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Distribution and Significance of Fecal Indicator Organisms in the Upper Chesapeake Bay

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Total viable aerobic, heterotrophic bacteria, total coliforms, fecal coliforms, and fecal streptococci were enumerated in samples collected at five stations located in the Upper Chesapeake Bay, December 1973 through December 1974. Significant levels of pollution indicator organisms were detected at all of the stations sampled. Highest counts were observed in samples collected at the confluence of the Susquehanna River and the Chesapeake Bay. The indicator organisms examined were observed to be quantitatively distributed independently of temperature and salinity. Counts were not found to be correlated with concentration of suspended sediment. However, significant proportions of both the total viable bacteria (53%) and fecal indicator organisms (>80%) were directly associated with suspended sediments. Correlation coefficients (r) for the indicator organisms examined in this study ranged from $r = 0.80$ to $r = 0.99$ for bottom water and suspended sediment, respectively. Prolonged survival of fecal streptococci in most of the sediment samples was observed, with concomitant reduction of the correlation coefficient from $r = 0.99$, fecal streptococci to total coliforms in water, to $r = 0.01$, fecal streptococci to fecal coliforms in sediments. The results of this study compared favorably with fecal coliforms: fecal streptococci ratios for the various sample types. Characterization of organisms beyond the confirmed most-probable-number procedure provided good correlation between bacterial indicator groups.

The Chesapeake Bay, including its tributary tidal drainages, represents the largest estuarine system on the U.S. Atlantic Coast. The Upper Chesapeake Bay, extending from Annapolis, Maryland, to Havre de Grace, Maryland, is a dynamic estuarine habitat greatly influenced by the Susquehanna River, which contributes 50% of the total freshwater input into the Bay proper and represents the major source of suspended material entering the Upper Bay (3). The Upper Bay varies from a completely freshwater habitat at Havre de Grace, at the head of the Bay, to approximately 12‰ salinity in the Annapolis region.

Shellfish industries are located within the boundaries of the Upper Bay; in addition, the Upper Bay also serves as a major recreational facility for the surrounding metropolitan areas. The bacteriological quality of Chesapeake Bay water strongly affects such uses. Sewage disposal projects and dredging activities involving Baltimore Harbor sediments have the potential for seriously degrading the water quality, thereby effectively reducing the resource potential of the Upper Bay.

Comparatively few studies on the distribution and flux of fecal indicator organisms in estuarine waters have been reported, arising, in part, from the fact that estuarine water is not presently a source of potable water. Therefore, ingestion of contaminated water is not a health hazard involving estuaries, in general. Nevertheless, high levels of fecal indicator organisms present in Chesapeake Bay for sustained lengths of time and found to be associated with the presence of pathogenic organisms is clearly an unacceptable situation.

The presence of coliform organisms in water has been attributed to influxes of allochthonous bacteria from waste discharges and surface water drainage. The proportion of fecal coliforms (FC) comprising the total coliform (TC) population or the total viable bacterial population can be used as an index of pollution from sanitary wastes of human or other animal origin (10, 13). The FC:fecal streptococci (FS) ratio has been suggested as an indicator of human versus animal origin of sanitary wastes and of the time interval between initial contamination and bacteriological sampling (5).

Various indexes can be derived from measuring specific bacterial populations and their quantitative comparison. However, several environmental variables affect the numbers of

TC, FC, and FS in the aquatic environment, namely predatory organisms (8), temperature, toxic chemicals and chelating agents (11), sediment (6), salinity and nutrient richness (7), and tides and frequency of sampling (2). The methods used for quantitative estimation of indicatory organisms are important, as is the extent to which suspected indicator organisms are characterized, i.e., the validity of the confirmed test for the given indicator species.

This study, therefore, was undertaken to investigate the environmental quality of the Upper Chesapeake Bay and to study those parameters influencing the microorganisms classically employed as indicators of public health significance.

MATERIALS AND METHODS

Samples. Samples were collected from four stations located in the Upper Chesapeake Bay and from one station on the Susquehanna River below Conowingo Dam (Fig. 1). The majority of the Chesapeake Bay samples were collected from the R/V RIDGELY WARFIELD. However, samples collected at station 1a, at the northern end of the Bay, were taken from the R/V PRITCHARD. All of the water and suspended sediment samples for bacteriological analysis were collected with sterile Niskin sampling bags. A Ponar grab was used for sampling sediment.

Dissolved oxygen (YSI oxygen meter, model 51A, Yellow Springs Instrument Co., Yellow Springs, Ohio), salinity, and temperature (Beckman Salinometer, model RS 5-3, Beckman Instruments Inc., Cedar Grove, N.J.) were determined concurrently with the sampling of water and sediment.

Media. An estuarine salts solution (6.7‰ salinity) composed of NaCl, 5.0 g/liter, KCl, 0.16 g/liter, and $MgSO_4 \cdot 7H_2O$, 1.5 g/liter, was used for dilution and resuspension of Upper Bay suspended sediments, sediments, and water. Upper Bay yeast extract agar was prepared by adding yeast extract ((Difco Laboratories, Detroit, Mich.), 1.0 g/liter, proteose-peptone (Difco), 1.0 g/liter, and agar (Difco), 20.0 g/liter, to the estuarine salts solution. Lactose broth (Difco), brilliant green lactose bile broth (Difco), EC broth (Difco), and eosin-methylene blue agar (Difco) were employed in the enumeration of TC and FC. Azide dextrose broth (BioQuest Laboratories, Cockeysville, Md.), ethyl violet azide broth (BioQuest), and M-enterococcus agar (BioQuest) were employed for enumerating FS.

Bacterial enumeration. A flow diagram describing the complete bacteriological analysis of a given sample is depicted in Fig. 2.

All samples collected were processed aboard ship immediately after sampling. Total viable counts of aerobic heterotrophic bacteria were performed employing Upper Bay yeast extract agar. Appropriate dilutions of individual samples were spread on agar plates, and the inoculated plates were held at room temperature and returned to the laboratory for incubation, usually 12 to 36 h after sampling and plating. Plates were incubated at 15 C for 4 weeks and were counted routinely at weekly intervals.

