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# A STUDY OF THE CONTAMINATION OF SUSPENDED FLUVIAL SEDIMENTS WITH ENTERIC BACTERIA IN AGRICULTURAL DRAINS

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# A STUDY OF THE CONTAMINATION OF SUSPENDED FLUVIAL SEDIMENTS WITH ENTERIC BACTERIA IN AGRICULTURAL DRAINS

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## ABSTRACT

The pollution of bathing beaches on the Great Lakes and inland waters occurs often as a result of the discharge of rivers and streams into near-shore waters. Depending on wind direction and speed, plumes of turbidity can be observed to be impacting directly on bathing beaches. The immediate impairment of the beach is the aesthetics, when water clarity diminishes to only a few centimetres. However, the major water quality parameters which increase are fecal coliforms and *Escherichia coli*. Total viable bacteria increase in conjunction with these parameters.

This study was initiated to determine to what extent the particulates contributing to the turbidity of the water were perhaps transporting bacteria to the beach waters.

Samples were collected from three agricultural drains suspected of impacting bathing beaches with elevated bacterial levels. The samples were analyzed by electronic zone sensing to size and count the suspended particulates. Analyses of the samples involved direct viable cell count and *Salmonella* determinations using epi-fluorescence and immunofluorescence microscopy to establish the numbers of bacteria sorbed to the suspended particulates of the agricultural drains.

In addition, a bacterial transport study was conducted. An antibiotic-labelled *Escherichia* coli was sorbed to agricultural drain sediment in the laboratory. This bacteria-particulate mixture was inserted into the Desjardine Drain 5 km from the discharge of the drain to the Old Ausable River.

Results of particulate analysis showed that four basic size ranges existed. Microscopic analyses of the size ranges of particulates colonized by bacteria were shown to range from $10^3$ to $10^5$ bacterial cells per $mm^2$ of particulate surface area. Some differences were observed between the agricultural drains studied, which may be related to the

clay content of the sediments of the specific drain. Some seasonal differences were also detected. The summer and autumn had the highest degree of particulate colonization while the particulates analyzed during the spring studies possessed fewer bacteria.

The results of the bacterial transport study demonstrated that the fecal associated bacterium, *Escherichia* coli, could travel 5 km from the point of insertion to the beaches of Lake Huron and that the bacteria remained viable in the drain for at least eighty-five days.

In conclusion, suspended particulates, contributing to the turbidity of agricultural drains, serve to transport high levels of bacteria to the receiving waters and often travel many kilometres from the point of entry of the bacteria into the drain.

# TABLE OF CONTENTS

## FIGURE CAPTIONS

Figure

1. Map of the Arthur Vanatter Drain study site.

2. Map of the Central School Drain study site.

3. Map of the Desjardine Drain study site.

4. Map showing grab and swab sampling sites during the bacterial transport study.

5. Size distribution of particulates in a typical sample of suspended sediments, based on diameter, from the Elzone 180 XY.

6. Levels of total viable bacteria and *Salmonella*/$mm^2$ surface area, adsorbed on particles 30 to 70 µm in diameter in the Arthur Vanatter Drain.

7. Levels of total viable bacteria/$mm^2$ surface area, adsorbed on particles 10 to 30 µm in diameter in the Arthur Vanatter Drain.

8. Levels of total viable bacteria/$mm^2$ surface area, adsorbed on particles 5 to 10 µm in diameter in the Arthur Vanatter Drain.

9. Levels of total viable bacteria and *Salmonella*/$mm^2$ surface area, adsorbed on particles 1 to 5 µm in diameter in the Arthur Vanatter Drain.

10. Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 30 to 70 µm in diameter in the Arthur Vanatter Drain.

11. Levels of total viable bacteria which are free-floating and associated with particles 10 to 30 µm in diameter in the Arthur Vanatter Drain.

12. Levels of total viable bacteria which are free-floating and associated with particles 5 to 10 µm in diameter in the Arthur Vanatter Drain.

13. Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 1 to 5 µm in diameter in the Arthur Vanatter Drain.

14. Levels of total viable bacteria and *Salmonella*/$mm^2$ surface area, adsorbed on particles 30 to 70 µm in diameter in the Central School Drain.

15. Levels of total viable bacteria/$mm^2$ surface area, adsorbed on particles 10 to 30 µm in diameter in the Central School Drain.

16. Levels of total viable bacteria/$mm^2$ surface area, adsorbed on particles 5 to 10 µm in diameter in the Central School Drain.

17. Levels of total viable bacteria and *Salmonella*/$mm^2$ surface area, adsorbed on particles 1 to 5 µm in diameter in the Central School Drain.

18. Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 30 to 70 µm in diameter in the Central School Drain.

19. Levels of total viable bacteria which are free-floating and associated with particles 10 to 30 gm in diameter in the Central School Drain.

20. Levels of total viable bacteria which are free-floating and associated with particles 5 to 10 µm in diameter in the Central School Drain.

21. Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 1 to 5 µm in diameter in the Central School Drain.

22. Levels of total viable bacteria and *Salmonella*/$mm^2$ surface area, adsorbed on particles 30 to 70 µm in diameter in the Desjardine Drain.

23. Levels of total viable bacteria/$mm^2$ surface area, adsorbed on particles 10 to 30 µm in diameter in the Desjardine Drain.

24. Levels of total viable bacteria/$mm^2$ surface area, adsorbed on particles 5 to 10 µm in diameter in the Desjardine Drain.

25. Levels of total viable bacteria and *Salmonella*/$mm^2$ surface area, adsorbed on particles 1 to 5 µm in diameter in the Desjardine Drain.

26. Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 30 to 70 µm in diameter in the Desjardine Drain.

27. Levels of total viable bacteria which are free-floating and associated with particles 10 to 30 µm in diameter in the Desjardine Drain.

## TABLE CAPTIONS



## PLATE CAPTIONS

# I. INTRODUCTION

The pleasure of utilizing the bathing beaches situated on the Great Lakes, as well as the inland beaches, in the province of Ontario, Canada, has been greatly curtailed during the past eight years because of excessively high levels of fecal bacteria. Detailed investigations into the reasons for bacterial contamination of recreational waters have revealed a set of circumstances that appears to be common to many of the beaches. The sources of fecal bacteria are often associated with agricultural runoff resulting from mismanagement of manure.' In more developed areas of the province, urban wastes, comprised of sewage treatment plant wastes, storm sewer discharges and specific industrial wastes, constitute the sources of fecal-associated bacteria in the majority of cases.

In evaluating how the bacteria affect water quality of bathing beaches, it was evident that the rivers and streams carrying bacteria from their points of entry into the receiving streams were contaminated with fecal associated bacteria at certain times and not at others. The routine sampling of the rivers impacting on the beaches indicated that the total and fecal bacterial loadings were actually quite infrequent. The bacterial quality of the beaches themselves fluctuated greatly, making management of the beaches for recreational activities very difficult. Specifically, fecal coliform and *Escherichia* coli levels could exceed the bathing beach guideline for 100 cells per 100 mL of water by one order of magnitude on one day, while the next day the levels could be less than 10 cells per 100 mL of sample. It was

observed that coincident with high bacteria levels were high turbidity levels. It appeared that when the waters were rough, with the wave height exceeding 60 cm, the bacterial levels were excessive, as were the particulate levels in the water. The impact of high turbidity levels in rivers on the beaches of Lake Huron is displayed in Plates 1 and 2. Conversely, when the beach waters were calm, bacterial levels were significantly lower (i.e., below the standard and the waters were clear (free of particulates)[2].

The association of bacteria with soil particulates was easily observed with farm drainage, whether the runoff was surface or subsurface. Sub-surface drainage from tiled fields, which had manure applied, contained bacteria and soil particulates. Recent studies conducted by Dean et al.[3], have demonstrated rapid infiltration of liquid manure applied to fields through to the underlying tiles. This occurred as liquid manure, containing levels of fecal coliforms and *E. coli* at $10^6$ cells per 100 mL of manure, penetrated macropores in the surface soil horizons. The drainage exiting the field tile into agricultural drains, which discharge into rivers impacting bathing beaches, contained levels of fecal bacteria and soil particulates ranging from $10^3$ to $10^5$ cells per 100 mL. The association of these bacteria with the soil particulates has been, until recently, only speculated. The lengthy survival of fecal 3 bacteria in soil however, has been known for some years[4,5] and is now being related to the transport of total and fecal bacteria in farm drainage.

**Plate1.** Turbidity plume of the Old Ausable river discharging to Lake Huron.

**Plate 2.** Turbidity plume of the Ausable river discharging to Lake Huron.

The transport of soil particulates resulting from erosion and runoff from farmland has been studied in detail by sedimentologists[6]. The transport of chemical constituents of soil and sediment particulates, including pesticides and metals, has been described. It is realized that soils containing high concentrations of clay (montmorillonite with a high cation exchange capacity) and organic matter have the ability to sorb significant quantities of chemical components. Some research has shown that viruses, such as bacteriophage, also tend to be attracted to these same constituents of soil because of surface charges on the bacteriophage and the clay and organic matter[7].

Preliminary studies have shown that various size fractions of suspended particulates in streams also conduct the transport of bacteria downstream. It was shown that certain size fractions of suspended particulates tended to be highly colonized by bacteria at specific times of the year. Specifically, at the headwaters of the Desjardine Drain during the summer months, particulates become colonized with bacteria at a concentration of 1 bacterium per 4 $\mu m^2$ of surface area or 2.1 x $10^5$ bacteria per $mm^2$ of particulate surface area. Characterization of particulates found at the discharge of the drain to those detected at a beach 18 km from the headwaters show a similar degree of colonization. One bacterium was found for every 1.6 $\mu m^2$ of surface area. The mean concentration was to be 6.5 x $10^5$ bacteria per $mm^2$. The particulates colonized ranged from 2 to 5 µm in diameter[8]. During the late fall, the examination of the particulates in the water showed a significant change in condition. At the headwaters of this agricultural drain, 1 bacterium per 2.2 x $10^3$ $\mu m^2$ of particulate surface area was detected. This equates to 450 bacteria per

$mm^2$ surface area. The Grand Bend beach which is impacted by the Desjardine Drain also exhibited a decrease in bacterial colonization of suspended particulates as 1 bacterium per 5.1 x $10^3$ $\mu m^2$ surface area was observed, or 195 bacteria per $mm^2$. Most bacteria were observed on particulates ranging in diameter from 2 to 5 µm. The average percent viability of the sorbed bacteria declined from 50 in the summer to 17 in the fall.

These observations provide some evidence as to the rate of colonization of suspended particulates in an agricultural drain that impacts on a bathing beach of the Great Lakes.

The focus of this study was to investigate the degree of colonization of suspended particulates by total viable bacteria and *Salmonella.* Three different agricultural drains were studied during the summer, fall and spring seasons, using the latest techniques in epi-fluorescence and immunofluorescence microscopy for detecting viable bacteria in sediment particulates [15-17].

In addition, a bacterial transport study was conducted where sediment-bound *E. coli,* labelled with nalidixic acid resistance, were introduced into the stream and were monitored to determine the distance transported on indigenous sediments of the agricultural drains. These results were then compared to similar studies done with nonparticulate-bound bacteria.

# II. METHODS AND MATERIALS

## A. Sampling Sites

The three agricultural drains were chosen because they represented different degrees of impact by rural land-use activities and they each affected the quality of their respective beach water.

The first location was the Arthur Vanatter Drain near the village of Kintore in Oxford County, as shown in Figure 1. The upstream location was approximately 300 meters north of the main site, a culvert on Concession Road 10. The downstream site was located 200 meters south of the concession road. Corn was planted along the edge of the drain (within 4 meters) and manure was applied as liquid swine waste only on the south side of the concession road, usually in the spring. The drain discharges to the middle branch of the Thames River.

The second sampling location, along the Central School Drain near Shakespeare in Perth County, was also comprised of three study sites, as shown in Figure 2. The sites were accessed by Concession Road 3. The upstream site, 100 meters north of the road, was bounded by pasture on which cattle are able to water in the drain. The main site location, at the road culvert, was also impacted by the cattle, but, in addition, a field tile discharged into the drain which contained field-applied manure and milkhouse

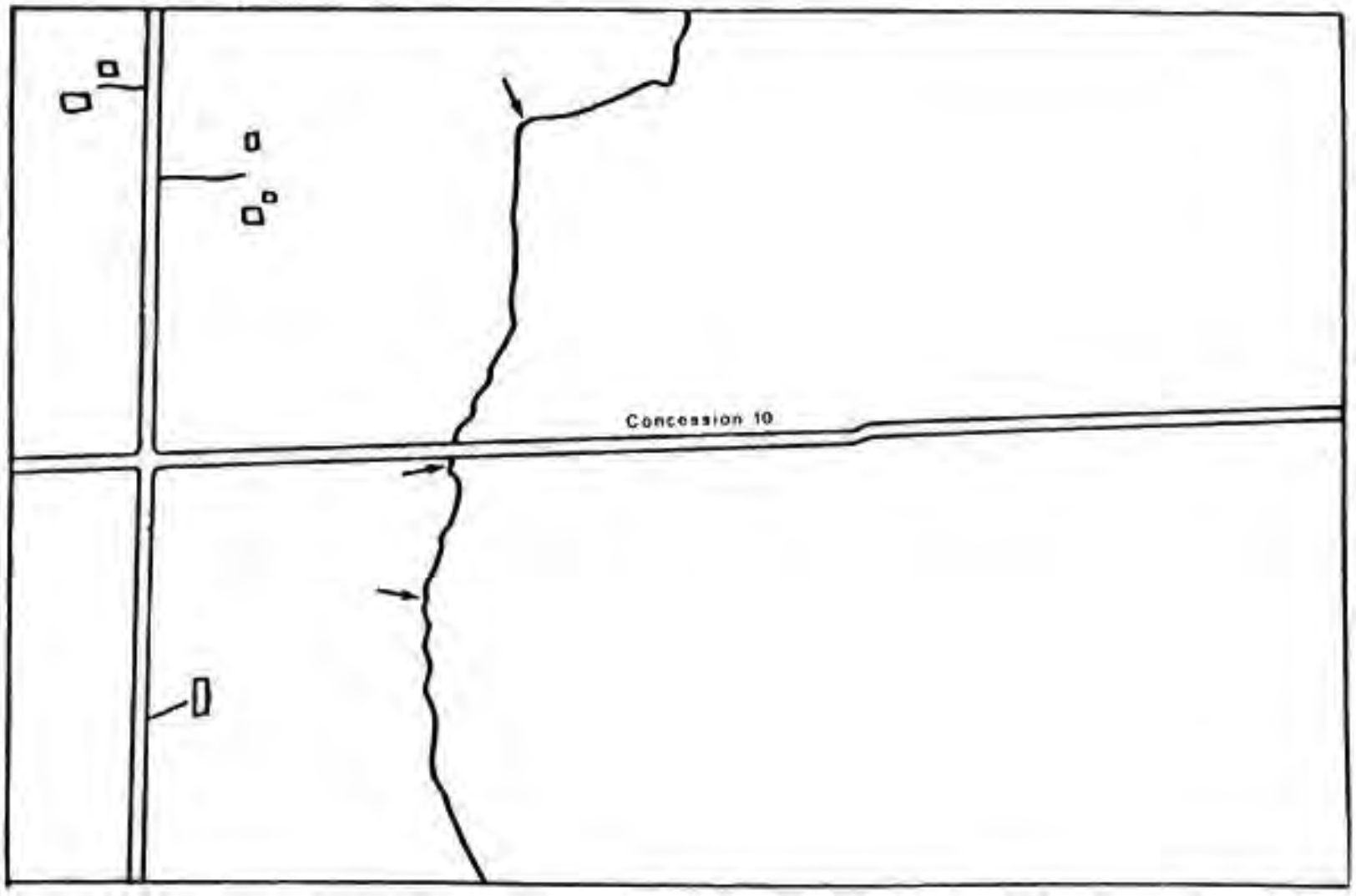


**Figure 1.** Map of the Arthur Vanatter drain study site.

wastes. The downstream site was located 150 metres from the road. The drain discharges to the Avon River, approximately 1 km to the south.

The third sampling location was located on the Desjardine Drain near Grand Bend in Huron County, as shown in Figure 3. The upstream site was situated 800 m east of the Playhouse Road. This site received farm wastes from upstream farming operations but was located in a marshy woodlot. The main site, to the west, was located at the access road. The surrounding land activities include beef farming and six or seven homes, all of which have septic tank tile beds adjacent to the drain. The downstream site was located 800 m west and was also impacted by beef cattle and septage from rural septic tanks. The Desjardine Drain discharges into the Old Ausable River which, in turn, discharges to Lake Huron at Grand Bend Beach.

## B. Sampling Protocol

The study commenced in the summer of 1991, with the manual sampling of the three sites in each of the three locations described above.

The samples for suspended particulates were taken in sterile, 500 mL wide-mouth plastic bottles placed in the main stream flow. The bottle design provided for rapid filling of suspended particulates with minimal shearing of the delicate aggregates.

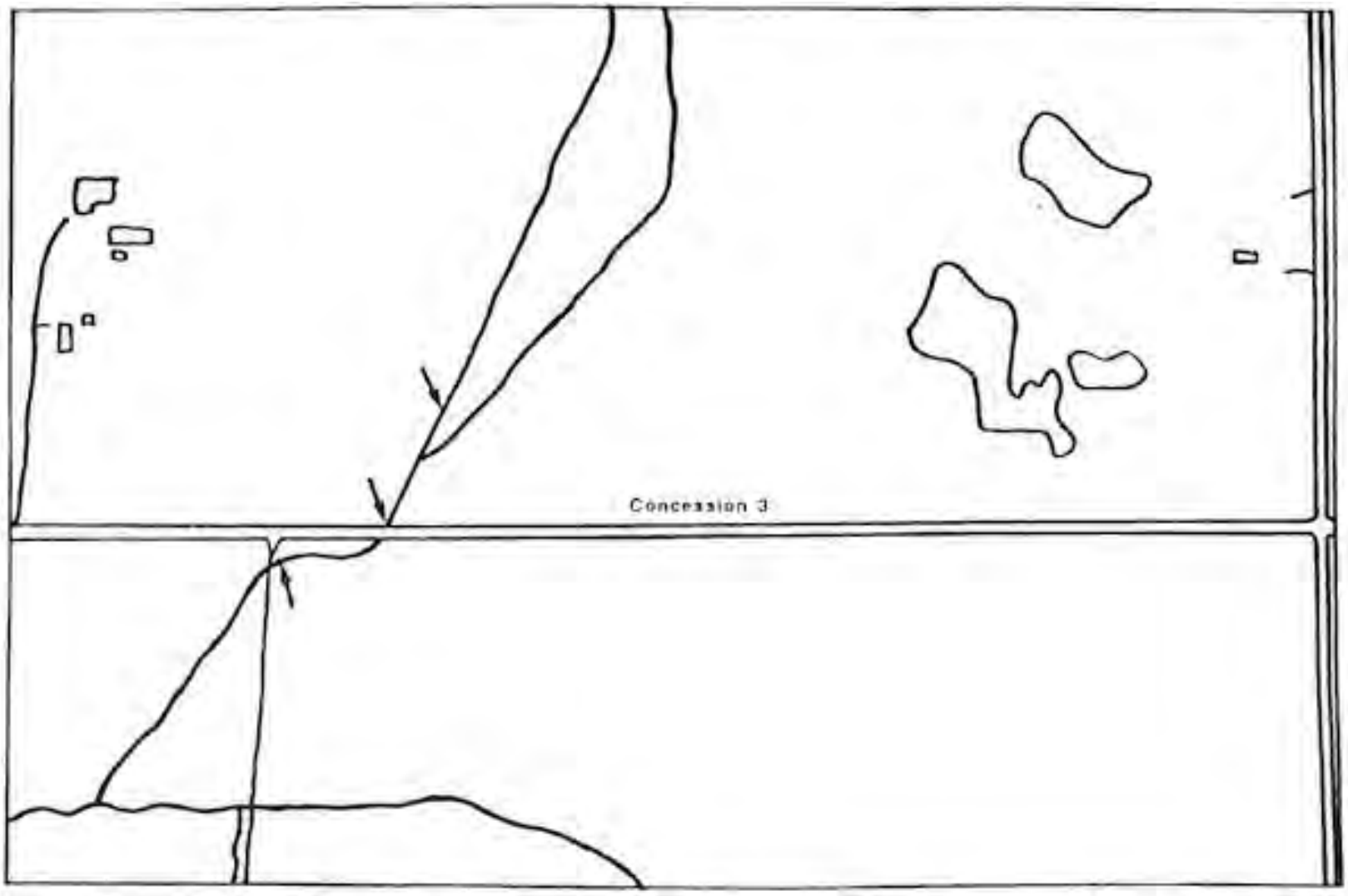


**Figure 2.** Map of the Central School drain study site.

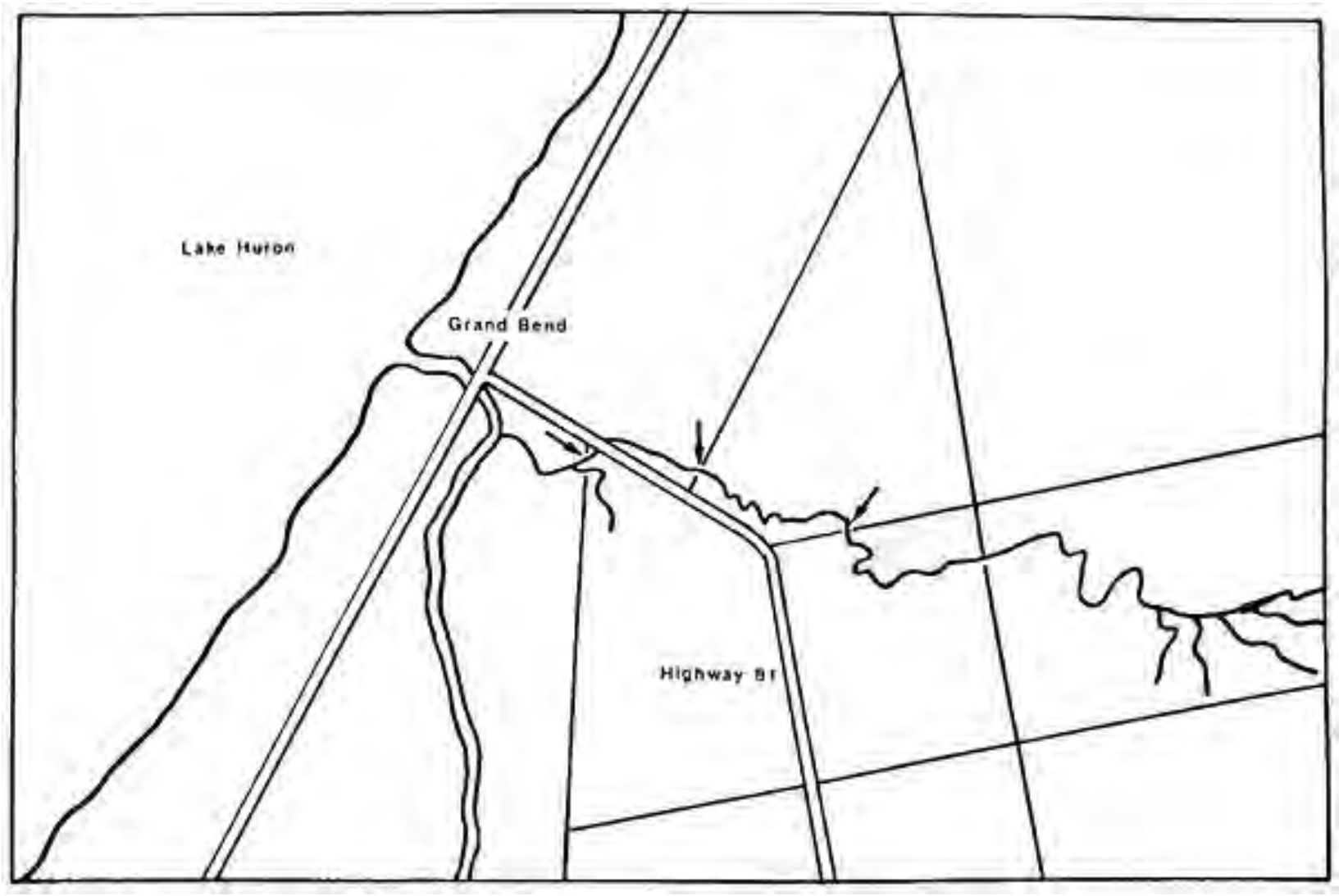


**Figure 3.** Map of the Desjardine drain study site.

Samples for the standard bacterial indicator parameters, *Escherichia coli* and fecal streptococci, were taken in sterile glass milk dilution bottles.

