MICROORGANISMS IN STORMWATER--

A SUMMARY OF RECENT INVESTIGATIONS

By Gail E. Mallard

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CONVERSION FACTORS AND ABBREVIATIONS

multiply inch-pound units	by	to obtain SI[1/] (metric) units
foot (ft)	0.3048	meter (m)
ounce (oz)	29.57	milliliter (mL)
pound (lb)	0.4536	kilogram (kg)
gallon (gal)	3.785	liter (L)

degree Fahrenheit (°F) = 1.8 degree Celsius (°C) + 32

1/ International System of Units

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ABSTRACT

The major concepts and considerations in microbiological analysis of storm runoff are summarized and documented to provide a basis for use by community planners, water managers, and others interested in studies of stormwater. Twenty-seven published reports dating since 1964 are cited.

All storm runoff contains a variety of bacteria, including total coliforms, fecal coliforms, and fecal streptococci, which are derived from the land over which the water flows. Most of the total coliforms are native soil organisms, whereas the fecal coliforms and fecal streptococci originate from the feces of wild and domestic animals. Urban runoff has been reported to contain pathogenic organisms, but this probably presents little direct threat to human health because the runoff is not ingested. Runoff water can, however, have other negative effects such as contamination of surface water, which may result in beach closures, or contamination of shellfish. This type of contamination is generally of short duration because indicator bacteria and pathogens die out rapidly in the aquatic environment. Similarly, bacteria and viruses deposited on soil by stormwater are inactivated by drying, competition from soil microflora, and a variety of other processes.

Every storm producing runoff is unique in the number and type of microorganisms because these vary from site to site, from storm to storm, and during the course of the storm. Stormwater to be examined for microorganisms must be collected in sterile containers and processed immediately.

INTRODUCTION

Any attempt to find microorganisms in stormwater generally will meet with success. Bacteria and viruses are normal inhabitants of soil, water, human and animal skin and gut, plant surfaces, and indeed almost every place on earth. Only certain extremely hostile environments and the internal tissues of plants and animals are free of microorganisms. Stormwater running over the land surface will inevitably become contaminated, but this should not be a cause for concern unless it threatens human health or well-being. Does stormwater present a serious threat? Unfortunately, no unequivocal answer can be given. Stormwater carrying microorganisms may enter a drinking-water source and cause an outbreak of disease, or it may lead only to the temporary shutdown of recreation facilities such as beaches. Depending on local conditions, the study of microbial contamination of runoff may be of real importance to a community or it may be of only academic interest.

Purpose and Scope

This summary presents general information gained from recent investigations and describes problems that may arise in future investigations. It is hoped that this information will be of value in planning studies that involve microorganisms in stormwater. This report was prepared for the National Urban Runoff Program in cooperation with the Long Island Regional Planning Board.

BASIC CONCEPTS IN MICROBIOLOGY

All scientific and technical fields have concepts, procedures, assumptions, and terminology that are unfamiliar to the nonspecialist. Although many of the terms and concepts of microbiology are familar to the general public, the exact meanings and limitations may be poorly understood. For this reason, the main discussion of microorganisms in storm runoff is preceded by a brief introduction to some of the principles of microbiology. A glossary of biological and microbiological terms is provided by Greeson and others (1977).

Indicator Organisms

Water that has been polluted by human sewage is hazardous because several diseases are transmitted via the fecal-oral route, such as typhoid, cholera, dysentery, and hepatitis. When presented with a water sample that is suspect, the microbiologist typically does not try to isolate and identify the pathogens, but instead determines numbers of "indicator" organisms--total coliforms, fecal coliforms, and fecal streptococci. These bacteria are known as indicator organisms because they indicate the presence of sewage and ideally are correlated with the number of pathogens in a water sample.

The growth characteristics and metabolic reactions of the indicator organisms have been investigated for many years, and relatively easy and straightforward methods for their detection and enumeration are available (American Public Health Association, 1975; Greeson and others, 1977). In contrast, methods for detection of pathogens are complicated, tedious, and time consuming.

In addition to being easier to isolate and study in the laboratory than pathogens, indicators are used because water contaminated by human waste will typically contain many more of these organisms than pathogens. Indicator organisms are present at relatively high levels in all members of the population and are being shed at all times. In contrast, only a few clinical cases and carriers will be shedding pathogens at any given time.

Most of the characteristics of an ideal indicator organism are listed in "Drinking Water and Health" (National Research Council, 1977, p. 71) and are quoted below:

1. Applicable to all types of water.

2. Present in sewage and polluted waters when pathogens are present.

3. Number is correlated with the amount of pollution.

4. Present in greater numbers than pathogens.

5. No aftergrowth in water.

6. Greater survival time than pathogens.

7. Absent from unpolluted waters.

8. Easily detected by simple laboratory tests in the shortest time consistent with accurate results.

9. Has constant characteristics.

10. Harmless to man and animal.

Although no organism or group of organisms is a perfect indicator, coliforms are ideal in most ways and have been used by water microbiologists and others concerned with the public health for many years. The U.S. Environmental Protection Agency's safe drinking-water standards are written in terms of coliforms rather than pathogens (American Public Health Association, 1975) as are standards for recreational waters. As recently as 1977, the use of coliforms as indicators of fecal contamination was endorsed by the Safe Drinking Water Committee of the National Research Council (1977). Yet, the popularity of coliforms as an indicator does not preclude critical reviews of their use (Dutka, 1973) or suggestions that other organisms or groups would provide more accurate determinations (Carberry and Stapleford, 1979). Additionally, continuing efforts are being made by many investigators to refine the methods used to detect indicator organisms.