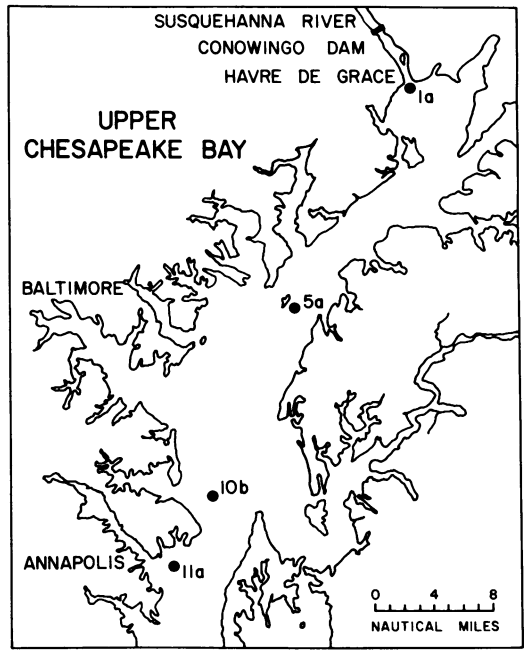

FIG. 1. *Upper Chesapeake Bay sampling stations.*

TC were estimated using a three-dilution, five-tube replication of lactose broth in a standard most-probable-number (MPN) series. Sample volumes of 10.0, 1.0, and 0.1 ml of bottom water or a 1/10 dilution of sediment were inoculated into the respective dilution tubes. Lactose MPN tubes were incubated at 35 C for 48 h, at which time they were examined for growth and production of gas. Lactose broth tubes positive for gas were reinoculated into brilliant green lactose bile broth and incubated for an additional 48 h at 35 C. Tubes showing acid and gas production were recorded as confirmed for TC and were included in the final MPN index of TC.

Positive lactose broth tubes were used to inoculate tubes of EC broth, incubated after inoculation in a constant temperature bath at 45.5 ± 0.5 C. After 24 h, tubes showing positive growth and gas production were recorded as confirmed FC for the purpose of the MPN computation.

Gas-producing and non-gas-producing cultures from lactose broth, brilliant green lactose bile broth, and EC broth were purified on eosin-methylene blue agar. IMViC test were run on all of the isolates.

Presumptive FS MPN were obtained following American Public Health Association (APHA) procedures (1). Three 5-ml tube replicates of azide dextrose broth were inoculated with 50.0-, 5.0-, and 0.5-ml samples. Using a modified APHA procedure, 50-ml samples were filtered through 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.), which were rolled up after filtration and placed in single-strength broth. Tubes showing growth after

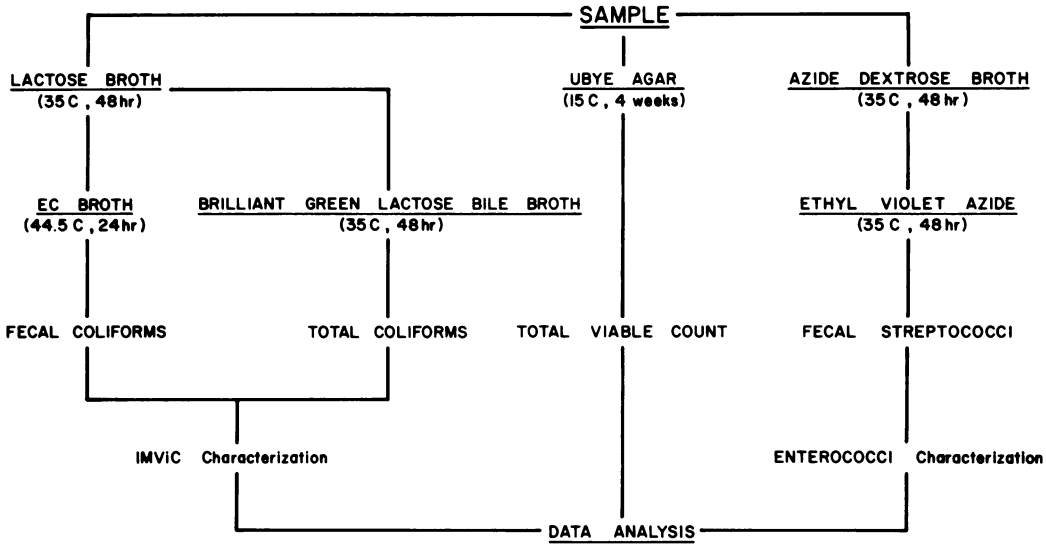

FIG. 2. *Scheme for the bacteriological examination of Upper Chesapeake Bay samples.*

incubation for 48 h at 35 C were inoculated into 10 ml of ethyl violet azide broth. After additional incubation for 48 h at 35 C, ethyl violet tubes showing turbidity and a pellet of purple sediment were recorded as confirmed FS.

Aliquots were taken for further testing from positive ethyl violet cultures. These were streaked on M-enterococcus agar. Small pink-to-red colonies appearing after incubation at 35 C were subcultured in brain heart infusion broth (Difco). Each isolate was purified and tested for morphology, Gram reaction, catalase, hippurate hydrolysis, starch hydrolysis, and growth at pH 9.6 and in 6.5% NaCl. Hippurate tests were performed according to the method of Facklam et al. (4). Starch hydrolysis was tested using 0.2% soluble starch incorporated in a nutrient agar (Difco) overlay which was flooded with Gram's iodine solution after visible growth appeared. Growth in nutrient broth buffered with 0.05 M Na_2CO_3 and brought to pH 9.6 in 6.5% NaCl was recorded.

Suspended sediment analysis. Suspended sediments collected for bacteriological analysis were harvested from fresh, aseptically collected water samples. Water was placed in sterile, polypropylene, screw-capped centrifuge tubes and centrifuged at a relative centrifugal force of $2,100 \times g$ for 15 min. The supernatant solution was aspirated, and the resultant brown flocculant pellets were resuspended in sterile estuarine salts solution. Total viable counts (TVC), FC, and FS were enumerated for both the water and the pellet suspension to determine the bacterial groups associated with suspended sediment.

Suspended sediment concentrations of overlying waters were determined by filtering 400 to 500 ml of Chesapeake Bay water through desiccated (48 h), tared (Mettler H analytical balance) to five decimal places, 0.6-μm cellulose membranes (Nucleopore Corp., Pleasanton, Calif.). Filters were rinsed with 3 volumes of distilled water to remove salts and were desiccated for 48 h. Membranes were reweighed and the suspended sediments were reported as milligrams per liter.

Water and suspended sediment samples were taken 2 m above the bottom. Sediment samples included the top sediment layer and approximately 10 cm of underlying sediment. During this course of sampling (December 1973 to December 1974), 102 samples of water and sediment were collected and analyzed for five Upper Chesapeake Bay stations (Fig. 1). Station C, Conowingo Dam, was included in the Upper Bay sampling schedule midway through the year, in an effort to determine the impact of the Susquehanna River upon the bacterial quality of the Upper Bay. Stations 1a, 5a, 10b, and 11a are transitional from a strictly freshwater habitat, influenced primarily by the Susquehanna River, to a classical estuarine habitat at Tolly oyster bar near Annapolis, Md. Various physical and chemical features of the samples collected from each station are given in Table 1. Marked temperature and dissolved oxygen differences between the various sampling stations were not noted. As expected, salinity increased proceeding down the Bay, from station 1a to stations 1b and 11a. Suspended sediment concentrations were significantly lower in the southern end of the Upper Bay, compared with stations 1a and 5a, both of which are directly impacted by suspended material carried by the Susquehanna River (3).