The bacterial transport study involved sampling, in duplicate, using conventional bacteriological sterile glass milk dilution bottles. Composite samples were taken using specifically constructed cotton swabs.

## C. Particulate Characterization Procedure

The sizing and counting of suspended particulates was conducted using an Elzone 180 XY Particle Analyzer, manufactured by Particle Data Inc., Elmhurst, Illinois. Preparation for analyses included filter screening each sample with 212 µm mesh. The particulates known to be transported in streams and rivers and known to act as sorbents for microorganisms are less than 200 µm in diameter.

The filtrate was collected and diluted in sodium hexametaphosphate (Calgon) according to the requirements of the instrumentation[18]. The Calgon-suspending diluent provided stabilization of the particulates from aggregation or deflocculation.

The Elzone 180 XY Particle Analyzer functions on the Coulter Principle. The system is comprised of a glass tube with an orifice at one end, which may vary in size from 12 to 900 gm, two electrodes and a vacuum pump. One electrode is inside the tube and the other is located on the outside. The tube is placed in

an aqueous medium containing electrolytes and the particles to be sized and counted. The vacuum pump draws the suspended medium through the orifice. As the particles pass by the electrodes, they cause an increase in current resistance. The change in resistance is directly proportional to the volume of the particle passing between the electrodes. The particle is then counted and sized. With this information, the Elzone 180 XY calculates particle diameter, volume and surface area in the appropriate units of measurement.

Since the particles to be counted would vary considerably in size, different orifice tubes were used. An orifice with a diameter greater than 60% of that of the largest particles to be sized was employed. To obtain optimal resolution of particle sizes, three orifices were used with orifice diameters of 380, 95 and 24 µm. The operating size range for the 380 µm orifice tube was from 2 to 150 µm. For the 95 µm orifice tube, the operating range was 2 to 38 µm. The range for the 24 µm tube was from 2 to 10 µm. Each orifice provided a higher degree of resolution of the particle sizes for its respective operating range.

After the 380 µm orifice was used, the remainder of the sample was filtered through a 53 µm mesh size for analysis with the 95 µm orifice tube.

In preparation for the 24 µm orifice tube, the sample was finally filtered through a 12 µm mesh to remove particulates greater than the optimal size for the orifice tube.

Once preliminary analyses were conducted, the predominate particle sizes were assessed as to diameter and surface area. This information allowed for the selection of filters with pore sizes slightly smaller than each of the predominate particle size diameter ranges. As a result, filters with 30, 10, 5 and 1 µm pore sizes were chosen from the particle sizing data. The purpose of this was to selectively isolate each predominate group of particles, based on their diameter, for direct microscopic observation.

Particles were also measured microscopically to confirm the observations from the particle analyzer. This was accomplished by first filtering a sample of suspended particulates with a standard vacuum filtration apparatus which uses 47 mm diameter filters. Initially, a 30 µm nylon mesh filter was used. The filtrate was captured and re-filtered on a 10 µm pore size Nuclepore filter. This was repeated consecutively for 5 and 1 µm pore size filters. A very slight vacuum was used in order to preserve the integrity of the particulates.

The size of the particulates was determined by using an area-calibrated graticule in an ocular of a microscope and, through that, estimating the area covered by the particulate.

## D. Bacterial Water Quality Methods

The *E. coli* and fecal streptococci water quality parameters were measured according to the methods of H.A.M.E.S [19].

## E. Epi-fluorescence and Immunofluorescence Microscopic Methods

The epi-fluorescence and immunofluorescence microscopic methods of analyses for total viable bacteria and *Salmonella* associated with particulates (sorbed and free-floating) are as follows:

i. *Viability Determination*

An aliquot of sample containing suspended particulates was incubated with 1 mL of 0.4 percent 2-p-iodophenyl-3-p-nitrophenyl tetrazolium chloride; 1 mL of 0.25 percent yeast extract; and 1 mL of 0.001 percent lomefloxacin (Searle Pharmaceuticals) per 10 mL of sample at 20°C for 4 hours, in the dark. Following the 4 hour incubation period, the sample was fixed with 0.6 mL of 37% formaldehyde per 10 mL of sample. Each sample was prepared as above.

*ii. Staining Technique*

A double staining process, as described by Hoff[20] was used to detect both total viable bacteria and *Salmonella sp.* employing a combination of 4',6-diamidino-2-phenylindole (D.A.P.I.) and fluorescein isothiocyanate fluorescent antibody (F.I.T.C.-F.A.).

The treated samples were filtered onto 25 mm diameter Nuclepore polycarbonate black membranes with the appropriate pore size.

Once the sample was filtered, 2 mL of 0.001% solution of the fluorescent stain (D.A.P.I.) was added to the membrane filter in order to stain both the free-floating bacteria and those sorbed to the particulates. D.A.P.I. was left on the membrane for 10 minutes, in the dark, after which the D.A.P.I. was gently vacuumed from the membrane. The membrane was then rinsed three times with filter-sterilized distilled water.

Once the filter was washed free of D.A.P.I., 0.2 mL of F.I.T.C.-F.A. (DIFCO) was added to the filter and left to incubate at room temperature, in the dark, for 30 minutes. After the incubation period, 2 mL of F.A. buffer (DIFCO), pH 9, were added to the filter, which remained in the filter funnel. It was immediately vacuumed gently from the filter. To further de-stain the filter, 2 mL of F.A. buffer were added to the filter and were left for 3 min. before gently vacuuming from the filter. This rinsing procedure was repeated two more times. After de-staining, with F.A. buffer, the filter was further rinsed three times with filter-sterilized distilled water.

The filter was removed aseptically and placed on a drop of glycerol on an acid-washed microscope slide. A drop of glycerol containing phenylenediamine was placed on the slide followed by a coverslip. The coverslip was sealed with nail polish. The slide was kept in the dark until examined. The phenylenediamine, added to the glycerol, retards fading of the fluorescence from D.A.P.I. and F.I.T.C.-F.A [21].

*iii. Microscopy*

The D.A.P.I. and F.I.T.C.-F.A. stained cells were examined using a Nikon Optiphot-2 microscope equipped with an episcopic fluorescence attachment EF-D using an UV-F (fluor) glycerine 100x objective. Photomicrographs were taken with a Nikon Microflex UFX-2 camera using Kodak Ektapress Gold 400 ASA film.

## F. Bacterial Particulate Transport Experimental Procedure

The distance the particulate-bound bacteria travelled was determined by the following method.

A 30 kg sample of the sediment was removed from a 3 cm depth at the sediment-water interface of the Desjardine Drain. The sediment was course-filtered to remove any leaves or other debris and then stored at 4°C. Samples of sediment were analyzed for percent sand, silt, organic matter and clay, the pH and the cation exchange capacity to establish the characteristics relevant to sediment sorption. The sediment was transferred to a 50 L carboy, after which 10 L of $10^7$ *E. coli* nalidixic acid resistant (NAL) per mL were added. To assist in the acclimatization of the bacteria to the sediment, 50 mg of glucose per kg of sediment were also added. The mixture was allowed to incubate for one week at 4°C. Using the microscopic direct viable cell count technique, a sample of the sediment was checked to assess the degree of sorption of *E. coli* (NAL) to the sediment, after the one week incubation period.

The bacterial transport experiment commenced with the discharging of two 50 L carboys into the Desjardine Drain. Flow measurements made in the drain indicated the approximate travel time of the leading edge of the bacterial-particulate plume at the specific downstream sites, as shown in Figure 4.

To assist in detecting the leading edge of the plume, fluorescein dye was added to the drain at the time the bacterial-particulate mixture was discharged.

Sampling was conducted in a manner designed to detect the leading edge of the plume, the main section of the plume and the trailing edge. In addition, to duplicate the grab samples being taken, swab samples were located at strategic sites in the drain as indicated in Figure 4.

Samples were collected twice a day for two days and then once a day for one week. Sampling continued once a week for another ten weeks. During the final week of study, sediment samples of the Desjardine Drain were taken at the four swab sampling stations. The method of bacterial extraction from the sediments and the enumeration procedure were described by Palmateer *et al.*

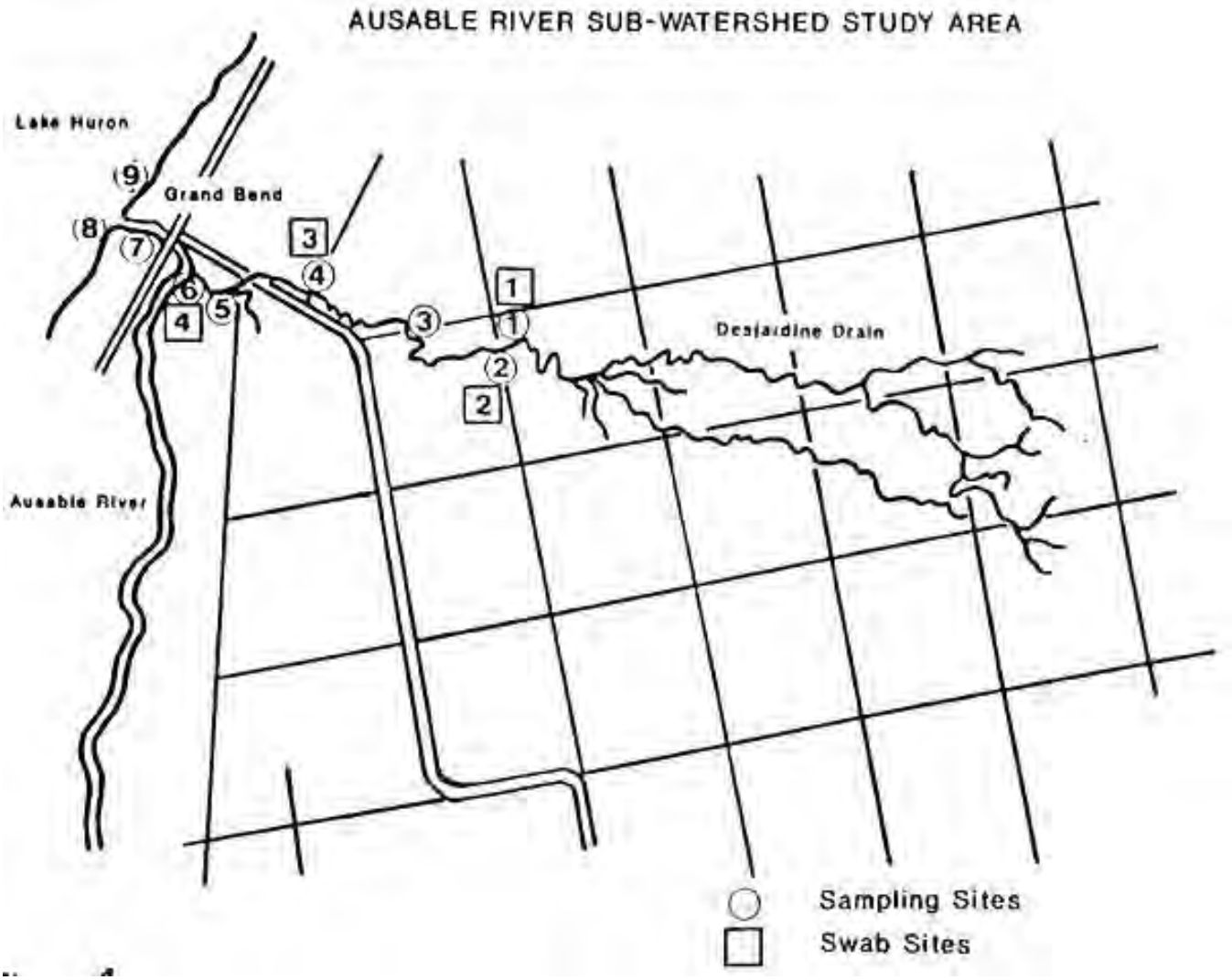


**Figure 4.** Map showing grab and swab sampling sites during the bacterial transport study.

# III. RESULTS

The bacterial water quality, with respect to fecal pollution, is shown in Table 1 for the three agricultural drains investigated during the summer, fall and spring seasons.

The geometric means indicated degraded water quality during the low-flow period of the summer. The fall also had poor bacterial water quality. *E. coli* and fecal streptococci fluctuated greatly from season to season; however, the spring geometric means were consistently lower than those of the previous fall period for all three drains.

These data typify the bacterial water quality at the beaches where each drain was known to impact. The variation in geometric means from season to season reflected the fluctuating bacterial water quality in the water column. To understand the reasons for these fluctuations, beyond the land-use activities that affect each drain, it was necessary to further investigate the suspended particulate load that occurred.

The bottom sediment, at the sediment-water interface, was sampled and characterized as shown in Table 2 for two seasons of the study. The Arthur Vanatter and the Central School Drains had similar results for most of the parameters. The Desjardine Drain had much less sand and considerably more silt, clay and organic matter in the bottom sediment than did the previous two drains. The Desjardine Drain also had a significantly higher cation exchange capacity. The potential for the sediments of the Desjardine Drain to attract bacteria, as well as fungi and viruses, was much greater than the sediments of the other two drains.

**Table 1.** Geometric mean levels of E. coli and fecal streptococci in the Arthur Vanatter, Central School and Desjardine drains during the three study seasons.

| | E.coli Geometric Mean / 100 ml | | | Fecal Streptococci Geometric Mean / 100 ml | | |
|---|---|---|---|---|---|---|
| Site | Summer | Autumn | Spring | Summer | Autumn | Spring |
| Arthur Vanatter Drain | 606.3 | 59.1 | 2.5 | 1,771.3 | 155.5 | 7.1 |
| Central School Drain | 2,546.2 | 122.8 | 22.5 | 1,326.2 | 1,795.9 | 60.2 |
| Desjardine Drain | 401.7 | 699.2 | 15.5 | 391.1 | 1,551.3 | 28.0 |

**Table 2.** Characteristics of sediments relevant to adsorption processes at the three study locations during the summer and spring.

| | Drain Locations | | | | | |
|---|---|---|---|---|---|---|
| | Arthur Vanatter | | Central School | | Desjardine | |
| Test | Summer | Spring | Summer | Spring | Summer | Spring |
| % Sand | 85 | 77 | 88 | 28 | 32 | 69 |
| % Silt | 8 | 15 | 4 | 54 | 40 | 16 |
| % Clay | 7 | 8 | 8 | 18 | 28 | 15 |
| % Organic Matter | 1.8 | 1.9 | 2.5 | 4.8 | 3.7 | 1.5 |
| Cation Exchange Capacity | 15 | 19 | 18 | 27 | 28 | 23 |
| pH | 8.1 | 7.8 | 7.7 | 7.6 | 7.9 | 7.9 |

The charge on the surface of the organic matter and the clay, as indicated by the cation exchange capacity, has shown to be partially responsible for sediment and soil sorption [12].

A typical analysis of the suspended particulates, using the Elzone 180 XY Particle Analyzer, is shown in Figure 5. The diameters of the particulates counted and sized, for each of the three orifice tubes that were utilized, are displayed. The predominance of particulates with specific diameters can be observed. This allowed for picking the filters with the pores sized slightly smaller than the diameter of each of the specific particulates, so that they could be removed from the suspended particulate population and be examined microscopically.

To visualize the relationship of the bacteria to the particulates, the following photomicrographs (Plates 3 and 4) are included.

The free-floating bacteria and the bacteria sorbed to the particulates were observed microscopically. The particulates absorb the fluorochrome to the extent that they can easily be observed in relation to the bacteria. This characteristic facilitates in counting the bacteria and in determining the surface area of the particulates.

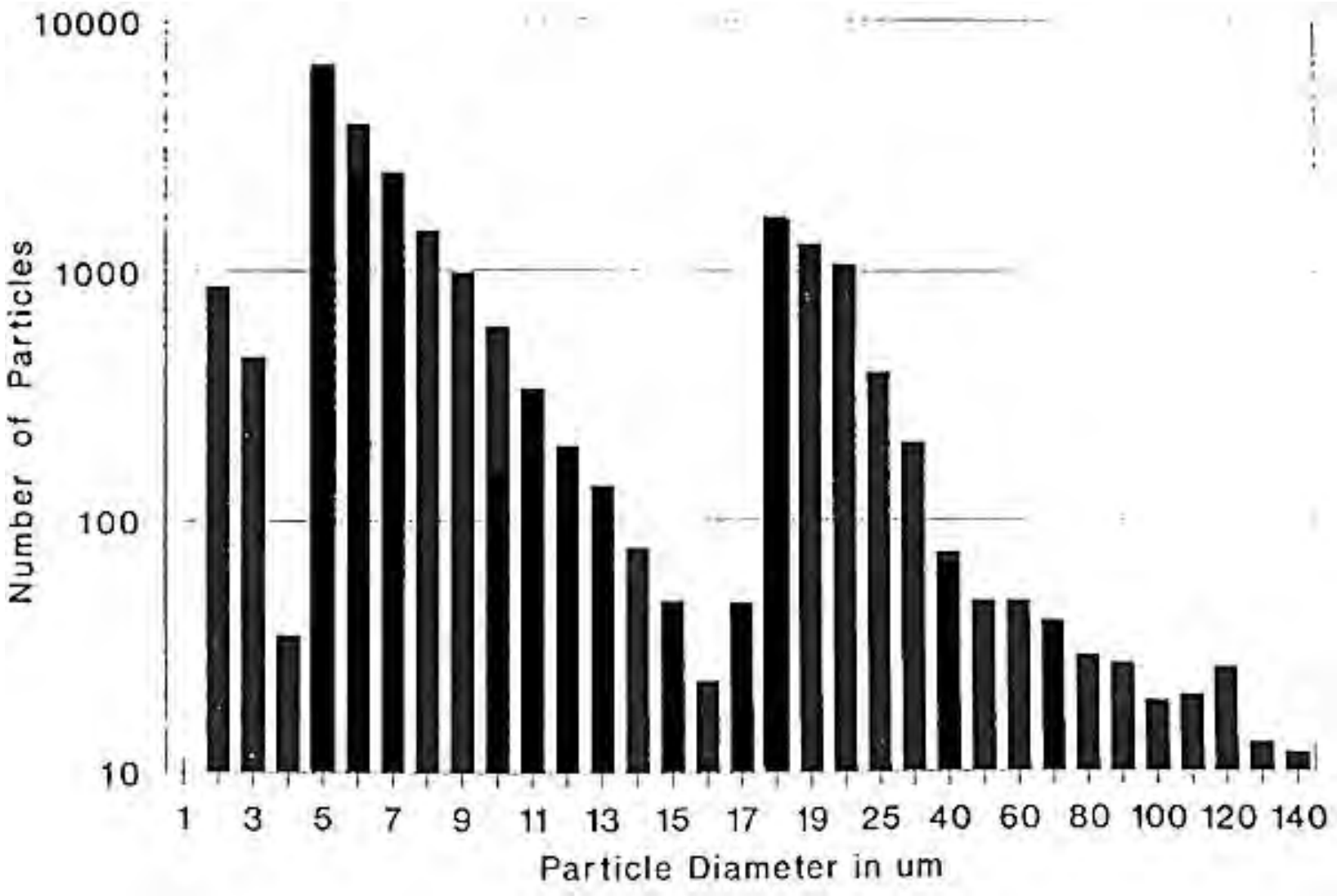


**Figure 5.** Size distribution of particulates in a typical sample of suspended sediments based on diameter from the Elzone 180XY.