The effectiveness of total coliforms, fecal coliforms, and fecal streptococci as indicators of viral contamination is particularly suspect. Several reports indicate that bacterial indicators are inadequate predictors of viruses in ground water (Marzouk and others, 1979; Vaughn and others, 1978) as well as fresh and marine surface waters (Vaughn and others, 1979). This lack of a reliable indicator for enteroviruses presents a serious problem because detection of viruses in water samples requires expensive and time-consuming procedures. Studies of viruses in the environment are, however, becoming more commonplace, and methods for their detection and enumeration are being improved.

The use of indicator organisms in stormwater-runoff studies may pose a special problem. Most of the fecal coliforms and fecal streptococci in stormwater are derived from animal feces, so that the level of these indicator organisms may be high at all times, regardless of the number of pathogens in the water sample. Olivieri and others (1977) found little or no correlation between indicator bacteria and either pathogenic bacteria or enteric viruses in storm samples.

Ratios of Fecal Coliforms to Fecal Streptococci

Aside from the question of the reliability of indicator organisms, a significant question remains unanswered--how is human fecal contamination differentiated from other fecal contamination? Since fecal material from many warm-blooded animals contains coliforms and streptococci, the presence of these groups does not mean that human waste was the source of contamination. Geldreich and Kenner (1969) approached this problem by examining feces from various warm-blooded animals (including humans), domestic wastewater, stormwater, food-processing wastes, agricultural waters, and recreational waters. They concluded that the ratio of fecal coliforms to fecal streptococci (FC:FS) in human feces and in water polluted with human waste is always greater than 4.0, whereas the ratio of fecal coliforms to fecal streptococci in feces from farm animals, cats, dogs, and rodents, and in separate stormwater systems and farmland drainage, is less than 0.7.

Although the ratio of fecal coliforms to fecal streptococci in a water sample gives some indication of the source of the contamination, these ratios must be interpreted with caution. First, the water samples must be taken near the source of contamination because once the organisms have entered the receiving body of water, variables such as temperature, pH, metal concentration, nutrient availability, and other environmental factors will alter the interrelationship between the two indicator systems. McFeters and others (1974) investigated the question of survival of indicator bacteria in natural waters and found that the initially high FC:FS ratio of human sewage decreased with time, whereas the initially low ratio for waste from domestic livestock increased. This reinforces the advice given by Geldreich and Kenner (1969, p. R349) that "the use of a ratio relationship for stream samples would be valid only during the initial 24-hour travel downstream from point of pollution discharge into the receiving stream."

Even if used correctly, the FC:FS ratio should not be regarded as a "magic number," especially for samples that contain water from a mixture of nonpoint sources. For example, if most of the contamination in a sample were from nonhuman sources, a small amount of human sewage might not be sufficient to shift the overall ratio upward enough to cause concern. As a result, the presence of human pathogens in the human sewage would be masked by the indicator ratio characteristic of animal waste, and a real danger would go undetected.

MICROORGANISMS IN STORMWATER

Bacterial Load of Stormwater

Rainwater contains very few bacteria before it reaches the ground. Geldreich and others (1968) collected rainwater from 49 storms in sterile collectors and tested for total coliforms, fecal coliforms, and fecal streptococci. In 42 of the samples, counts of these bacteria were less than one organism per 100 mL; in the remaining seven samples, total coliform densities ranged between 1 and 92 per 100 mL. When this "contaminated" rainwater was filtered and the filter examined microscopically, the authors observed soil particles and insect and vegetation fragments, which they believed were the source of the bacteria. Whenever rainwater comes in contact with land, it becomes contaminated with bacteria. Counts of total coliforms, fecal coliforms, and fecal streptococci in stormwater, compiled from several references (table 1), range as follows: 10^3 to 10^5 total coliforms per 100 mL, 10^2 to 10^4 fecal coliforms per 100 mL, and 10^2 to 10^5 fecal streptococci per 100 mL.

Sources of Bacteria in Stormwater

Geldreich and others (1968) examined 843 stormwater samples and observed that fecal coliforms constituted an average of 8.6 percent of the median total coliforms present. After examining over 7,000 separate strains of coliforms and determining their physiological type, they concluded that the remaining 91.4 percent of the total coliforms came from the soil. The distribution of strains was found to be the same in soil, surface waters, and stormwater. This exhaustive analysis leaves little doubt that most total coliforms in stormwater are native soil organisms that are washed off soil particles by water running over the land surface.