Statistical analysis. MPN were computed from standard APHA (1) tables. Since MPN estimates fall within wide confidence limits, no attempts were made to test individual MPN estimates for significance.

Seasonal data were entered and stored in the

TABLE 1. *Physical and chemical characteristics of the stations in Upper Chesapeake Bay that were sampled in this study*

Station	No. of measurements	Depth (meters)	Temp (C)		Salinity (‰)		Dissolved oxygen (mg/liter)		Suspended sediments (mg/liter)		
			Avg	Range	Avg	Range	Avg	Range	No. of measurements	Avg	Range
1a	8	8.5	13.2	2.5–26.8	0.0	0.0	8.9	6.3–13.0	7	27.0	12.7–43.3
5a	10	7.0	12.0	2.2–27.2	3.8	1.3–6.8	10.8	7.5–14.8	4	41.7	24.9–62.6
10b	10	10.5	12.5	4.5–23.8	14.0	5.0–17.4	7.9	4.2–11.7	6	15.6	1.2–37.6
11a	10	6.0	12.2	3.3–24.2	10.4	5.7–14.1	9.4	3.2–12.4	5	6.1	3.7–8.6

Cybernet 2000 data bank (Control Data Corp., Gaithersburg, Md.). Multiple correlation coefficient matrixes were generated on a CDC 6600 computer using a BASIC Stat 21 multiple linear regression program. Correlation coefficients (r) were tested for significance at the 95% confidence level by comparing computed r values to tabulated critical r values, as suggested by Rohlf and Sokal (12).

RESULTS AND DISCUSSION

TVC. TVC can be interpreted as reflecting input of microorganisms from extra-aquatic sources, as well as describing trophic conditions of a given habitat, i.e., availability of growth-supporting organic matter and micronutrients. The fluctuations in TVC at the sampling stations observed in this study may be a result of either or both conditions. A bimodal distribution in total viable counts was found to occur in the bottom water at all stations (Fig. 3). Highest TVC were obtained during the winter and spring months, with the lowest TVC recorded during July, September, and October. Sediment TVC at stations 5a, 10b, 11a, and 1a followed essentially the same distribution as that of the bottom water samples. TVC of the sediment at station 1a followed an anti-coincidental distribution, compared with overlying bottom water; i.e., peak-sediment TVC occurred during June, July, and September, with low-sediment TVC measured at station 1a in the winter and early spring.

The highest recorded counts for the year were at station 1a, approximately 10 to 100 times greater than stations 5a and 10b. In only three instances, i.e., the March water and sediment samples and May water samples, were counts at station 11a higher than station 1a. A comparison of total viable counts for water samples collected at station 1a and at Conowingo (Fig. 3) suggested that microorganisms enter Chesapeake Bay from the Susquehanna River at station 1a. TVC at Conowingo exceeded TVC at station 1a by 50% in September, whereas all other recorded values for Conowingo water were less than, or equal to, 1a water.

MPN index of indicator organisms. The counts of TC, FC, and FS fluctuated seasonally and with distance from stations 1a to 11a (longitudinally) in samples of Chesapeake Bay water examined in this study (Fig. 4). In general, MPN values decreased from winter to summer, with a sharp increase in the number of indicator organisms in December. The MPN trend for station 11a, however, was characteristically higher in the summer months, June through September. TC levels, representing both human and nonhuman sources of coliforms, were higher than FC and, in most cases, FS. FS in bottom water closely paralleled FC trends except at station 11a, where FS rose dramatically during the summer months, whereas FC remained low. Highest MPN values were obtained for water samples from station 1a in all cases. The entry of TC to the Upper Bay via the Susquehanna River appears to be significant, judging from the high TC MPN values measured at Conowingo Dam. The high FC and FS levels at Conowingo Dam paralleled the distribution of these fecal indicators at station 1a. The sharp peaks in TC and FC counts noted in December 1974 at station 5a most likely reflect a point source of pollution. (Unfortunately, the R/V RIDGELY WARFIELD sanitary holding tanks automatically discharge when full and a discharge occurred just prior to arrival at station 5a. The sharp MPN increase observed at station 5a clearly reflects the sewage input.) The sharp increase in FC in the water at station 5a was not observed for sediment samples at 5a (Fig. 5). Adsorption or deposition of coliform bacteria onto sediment would not have occurred within the time of discharge and sampling (approximately 1 h).

A significant irregularity occurred in MPN values for water and sediment (Fig. 4 and 5) at station 1a. A substantial (100-fold) increase in TC and FC occurred in the sediments at station 1a during the summer of 1974. FS levels remained at a relatively uniform level in the summer months, although a 100-fold fluctuation occurred seasonally. Also, it should be