**Plate 3.** Photomicrograph of bacteria sorbed to particulates and free-floating at 2200 X magnification.

**Plate 4.** Photomicrograph of bacteria sorbed to particulates and free-floating at 2200 X magnification.

## A. Arthur Vanatter Drain

The levels of bacterial attachment to suspended particulates in the Arthur Vanatter Drain are displayed in Figures 6 through 9. The bacterial sorption in the summer was relatively constant in the 30 to 70 µm diameter range. The sorption decreased by approximately one logarithm in the 10 to 30 µm diameter range and again increased to $10^4$ in the 5 to 10 µm diameter range. It is notable that the level of colonization decreased to $10^3$ with the 1 to 5 µm diameter range.

The autumn data, when compared to the summer data, showed bacterial decreases again for most of the size ranges.

During the spring, the level of bacterial colonization remained the same as the autumn level. No significant differences exist between the upstream and the downstream sites.

The free-floating bacteria associated with the 30 to 70 µm diameter particulates were only detected in the summer and autumn, as shown in Figures 10 through 13. The levels were considerably lower than the levels of bacteria sorbed to the particulates for this size range. In comparison, the 10 to 30 µm diameter size did have free-floating bacteria, which was also the case for the 5 to 10 µm and 1 to 5 µm diameter size ranges. However, the concentration of bacteria did decrease in the spring as compared to the summer and autumn. All particulate size ranges indicated that there was an increase in the bacterial levels in the fall, which is unique to the free-floating bacteria associated with the various size ranges.

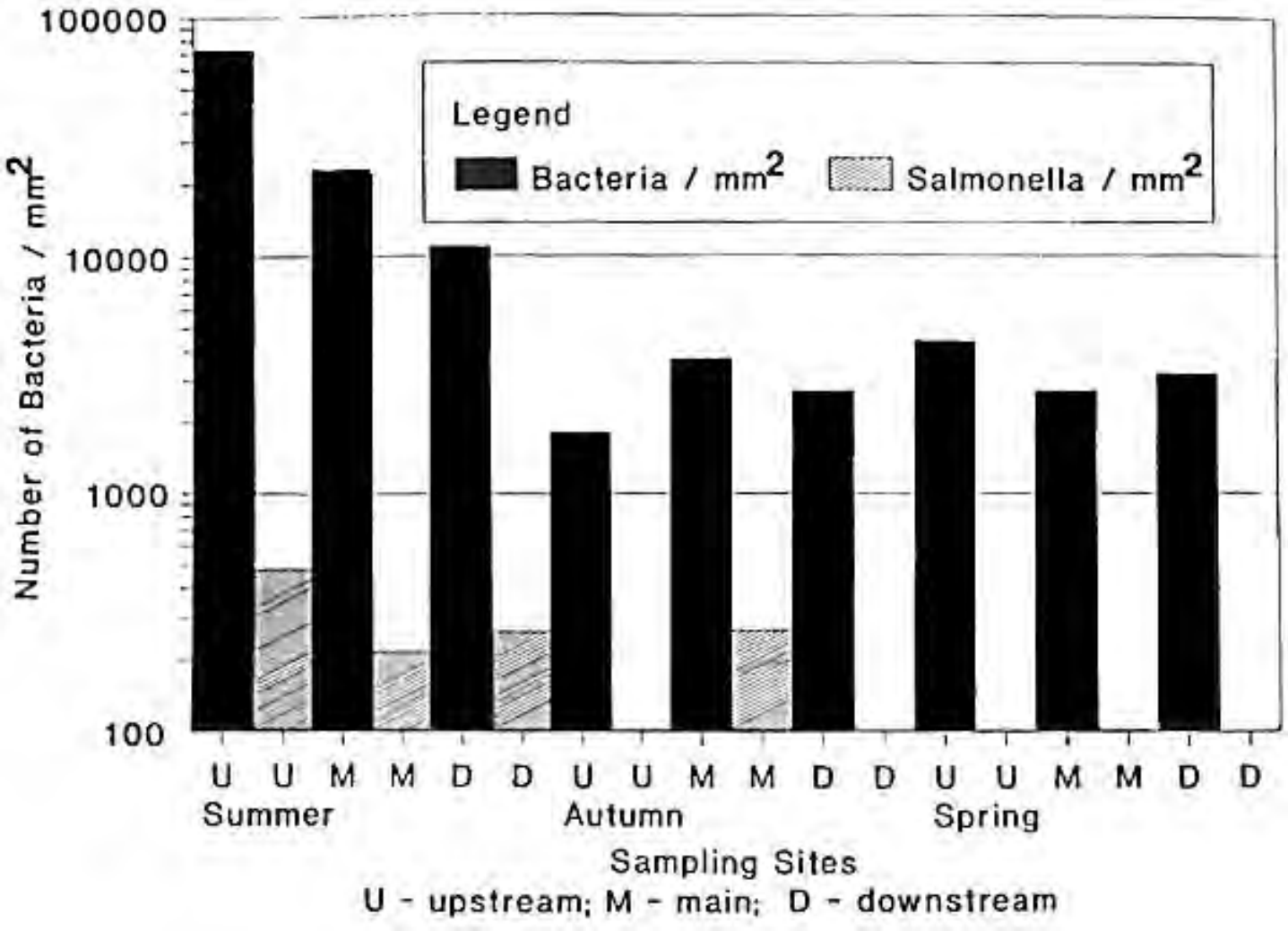


**Figure 6.** Levels of total viable bacteria and *Salmonella* / $mm^2$ surface area, adsorbed on particles 30- 70 µm diameter in the Arthur Vanatter Drain.

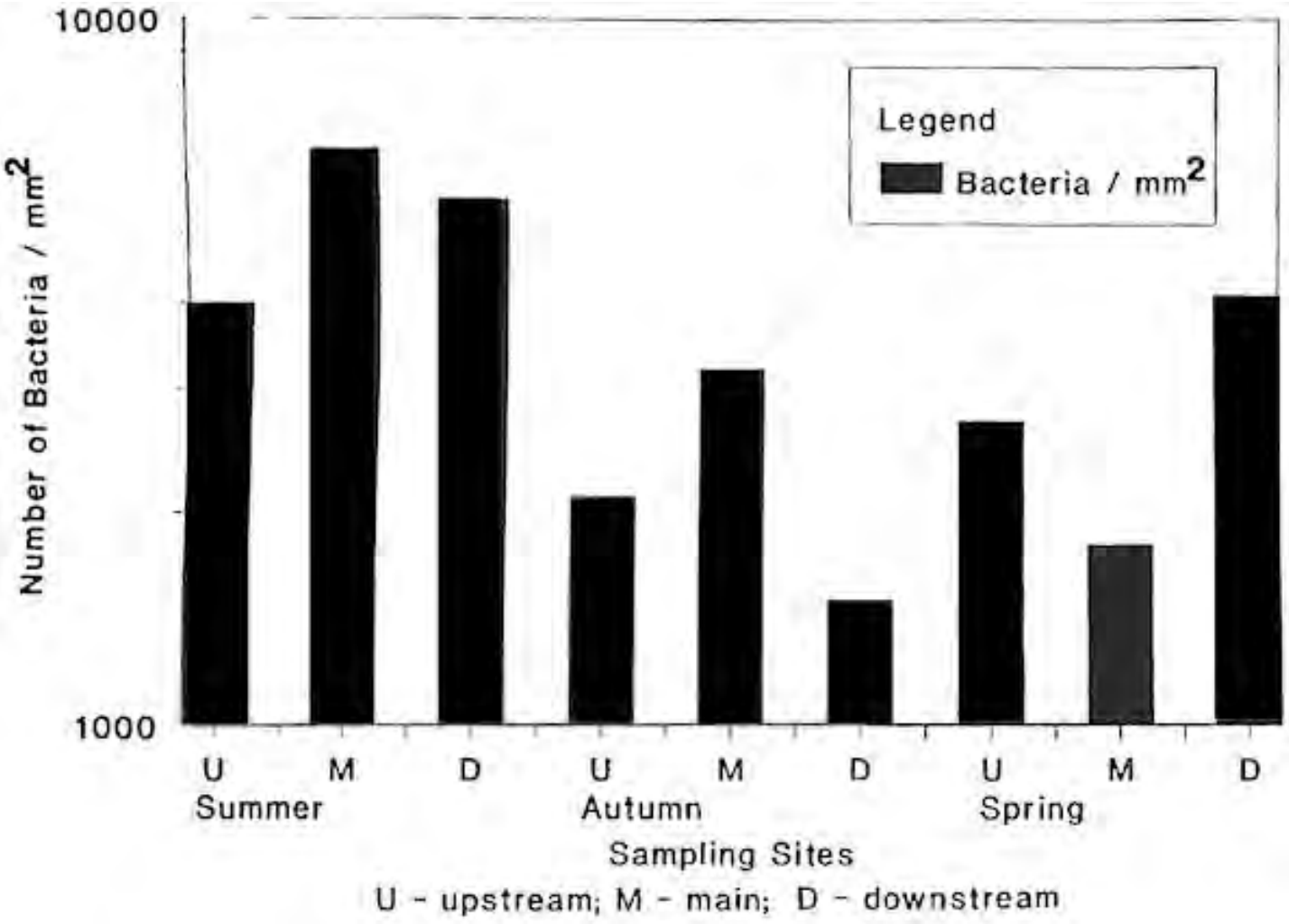


**Figure 7.** Levels of total viable bacteria /$mm^2$surface area, adsorbed on particles 10-30 µm diameter in the Arthur Vanatter Drain.

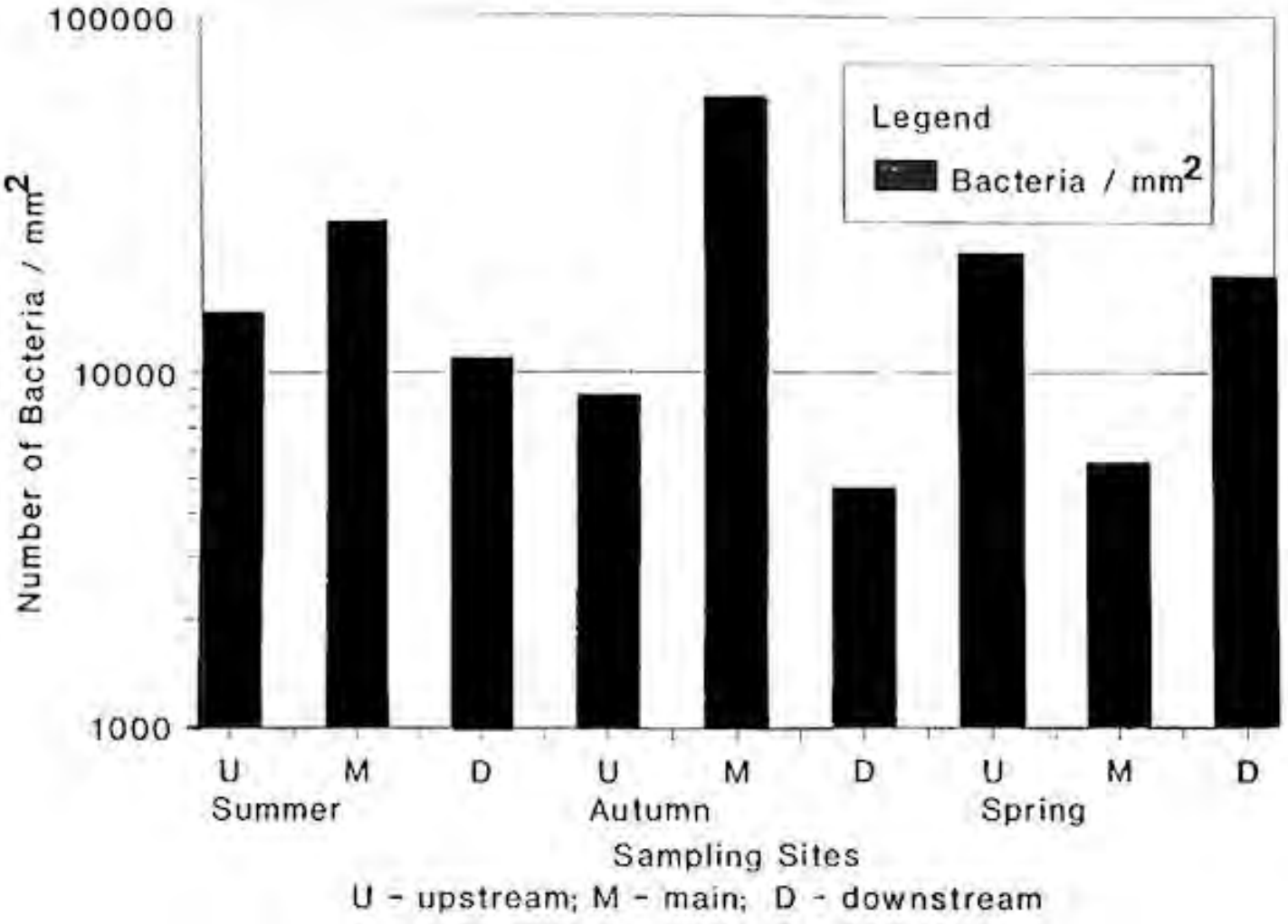


**Figure 8.** Levels of total viable bacteria /$mm^2$ surface area, adsorbed on particles 5-10 µm diameter in the Arthur Vanatter Drain.

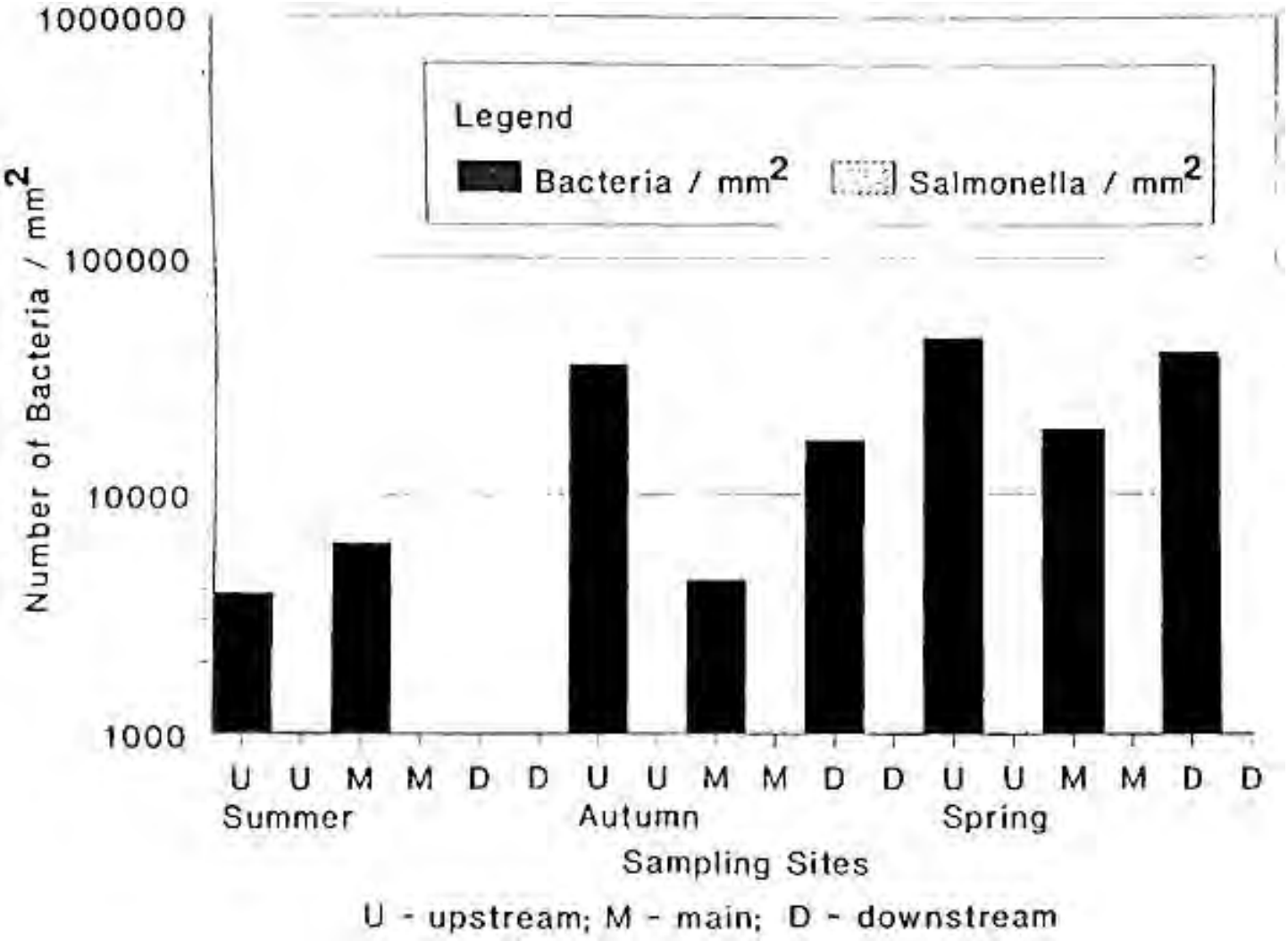


**Figure 9.** Level of total viable bacteria and *Salmonella*/mm$^2$ surface area, adsorbed on particles 1- 5 µm diameter in the Arthur Vanatter Drain.

*Salmonella* bacteria recovery at the Arthur Vanatter drain was observed in the summer period sorbed to particles in the 30 to 70 µm diameter range; otherwise, *Salmonella* were undetectable on any of the other particulates examined. *Salmonella* were again recovered during the autumn at $10^2$ per $mm^2$ of particulate surface area. No *Salmonella* were observed in the spring.

Free-floating *Salmonella* were detected on only three occasions. The levels ranged from $10^2$ to $10^3$ cells per mL, as shown in Figures 10 through 13.

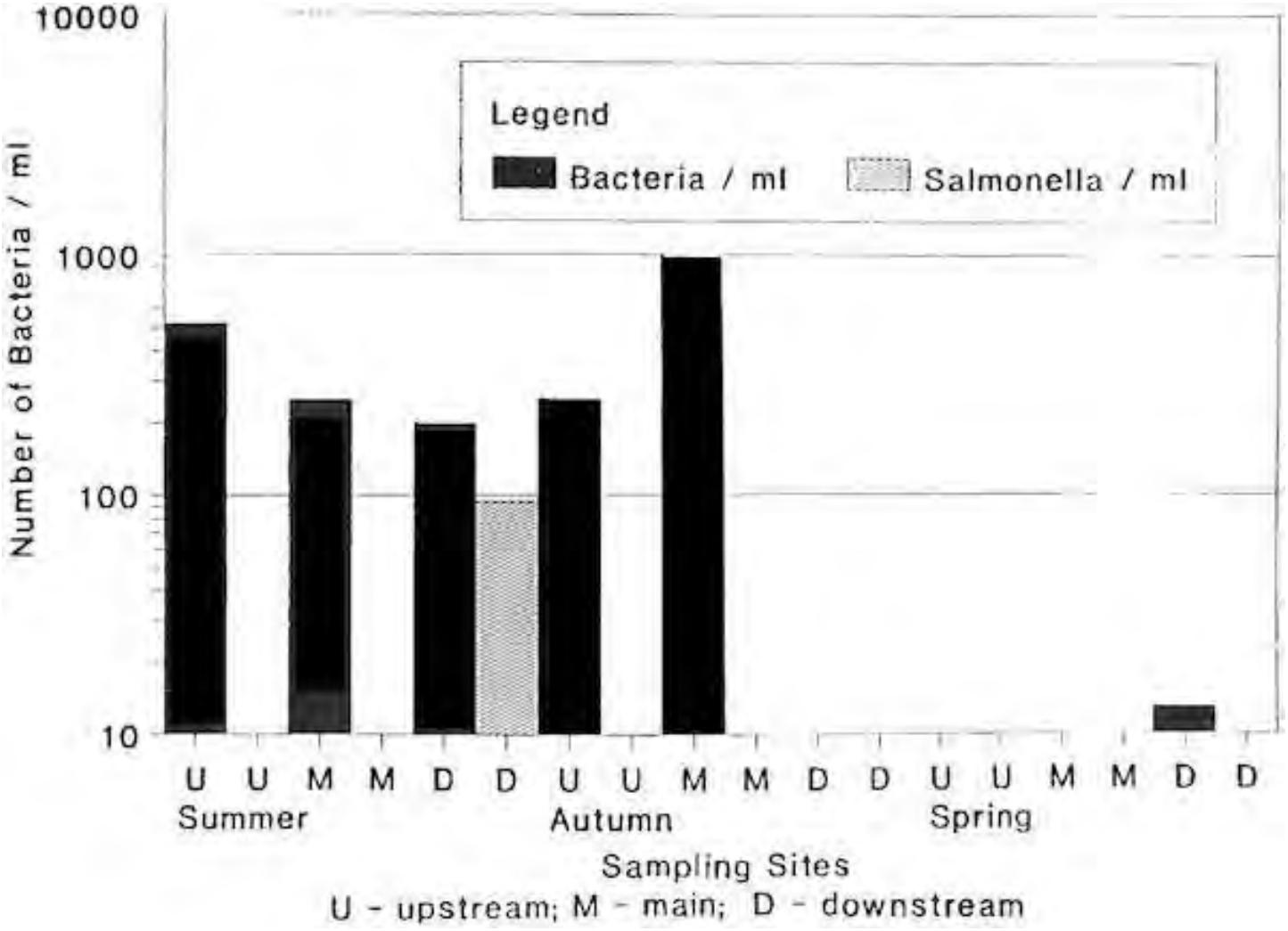


**Figure 10.** Levels of total viable bacteria and *Salmonella* which are free floating and associated with particles 30-70 µm diameter in the Arthur Vanatter Drain.

