Microbiological Monitoring for Water-Quality Assessment

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ABSTRACT

The weakest link in the chain of events leading to production of reliable microbiological-monitoring data is a poor or inadequate sample. This results primarily from diversity of environmental conditions from which a sample must be collected. In surface waters, affinity of microbiological organisms for suspended particles necessitates that sampling procedures be designed to collect a representative sample of the water-sediment mixture. The key problem and the challenge to microbiological monitoring is production of a sterilizable, depth-integrating sampler that will accommodate the disparity of sediment changes in streamflow.

Implicit in these definitions is measurement of some environmental factor over time. For the purpose of this paper, “monitoring” is defined as repetitive measurement or sampling for whatever the intended purpose. Excluded from this definition is the concept of biotic monitoring, the term applied to a wide variety of techniques that use the response of organisms to environmental conditions.

NEED FOR STANDARDIZATION

Because of the involvement of many persons representing many groups and agencies, any program or venture that involves microbiological monitoring requires standardization so the data obtained from all areas and sources will be comparable. The necessity for standardization for both sample collection and sample analysis is emphasized by enactment of several public laws, existence of national water-quality networks, and presence of centralized storage and retrieval data systems.

There currently exists the National Water Data System consisting of all generally available water data including those collected by both Federal and non-Federal entities. According to the 1976 Catalog File of the U. S. Geological Survey's Office of Water Data Coordination, 33,408 water-monitoring stations were operated in the United States during the 10-year period of 1966-1976. Of these stations, bacteria were monitored at 12,902 stations by 132 Federal and State agencies. Principal Federal agencies included the U.S. Geological Survey with 2,233 stations, Environmental Protection Agency with 609 stations, U.S. Army Corps of Engineers with 468 stations, and the U.S. Forest Service with 264 stations. Currently bacteria are being monitored at 9,964 stations; 7,531 stations are on surface waters and 2,433 stations are on ground waters.

Principal national networks involved with the monitoring of water quality, including microbiological quality, are the National Stream-Quality Accounting Network (NASQAN); the Benchmark Network...
both operated by the U.S. Geological Survey; and the National Water-Quality Surveillance System (NWQSS) (27,28) operated by the Environmental Protection Agency. NASQAN, presently consisting of 345 stations and an anticipated size of 540 stations, is designed to provide information on year-to-year variations in water quality and quality and to document changes with time in water quality throughout the Nation. The Benchmark Network consists of 56 measuring sites in small basins where the hydrology is relatively unaffected by man and thus is not likely to change over the years. It is designed to document the range of "natural" streamflow and water-quality conditions and to provide a basis for understanding the natural forces controlling them.

NWQSS is designed to monitor the progress in the Nation's effort to abate water pollution. The approximately 120 stations of the network are situated in paired configurations to observe changes in quality of water passing through municipal-industrial and agricultural areas.

The Environmental Protection Agency recently has proposed a basic water-monitoring program for the United States (7,26) which includes the three forementioned networks. The new program will emphasize those activities that aid (a) development of national trend assessments, (b) control of toxic substances, (c) waste treatment facilities process, (d) compliance assurance within the National Pollutant Discharge Elimination System, (e) assessment of non-point source pollution, (f) State water-quality management planning, and (g) State monitoring programs (7).

The national water-quality data systems receive information from a multitude of individuals and agencies. Well-known systems include the storage and retrieval system or STORET of the Environmental Protection Agency and the water storage and retrieval system or WATSTORE of the U.S. Geological Survey. In addition, the National Water Data Exchange, called NAWDEX, recently has been established under the auspices of the U.S. Geological Survey. NAWDEX is an interagency program to assist users of water information in the identification, location, and acquisition of needed data (6).

The reasons for standardization need not be dwelled upon in that it is paramount in the minds of all individuals involved with the collection and analysis of water-quality samples. Results of several attempts at standardization in aquatic microbiology are available.

In 1969, on the recommendation of the Coordinating Council of the International Hydrological Decade, an Inter-Agency Panel on Standardization in Hydrology was established. The most well-known product from the Inter-Agency Panel was the International Standards of Drinking Water (32). Despite international attempts at standardization, the World Meteorological Organization (33) recently commented, "A lot of work toward international standardization in hydrology and its related fields has been carried out already, but much remains to be done, even for simple basic measurements."

In 1972, a report entitled, Recommended Methods of Water Data Acquisition (27) was released by the Federal Inter-Agency Work Group on Designation of Standards of Water Data Acquisition. The interagency activity and the document were endorsed by the Federal Advisory Committee on Water Data. The manual currently is being rewritten as the National Handbook for Water Data Acquisition with extensive review by the nonfederal sector.