If most of the total coliforms come from the soil, what is the source of the other two pollution indicators--fecal coliforms and fecal streptococci? By definition these are contributed by warm-blooded animals. Because the ratio of fecal coliforms to fecal streptococci in urban stormwater is usually much less than 1 (see table 1), it is assumed that most of the bacteria in stormwater are of nonhuman origin. In an urban environment, this pollution is most likely due to fecal material from dogs, cats, rodents, and other small animals, whereas in rural areas, larger domestic animals would make a significant contribution. In either case the amount of waste generated by animals should not be underestimated. For example, Geldreich (1976, p. 361) cites an estimate that "the 500,000 owned dogs in New York City deposit about 150,000 pounds of feces and 90,000 gallons of urine each day on streets, sidewalks, and park areas." Even a small number of farm animals can have a significant impact because one cow will generate as much manure as 16.4 humans, a pig will produce as much as 1.9 humans, and 12 chickens as much as one human (Geldreich, 1976). Pollution from wild animals must also be considered. A beach in Madison, Wisconsin, was closed to swimming because of high bacterial counts in 1978. An investigation revealed that fecal coliforms deposited by wild ducks had multiplied in the beach sands and were then carried into the water by storm runoff (Standridge and others, 1979).

Variation in Number of Microorganisms in Stormwater

The microbiological character of runoff from any given storm and any given area is likely to be unique. This presents obvious problems if one is to compare runoff between areas or within the same area over time. First, the land use and topography of the drainage area will have a significant effect on quality of the runoff. For example, urban stormwater and runoff from a feedlot will be very different from each other. Even within the general category of urban runoff, water from residential areas is likely to differ considerably from water from commercial areas in terms of microorganisms.

Table 1.--Densities of indicator bacteria in stormwater.

Source of stormwater	Total coliforms per 100 mL	Fecal coliforms per 100 mL	Fecal strep per 100 mL	FC:FS Ratio	Reference
Detroit - urban street catch basins, 1949	25,000 - 930,000	not measured	not measured	not measured	Weibel and others, 1964
Detroit - urban street catch basins, 1950	2,300 - 430,000	do.	do.	do.	do.
Seattle - street gutters 1959-1960	up to 16,100	do.	do.	do.	do.
Stockholm, Sweden - streets and parks, 1945-1948	median: 4,000 high: 200,000	do.	do.	do.	do.
Pretoria, South Africa - residential, park, school, sports ground areas	240,000	do.	do.	do.	do.
Pretoria, South Africa - business and flat area	230,000	do.	do.	do.	do.
Cincinnati, Ohio - residential; 1962-1963	median 58,000	median 10,900	median 20,500	0.53	do.
Cincinnati, Ohio - 1962-1964					Geldreich and others, 1968
Wooded hillside					
Spring	2,400	190	940	0.20	
Summer	79,000	1,900	27,000	0.70	
Autumn	180,000	430	13,000	0.03	
Winter	260	20	950	0.20	
Street gutters					do.
Spring	1,400	230	3,100	0.07	
Summer	90,000	6,400	150,000	0.04	
Autumn	290,000	47,000	140,000	0.34	
Winter	1,600	50	2,200	0.02	
Business district					do.
Spring	22,000	2,500	13,000	0.19	
Summer	172,000	13,000	51,000	0.26	
Autumn	190,000	40,000	56,000	0.71	
Winter	46,000	4,300	28,000	0.15	
Rural					do.
Spring	4,400	55	3,600	0.02	
Summer	29,000	2,700	58,000	0.05	
Autumn	18,000	210	2,100	0.10	
Winter	58,000	9,000	790,000	0.01	
Baltimore - Inner city, residential commercial - Pop. density 92.5/acre, 1974-1975	mean of 24 storms 380,000	mean of 24 storms 83,000	mean of 24 storms 560,000	90% less than 4.0; 80% less than 1.0	Olivieri and others, 1977.
Baltimore - Residential/shopping center - Pop. density 26.6/acre, 1974-1975	mean of 21 storms 38,000	mean of 21 storms 6,900	mean of 21 storms 50,000	90% less than 4.0; 80% less than 1.0	do.
Burlington, Ontario - single-family residential	10,000	4,000	4,000	1.0	Qureshi and Dutka, 1979a

If runoff from only one area is considered, time is the most significant variable because bacterial counts vary from storm to storm (Davis and others, 1977) and with season (see table 1). However, the amount of time elapsed between storms does not seem to affect microbiological quality, as indicated by Olivieri and others (1977, p. 88), who, after studying storms in Baltimore, Maryland, for a 12-month period, concluded that "the levels of fecal coliforms observed in the storm runoff appear to be independent of the time between storms." Upon first consideration, this conclusion may seem unlikely because waste material will tend to accumulate between storms. However, fecal organisms begin to die as soon as they leave their normal habitat, the gastrointestinal tract of warm-blooded animals, so that only freshly deposited fecal bacteria are likely to affect the counts in runoff.