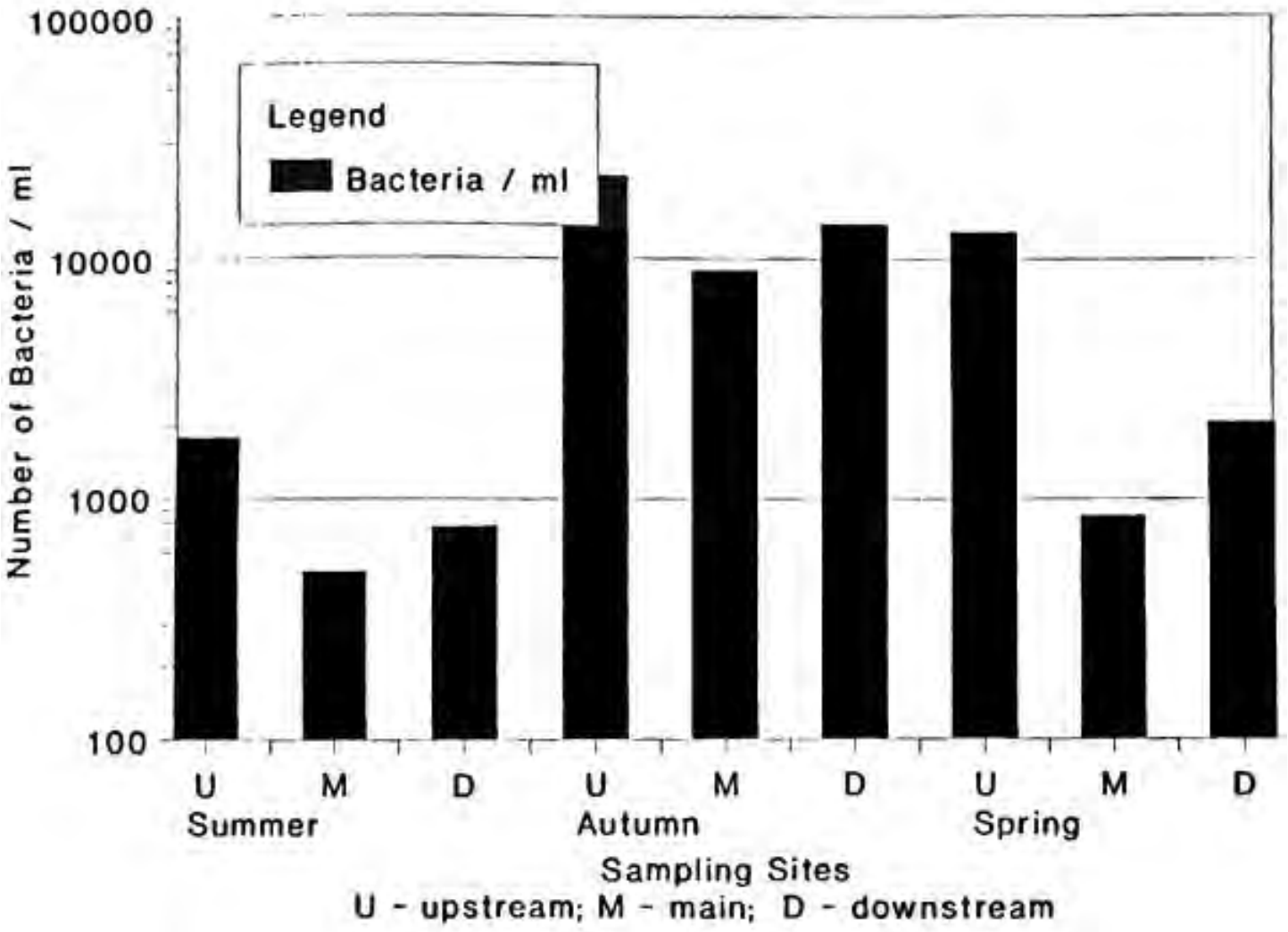


**Figure 11.** Levels of total viable bacteria which are free-floating and associated with particles 10-30 µm diameter in the Arthur Vanatter Drain.

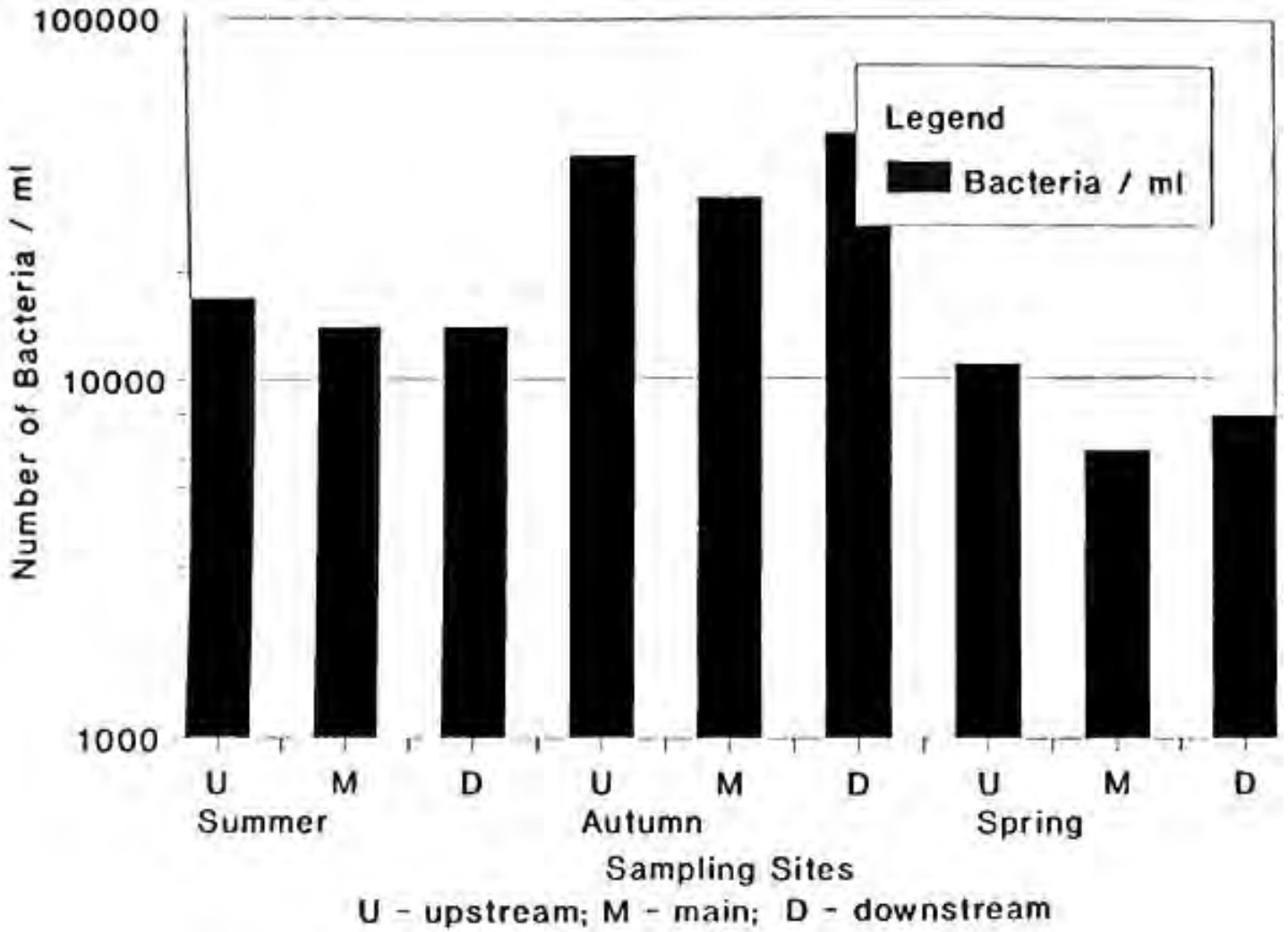


**Figure 12.** Levels of total viable bacteria which are free-floating and associated with particles 5-10 µm diameter in the Arthur Vanatter Drain.

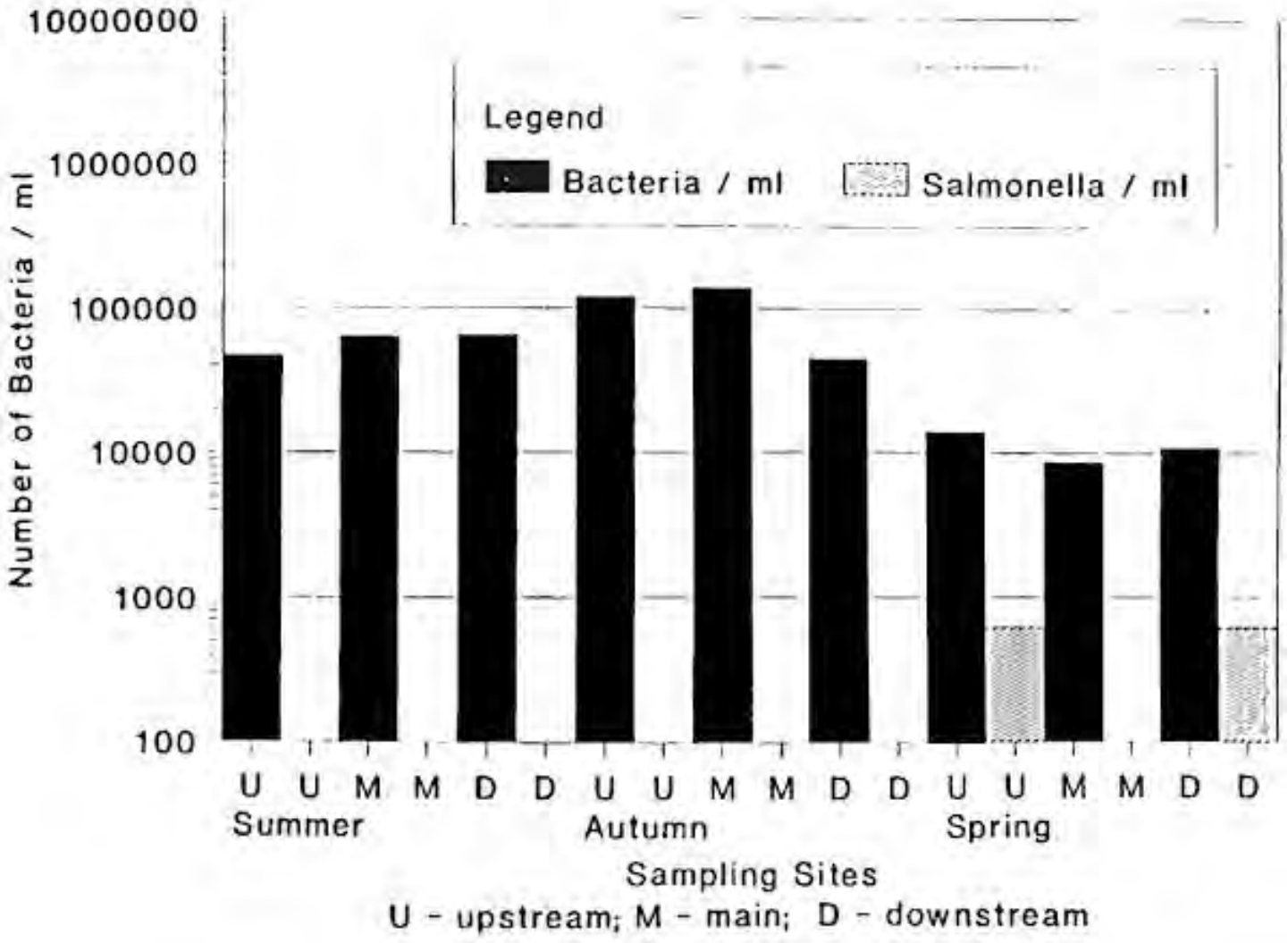


**Figure 13.** Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 1-5 µm diameter in the Arthur Vanatter Drain.

## B. Central School Drain

The levels of bacteria during the summer were found to vary between $10^3$ and $10^4$ bacteria per $mm^2$ of particulate surface area for the 30 to 70 gm diameter range (Figures 14 through 17). A decrease to $10^3$ bacteria per $mm^2$ was observed for the 10 to 30 µm diameter range. Both the 5 to 10 µm and the 1 to 5 µm diameter ranges increased to $10^4$ bacteria per $mm^2$. During the autumn, the results show a decrease from $10^4$ to $10^3$ bacteria per $mm^2$ for the 30 to 70 µm diameter range. The other size ranges examined remained the same as they were during the summer months. The spring sampling results revealed bacterial sorption to suspended particulates to be similar to the summer and autumn sampling periods.

In Figures 18 through 21, fluctuating levels of free-floating bacteria can be detected. Figure 20 shows that no bacteria were recovered, during the spring, which were associated with the larger particulates (30 to 70 µm diameter). This was typical of the results.

The levels of bacteria detected free-floating with the 10 to 30 µm, 5 to 10 µm and 1 to 5 µm diameter size ranges show levels from $10^3$ to $10^5$ per mL. Significant levels were detected during the spring as well as during the summer and autumn.

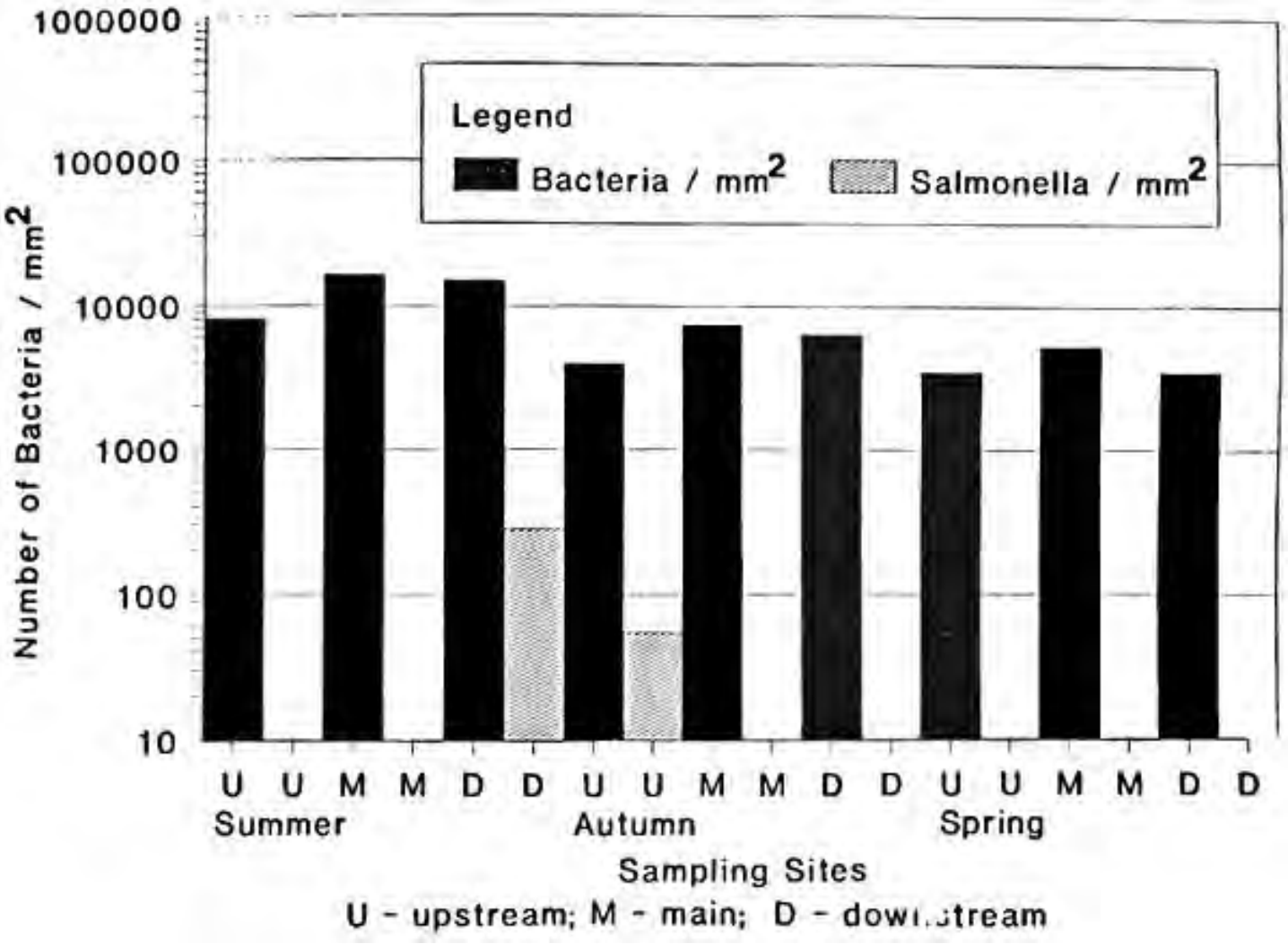


**Figure 14.** Levels of total viable bacteria and *Salmonella*/ $mm^2$ surface area, adsorbed on particles 30- 70 µm diameter in the Central School Drain.

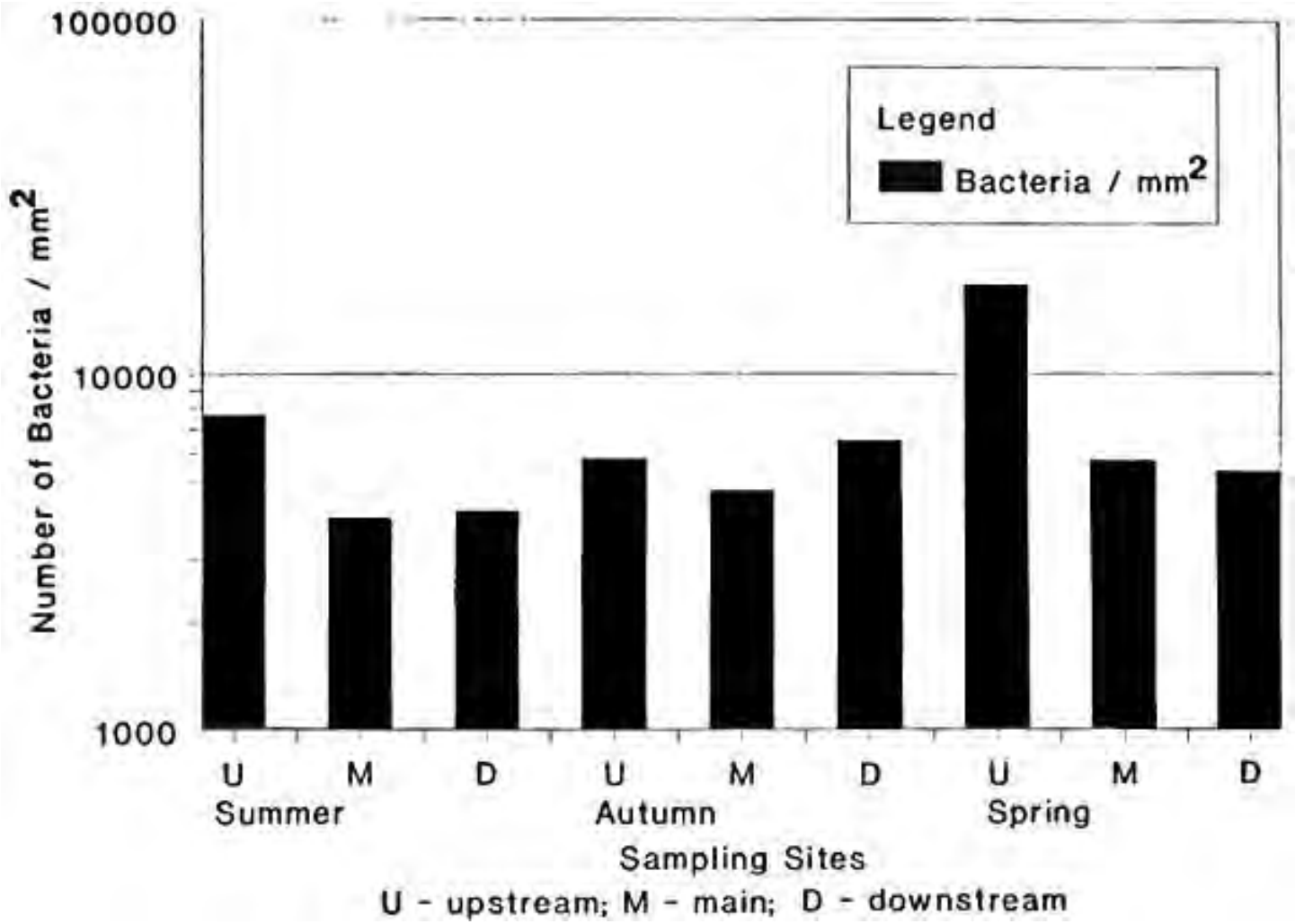


**Figure 15.** Levels of total viable bacteria / $mm^2$ surface area, adsorbed on particles 10-30 µm diameter in the Central School Drain.

