class B, good stream, and may even qualify as a Class A, excellent stream. (Plate VIA and VIB). Above the Wilson Creek confluence the Yakima River is a Class A excellent stream. To be classified as a Class B body of water, the total coliform count shall not have a median value which exceeds 1,000 bacteria per 100 milliliters of sample and shall

WASHINGTON STATE WATER CLASSIFICATION SYSTEM

	Class AA Extraordinary	Class A Excellent	Class B Good	Class C Fair
Total Coliform Count	Median value shall not exceed 50/100 ml. with less than 10% of samples not exceeding 230/100 ml. when associated with fecal source.	Median value shall not exceed 240/100 ml. with less than 20% of samples not exceeding 1000 when associated with fecal source.	Median value shall not exceed 1000/100 ml. with less than 20% exceeding 2,400 when associated with any fecal source.	Median shall not exceed 1000/100 ml. when associated with any fecal source.
Dissolved Oxygen	Shall exceed 9.5 parts per million.	Shall exceed 8.0 parts per million.	Shall exceed 6.5 parts per million.	Shall exceed 5.0 parts per million.
Temperature	Shall not exceed 60° F.	Shall not exceed 65° F.	Shall not exceed 70° F.	Shall not exceed 75° F.
pH	Shall range between 6.5 to 8.5.	Shall range between 6.5 to 8.5.	Shall range between 6.5 to 8.5.	Shall range between 6.0 to 9.0.
Turbidity	Shall not exceed 5 Jackson Turbidity Units.	Shall not exceed 5 Jackson Turbidity Units.	Shall not exceed 10 Jackson Turbidity Units.	Shall not exceed 10 Jackson Turbidity Units.
Aesthetic Value	Shall... not offend the senses.	Shall not... offend the senses.	Shall not... affect water usage or taint the flesh of edible species.	Shall not... be interfered with by obnoxious wastes.

(19:3-6).

PLATE 6a

CONDITIONS OF STUDY WATERS

	Yakima River	Wilson Creek
Total Coliform Count	Median number 65/100 ml. (August, 1968 only)	Median number 240/100 ml. (August, 1968 only)
Dissolved Oxygen	9.0 to 10.3 parts per million.	7.1 to 10.1 parts per million.
Temperature	44° F to 60° F. (7° C to 16.5° C)	48° F to 65° F. (8° C to 18° C)
pH	7.5 to 8.2.	8.0 to 8.2.
Turbidity	----	----
Aesthetic Value	Did not offend the senses.	Did offend the sense of sight and touch.

PLATE 6b

not exceed 2,400 when associated with any source of fecal material. (19:5).

On the basis of samplings made in October, 1967, the median coliform bacteria count was 460 per 100 milliliters of sample. This is well within the limits of the Class B, good stream requirements. (19:5).

The dissolved oxygen must be at least 6.5 parts per million to be classified as Class B water and 8.0 parts per million to be Class A. At no time did the dissolved oxygen drop below 9.0 parts per million.

Temperature must not exceed 70° F (22° C) to qualify for Class B water or 65° F (18.5° C) for Class A. At no time did it exceed 60° F (16.5° C).

To be classified as Class A or B water, the pH should be within the range of 6.5 to 8.5. The ranges of the pH sampled with the Beckman portable pH meter ranged from 7.5 to 8.2, which is well within the allowable ranges for both classes.

As previously mentioned, the density of enterococci in water is seven to eight times greater than the density of the coliform bacteria. During the month of August, 1968, samples were collected to determine the density of the enterococci and to see if there was any human contamination present. The median number of fecal streptococci for the same month in the Yakima River below its confluence with

Wilson Creek was 460 colonies per 100 milliliters of water. This meant that the median coliform count for the same period was about 65 coliform colonies per 100 milliliters of water sampled. (1/7th the number of enterococci present). This would place that section of the Yakima River in Class A, excellent water.

On the other hand, Wilson Creek had a median enterococci density of 1675 colonies per 100 milliliters water for the same period. This would place its coliform count for August, 1968 at 240 colonies per 100 milliliters water. This would make Wilson Creek a Class B, good stream.

During this same period, samples were collected in order to check the presence of human fecal streptococci. The median value for the number of colonies isolated by the heat and tellurite method was 240 colonies per 100 milliliters water for the Yakima River below its confluence with Wilson Creek, and 790 colonies per 100 milliliters for Wilson Creek.

The median total fecal streptococci value for the Yakima River above its confluence with Wilson Creek was 70 colonies per 100 milliliters of water. The median human fecal streptococci for the Yakima River above Wilson Creek was 19 colonies per 100 milliliters water. (This is based on the August, 1968 samples only). This shows that the water of Wilson creek increases both the total and human fecal streptococcal count by approximately ten fold. Therefore, this

shows that Wilson Creek is a major source of pollution in the Yakima River drainage system.

CHAPTER VI

CONCLUDING REMARKS

The Yakima River is a Class A body of water. However, it is becoming degraded by the waters of Wilson Creek. If Wilson Creek continues to become contaminated with organic material, and other pollutants, so will the Yakima River. In time, the Yakima River may become useless as a recreational facility unless steps are taken to eliminate the sources of degradation of Wilson Creek and thereby eliminate a major source of degradation of the Yakima River. Human as well as non-human pollutants are reaching the Yakima River via Wilson Creek. Due to the wide ranges between the control plates made from sewage samples and the results from the heat and tellurite treated sewage specimens, it was not possible to quantitatively relate the numbers of human fecal streptococci to those from bovine sources. With further improvement of the technique and additional data, perhaps a quantitative relationship could be obtained.

Furthermore, the same or similar treatment of fecal samples from other organisms, such as sheep, horses, dogs, poultry, etc., should be compared with human fecal streptococci to be sure that none of these sources can survive a heat and tellurite treatment, thus assuring that only human fecal streptococci are being counted.

Lastly, before any major plan for the development or conservation of the Yakima River can be definitely decided upon, a descriptive study of the condition of the Yakima River from its head waters to its confluence with the Columbia River should be undertaken. This study should describe the present conditions of the river according to the guidelines outlined in the State Water Quality Guide. Such a descriptive paper would be immensely helpful for any further study dealing with almost any aspect of the Yakima River.

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