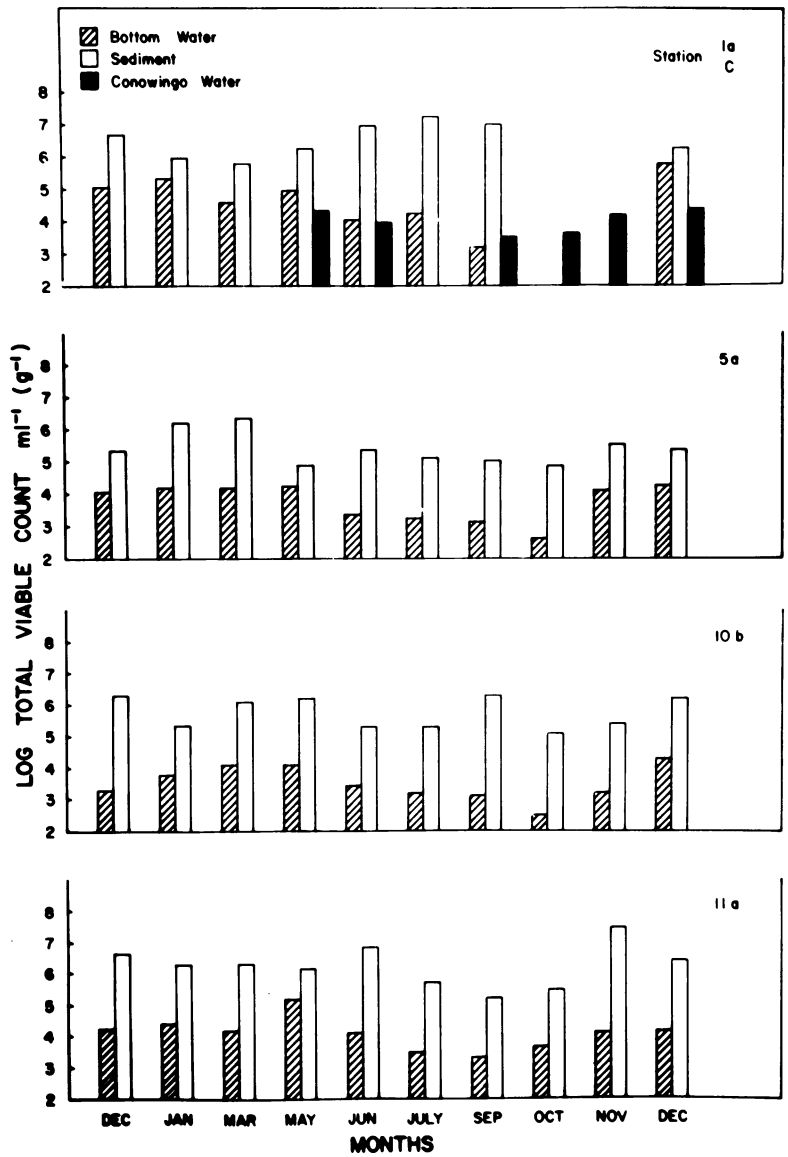

FIG. 3. *Comparison of the temporal distribution of total viable bacteria in water and sediment among Upper Chesapeake Bay samples.*

noted that the FS counts in December 1973 and December 1974 were not significantly different. In general, the counts of indicator organisms at all of the sampling stations were lower for sediment than for water samples. Also, the sediment data indicated less seasonal fluctuation. FS counts were frequently higher than sediment TC and, in general, were also higher than sediment FC. It is obvious from these results that frequent sampling over a complete seasonal cycle is required for adequate measurement and interpretation of the incidence and distribution of indicator organisms, i.e., their flux within a given aquatic habitat.

Several generalizations can be drawn from the data given in Fig. 3 through 5. Higher coliform levels occurring during the winter months suggest survival of the coliforms until the water temperature drops significantly, at which time the coliforms die off. Selection of microbial groups on the basis of longer survival time and increased nutrient flux from decomposition of summer productivity and watershed drainage may also contribute to the November and December increase in numbers. FS survival appears to be enhanced in sediment at all

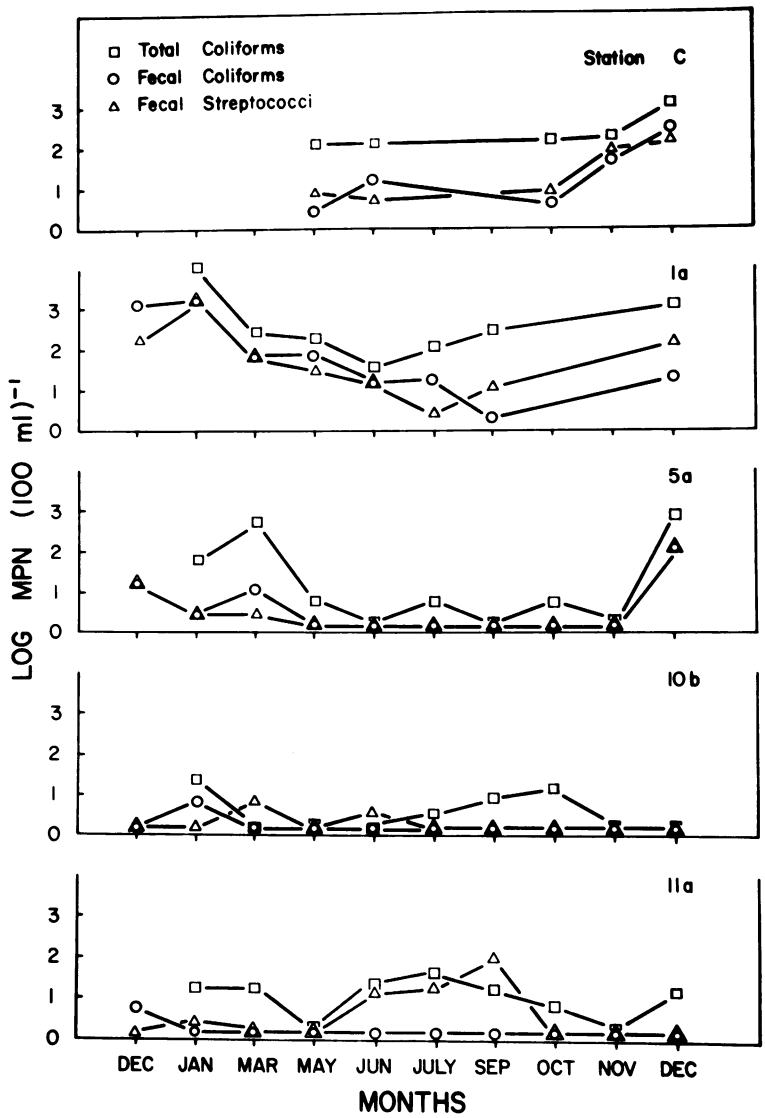

FIG. 4. *Comparison of the temporal distribution of fecal indicator organisms in the bottom water among Upper Chesapeake Bay samples.*

stations at which the FS and FC populations were maintained at consistently low numbers.

Efficiency of identification of indicator organisms. FC and FS were isolated, purified, and characterized to test the accuracy of the methods employed for enumeration (Tables 2 and 3). Approximately 80% of the FC were classified as *Escherichia coli* type I, considered to have come from warm-blooded animals. Samples from some stations yielded more false-positive coliforms than others, and these, in pure culture, proved to be a number of intermediate coliform chemotypes, as well as noncoliform organisms. Station 10b more commonly gave false-positive coliform reactions in the MPN determination. This finding underlines the need to define the MPN beyond the presumptive stage of characterization. FS isolates were examined using a number of selected tests, and the results indicated that more than 80% were enterococci. Although not exclusively of fecal origin, the majority of these enterococci are considered to originate from domestic sewage.