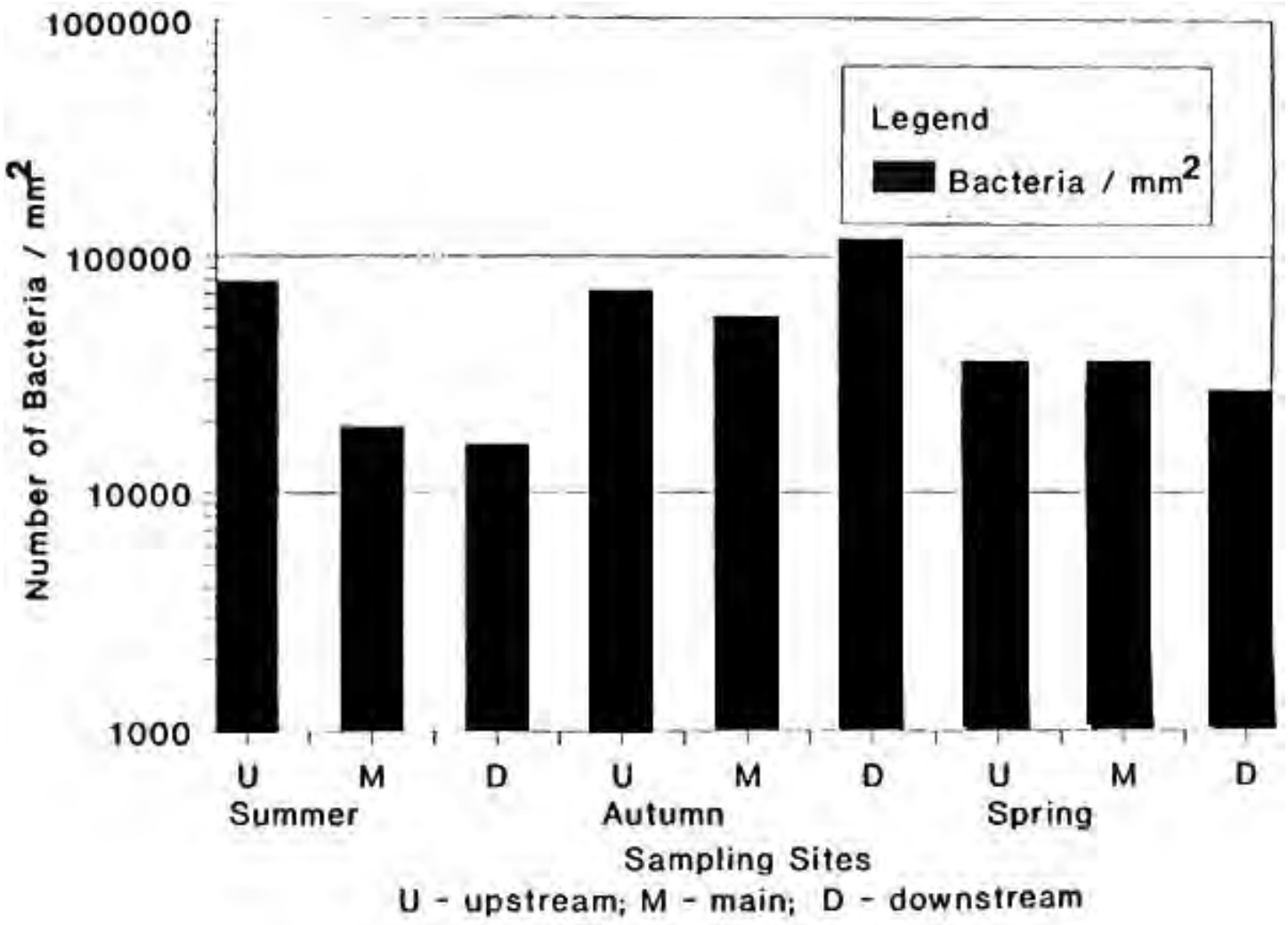


**Figure 16.** Levels of total viable bacteria /$mm^2$surface area, adsorbed on particles 5-10 µm diameter in the Central School Drain.

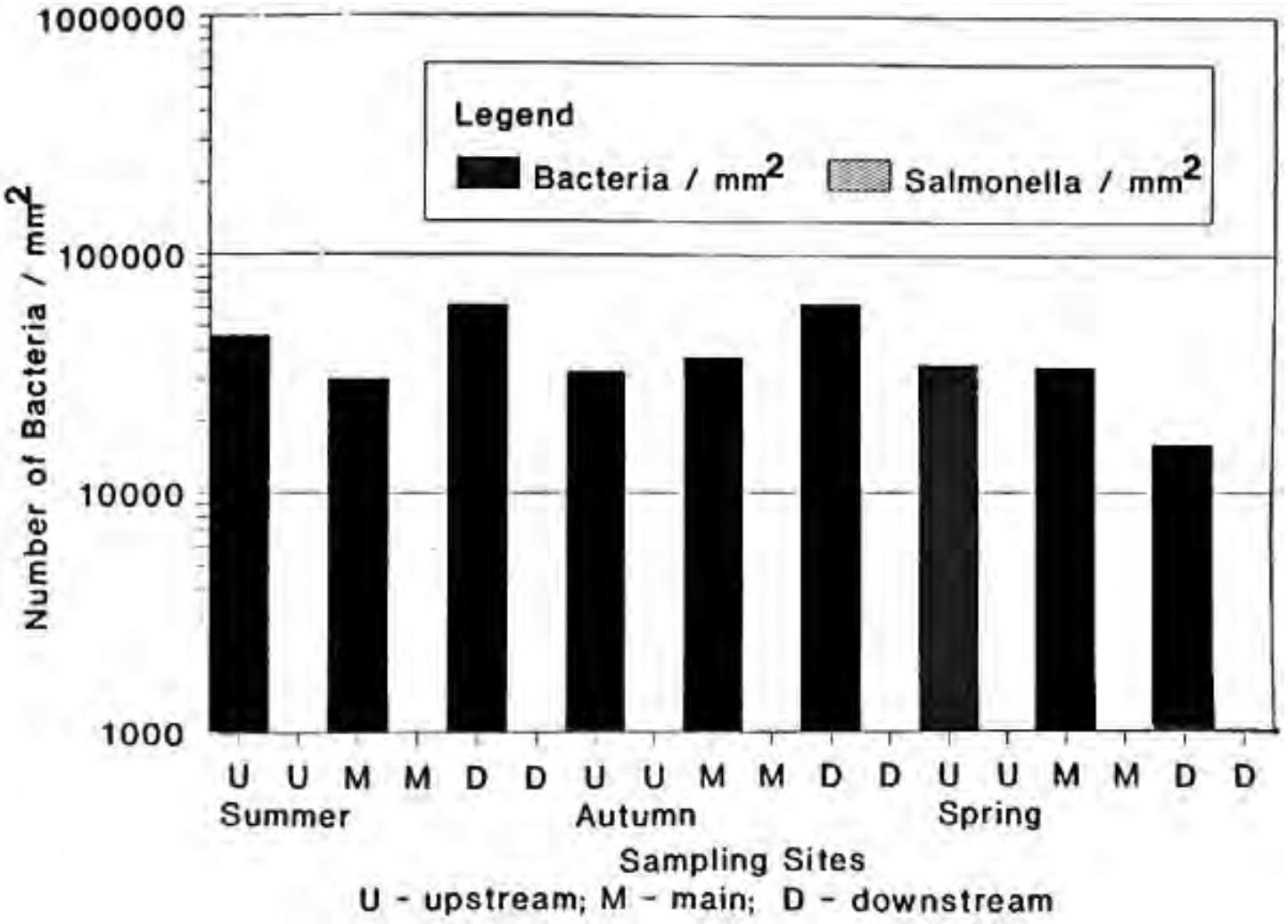


**Figure 17.** Levels of total viable bacteria and *Salmonella* /mm$^2$ surface area, adsorbed on particles 1- 5 µm diameter in the Central School Drain.

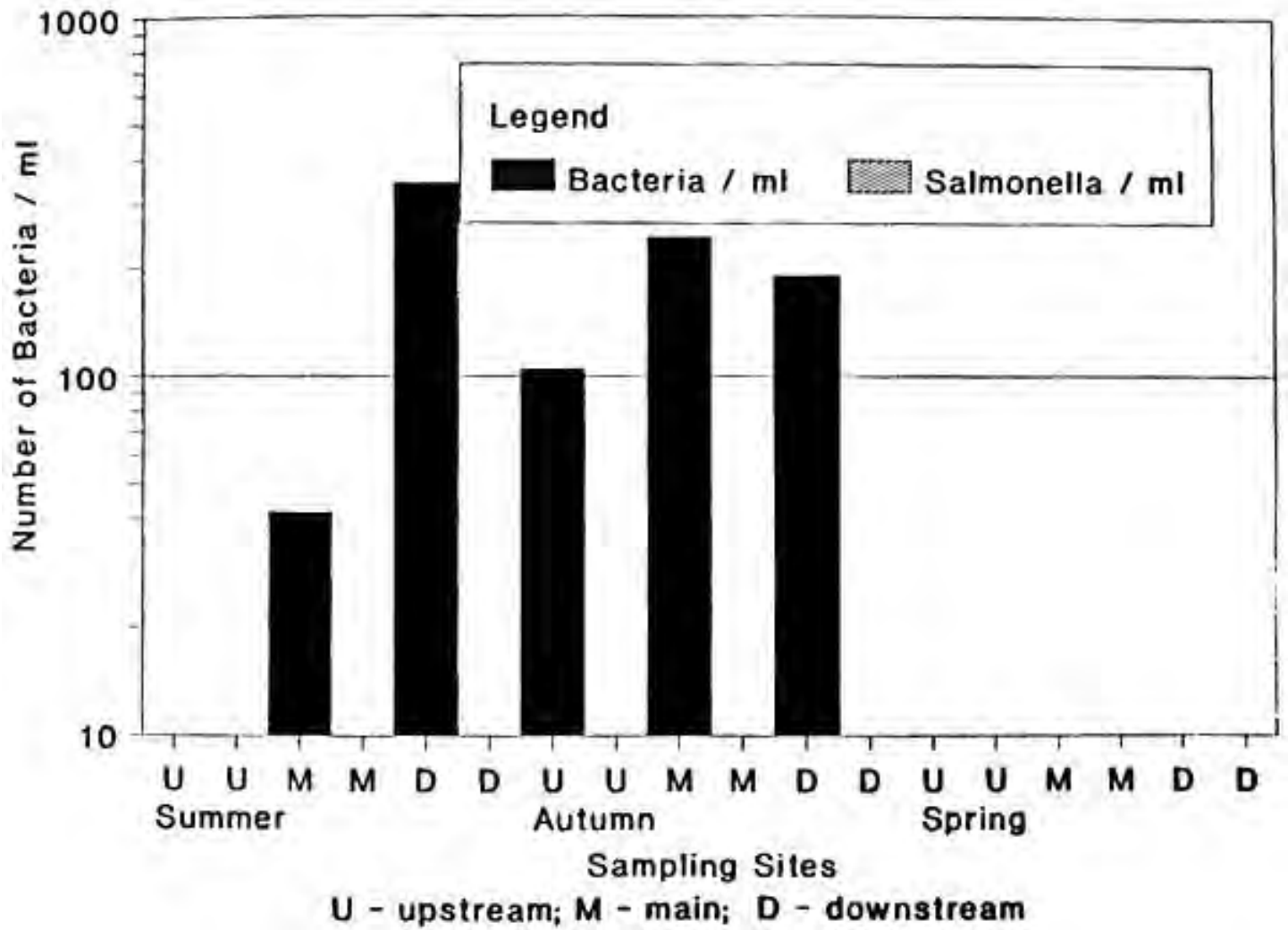


**Figure 18.** Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 30-70 µm diameter in the Central School Drain.

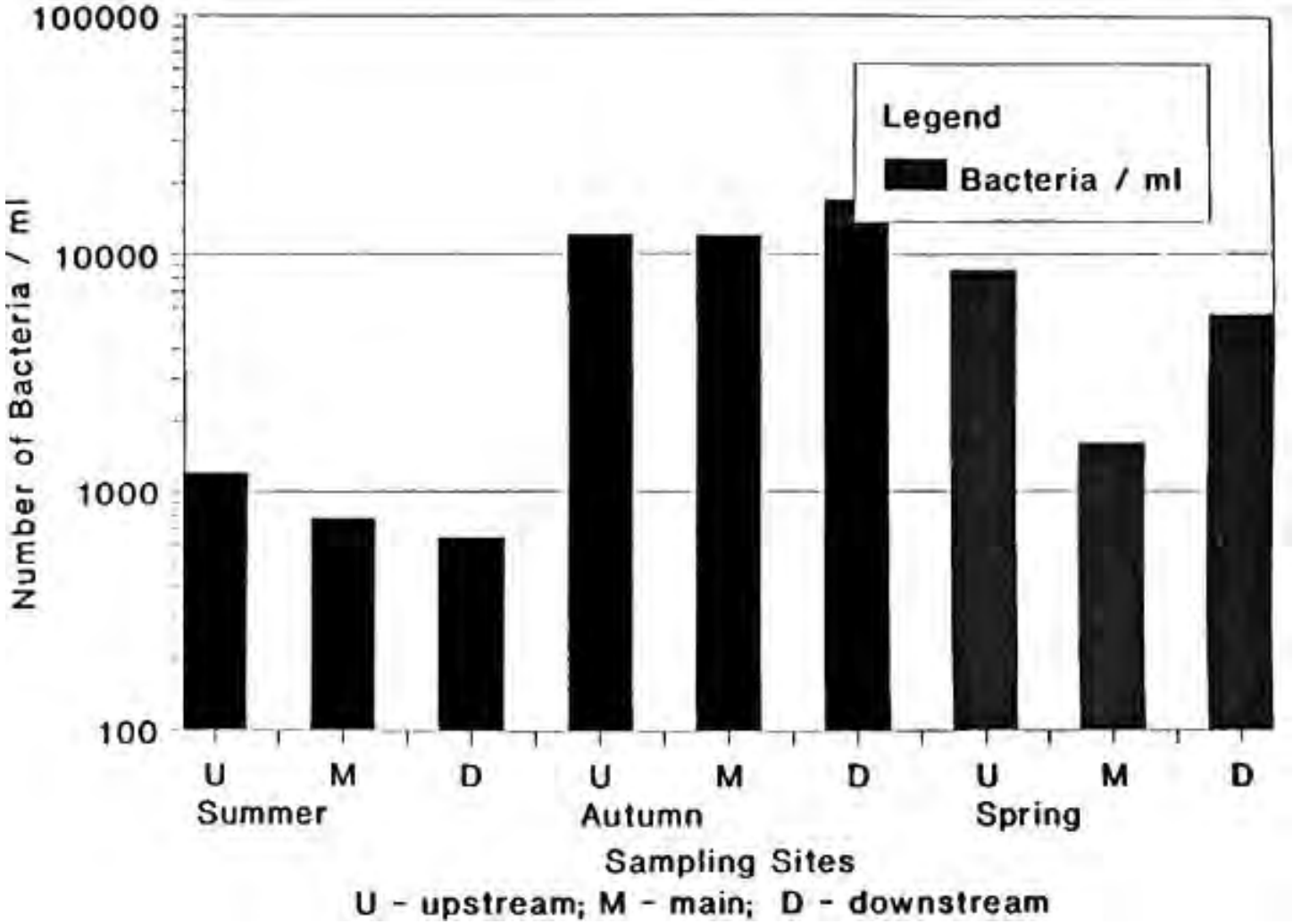


**Figure 19.** Levels of total viable bacteria which are free-floating and associated with particles 10-30 µm diameter in the Central School Drain.

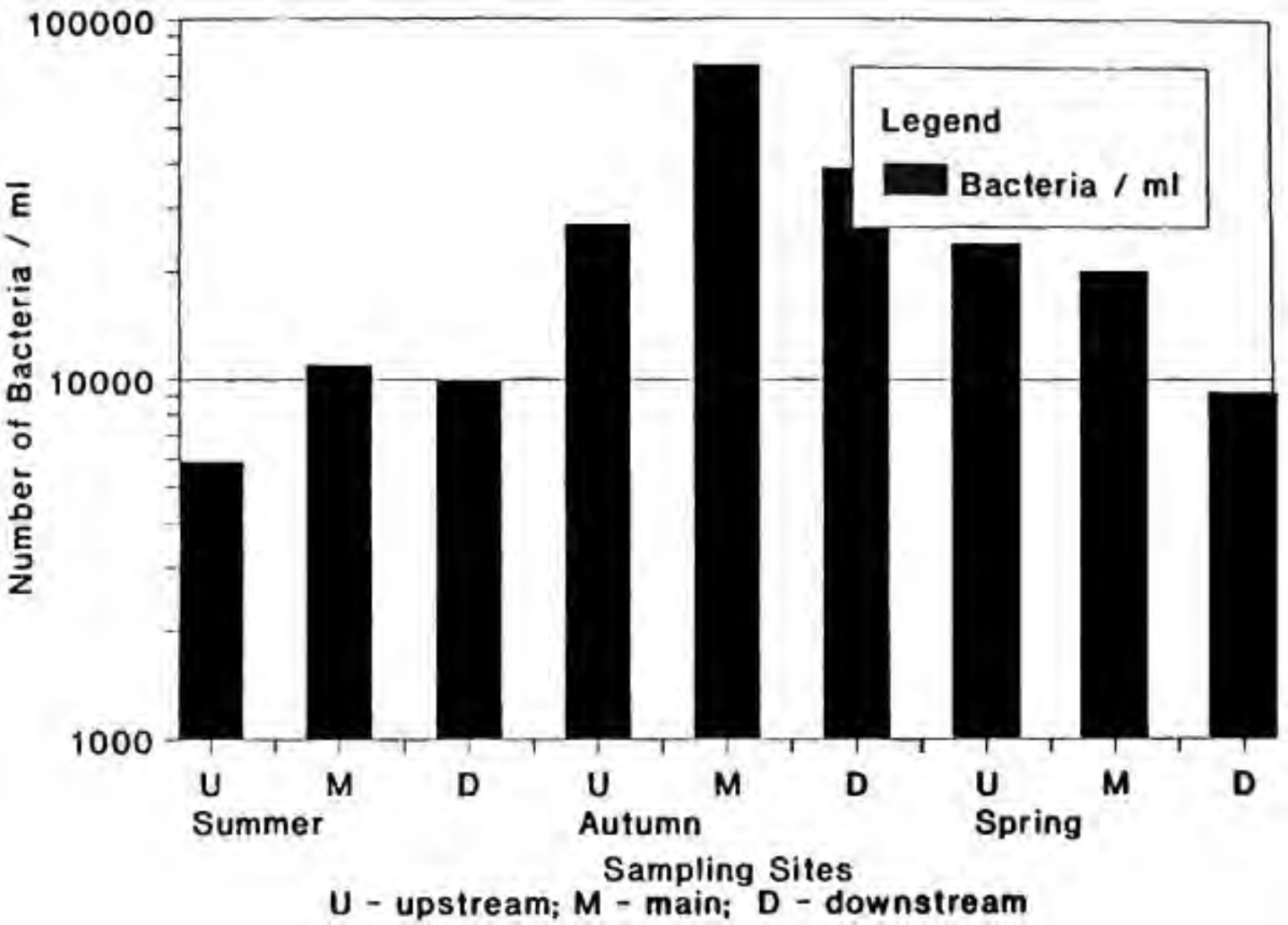


**Figure 20.** Levels of total viable bacteria which are free-floating and associated with particles 5-10 µm diameter in the Central School Drain.

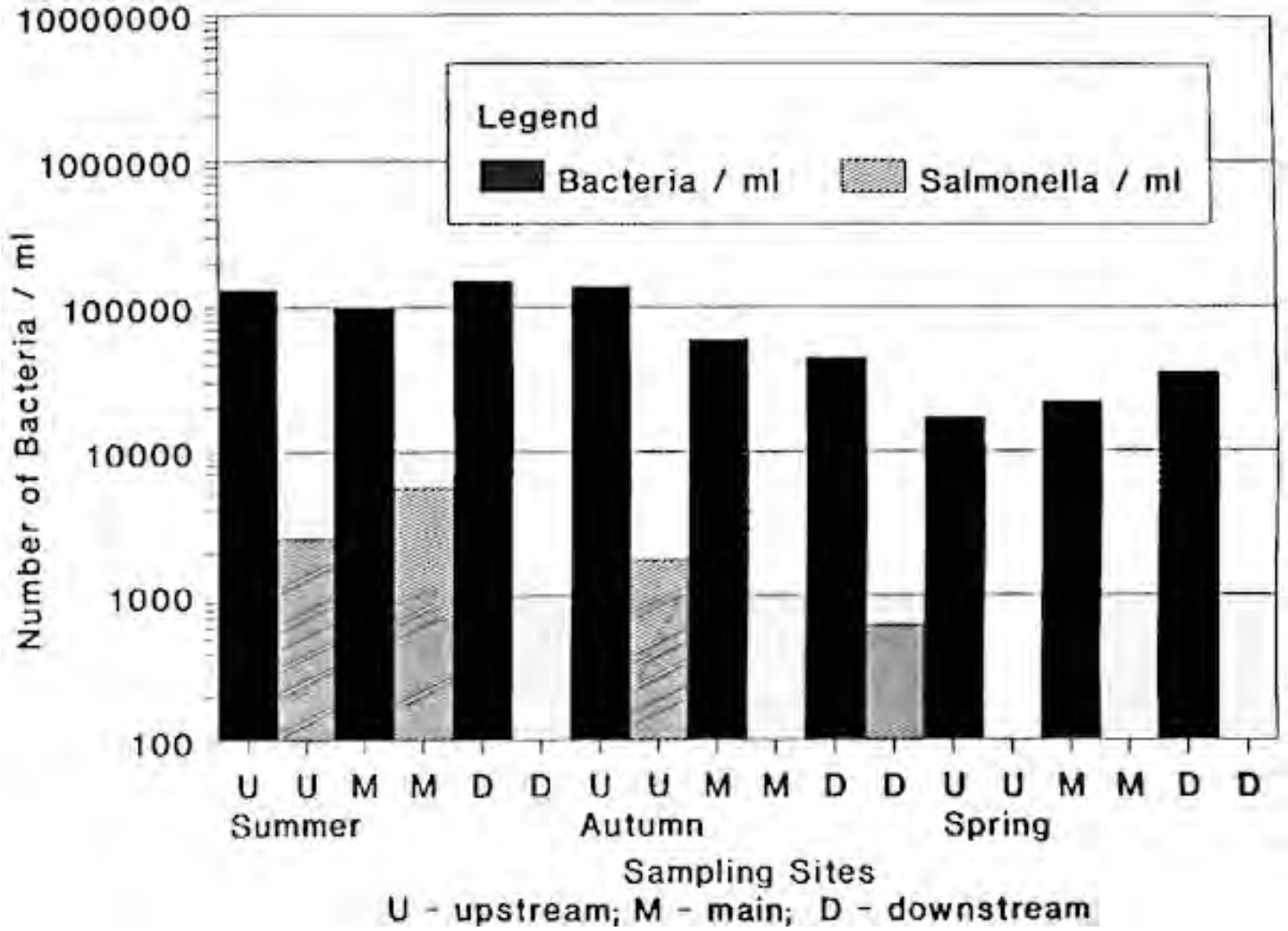


**Figure 21.** Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 1-5 µm diameter in the Central School Drain.

*Salmonella* were recovered from samples taken during all three seasons. They were recovered free-floating and were associated with the 1 to 5 µm diameter particulates.