Other notable attempts at standardization include authoritative, widely used references such as Standard Methods for the Examination of Water and Wastewater, published jointly by the American Public Health Association, the American Water Works Association, and the Water Pollution Control Federation (1); and the Book of ASTM Standards. Part 31 Water revised and published annually by the American Society for Testing and Materials (2). In addition, several publications by Federal agencies are in widespread use. The list includes Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples (12) by the U.S. Geological Survey and the Handbook for Evaluating Water Bacteriological Laboratories (11) by the Environmental Protection Agency. Soon to be released will be EPA's Microbiological Methods for Monitoring the Environment. I. Water and Wastes (4).

The most recent, and perhaps most notable, move for standardization in the United States was the recently published Water Programs—Guidelines Establishing Test Procedures for the Analysis of Pollutants—Amendments (25). It recommends the specific procedures by which measurements of 115 physical, chemical, and biological characteristics (including five for microbiological determinations) of water will be made. The document was prepared pursuant to section 304(g) of the Federal Water Pollution Control Act Amendments of 1972 (Public Law 92-500, October 18, 1972).

The standardization of sampling and analytical techniques is necessary for acquiring the valid and interrelated data needed for meaningful assessments of the occurrence, distribution, and fate of water quality constituents. Although the precision, reproducibility, and quality control used in doing laboratory analyses is of a high degree, the reported data are no better than the confidence that can be placed in the representativeness of the sampling (9).

Use of adequate microbiological samplers and sampling techniques has not been emphasized in the technical literature. We are all familiar with the common statement that appears in methods manuals, "Take a representative sample." Undoubtedly, the weakest link in the chain of events leading to the production of microbiological data is an inadequate sample.

The problem is complex and has many pitfalls. This results primarily from the diversity of environmental
conditions from which a sample must be collected. A sampling procedure used in a flowing stream is not suitable for sampling a well; a sampling procedure used in lakes and reservoirs is not suitable for sampling a treated public water supply or industrial and municipal wastes effluents.

Even if it were technically possible to define or set minimum standards for collection of a representative sample from all possible hydrological situations, it is beyond the constraints of this paper to discuss the many fine points. Rather, it is the intention to convey some general guidelines and basic understandings necessary for developing the proper procedures for the collection of representative microbiological samples.

**MICROBIOLOGICAL SAMPLING**

Microbiological sampling can be defined as all the acts and procedures that must be done before delivery of a water sample to the analyzing laboratory or all the procedures that must be done before delivery of a water sample to the analyzing laboratory or all the methods by which data are generated under field conditions. This includes site selection, field instrumentation, sample collectors, sample collection, and preservation.

Underlying and paramount to all the approaches to microbiological sampling is the intended use of the data after they are acquired. For example, data obtained for defining quality of a mass of water generally are not suitable for documenting spatial and temporal variations. In addition, data collected for documenting trends in a body of water are usually unsuitable for determining conformance of waste discharges with pollution control standards.

The initial step, then, begins with a statement of the problem or intent. After the objective has been clearly stated, the data needed to fulfill the objective can be determined. Determination of the data needs is followed by a designed approach to include such items as the number of required samples, sampling locations, sampling frequency, and sampling techniques. Realistically, the final step in this orderly process is a consideration of cost constraints.

When a new water quality station is established, its general location and the frequency of sample collection is set by the data needs, type of investigation, purpose of the study, and anticipated variation in microbiological characteristics. Selection of the exact sampling site will probably depend upon a combination of factors including accessibility, availability of other information, and uniformity of water quality at the site. Even though an individual station is established to meet a specific need for information, the possibility of placing and operating it to supply data for other studies should not be overlooked.

Water samples are collected and analyzed to ascertain characteristics of a body or mass of water. The sample is usually only an infinitesimal part of the total volume and, therefore, is representative of the total mass only to the degree that uniformity of composition exists within the total mass. Uniformity of composition can be assumed for many sources of water including public water supplies and municipal and industrial wastes effluents. Methods for sampling of these water are sufficiently documented by the American Public Health Association and others (1), and Greeson et al. (12).

In their natural state, surface waters are subjected to forces that promote mixing and homogeneity. The fact that such tendencies exist, however, is not sufficient cause for assuming that a body of water is so well mixed that no attention to sampling techniques is required. In most instances, a body of water may not have uniform composition because of local conditions.

Customarily, surface waters have been sampled by filling a container held just beneath the surface of the body of water. The sample is commonly referred to as a dip sample or grab sample. If the microbiological characteristics are homogeneous throughout the cross-section of a stream, one dip sample taken anywhere in the cross section will adequately define water quality.

If the microbiological characteristics are not uniform throughout the cross section, a sample representing the average composition of the stream must be taken. In addition, and in further emphasis of this procedure, it is known that many microbiological constituents are transported in streams attached to suspended particles. Jannasch (14) found that in the Nile River only 0.02-0.04% of the contained bacteria were truly planktonic or freeliving. Similar findings were observed by Wuhrmann (34) who found that only 0.005% of the bacteria in an experimental artificial stream were free-living, and when sewage was introduced into the stream, only 0.9% of the bacteria were free-living. Bardtke (3) observed that 10-30% of the bacteria were free-living in the lakes of the Stuttgart region.

