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FECAL COLIFORM ANALYSES - METHOD EVALUATION FOR STRESSED ORGANISMS

by

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ABSTRACT

No significant difference was found between two tests for fecal coliform densities using water samples from the treated sanitary waste outfalls at the Savannah River Plant, a nuclear materials production site located near Aiken, S. C. These two methods of concern were the most probable number index (MPN) and the membrane filtration procedure (MF). The MPN method is the accepted method for determining fecal coliform densities in chlorinated effluents, but requires more work than the MF procedure. Per Microbiological Methods for Monitoring the Environment (1978) by EPA, any decision to use the MF test for stressed organisms requires parallel testing with the MPN test. The MPN index is the number of fecal coliform bacteria that, more probably than any other number, would give the results shown by laboratory examination. It is not an actual count of coliform bacteria. The MF procedure is a direct plating method and the colonies are directly counted.

INTRODUCTION

The Savannah River Plant is a controlled-access area of approximately 780 square kilometers (192,700 acres) near Aiken, South Carolina. It is a major installation of the Department of Energy established in the early 1950's for the production of nuclear materials for national defense. Plant facilities which can be characterized as heavy industry, consist of five production reactors (4 operational, one in standby status), electrical and steam generating plants, two chemical separation facilities, fuel and target fabrication facilities, research laboratories, and support and administrative facilities.

Water quality monitoring has been an integral part of the environmental monitoring program since analysis of Savannah River water began in 1959. A new National Pollutant Discharge Elimination System (NPDES) permit (SC 0000175) was issued to SRP by the South Carolina Department of Health and Environmental Control (SCDHEC). The single, site-wide permit gives state approval for discharges of both sanitary waste water and industrial waste water. The new permit became effective January 1, 1985 and supersedes two individual permits issued previously.

Monthly sampling for fecal coliform of each sanitary waste water outfall is required. Disinfection of the sanitary waste water outfalls is achieved by chlorination or bromination. Chlorination and bromination create a stressful environment for fecal coliform organisms.

MOTIVATION FOR EVALUATION

The Environmental Protection Agency (EPA) requires parallel testing of the MF procedure with the MPN procedure if stressed microorganisms are checked. EPA feels that the single-step MF fecal coliform procedure may produce lower results than those obtained with the multiple-tube procedure. The disinfection process may affect recovery of fecal coliforms on the membrane filter [1].

Fecal coliform organisms are usually considered to be non-pathogenic intestinal organisms; however, they indicate the possible occurance of pathogenic organisms. Fecal coliform density was chosen as an indicator for water quality because of

- statistical correlations among coliform density, salmonella density, and typhoid death rate.
- survival factor in natural waters [2].

It is SRP policy to protect the environment. At SRP, eleven sanitary waste treatment plants empty into natural streams. The sanitary waste treatment outfalls are monitored for fecal coliform organisms each month.

The purpose of the method evaluation was to ensure that analytical results adequately reflected the quality of the treated water from the sanitary waste plants.

DESCRIPTION OF METHODS

SAMPLING

Grab samples were taken consecutively in discrete sterile containers on the days indicated in the Tables. Samples were placed on ice and analyzed the same day of collection.

MF METHOD

The membrane filtration method involves the use of enriched lactose medium and depends on temperature for selectivity. Different volumes of water sample were filtered through a membrane filter. The filter was placed in a culture dish containing 2 ml MFC medium and incubated for 24 $\pm$ 2 hrs at 44.5 $\pm$ 0.2 ^{o}C. Only blue colonies were counted [4]. Reagent blanks and a positive control ensured the quality of the test. Calculations followed guidelines established by EPA [1].

MPN METHODS

The Presumptive and Confirmed Tests were used for the MPN Method. Culture tubes containing inverted vials were used for gas production checks. In the Presumptive Test, culture tubes were inoculated with 10, 1, and 0.1 ml of sample (5 tubes for each, 15 total). The tubes were incubated at 35^{o}C and then examined after 24 and 48 hours for gas formation and/or growth. As soon as gas formation was detected, positive tubes were inoculated into Confirmed Test (EC) media at 44.5 ^{o}C. The confirmed test was examined after 24 and 48 hours for gas production and/or growth. Blanks and positive controls ensured the quality of the test. Calculations were based on Table 908:V of Standard Methods [5].