FC-FS ratios. FC-FS ratios provide an index of occurrence which can be interpreted as describing the proportion of these fecal indicator

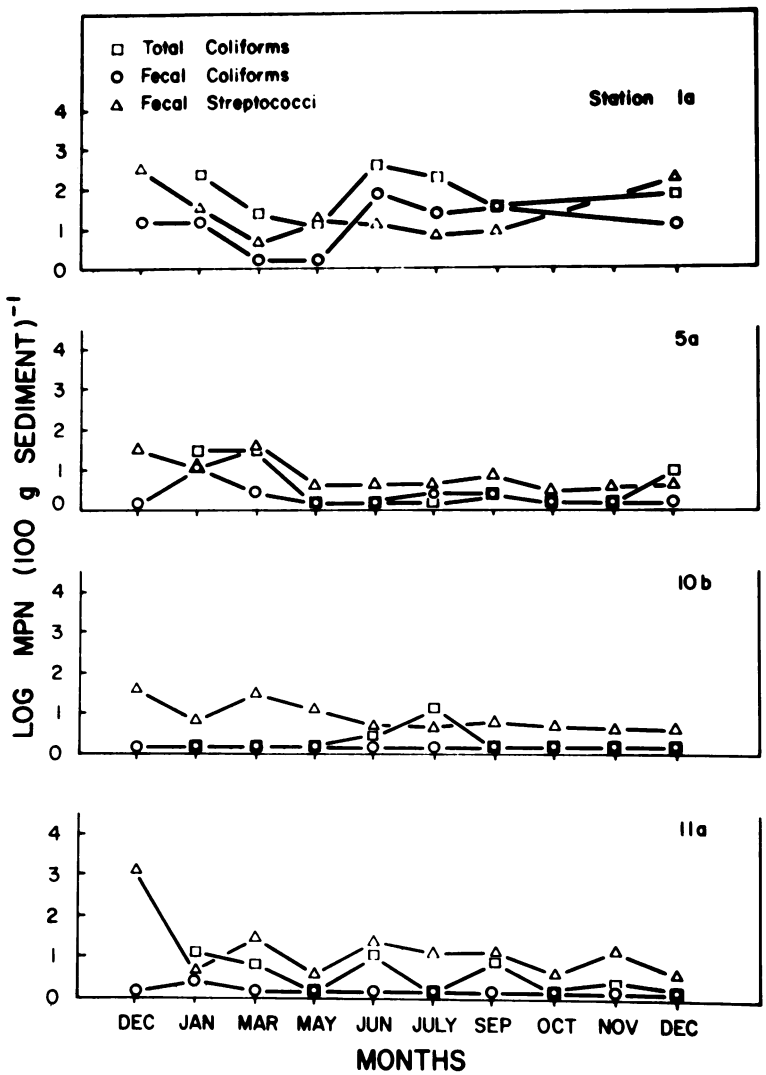

FIG. 5. *Comparison of the temporal distribution of fecal indicator organisms in the sediment among Upper Chesapeake Bay stations.*

organisms within a given population. Geldreich and Kenner (5) suggested that the FC-FS ratio is characteristic for given types of pollution or sources of pollution. The ratio itself is not a quantitative indication of pollution but a qualitative pollution index. Thus, domestic sewage yields FC-FS ratios greater than 4.0, whereas ratios less than 0.7 indicate that the pollution derives from warm-blooded animals other than humans. Lear and Jaworski (9) reported that, from data collected for the upper Potomac River estuary, FC-FS ratios in excess of 4.0 could be calculated. FC-FS ratios greater than 4.0 were observed at all of the sampling stations included in this study, except station 5a (Table 4).

Data for station 1a showed more (33%) FC-FS ratios greater than 4.0, compared with data for Conowingo, 10b, and 11a samples, each of which yielded one sample (<16% of the samples) greater than 4.0. It is notable that suspended sediment samples for station 1a, on three occasions, greatly exceeded the FC-FS ratio of 4.0, whereas one of the suspended sediment samples from station 11a revealed FC-FS ratios greater than 4.0. Sediment samples at stations 5a, 10b, and 11a all yielded low FC-FS ratios, indicating nonhuman sources of contamination occurring at or near those stations. Since differential rates of survival exist among FC and FS in aged samples, lower FC:

TABLE 2. *Identification of organisms isolated in the coliform MPN determination procedures*

Growth response[a]			Total no. of cultures[b]	No. of *E. coli* type I[c]	% *E. coli* type I
Lactose	BGLB	EC			
+	−	−	24	4	16.7
+	+	−	17[d]	1	5.9
+	+	+	81	64	79.0
+	+	(+)	2	1	50.0

[a] A positive response indicates growth and gas production. (+) Indicates growth without gas production. Lactose, lactose broth; BGLB, brilliant green lactose bile broth; and EC, EC broth.

[b] Isolated from colonies appearing on EMB agar which had been streaked onto the EMB agar from positive MPN tubes.

[c] Gram-negative asporogenous rods fermenting lactose; indole (+), methyl red (+), acetoin (−), and citrate (−), typically of human fecal origin (IMViC).

[d] Intermediate chemotypes (− + − +).

FS ratios may reflect time and/or geographical distance relative to the pollution source and not only the type of pollutant. Thus, the FC:FS ratio has definite limitations in its application to environmental monitoring.

Occurrence of indicator organisms relative to TVC. To determine the contribution of the population of indicator organisms to the total viable bacterial population, an estimation of the indicator organisms, MPN per million TVC, was computed (See Fig. 6). The relative proportions of FC and FS in the TVC for the Chesapeake Bay water samples decreased southwards, from station 1a to station 11a, during the winter months. During the spring months, the proportion approached uniformity among sampling stations. However, beginning in June at station 11a, a gradual rise in the relative abundance of indicator organisms in the water column occurred (Fig. 6). This in-

TABLE 3. *Identification of organisms isolated from fecal Streptococcus MPN determinations*

Source[a]	Total no. of cultures	%[b]				
		Catalase (+)	Growth		Starch hydrolysis	Hippurate hydrolysis
			6.5% NaCl	pH 9.6		
I. Direct plating on M-enterococcus agar	14	7.14	92.85	78.57	ND[c]	7.14
II. Ethyl violet azide broth	22	31.82	77.27	86.36	ND	13.64
III. Ethyl violet azide broth to M-enterococcus agar	123	10.60	86.90	83.50	0.2	0.01

[a] Two methods for the isolation of FS were used: direct plating on M-enterococcus agar (I) or the azide dextrose-ethyl violet azide broth MPN sequence (II and III). The latter two procedures differed in the way cultures were isolated from ethyl violet (+) tubes. In method II, cultures were isolated by streaking onto a nonselective agar medium, whereas in method III, M-enterococcus agar was inoculated by streak plate procedure.

[b] FS of human origin ("enterococci") typically grow in 6.5% NaCl and pH 9.6 broths, but do not hydrolyze starch or hippuric acid.

[c] ND, Not determined.