Changes in bacterial counts during a storm have been documented by Davis and others (1977), who studied an area near Houston, Texas, that was being developed into a planned community. Figure 1, which shows a hydrograph and bacteriological data from one storm at a small stream site, indicates that the densities of both fecal coliforms and fecal streptococci rise sharply at the beginning of the storm and gradually decay over the next 60 hours. The time from 20-50 hours represents the bulk of the hydrograph. During this period the fecal coliform density decreases by slightly less than 1 log unit (base 10), while the fecal streptococcal density decreases by just over 1 log unit. Qureshi and Dutka (1979a) conducted a similar study of stormwater draining a residential area in Burlington, Ontario, and emptying into a small creek. In that study, samples of runoff were collected at the outfall before entering the stream. Changes in bacterial counts at this site (arithmetic mean of 12 storms) are shown in figure 2. Data for individual storms show no predictable pattern for maximum bacterial populations (Dutka, 1977; Qureshi and Dutka, 1979b). In a report on the microbiological quality of stormwater from residential and commercial areas (3 sites), Qureshi and Dutka (1979b, p. 977) concluded:

> There appeared to be little relationship between the duration, intensity, and amount of rainfall and the occurrence of peak microbial populations. As a result, no typical pattern of time-related distribution of indicator and pathogenic bacteria could be established in this investigation.

The amount of time microorganisms spend in the stormwater before collection and analysis has a significant effect on the final counts obtained. In general, storm runoff is a hostile environment for fecal indicator organisms and pathogens because these organisms require high nutrient levels and warm temperatures for growth. Furthermore, salts, organic constituents, and other chemicals that may be in stormwater will have an adverse effect on microorganisms, so that the number of organisms in stormwater will decline with time. In addition, the relative concentrations will change with time because the strains die off at differing rates. A more complete discussion of the importance of prompt analysis is presented in the section "Methods and Procedures" (p. 13).

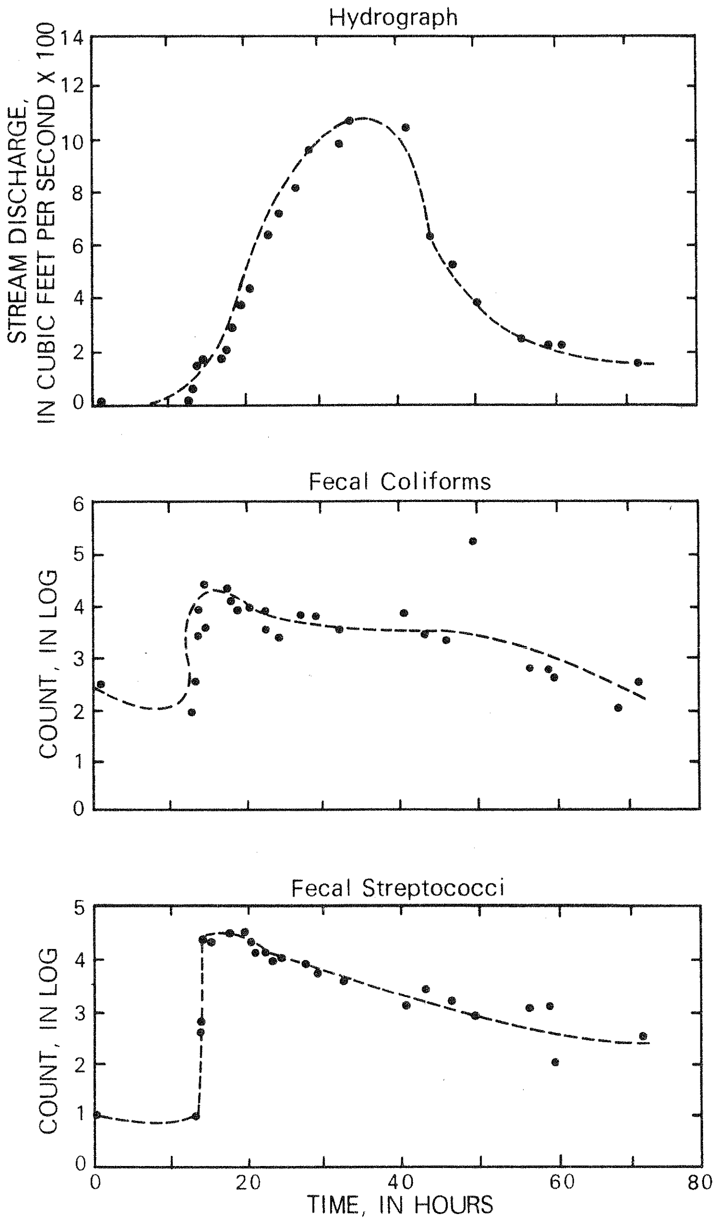

Figure 1. Hydrograph and densities of fecal coliform and fecal streptococcal bacteria in a small stream near Houston, Texas, during a storm. (Modified from Davis and others, 1977; reproduced with permission of Water Resources Bulletin).