*Salmonella* bacterial contamination of suspended particulates was observed to be minimal as they were recovered only twice during the three periods of the study.

## C. Desjardine Drain

Figures 22 through 25 display the results of total viable bacterial concentrations on the particulates for the Desjardine Drain in the format of mean numbers of sorbed bacteria per $mm^2$ of particulate. The free-floating bacterial concentrations are also exhibited, based on the number of bacteria per mL for each size range.

The results of the *Salmonella* determinations per $mm^2$ of particulate surface at the three sampling sites for each season during the Desjardine Drain study are also shown in Figures 22 through 25.

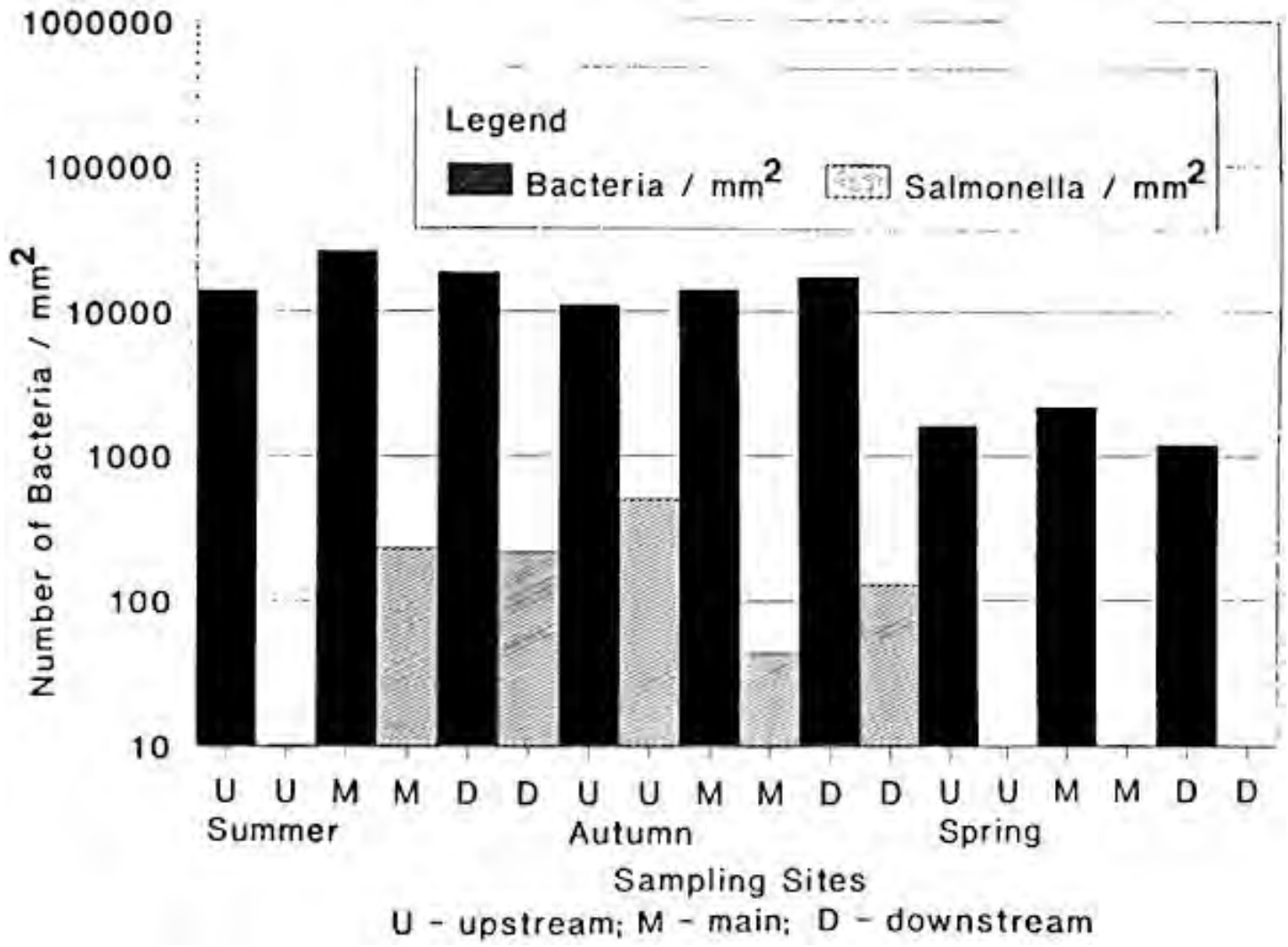


**Figure 22.** Level of total viable bacteria and *Salmonella* / $mm^2$ surface area, adsorbed on particles 30- 70 µm diameter in the Desjardine Drain.

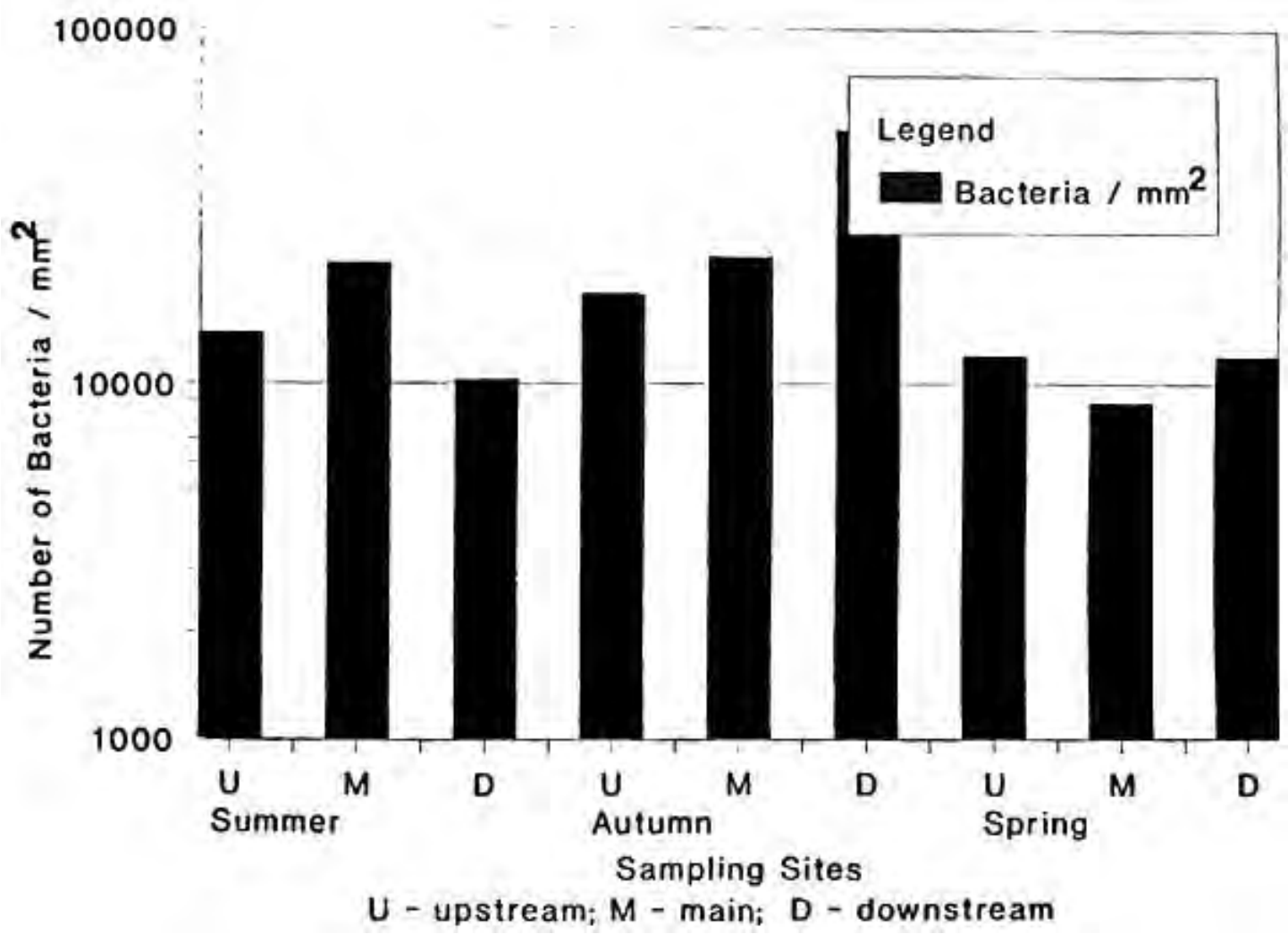


**Figure 23.** Levels of total viable bacteria / $mm^2$ surface area, adsorbed on particles 10-30 µm diameter in the Desjardine Drain.

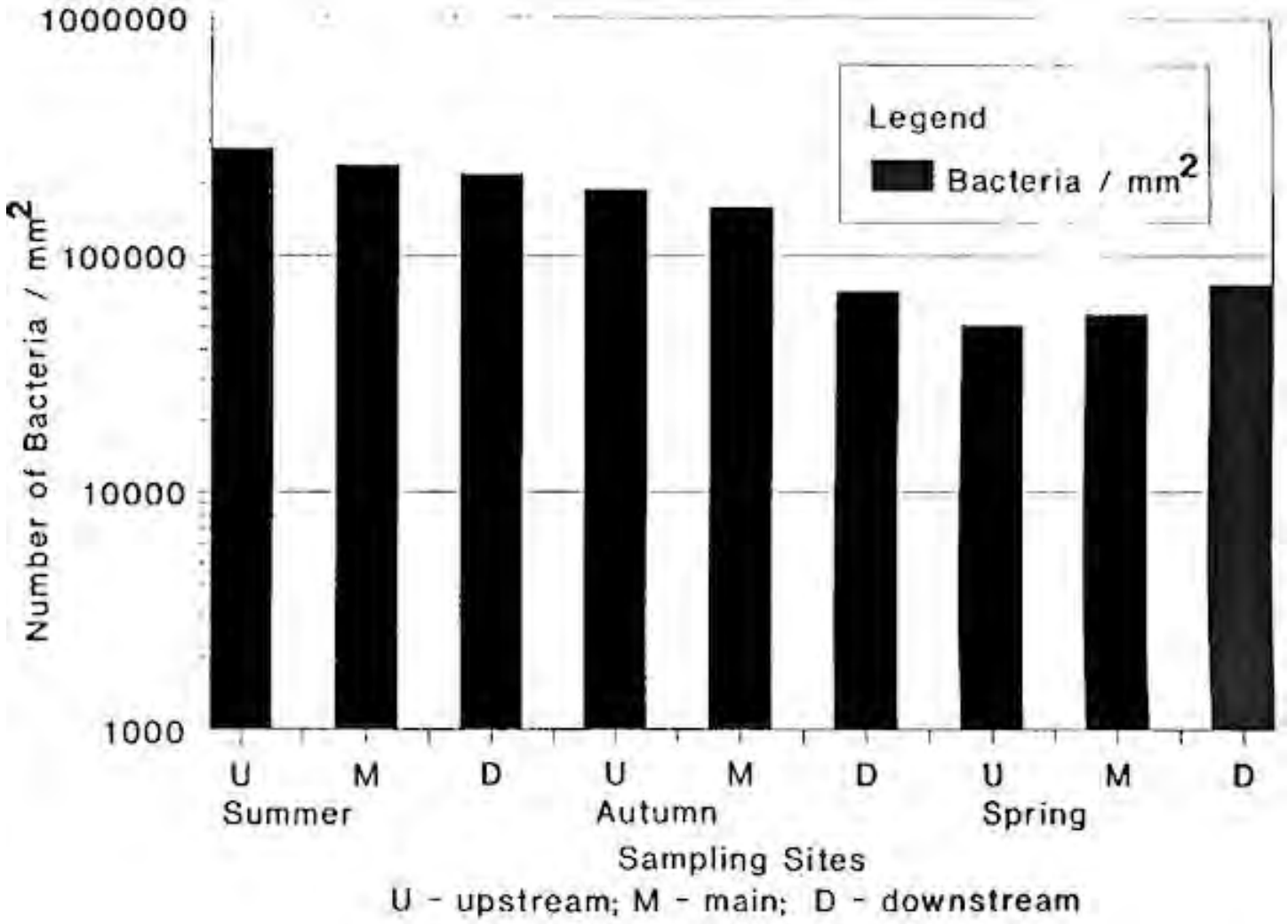


**Figure 24.** Levels of total viable bacteria / $mm^2$ surface area, adsorbed on particles 5-10 µm diameter in the Desjardine Drain.

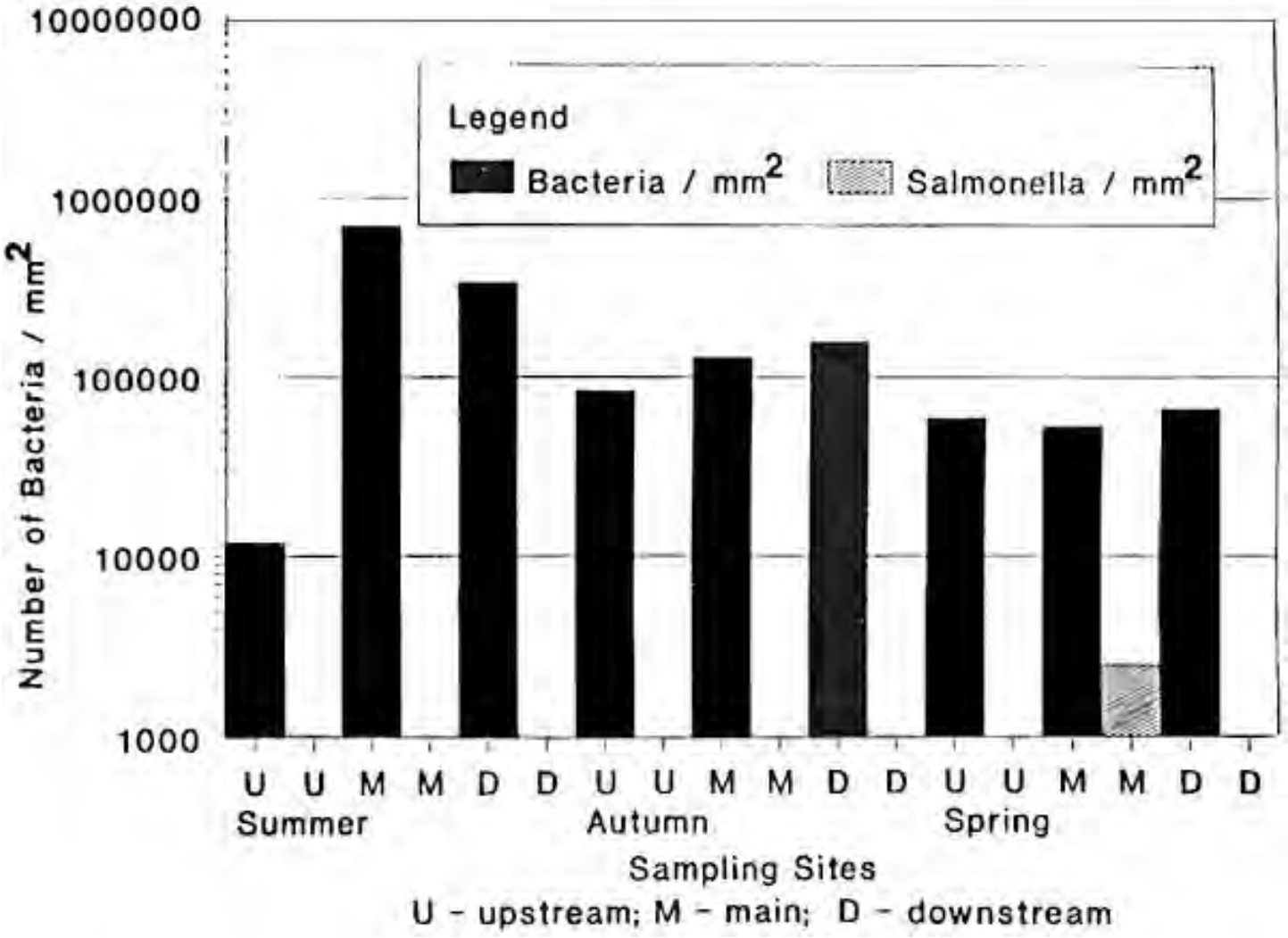


**Figure 25.** Level of total viable bacteria and *Salmonella* / $mm^2$ surface area, adsorbed on particles 1- 5 µm diameter in the Desjardine Drain.

It is evident that the large particulates in both the 30 to 70 µm and the 10 to 30 µm diameter ranges were colonized by the total viable bacteria to approximately $10^4$ cells per $mm^2$ of surface area during the summer and autumn months, with little variation. The exception occurred during the spring when the levels declined to approximately $10^3$ cells per $mm^2$.

The *Salmonella* concentrations, as expected, were considerably lower than those of the total viable bacteria, but were still easily detectable at all of the study sites on the Desjardine Drain on particulates 30 to 70 µm in diameter.

In contrast, the levels of total viable bacteria show an increase of 1 logarithm to $10^5$ cells per $mm^2$ of particulate surface area. Again, a decrease in the levels of bacteria was observed in the spring as compared to the summer and autumn levels.

*Salmonella* were only observed on particulates in the 1 to 5 µm diameter range, as shown in Figure 25.

Figures 26 through 29 show that the free-floating bacterial concentrations per mL vary significantly, from $1.1 \times 10^3$ to $1.8 \times 10^5$.