TABLE 4. *FC-FS ratios calculated from samples collected at the Upper Bay sampling stations*

Station	Dec.	Jan.	Mar.	May	June	July	Sept.	Oct.	Nov.	Dec.	Avg
Conowingo											
Water				0.5	2.5		0.8	2.2	5.0	1.9	2.3
1a											
Water	7.3	1.1	0.9	1.8	1.4	5.0	0.3			0.2	2.2
Sediment	0.03	0.4	0.3	1.0	5.4	4.5	5.8			0.6	2.3
Suspended sediment	0.04	0.1	11.3	0.3	3.0	8.2	10.8			1.8	4.4
5a											
Water	1.5	1.1	2.8	1.1	1.3	1.3	2.5	2.7	2.7	1.0	1.8
Sediment	1.9	0.7	0.1	0.3	0.3	0.3	0.5	0.5	3.0	0.3	0.9
10b											
Water	2.5	1.1	11.1	1.0	1.0	0.4	3.0	0.8	3.0	1.4	2.3
Sediment	0.03	0.3	0.04	0.1	0.3	0.3	1.9	0.3	0.3	0.3	0.4
11a											
Water	5.2	0.7	1.0	1.0	0.13	0.07	0.02	1.1	3.0	1.5	1.6
Sediment	0.001	0.8	0.04	0.3	0.6	0.1	0.1	0.3	0.08	0.3	0.2
Suspended sediment	3.3	0.6	0.3	0.3	0.5	0.3	0.2	0.3	0.004	7.5	1.5
Average	2.2	0.5	2.8	0.7	1.5	2.0	2.4	1.0	2.1	1.5	

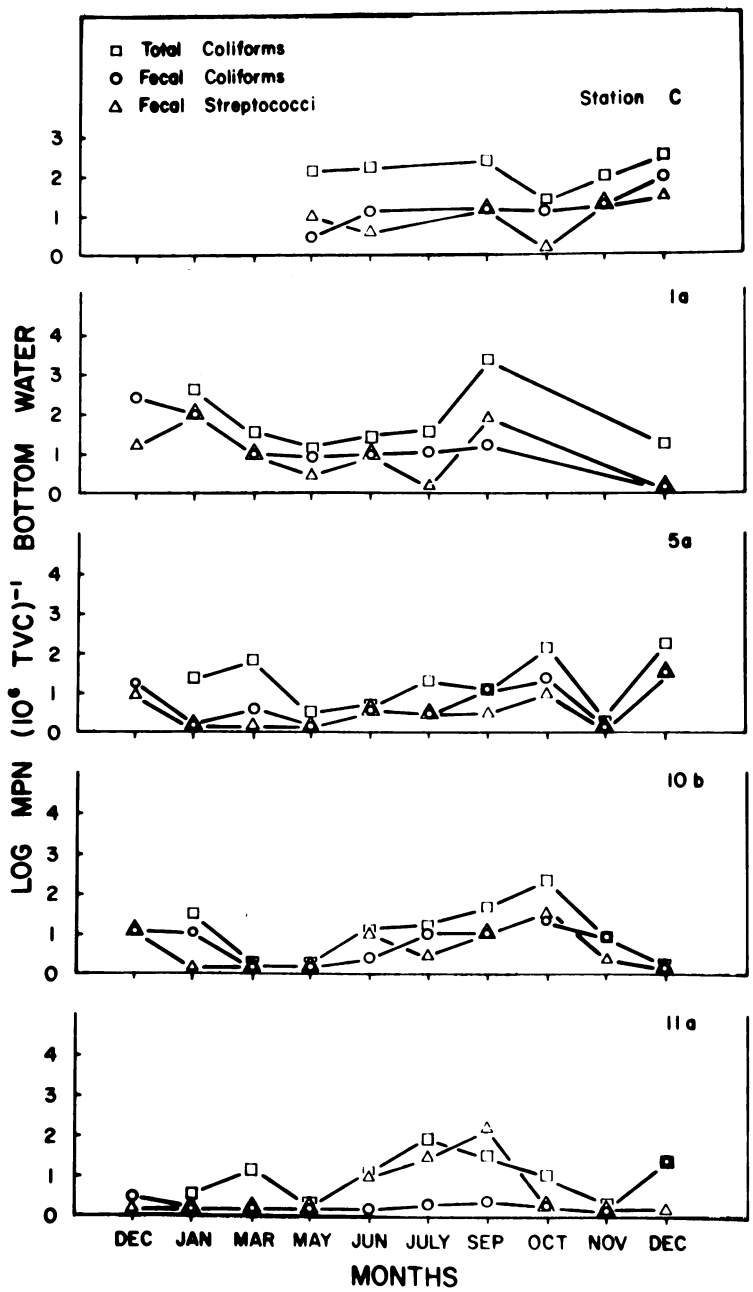

FIG. 6. *Comparison of waterborne indicator organisms in relation to the temporal distribution of total viable bacteria among Upper Chesapeake Bay samples.*

crease was mirrored at the other sampling stations at progressively later dates from stations 11a and 1a. This pattern of occurrence of indicator organisms was not reflected in the bottom sediments (Fig. 7). Although station 11a water and sediments showed coincident peaks in the relative proportion of FC and FS making up the total viable, aerobic, heterotrophic bacterial population, the chronological progression in proportion of indicator organisms per population did appear in the sediments at stations from 1a to 11a. Station 5a and 10b sediment yielded relatively constant proportions of indicator organisms throughout the year, whereas the abundance of FC and FS showed greater fluctuation in 1a sediment samples. The pattern of