REVIEW OF DATA

Method evaluations were completed during 1982-1983 and again in 1985. Results are reviewed separately.

1982-1983

The MF procedure was an acceptable alternate method for analyzing fecal coliform organisms. Raw sample data are in Table I. Suspected outliers were checked using the critical T values for sets of 5 replicates at a 0.01 significance level (4 replicates for H). Since the calculated value for t was 1.76, the two-tailed t test demonstrated that the methods do not produce significantly different means.

1985

The data in Table II demonstrate the the MPN method does not produce significantly different results than the MF method for the samples checked. Because the MPN method detects stressed organisms [1], it is important to demonstrate that large populations of stressed organisms are not released in the effluent.

The original design of the test involved the use of the two-tailed t-test to evaluate differences of paired data. The set of paired data which demontrated measurable differences was not large enough for statistical evaluation. The treatment process of the sanitary waste treatment plants prevented a large population of fecal coliform organisms; however, prevention of fecal coliform organisms in the effluent is the intent of the treatment.

SUMMARY

The MF procedure adequately monitors chlorinated and brominated sanitary waste effluent from the Savannah River Plant.

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TABLE I

RAW SAMPLE DATA FROM THE ANALYSIS OF
CHLORINATED AND BROMINATED SEWAGE TREATMENT PLANT EFFLUENTS

SAMPLE	DATE	METHOD	REPLICATES IN COUNTS/100ml				
			I	II	III	IV	V
A	11/30/82	MF	2	12	6	< 2	4
		MPN	2	33	8	< 2	< 2
D	01/11/83	MF	<2	< 2	<2	< 2	< 2
		MPN	2	2	<2	< 2	< 2
F	01/18/83	MF	<2	< 2	<2	< 2	< 2
		MPN	<2	< 2	<2	< 2	< 2
G	01/25/83	MF	<2	< 2	<2	< 2	< 2
		MPN	<2	< 2	<2	< 2	< 2
H	02/01/83	MF	<2	< 2	4	70	TNTC
		MPN	<2	< 2	130	240	≥1600
K	02/08/83	MF	20	16	18	20	16
		MPN	<2	2	11	8	4
L	02/15/83	MF	2	< 2	<2	< 2	< 2
		MPN	<2	2	<2	< 2	< 2
P	02/22/83	MF	42	8	44	38	72
		MPN	50	21	34	23	23

TABLE II

RAW SAMPLE DATA FROM THE ANALYSIS OF CHLORINATED AND BROMINATED SEWAGE TREATMENT PLANT EFFLUENTS

SAMPLE	DATE	METHOD	REPLICATES IN COUNTS/100ml				
			I	II	III	IV	V
A	3/19/85	MF	<4	<4	<4	4	130
		MPN	2	2	<2	2	23
C	3/19/85	MF	<4	<4	<4	<4	<4
		MPN	2	<2	2	8	<2
D	4/29/85	MF	<4	<4	<4	<4	<4
		MPN	<2	2	2	<2	<2
F-I	5/20/85	MF	5000	2500	<4	<4	<4
		MPN	≥1600	900	<2	<2	<2
F-7	6/10/85	MF	<4	<4	8	<4	<4
		MPN	<2	<2	2	<2	<2
G	8/21/85	MF	<4	<4	<4	<4	<4
		MPN	<2	<2	<2	<2	<2
H	6/17/85	MF	<4	<4	<4	<4	<4
		MPN	<2	<2	<2	<2	<2
K	6/24/85	MF	<4	<4	<4	<4	<4
		MPN	<2	<2	<2	<2	<2
L	7/15/85	MF	<4	<4	<4	<4	<4
		MPN	<2	7	<2	<2	<2
P	7/29/85	MF	<4	<4	<4	<4	<4
		MPN	<2	<2	<2	<2	<2
S	8/26/86	MF	<4	<4	<4	<4	<4
		MPN	<2	<2	<2	<2	<2