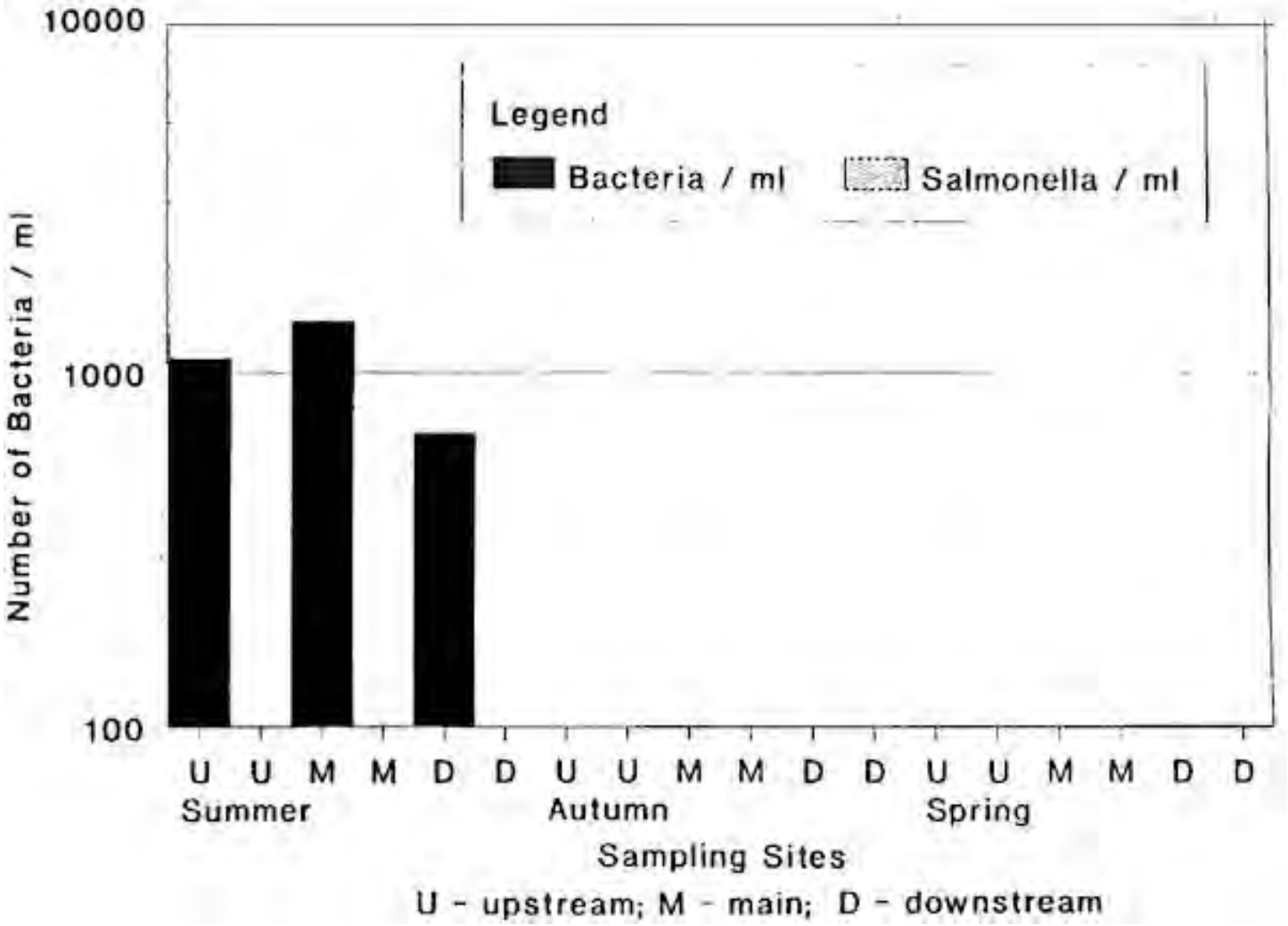


**Figure 26.** Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 30-70 µm diameter in the Desjardine Drain.

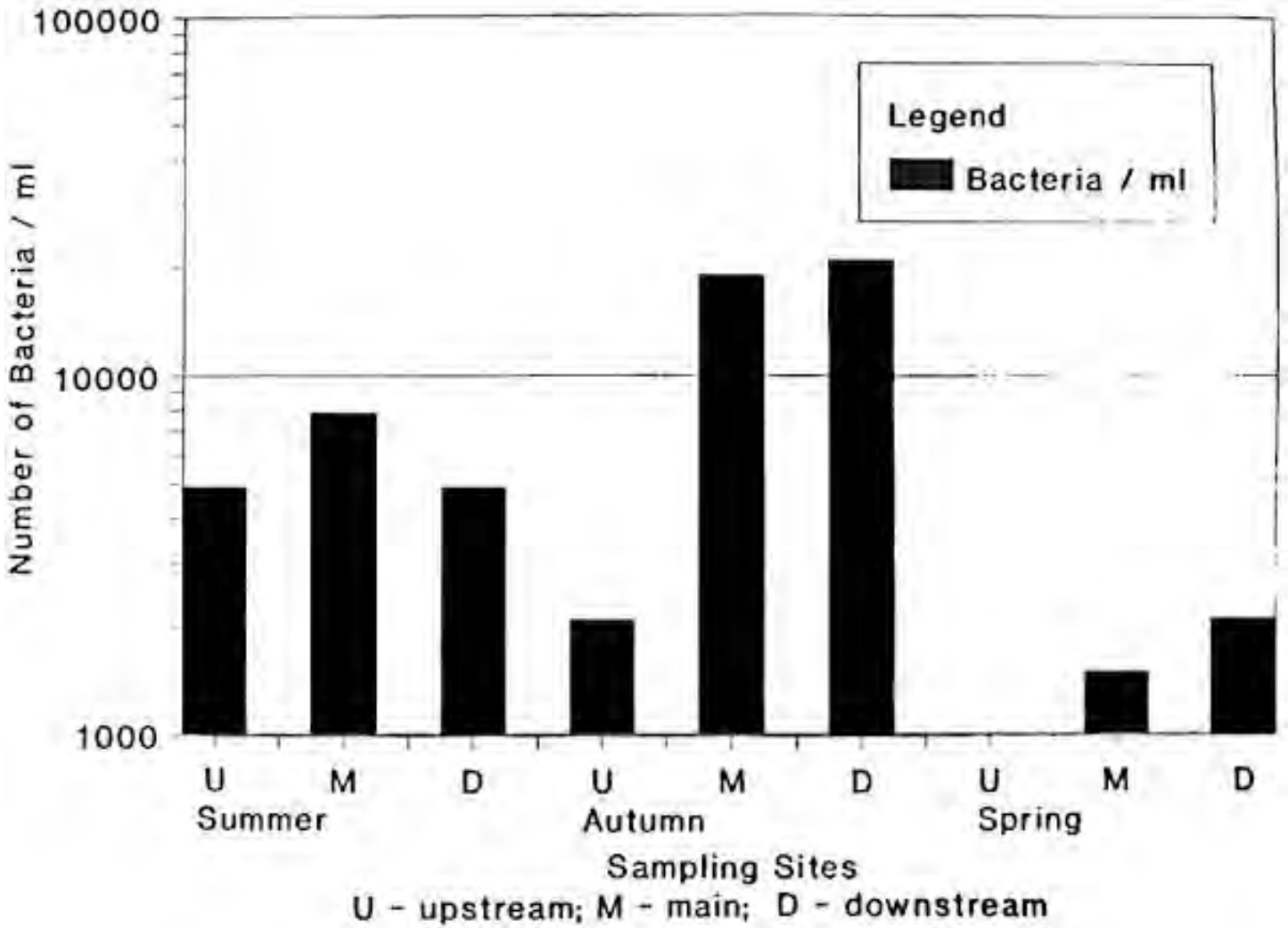


**Figure 27.** Levels of total viable bacteria which are free-floating and associated with particles 10-30 µm diameter in the Desjardine Drain.

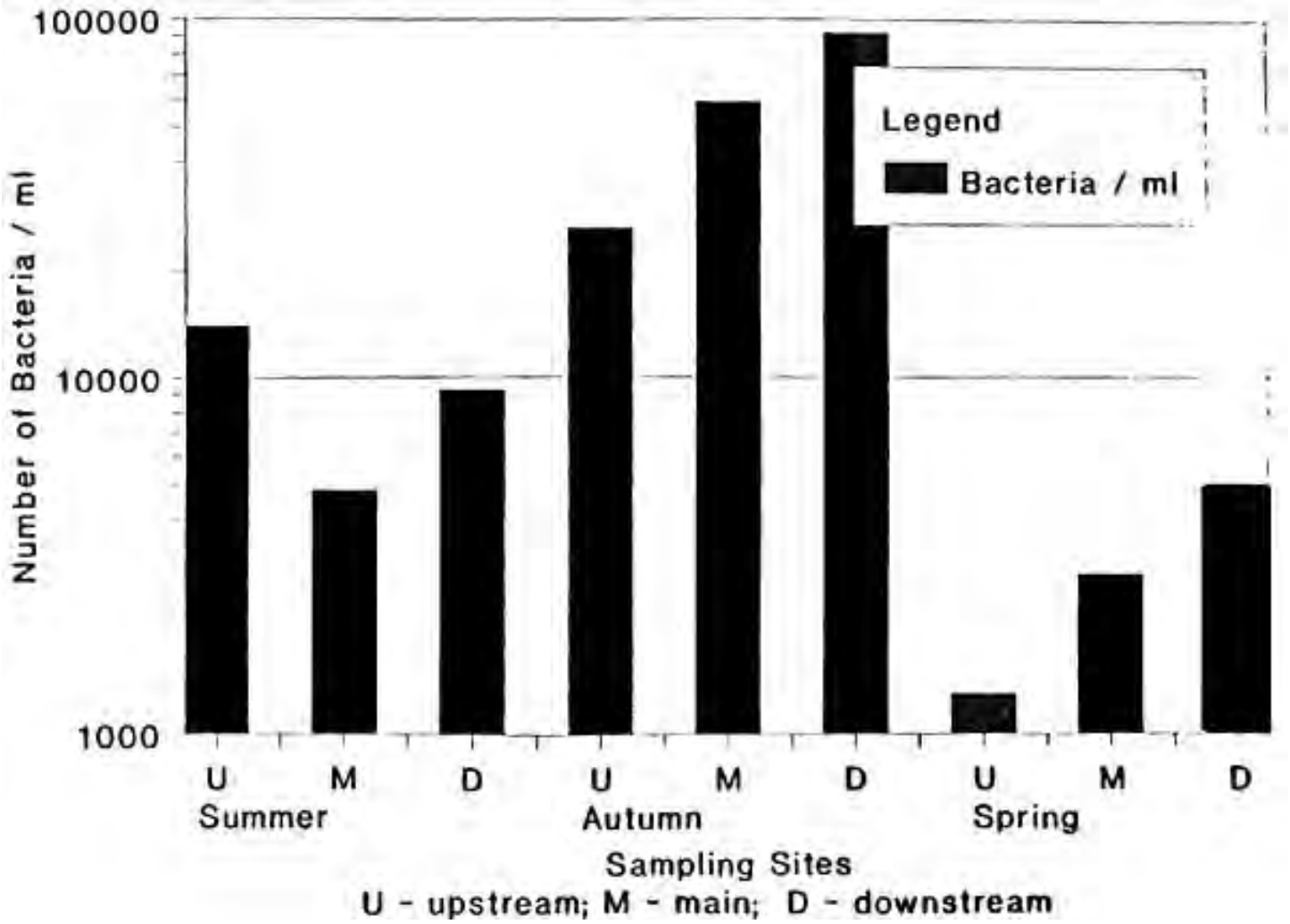


**Figure 28.** Levels of total viable bacteria which are free-floating and associated with particles 5-10 µm diameter in the Desjardine Drain.

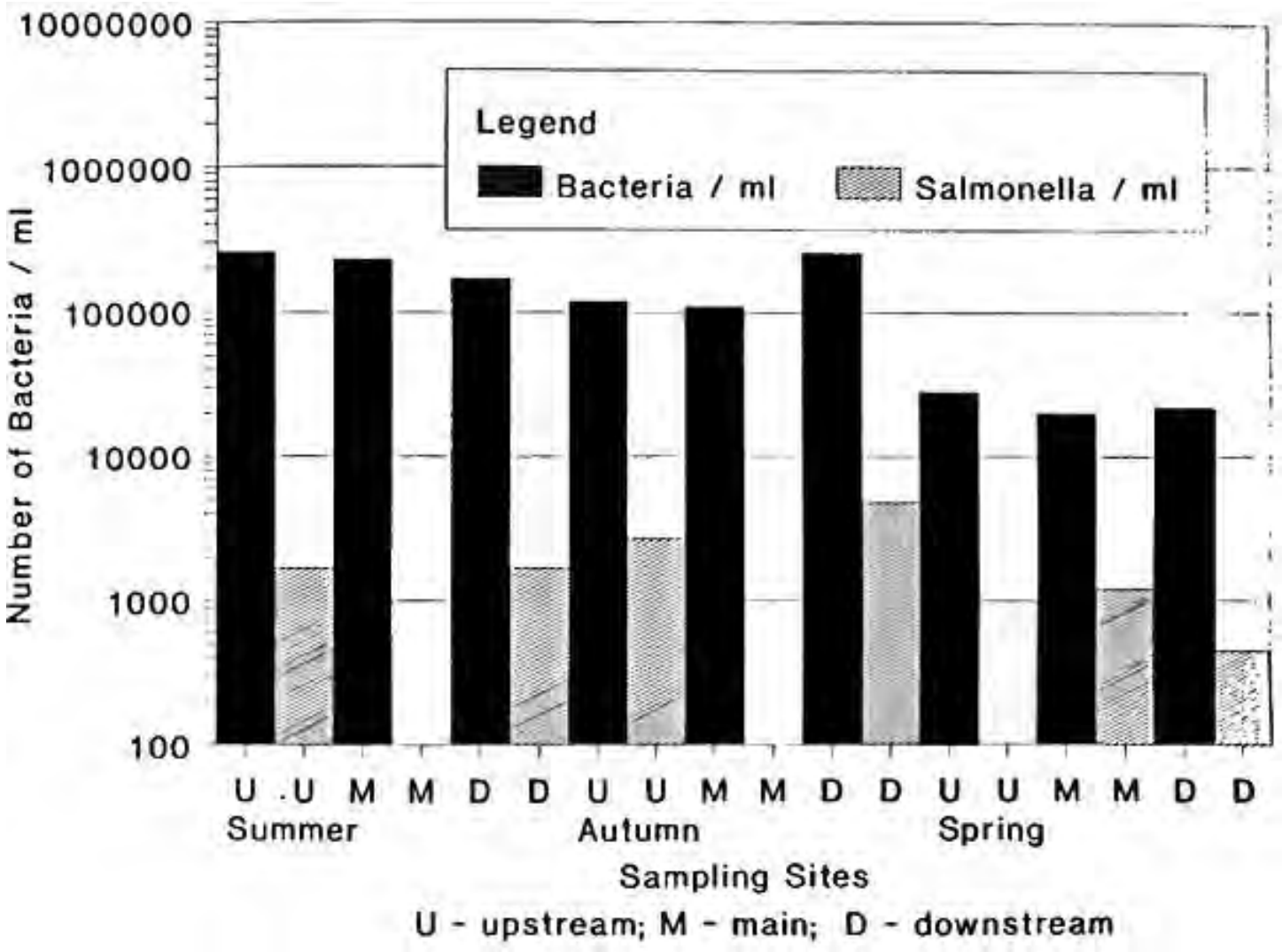


**Figure 29.** Levels of total viable bacteria and *Salmonella* which are free-floating and associated with particles 1-5 µm diameter in the Desjardine Drain.

Free-floating *Salmonella* were associated most frequently and found at higher concentrations with the 1 to 5 µm diameter particulates than with any of the other size fractions.

The percent viability of bacterial cells sorbed to particulates or free-floating and associated with various sizes of particulates was determined as an integral part of the bacterial analysis.

Table 3 shows the percent viability as the number of viable cells, divided by the total numbers of cells, multiplied by one hundred. This was calculated for both sorbed cells and free-floating. It is evident from the data that the percent viability varies with the drain being considered.

The highest percent viability, 59.2%, was observed with bacteria in the Desjardine Drain in the autumn. The average percent viability of 41.0 for the Desjardine Drain was equalled once by the average percent viability of the Arthur Vanatter Drain during the autumn. No trend was readily observed from the data other than that the average viability in the spring for each drain was the lowest while the highest average percent viability occurred in the summer for two of the three drains. The percent viability of the free-floating bacteria were generally lower than for the bacteria sorbed to particulates.

**Table 3.** Percent viability of total bacteria adsorbed to particulates and tree floating at the three study sites during the summer, autumn and spring seasons

| Particulate size in µm | Percent Viability of Total Bacteria | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Arthur Vanatter Drain | | | Central School Drain | | | Desjardine Drain | | |
| | Summer | Autumn | Spring | Summer | Autumn | Spring | Summer | Autumn | Spring |
| Adsorbed | | | | | | | | | |
| 30 | 29.8 | 32.5 | 19.1 | 30.0 | 34.5 | 19.2 | 32.2 | 30.7 | 23.2 |
| 10 | 35.3 | 38.3 | 20.7 | 34.8 | 25.8 | 23,6 | 33.7 | 32.6 | 24.9 |
| 5 | 38.2 | 46.7 | 26.6 | 44.5 | 38.9 | 27.0 | 38:9 | 35.4 | 26.8 |
| 1 | 27.7 | 45.3 | 46.4 | 35.2 | 35.8 | 19.7 | 59.2 | 32.3 | 35.1 |
| Average | 32.8 | 40.7 | 28.2 | 36.1 | 33.8 | 22.4 | 41.0 | 32.8 | 27.5 |
| Free Floating | | | | | | | | | |
| 30 | 52.4 | 30.9 | 8.25 | 21.0 | 14.9 | 0.0 | 34.2 | 0.0 | 0.0 |
| 10 | 26.6 | 43.3 | 15.9 | 32.9 | 33.0 | 21.4 | 35.9 | 56.2 | 11.9 |
| 5 | 21.5 | 31.5 | 21.3 | 36.0 | 31.3 | 18.7 | 34.5 | 44.3 | 24.0 |
| 1 | 30.6 | 41.3 | 17.5 | 23.5 | 39.1 | 15.8 | 81.7 | 31.9 | 20.0 |
| Average | 32.8 | 36.8 | 15.7 | 28.4 | 29.6 | 14.0 | 34.1 | 33.1 | 14.0 |

## D. Bacterial Transport Study

The *Escherichia coli* (NAL) was recovered from the Desjardine Drain at sites shown in Figure 30. The levels in both morning and afternoon, twenty-four hours after the discharge of the *E. coli* (NAL) laden sediments into the drain, were at $10^3$ cells per 100 mL. The exception occurred at the beach at Grand Bend where the cells underwent an enormous dilution when the Old Ausable River discharged into Lake Huron. The tracer bacterium remained at approximately $10^3$ for the afternoon of Day 2 of the study.

In Figure 31, the *E.* coli (NAL) concentration began to decrease at the point of insertion of the sediment-E. coli (NAL) mixture on Day 3. However, the levels remained high in the drain. In addition, higher levels of tracer bacteria were recovered from the south beach station on Lake Huron.

This dispersion of the tracer bacteria continued for approximately eight days before the levels declined in the Drain. This is depicted in Figures 32 through 34 for Station 2, the point of insertion and Stations 4 and 6 respectively for a period of eight-five days. The data in Table 4 substantiates the results obtained from the grab samples. The *E.* coli (NAL) was detectable in the Desjardine Drain from November 19, 1991, through to January 29, 1992, using the swabs as the method of recovery.