A number of the devices constructed and described for microbiological sampling were reviewed by Rodina (22). Some samplers have mechanical devices for removing a stopper at a desired depth and replacing it when the vessel has filled with water. Other samplers make use of capillary tubes, which are broken at a desired depth by a messenger, thus permitting the sample to be drawn into a sterile collecting device. A microbiological sampler designed by Niskin (19) consisted of a large metal hinge fitted with a sterile plastic bag and tube. When the sampler was tripped by a messenger, the tube opened aseptically as the hinge flipped open, and the water sample was drawn in.

Most commercially available microbiological samplers are based on the original design principle of ZoBell (35) as modified by Sieburth (23); that is, a sterile collapsed rubber bulb is lowered to a desired depth and tripped with a messenger. Water is drawn through a tube by the action of the rubber bulb.

The aforementioned samplers, while innovative in design, will collect a sample of water from a point at a predetermined depth. However, a point sample of this type is a grab sample and will not represent the average.
composition of a stream. The data obtained from such a sample can be misleading and erroneous as to the true bacterial density in a body of water.

Theoretically, a sample representing the average composition of a stream can be obtained by compositing several depth-integrated samples. Each sample should be of equal volume and should be collected at transects of equal flow in the cross-section.

For sampling throughout the vertical profile in streams, depth-integrating samplers are used. The simplest depth-integrating sampler may consist only of a mechanism for holding and submerging the container. When the container is lowered at a uniform rate, water is admitted throughout the vertical profile. One such device is simply a weighted glass bottle that can be lowered by a nylon rope.

If the person taking the sample could be assured that the bottle was lowered to the bottom and raised to the surface at a uniform rate, he would have roughly approached collection of what is known as a depth-integrated sample (9).

A true depth-integrated sample, however, is collected by means of a sampler which integrates discharge as a function of depth (13,24). The velocity of flow in a stream, as well as the size and distribution of sediment particles, vary both vertically and horizontally (6,30). Depth integration is used to collect a water-sediment sample that is weighted according to the velocity at each increment of depth.

One of the best sampling techniques currently accepted by hydrologists for use in such situations is the equal-transit rate (ETR) method (13). In this method, the standard suspended-sediment sampler is used to collect a discharge-weighted sample. Samples are taken at a number of equally spaced verticals in the cross-section. The transit rate of the sampler, which is the rate of movement of the sampler from the water surface to the streambed and back to the surface, should be the same at all verticals. Samples collected in each vertical are composited into a single sample that is representative of the entire flow in the cross-section. According to Feltz and Culbertson (9), the composite sample of the water-sediment mixture collected in this manner is a representative sample that is velocity- and discharge-weighted.

Several depth-integrating samplers are in widespread use in water-quality studies and have been described by the Subcommittee on Sedimentation (24). However, paramount problems exist with the use of currently available depth-integrating samplers for collection of microbiological samples. First, the samplers cannot be adequately sterilized and, second, equal-volume samples collected for centroids of equal flow in a cross section cannot be composited under aseptic conditions. The latter problem can be rectified by determining the density of bacteria in the individual samples and compositing the results. Though technically acceptable, the costs and manpower constraints would make this approach prohibitive or difficult at best.

The key problem and the challenge to microbiological monitoring is the production of a sterilizable, depth-integrating sampler designed to accommodate the disparity of sediment distribution as related to variations in depth and cross section and to changes in streamflow. Until such a sampler has been designed, tested, and made readily available, the data produced in microbiological-monitoring programs involving surface waters can be considered of questionable accuracy, regardless of the notable advances that are taking place in the state-of-the-art of analytical procedures.

CONCLUSIONS

As stated in Standard Methods (1), it is impossible to give directions for sampling techniques to cover all conditions which will be encountered, and the choice of the technique must be left to the sample collector. There is not now, nor is there ever likely to be, a single method of sampling which can be used to describe all microbiological aspects of the hydrologic environment. There are many different approaches to evaluating microbiological quality and each is dependent on the intended use of the data after they are collected.

By carefully following a few simple guidelines such as (a) defining the intended use of data before they are collected (b) evaluating the hydrologic situation from which the sample is to be collected (for example, homogeneity of flow and suspended sediment concentrations), (c) giving consideration to measurement of significant properties, and (d) using common sense, it will be possible to obtain a sample that is truly representative of the whole.

The state-of-the-art technology is a changing scene, in that many of the methods that are in use today may become obsolete tomorrow. In all instances, the quality of any data is no better than the method and representativeness of the sample.

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REFERENCES