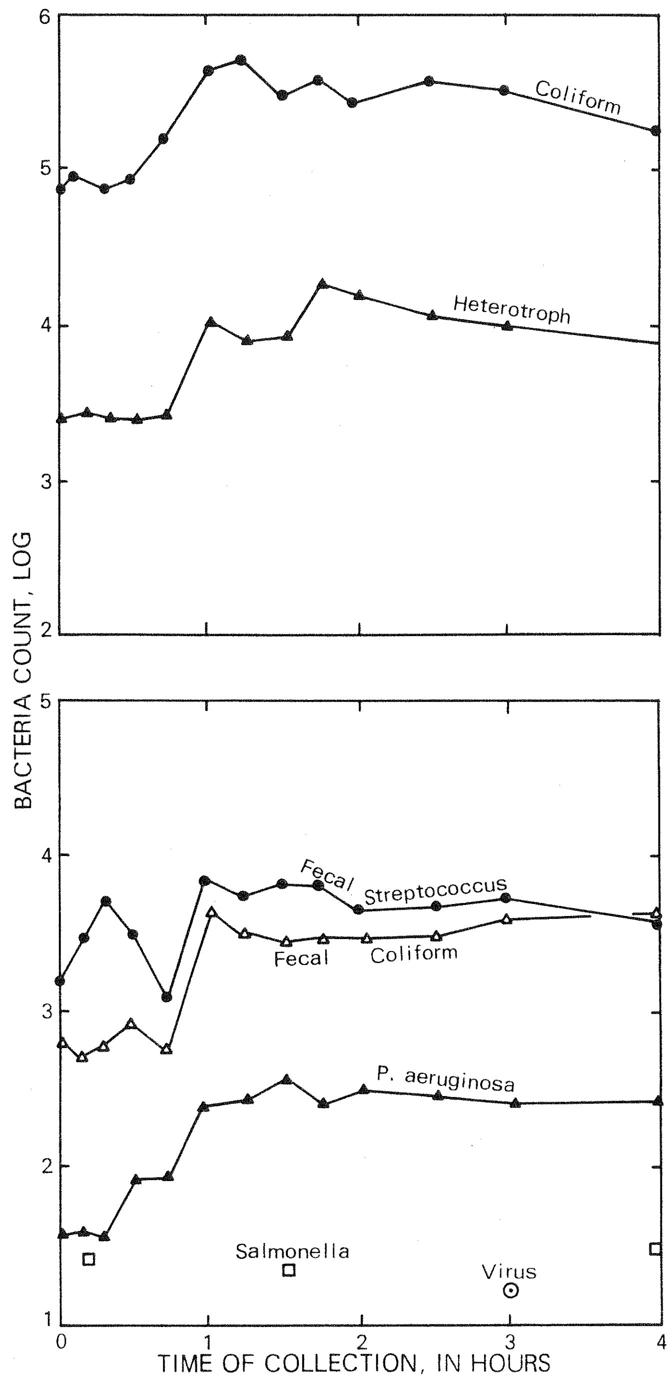

Figure 2. Bacterial populations in runoff samples taken at stormwater outfall, Burlington, Ontario. Arithmetic mean of bacteriological data based on 12 storms. (From Qureshi and Dutka, 1979; reproduced from Water and Sewage Works, v. 123, no. 3, March 1979, with permission).

Pathogens in Stormwater

Olivieri and others (1977) investigated the occurrence of pathogens in urban stormwater in Baltimore. An analysis of stormwater samples from a site not known to have any sewage overflows gave the results presented in table 2.

It is obvious that urban stormwater can contain pathogens, although at relatively low levels. Could this represent a threat to human health? One way to begin to answer this question is to consider the number of bacteria and viruses necessary to establish infection. Unfortunately, this number is dependent on the strain of the pathogen involved because some strains are more virulent than others. The susceptibility of a human population is also variable and depends on such considerations as age, general health, and degree of immunity. Nevertheless, rough estimates can be made from available data. A dose of 10^5 *Salmonella* may be necessary to establish infection, but in the case of some *Shigella* strains, 10^2 or 10^3 viable organisms is enough to infect (National Research council, 1977). The situation with viruses is not as well documented, but in general, from a conservative approach, one virus particle is considered sufficient to establish infection. It should be pointed out, however, that infection does not always lead to disease.

Table 2.--Densities of pathogens in stormwater from Baltimore, Maryland.[1/]

Pathogen	Percent occurrence in collected samples	Geometric mean densities
Salmonella species	52	5.7 per 10 L
Pseudomonas aeruginosa	100	592 per 100 mL
Staphylococcus aureus	82	12 per 100 mL
Animal virus	83	--
Polio virus	42	--
Coxsackie Virus B	50	--
Echovirus	33	--
other unidentified virus	8	--
Enterovirus	--	170 plaque-forming units per 10 L

[1/]Data from Olivieri and others (1977)

By combining the available information about densities of pathogens in stormwater with the infective doses just cited, one might be able to make an estimate of the direct health hazard of urban runoff. From this line of reasoning, the amount of stormwater an individual would have to consume to acquire a *Salmonella* infection would be on the order of hundreds of gallons. The danger from virus infection may be greater because dilution will not destroy infectivity. It is unlikely that any single individual will consume enough stormwater to insure virus infection, but if many individuals drink slightly contaminated water, it is possible that one or a few may become infected.