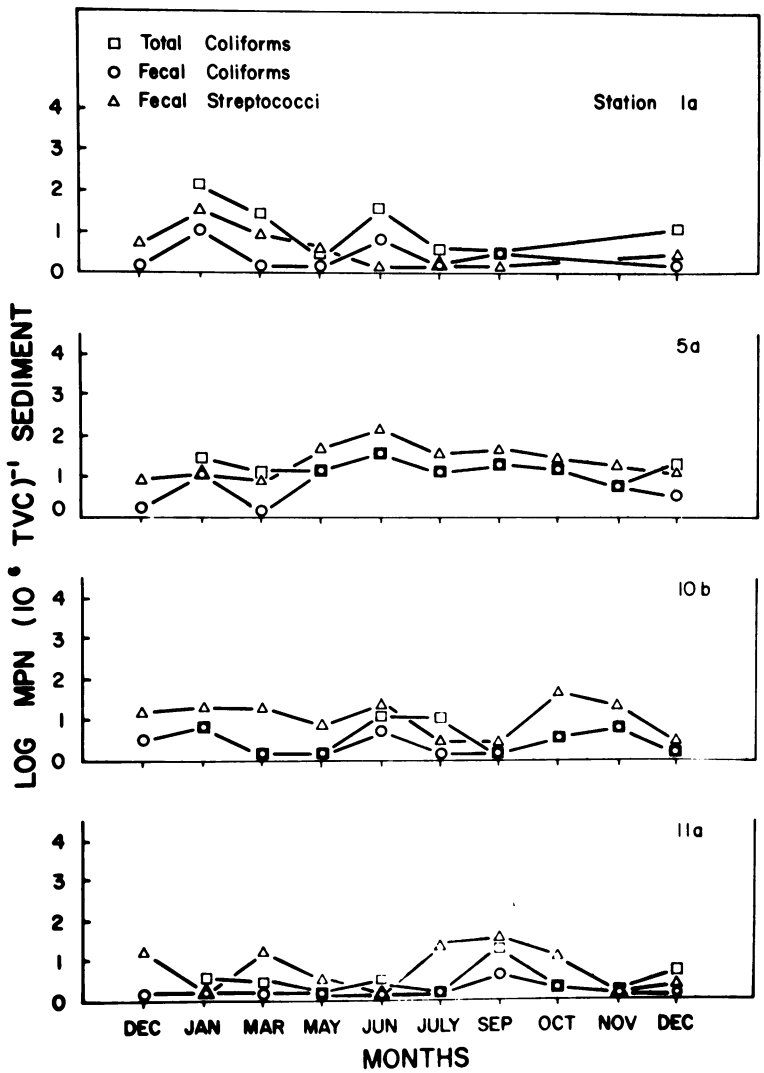

FIG. 7. *Comparison of sediment-bound indicator organisms in relation to the temporal distribution of total viable bacteria in Upper Chesapeake Bay sediments.*

water flow, including speed and direction, may contribute to this interesting observation. The data were found to be strongly correlated with those given in Fig. 4 and 5. It must be remembered that the TVC is nearly always significantly higher in sediments compared with the bottom water (Fig. 3). Thus, the relative abundance of indicator organisms would be expected to be lower in sediment samples. In several instances, a greater proportion of FS was observed in sediment samples collected at stations 10b and 5a, which can be interpreted as indicating enhanced survival of FS in sediments or distance and time elapsed from fecal contamination input and recovery of FS. It can be concluded, from the abundance of fecal indicators observed at station 1a, that a source of contamination exists in the vicinity of that station. When these data are examined in conjunction with FC:FS ratios for station 1a (Table 4), the overwhelming evidence leads to the conclusion that domestic sewage is present at station 1a. The source of contamination at station 1a is, unfortunately, partially masked by the dilution of Chesapeake Bay water with Susquehanna River water, the latter having high TC levels.

The progressive increase in numbers of fecal indicator organisms occurring from summer to fall at stations 11a through 1a may be linked to increased summertime activities in Chesa-

peake Bay related to recreational use. However, this is completely speculative and less likely than hydrological effects, since the pattern of peak occurrence of indicator organisms shows consistently later occurrence during the year southward from stations 1a to 11a.

Association of bacteria with particulates. A potential mechanism of transport for bacteria from water to sediment and vice-versa is provided by suspended particulate matter. A highly significant proportion, both of total viable bacteria and selected indicators of fecal pollution, were found to be associated with particulate matter in the water column (Table 5). Up to 53% of the total viable bacteria in station 11a water samples was found to be associated with particulate matter collected in the 2,100 × *g* centrifugation pellets. TVC found to be associated with particulates in station 1a samples exceeded 25% in three of five determinations. Similarly, the results show a significant proportion of the total MPN of the fecal indicators at station 1a, and a majority of the fecal indicators at station 11a were associated with the suspended particulates. However, an unequivocal correlation between concentration of suspended sediment and bacteria associated with particulates at a given station was not observed.

Results of a seasonal comparison of bacteria associated with suspended matter carried out at stations 1a and 11a (Fig. 8) showed that both the TVC and number of indicator organisms decreased from winter to spring at station 1a. An identical pattern in TVC distribution was observed at station 11a. However, the distribution of indicator organisms associated with suspended sediment rose from December to January and remained stable until September, at which time the numbers, both of TC and of FC, associated with the particulates declined, whereas the number of FS remained stable until December 1974. In general, counts of bacteria associated with particulates obtained for samples collected at station 1a were higher than at station 11a throughout the year.

Collectively, the above data show that the total numbers of FC and FS can vary temporally and spatially. Furthermore, transport via association with suspended sediments appears to be a significant mechanism operating in the aquatic environment. It must be emphasized that the evidence accumulated in this study points out the difficulties and pitfalls in placing total dependence on a single bacterial group for indication of a public health hazard arising from the presence of pathogens in suspended sediment. It is not at all unequivocal that the MPN for TC does, in fact, give a quantitative indication of the potential presence of *E. coli* type I or even qualitative support for the potential presence of human pathogens of the enteric group.

Statistical relationship between microbiological, chemical, and physical parameters. Multiple linear correlations for chemical, physical, and microbiological parameters measured in the Upper Chesapeake Bay at stations sampled from December 1973 to July 1974 were computed, and a correlation coefficient matrix was generated (Table 6). From these calculations, it is concluded that temperature and salinity were not correlated with other variables examined in this study. The concentration of suspended sediment at depths coinciding with depths of samples collected for bacteriological analysis revealed that there was good correlation between suspended sediment, TVC, TC, and FC, but poor, if any, correlation with FS. However, the correlations were not significant if comparisons were made with critical correlation coefficients for the same sample size as employed in the correlation analysis. Although the relationship cannot be concluded to be statistically significant because of the small sample size, the trend observed did strongly indicate that quantitative variations in numbers of

TABLE 5. *Association of indicator organisms with particulates*

Date	Station 11a				Station 1a			
	% of TVC[a] (2,100 × *g* pellet)	% of MPN[a]		SSED[b] (mg/liter)	% of TVC (2,100 × *g* pellet	% of MPN		SSED (mg/liter)
		FC	FS			FC	FS	
1/74	17.9	89.0	93.8	7.98	25.2	7.5		33.9
3/74	19.3			9.6	1.4	76.3	6.1	12.7
5/74	53.1		83.3	10.5	26.8	3.5	22.3	43.3
6/74	11.4	28.4	7.0	3.7	27.9	39.4	18.0	13.2
7/74		43.8	9.9	7.4	0.2	93.2	57.1	13.5
9/74	15.9	78.0	74.7	6.3				7.7

[a] Percentage of organisms removed from the water column by centrifugation at 2,100 × *g*.
[b] SSED, Suspended sediment.