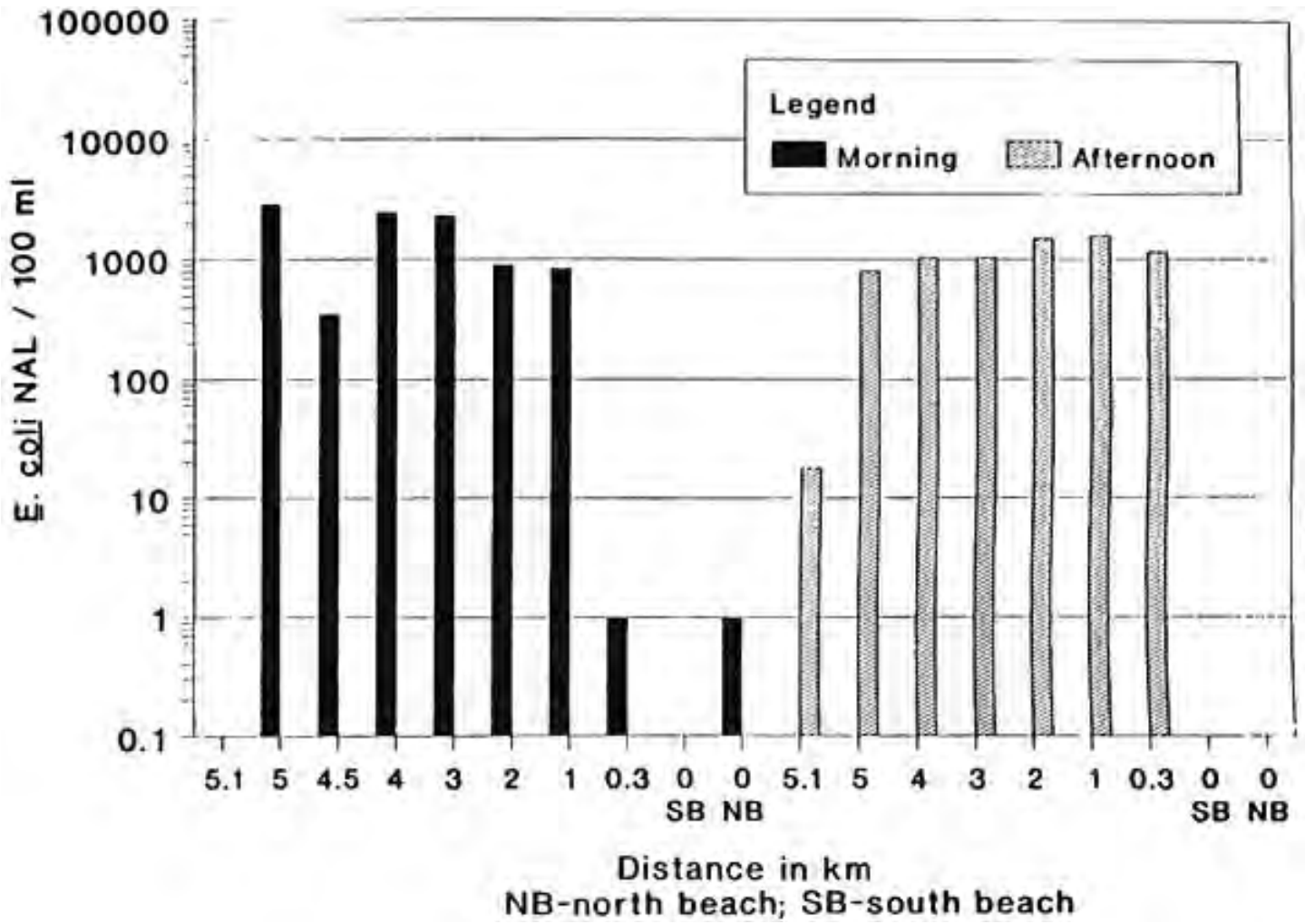


**Figure 30.** Levels of *E. coli* NAL recovered in the Desjardine Drain at 10 sites on November 20, 1991.

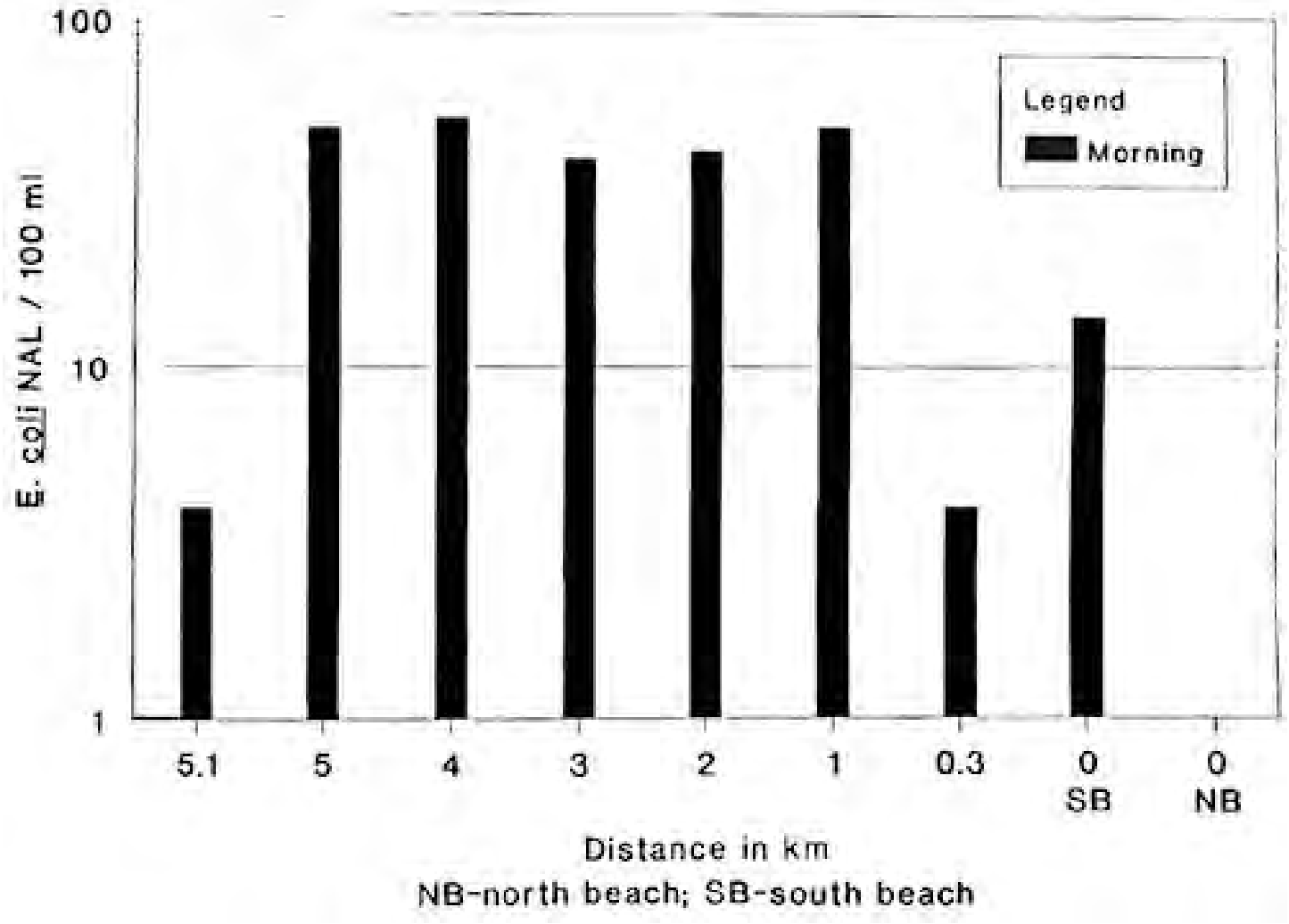


**Figure 31.** Levels of *E. coli* NAL recovered in the Desjardine Drain at 9 sites on November 21, 1991.

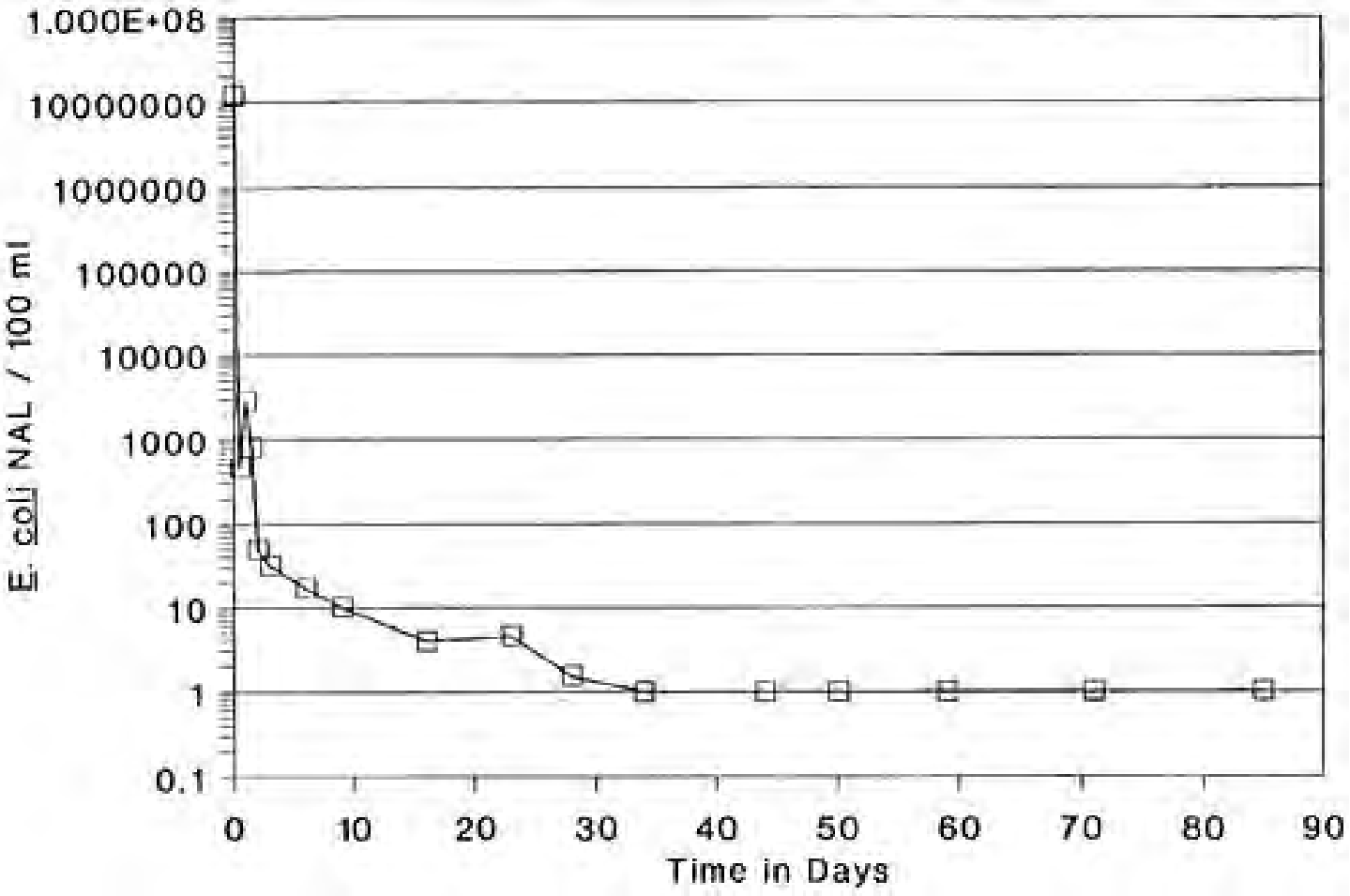


**Figure 32.** Levels of *E. coli* NAL recovered at site 2 during the entire study period of 85 days**.**

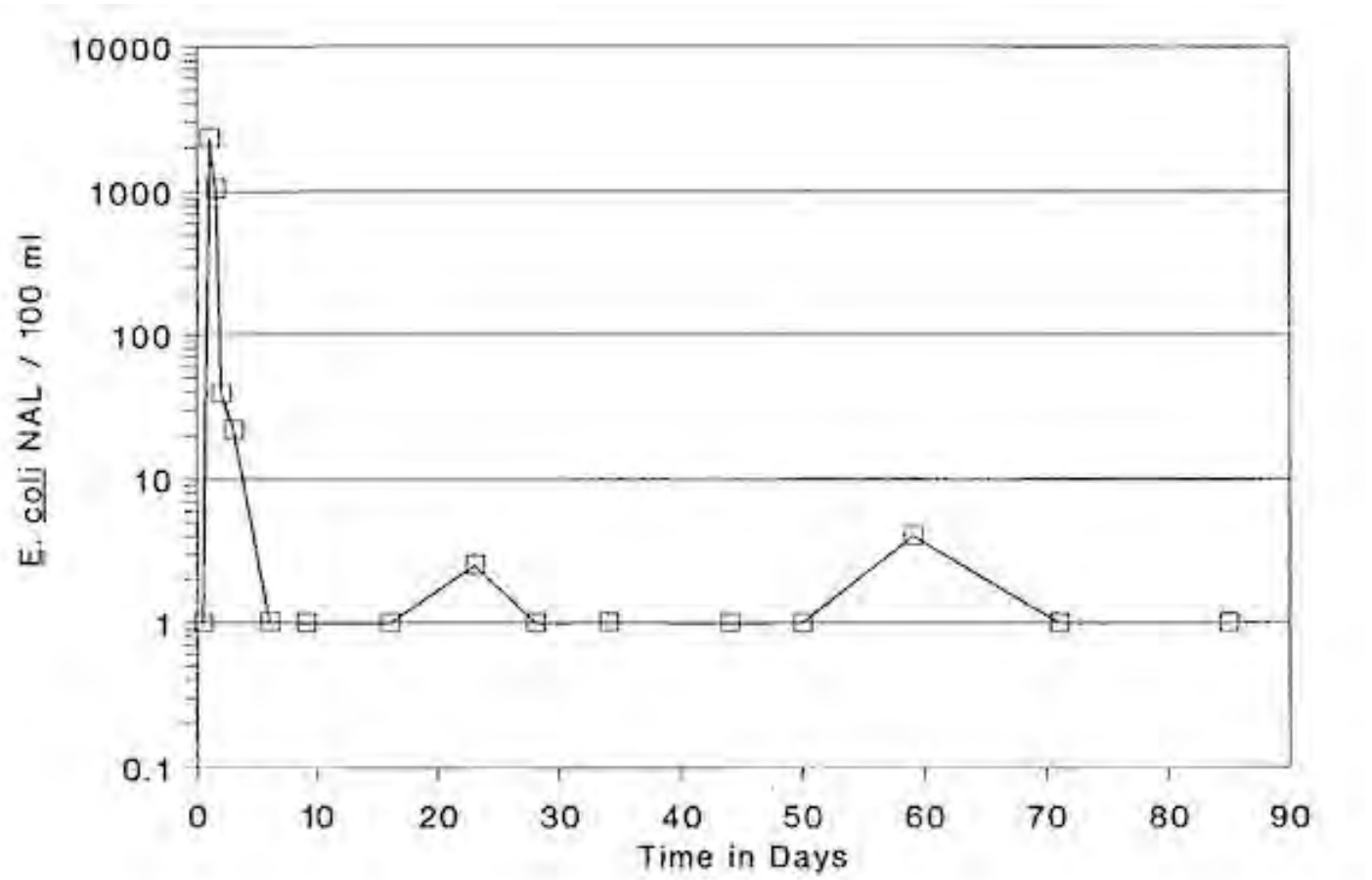


**Figure 33.** Levels of *E. coli* NAL recovered at site 4 during the entire study period of 85 days.

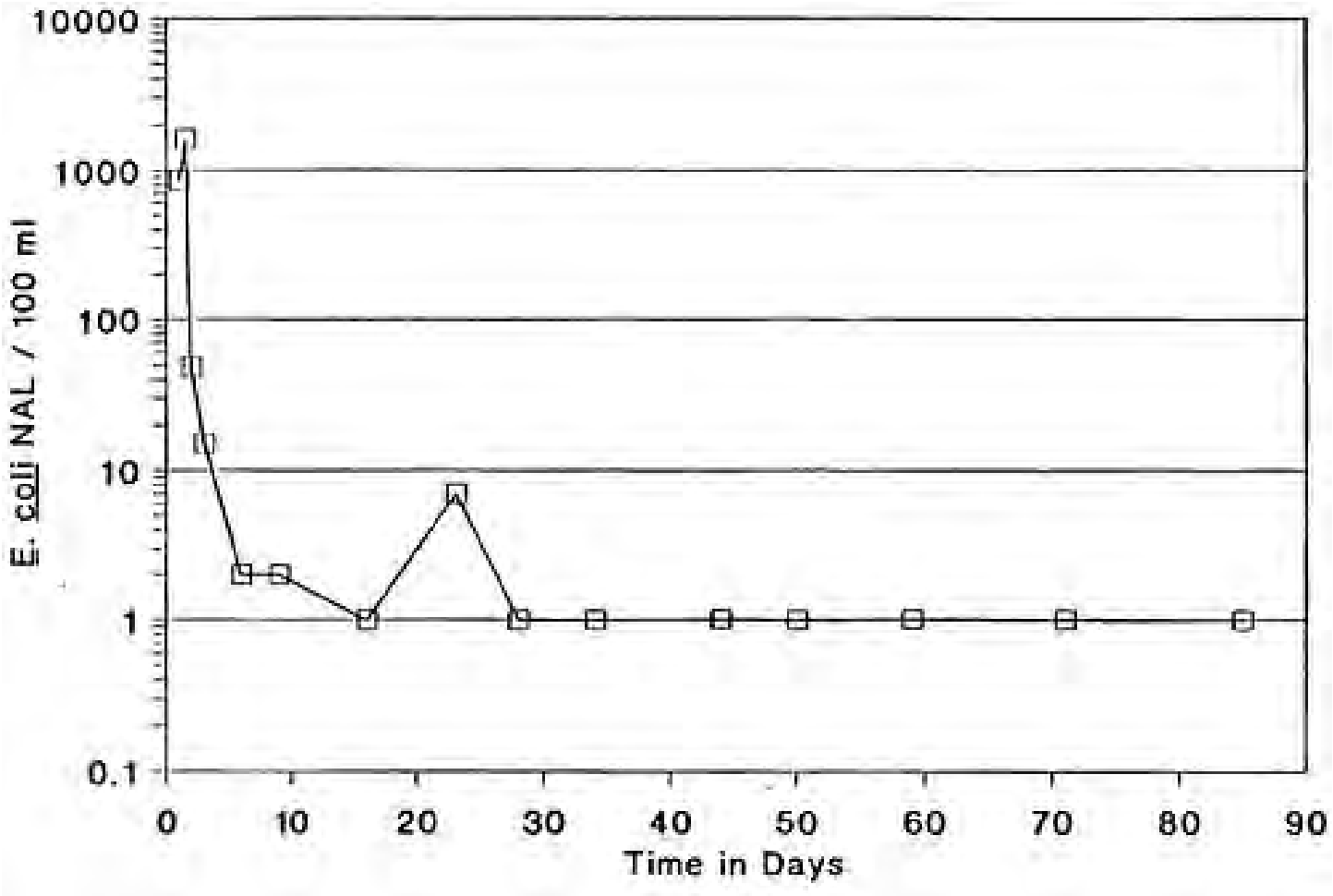


**Figure 34.** Levels of *E. coli* NAL recovered at site 6 during the entire study period of 85 days.

**Table 4.** Occurrence of *E. coli* NAL at 4 swab sites located in the Desjardine Drain.

| Results of Swab Analyses | Sample Sites | | | |
|---|---|---|---|---|
| Date | Swab 1 | Swab 2 | Swab 3 | Swab 4 |
| Nov. 19/91 | nd | + | - | nd |
| Nov. 20/91 | - | + | + | + |
| Nov. 21/91 | + | + | + | + |
| Nov. 22/91 | + | + | + | + |
| Nov. 25/91 | - | + | + | + |
| Nov. 28/91 | - | + | + | + |
| Dec. 5/91 | - | + | + | - |
| Dec. 12/91 | - | + | + | + |
| Dec. 17/91 | + | + | + | + |
| Dec. 23/91 | - | + | - | + |
| Jan. 2/92 | + | + | + | + |
| Jan. 8/92 | + | + | + | + |
| Jan. 17/92 | + | - | + | nd |
| Jan. 29/92 | - | + | + | nd |

However, sediment analyses were also conducted in the drain at the completion of the study and sediments from Sites 2, 4 and 6 contained viable *E. coli* (NAL) while the upstream control station remained negative.

# IV. DISCUSSION

## A. Agricultural Drains

This study showed that the concentration of the pollution indicator bacteria *E.* coli, and fecal streptococci were typical for agricultural drains in southwestern Ontario [23].

The bacterial water quality of the Arthur Vanatter Drain during the summer was poor, with respect to the 100 *E.* coli per 100 mL bathing beach water guideline. The *E. coli* mean of 606.3 and fecal streptococci mean of 1771.3 were both excessive. Remedial measures, such as introducing buffer strips and fencing cattle from the drain, did not reflect as benefits to the drainage water quality. A wild deer population in a wooded area bordering on the northeast side of the drain may have contributed to some of the fecal pollution in the drain near the sampling sites.

The Central School Drain's bacterial water quality was the poorest of the three study drains. Cattle regularly watered in the drain, from the upstream site to the main sampling site. In addition, a tile drain discharge at the main site was known to contain milkhouse waste water and subsurface manure runoff. The mean *E.* coli and fecal streptococci values during the summer and fall were above bathing beach bacterial water quality guidelines.

In contrast, during the spring, the improved means for both *E. coli* and fecal streptococci in the Central School Drain reflected the fact that the cattle had been fenced from the drain and that the milkhouse waste had been intercepted.

The Desjardine Drain bacterial water quality was slightly higher in comparison to the Central School Drain's. The mean levels of both bacterial indicators in the autumn were higher than the other two drains. Septage from poorly performing septic tanks and manure from cattle watering in the stream both contributed to the fecal bacterial loadings. Spring flows were high and likely diluted the concentration of fecal bacteria for the Desjardine Drain.

All three drains demonstrated significantly improved bacterial water quality in the spring.

The suspended particulates and bacterial sorption analyses, in contrast to the water quality data, did not reflect the same variation in bacterial concentrations as the fecal bacterial indicators. The levels of total viable and *Salmonella* bacteria sorbed to suspended particulates were consistently highest for the Desjardine Drain. Analyses of the bottom sediments for percent sand, silt, organic matter and clay showed that the surface sediment had three times the clay content than in either of the other two drains. In addition, bacterial concentrations were highest for the 1 to 5 am diameter size range, which is the size range nearest that of clay particulates. In reviews by both Stotzky[24] and Marshall,[9] bacterial sorption to clays, specifically montmorillonite, was

demonstrated to occur to a much greater extent than to soils with a low clay content.

Burton *et al.*, 25 found that a variety of pathogenic bacteria, including *Salmonella sp.,* survived for much longer periods when bound to clay sediments than compared to their survival in the overlying water column.

Suspended drainage sediments were a combination of water-saturated soil, present in the drain from upstream erosion, and sediments native to the bottom of the drain.

The percent clay and the organic matter, both of which act as sorbents for microorganisms, were found to be much higher in the Central School Drain than in the Arthur Vanatter Drain.

The levels of both the total viable bacteria and *Salmonella* are difficult to put into perspective due to the paucity of such data, using this methodology, in the literature. Tsernoglou and Anthony[26], using similar methods of analysis, found levels of bacteria sorbed to sediments of fresh water lakes to range from $3 \times 10^3$ to $1.5 \times 10^4$ per $mm^2$ of surface area, which compare with the results shown.

The total viable bacteria and *Salmonella* detection technique employed in this study were modifications of the methods used by Kogure *et al*.[18] and Xu *et al*.[17] and allowed differentiation of viable from non-viable bacteria. The bacterial

concentrations sorbed to particulates, or that were free-floating, indicate that significant levels of *Salmonella* exist in these drains. In comparison to previous sanitary surveys conducted on these drains, *Salmonella* were substantially underestimated because conventional culturing techniques were used. Xu *et al.*[27] demonstrated the survival and viability of non-culturable *E. coli* and *Vibrio cholerae* in aquatic environments.

Clearly, fluvial sediments can transport significant loads of bacteria on particulates that are small enough (<70 µm diameter in size) to be transported during moderate stream flow conditions.

Salomons[28] discussed various aspects of sediments and water quality and made the point that sediments that act as sorbents for various environmental pollutants serve to improve the water quality of the water column overlying the sediments. However, when these sediments become resuspended in the water column, as they often do during significant increases in the stream flow, the water quality rapidly deteriorates.

Matson *et al*.[29] also concluded that river sediments previously contaminated with fecal bacteria could be resuspended at some later time and cause significant deterioration in water quality of the overlying water during elevated flow periods.

## B. Bacterial Transport Study

*E. coli* (NAL) were sorbed to the sediment of the Desjardine Drain which was 22% clay and which had a cation exchange capacity of 25 milliequivalents per 100 g. This sediment was reintroduced into the Desjardine Drain. Within twenty-four hours, the bacterial-particulate mixture had reached the beaches of Lake Huron. In recent studies, Palmateer *et al*.[30] demonstrated that the *E. coli* (NAL) could travel the entire 18 km of the Desjardine Drain from its origin, which was field tile drainage, to the discharge of the Old Ausable River into Lake Huron at the Grand Bend beach.

In those studies, the bacterial suspension was added to the drain, with no immediate involvement of sediments. Five days from the time of insertion of the tracer bacteria, both water and sediment samples of the Desjardine Drain were found to be free of any viable *E. coli* (NAL). It was evident that the entire plume of tracer bacteria had passed through the Desjardine Drain to Lake Huron. If there was a residual, it was nonculturable.

In contrast, this study took eighty-five days for the same *E. coli* (NAL), sorbed to particulates, to eventually die-off and/or pass entirely from the drain.

The effect of bacteria being sorbed to nutrient-rich sediments in an agricultural drain is obvious.

# V. CONCLUSIONS

## A. Agricultural Drains

Suspended particulates in agricultural drainage carry high levels of bacteria at $10^3$ to $10^5$ cells per $mm^2$ as a result of the sorption processes.

## B. Bacterial Transport Study

The transport of the fecal-associated bacterium *E. coli,* once sorbed to suspended particulates, may travel kilometres downstream in agricultural drains to impact bathing beaches far from the point of entry of the fecal pollution in the drain.

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