Stormwater does not need to be ingested to pose a health hazard. It is perhaps ironic that contamination of salt water, which is not consumed, may be more dangerous than contamination of drinking water. For example, oysters and clams are known to concentrate viruses, and several well-documented outbreaks of hepatitis have been caused by virus-contaminated shellfish (Vaughn and Landry, 1977). Also, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are associated with eye, ear, and skin disease and might become a problem in areas where stormwater, or surface waters heavily contaminated with stormwater, are used for recreational activities.

All of the pathogens mentioned above are of human origin and, as expected, are present in urban stormwater in relatively low numbers. The report on the Baltimore study discusses the issue at some length and concludes that urban stormwater probably represents little threat to public health (Olivieri and others, 1977). However, Van Donsel and others (1967, p. 1362) cite a U.S. Public Health Service analysis of waterborne-disease outbreaks occurring in the United States and Puerto Rico from 1946 to 1960: "At least 29 outbreaks involving 9,233 cases were associated with storm-water runoff, caused either by rainfall washing human and animal feces or sewage into wells, springs, streams, reservoirs, and open water mains, or by wide-spread flooding of individual and public water systems."

Fate of Microorganisms in Stormwater

As long as stormwater containing bacteria and viruses is confined to gutters, ditches, and storm sewers, it poses little threat to humans; but when it enters the receiving body, the viruses and bacteria may become a problem. Fortunately, bacteria that are adapted to live in or on a human host (pathogens and fecal indicators) are not well adapted for competition and survival in the aquatic ecosystem. McFeters and others (1974) placed enteric bacteria in well water (temperature 9.5°C to 12.5°C) inside membrane dialysis chambers. The average time required for a 50-percent reduction in the initial population (half-time) was 17 hours for fecal coliforms and 22 hours for enterococci. Among a group of pathogens studied, the longest half-time was 26.8 hours (*Shigella* sp.) and the shortest, 2.4 hours (*Salmonella* sp.). The authors observed no growth of the bacteria studied.

Although viruses will not grow outside of their hosts, they apparently do not disappear as rapidly as bacteria. Studies cited by Vaughn and others (1979) indicate that human viruses may persist in aquatic environments for long periods of time, as much as 154 days in one example.

Van Donsel and others (1967) studied the survival of indicator bacteria in soil using tracer strains that could be identified. They found that in summer the fecal coliforms survived slightly longer than the fecal streptococci (3.3 vs. 2.7 days for 90 percent reduction), whereas in spring and winter, the fecal streptococci survived much longer than the fecal coliforms. In autumn, survival time was the same for both types, and the shortest survival time for both groups was at an exposed site in summer. There was some evidence of aftergrowth of nonfecal coliforms. Van Donsel and others (1967, p. 1362) pointed out that this aftergrowth might "contribute to variations in the bacterial count of storm runoff which have no relation to the sanitary history of the drainage area." The problem of aftergrowth of fecal coliforms deposited by ducks on a beach in Wisconsin (Standridge and others, 1979) has already been described.

Generally, soil is effective in removing bacteria and viruses. Gerba and others (1975) have recently presented an extensive review of this topic. Factors that affect the survival of bacteria in soil are moisture content and moisture-holding capacity, temperature, pH, sunlight, availability of organic matter, and competition and antagonism from soil microflora. Movement of bacteria into the ground water is limited by the straining of bacteria at the soil surface and by adsorption to clays in the soil. Deterioration of ground-water quality from the downward movement of microorganisms is likely to be a problem only where the water table is near land surface.

A recent study (Yeager and O'Brien, 1979) concluded that drying is the most important factor in the inactivation of viruses in soils. Virus removal by soils depends mainly on adsorption by clays (Gerba and others, 1975). However, viruses that are adsorbed to clays can retain their infectivity (Schaub and Sagik, 1975). Binding of virus particles to clay depends on the net charge of the virus, which is in turn dependent on pH. Unfortunately the viruses are not bound irreversibly to the soil particles and may be desorbed by rainwater (Landry and others, 1979). Wellings and others (1975) have isolated viruses from ground water under a recharge basin after a period of heavy rain.

The movement of pathogens to ground water may be a problem in any area where storm runoff is stored in basins or used for artificial recharge. The largest source of information on this hazard is the literature on artificial recharge of ground water and land treatment of sewage. Both practices present a greater potential contamination problem than storm runoff since pathogens will likely be applied to the same land area in greater numbers over a long period of time. Several studies indicate that ground-water contamination from artificial recharge is possible. For example, Vaughn and others (1978) found viruses and bacteria in ground water beneath recharge basins at two Long Island, N.Y., sewage-treatment plants where the depth to water was 18 and 30 feet, but found no viruses under basins that were 80 feet above the water table. However, even where viruses and bacteria were found in the ground water, the numbers had been considerably reduced during passage through the soil. Although the numbers of bacteria and viruses are likely to be much lower in urban stormwater than in treated wastewater, Vaughn and Landry (1977) recovered viruses from ground water beneath a stormwater-recharge basin on Long Island. The source of these viruses has not been definitely established; however, their presence suggests the possibility of ground-water contamination by stormwater.