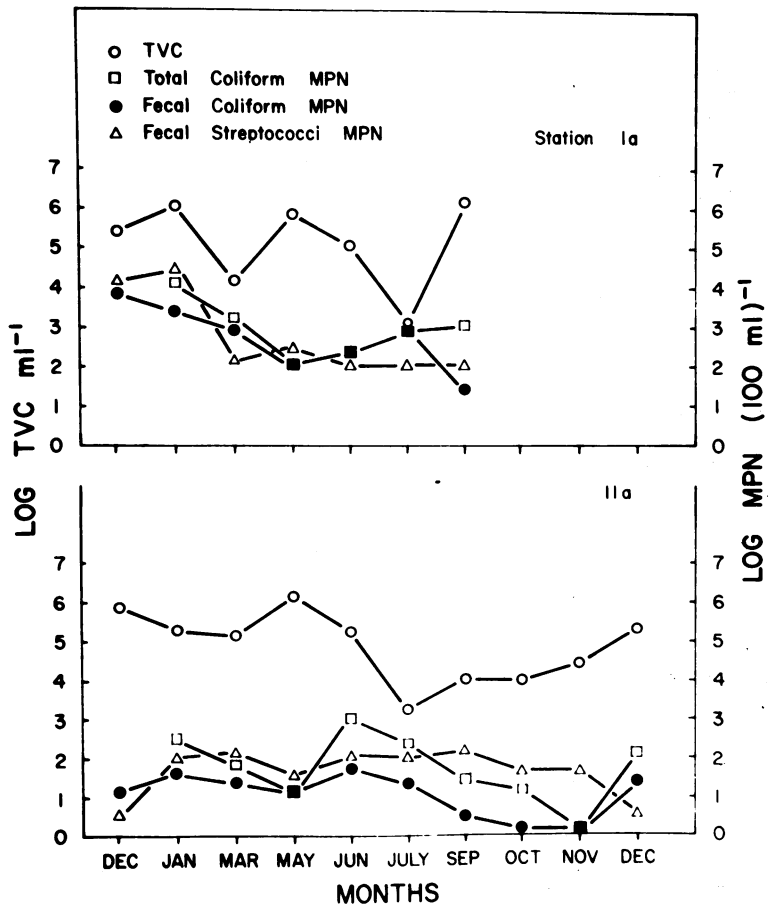

FIG. 8. *Comparative recovery of bacteria associated with suspended sediment at stations 1a and 11a in the Upper Chesapeake Bay.*

indicator organisms were independent of salinity, temperature, and suspended sediment concentration. However, a much larger sample size would be necessary to reject outright the null hypothesis that there is no functional relationship between numbers of bacterial indicator organisms and selected physical and chemical indexes. This point is being pursued by further studies underway in our laboratory.

A highly significant correlation was noted for the microbiological variables (Table 6). The highest correlation was found for TC and FS concentrations in samples of bottom water. Assuming the TVC to be the independent variable and TC, FC, and FS to be dependent variables, 62 to 72% of the variation in numbers of indicator organisms may be attributed to variations in the TVC.

The strong correlation between the TVC and numbers of indicator organisms was not observed when data for the entire sampling year were treated (Table 7). Also, the correlation between TVC and TC, FC, and FS was weakened when all sample types were included in the analysis. Hence, the variation arising from seasonal influences appears to reduce or eliminate the correlations observed for individual samples. The correlation observed for TVC and the number of indicator organisms in the suspended sediment samples was detectable but not statistically significant. However, significant correlation was observed for TVC and TC and FS in bottom waters and FC in sediment when all of the data collected throughout 1974 were subjected to analysis. Exceptionally high correlation coefficients ($r = 0.99$) between indicator organisms, i.e., TC, FC, and FS, were observed for the yearly data compiled for bottom water and suspended sediment samples (Table 7). These results are not unexpected, since graphical representation of MPN data presented in Fig. 4 and 8 suggested strong relation-

TABLE 6. *Correlation matrix of physical, chemical, and microbiological parameters for bottom water samples, December 1973 to July 1974*[a]

Temp	Salinity	Suspended sediment	TVC	TC	FC	FS	Determination
1.00	−0.06	−0.22	−0.39	−0.26	−0.34	−0.29	Temperature
	1.00	−0.37	−0.46	−0.23	−0.32	−0.25	Salinity
		1.00	0.27	0.20	0.32	−0.22	Suspended sediment
			1.00	0.79	0.85	0.82	TVC
				1.00	0.82	0.99	TC
					1.00	0.88	FC
						1.00	FS

[a] Critical $r = 0.42$, $\alpha = 0.05$.

TABLE 7. *Correlation coefficient matrixes for microbiological parameters of various sample types*

TVC	TC	FC	FS		Sample	Critical r (α = 0.05)
1.00	0.30	0.22	0.30	TVC		
	1.00	0.99	0.99	TC	Bottom water	.27
		1.00	0.99	FC		
			1.00	FS		
1.00	0.26	0.32	0.01	TVC		
	1.00	0.89	0.11	TC	Sediment	.27
		1.00	0.00	FC		
			1.00	FS		
1.00	0.24	0.30	0.26	TVC		
	1.00	0.94	0.99	TC	Suspended sediment	.44
		1.00	0.92	FC		
			1.00	FS		
1.00	−0.02	−0.03	−0.01	TVC		
	1.00	0.95	0.91	TC	Overall	.20
		1.00	0.85	FC		
			1.00	FS		

ships among indicator organisms. FS were found to be independent of both TC and FC in the sediment, substantiated by data presented in Fig. 5 and 7, which indicated a reversal in the abundance of FS in sediment compared with bottom water (Fig. 4 and 6).

The statistical analysis proved helpful in focusing on those bacterial parameters influenced by physical and chemical characteristics of the Upper Chesapeake Bay stations examined in this study and, more importantly, on those parameters for which there were strong correlations. Survival of FS in sediments is known to be of greater duration than that of FC (5). The observed correlations for FS and other indicator organisms included in this study were not statistically significant for the entire yearly sediment sampling data, which can be interpreted as indirect evidence of prolonged survival of FS in sediments.

The methods of analysis employed in this study offer useful applications, such as in the surveillance of dredge spoil and domestic dump sites for assessment of the impact of such activities in the estuarine and coastal zones.

From the results of the microbiological analyses employed in this study, it is concluded that TC MPN determinations are insufficiently precise indicators to be used without further analysis. The validity of coliform MPN estimates is sufficiently doubtful that the use of this procedure for determining the sanitary quality of estuarine and coastal water should be re-evaluated. In any case, it is recommended that coliform MPN determinations be reported in such a way that the incidence of false-positive (as indicated by Table 2) results are accommodated.

The indicator organism concept, as currently applied to environmental assessments, should be broadened to include indicators of environmental deterioration, i.e., increased presence of clostridia indicating increased contamination and potential anaerobiosis, enhanced presence of *Klebsiella* and *Aeromonas* spp. associated with waste waters, etc. Reliance on the coliforms, a relatively imprecise and nonspecific

indicator of the presence of potential pathogens, creates serious problems, both in measuring environmental quality and in calculating public health safety. Methods for direct isolation of specific pathogenic bacteria and viruses, coupled with the use of environmental quality indicators, will provide more accurate and certainly more significant information for public health policy and managerial decisions.

Finally, the results of this survey provide evidence of significant levels of pollution from human wastes in the water, sediment, and suspended sediment throughout the Upper Chesapeake Bay. There is little doubt but that further deterioration in water quality will seriously affect shellfish harvesting and recreational uses of the Upper Chesapeake Bay.

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