METHODS AND PROCEDURES

A detailed discussion of the methods used to grow and enumerate microorganisms in stormwater is beyond the scope of this paper. Other sources (American Public Health Association, 1975; Greeson and others, 1977) give much information on the procedures used to cultivate indicator bacteria (fecal coliforms and fecal streptococci). Pathogenic organisms are more difficult to work with, and great care must be exercised to avoid laboratory-acquired infections. Viruses must be grown inside a living host cell, and virus concentration and propagation are complicated processes that should be left to specialists in virology.

Setting aside any discussion of the methods used in processing a water sample, an important issue remains. If the sampling program is improperly designed or if the water sample is collected improperly, the data will be at best meaningless or at worst misleading. Some of the points that must be considered in sampling are addressed below.

Sample Collection

Stormwater

Water samples for microbiological analysis should be collected by hand in sterile containers by a trained individual (American Public Health Association, 1975). Two early reports (Burm and Vaughan, 1966; Weibel and others, 1964) mention allegedly sterile automatic-sampling devices for stormwater; however, the descriptions of the devices are somewhat incomplete and leave doubt as to the integrity of the sample. Almost all recent papers indicate that stormwater samples were collected by hand in sterile containers. This is obviously expensive and time consuming. It might be argued that since stormwater is already heavily contaminated, slight additional contamination from the sampling device will be negligible. However, this is circular reasoning, and the true level of contamination in the stormwater can never be established by this approach. Another argument might be that fecal coliforms and fecal streptococci are unlikely to be in the air or in washed bottles and automatic samplers. This argument may seem reasonable; nevertheless, microbiological data reported for samples collected under nonsterile conditions will always be suspect.

Receiving Waters

Collecting stormwater at the outfall of a storm drain will give the best estimate of the microbiological quality of the runoff. However, to judge the impact of stormwater on some environments, it may be necessary to sample a stream or other receiving water. In lakes, reservoirs, deep rivers, and estuaries, bacterial abundance may vary transversely, with depth, and with time of day. The time of day variable was pointed out by Davis and others (1977) in their study of a stream receiving storm runoff. In their study, bacterial densities were not constant in stream waters over a period of 24 hours, even during constant low-flow conditions. Their data showed highly variable batch flows, and they concluded that grab samples reflected the condition and content solely at the time at which the sample was taken. A possible solution to this problem might be to collect samples upstream from the discharge point to serve as a control for each stormwater sample taken.

Storage of Stormwater Samples

The rule when working with microorganisms is that the more rapidly the samples are processed, the more accurate the results will be. Bacterial samples must be refrigerated if held more than 1 hour before processing; the maximum transport time is 6 hours, and the samples must be processed within 2 hours after arrival at the lab (American Public Health Association, 1975; Greeson and others, 1977).

A measure of the potential for survival of various bacterial groups in stormwater is presented in figure 3, taken from Geldreich (1976). In that study, urban stormwater was collected and filtered to remove the "native" bacterial populations, and pure cultures were added to the water. The culture densities were then determined over a period of 2 weeks. Problems with this approach are that the laboratory cultures might have declined at a different rate than those occurring naturally in stormwater, and also that the added bacteria might have survived longer because they did not have to compete with the "native" population.

As figure 3 indicates, the apparent number of bacteria decreased significantly with storage time, and different types of bacteria died off at different rates. In general, the bacteria survived longer when stored at 10°C (fig. 3A) than at 20°C (fig. 3B), but even when stored at 10°C (fig. 3A), 50 percent of the *Salmonella typhimurium* died within 2 days. Fecal coliforms survived slightly longer, with 50 percent surviving for 3 days. The rate of decline of fecal streptococci depended on the species under consideration. *Streptococcus faecalis* died off relatively slowly, with 50 percent surviving for 14 days, whereas *Streptococcus bovis* (a component of animal feces but not human feces) disappeared within the first day at 10°C and within 3 days at 20°C. These data clearly support the general rule that rapid processing of microbiological samples is of utmost importance.

Design of Sampling Program

When designing a program to determine the microbiological quality of stormwater, one must consider the following points:

(1) The number and type of bacteria in runoff will be influenced by the intensity of the storm, duration of the storm, season of the year, and land use of the area being drained. Therefore, great care must be taken in any extrapolation from one area to another or from one storm to another.

(2) Provision must be made for a nearby laboratory to handle all samples. Samples must be processed within hours of collection.

(3) No good predictor or indicator for the presence of pathogens in stormwater is known. Pathogenic bacteria and viruses seem to occur in a random fashion throughout the storm.

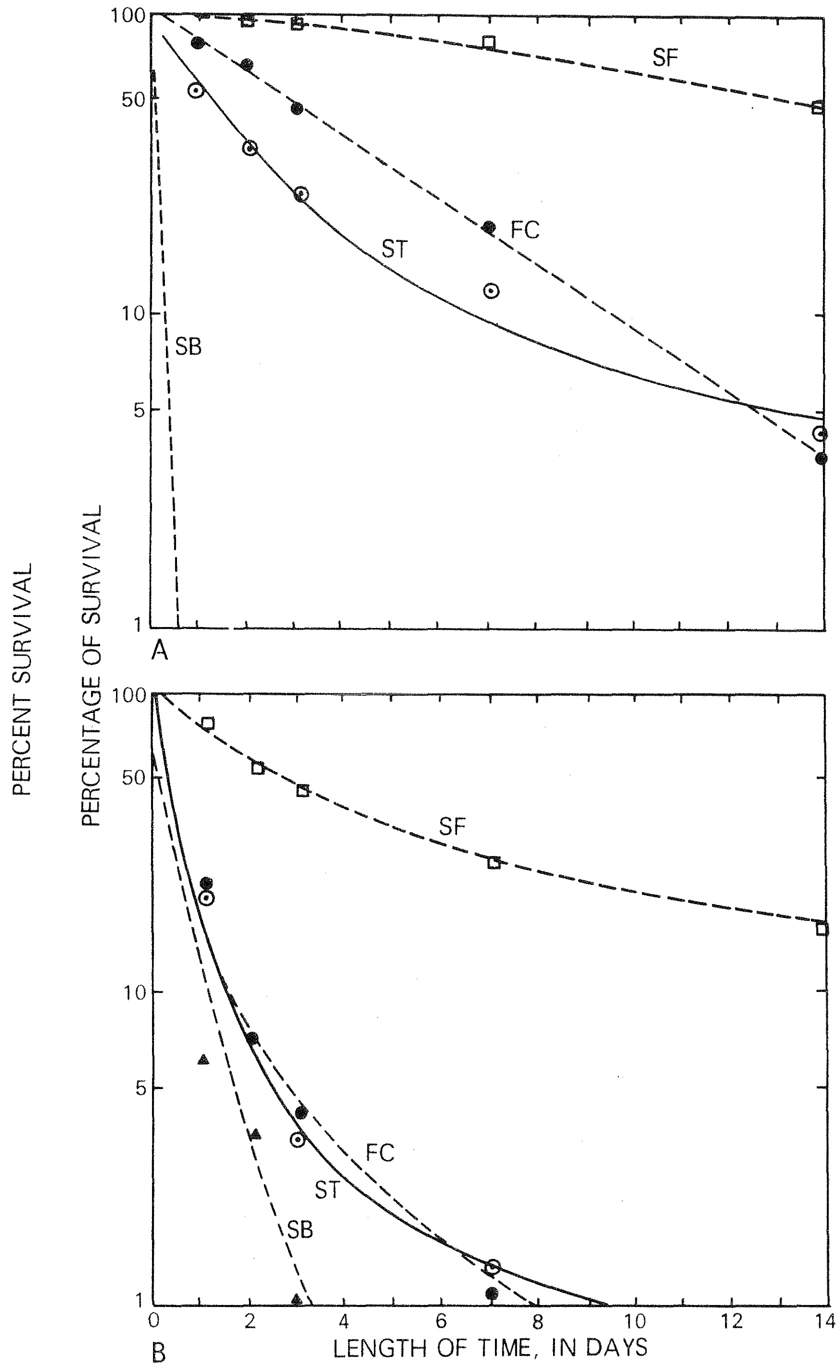

Figure 3.--Persistence of selected bacterial strains in stormwater: A, stored at 10°C; B, stored at 20°C. SF = Streptococcus faecalis, FC = fecal coliform bacteria, ST = Salmonella typhimurium, SB = Streptococcus bovis. (Modified from Geldreich, 1976; reprinted with permission from Critical Reviews in Environmental Control; copyright by The Chemical Rubber Co., CRC Press, Inc.).

SUMMARY AND CONCLUSIONS

Information obtained from recent investigations of microorganisms in stormwater has been reviewed and summarized with the intent of providing assistance to those planning studies of stormwater. The following conclusions are indicated by this review:

1. Most stormwater contains relatively large numbers of total coliforms, fecal coliforms, and fecal streptococci.

2. Most of the total coliforms in runoff are native soil organisms.

3. The source of most fecal coliforms and fecal streptococci in runoff is probably wild or domestic animals.

4. A report from Baltimore, Maryland has documented the occurrence of hazardous organisms in urban runoff. The source of this contamination is unknown.

5. Stormwater probably presents little direct threat to human health because it is not ingested, but it can produce other negative effects such as contamination of beaches or shellfish.

6. Contamination of surface water by bacteria in stormwater is of short duration because indicators and pathogens die out rapidly in the aquatic environment. Viruses apparently do not disappear as rapidly as bacteria and may be concentrated by shellfish.

7. Bacteria and viruses deposited on soil by stormwater are inactivated by drying, competition from soil microflora, and a variety of other processes. The movement of pathogens to ground water is likely to be a problem only where the water table is near land surface.

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