Bacteriological Water Quality Indicators in Natural Waters

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Abstract

A number of natural waters were analyzed for the presence of somatic coliphages, total and fecal coliforms (TC and FC), Escherichia coli (Ec), heterotrophic plate count (HPC) and fecal streptococci (FS). Sources sampled include permanent and intermittent streams, irrigation canals, potable water treatment plant influents and sewage treatment plant influents and effluents and receiving waters above and below those effluents.

Earlier studies in Puerto Rico have suggested that coliphages are only detected in natural waters contaminated with sewage(1). In this study most sources had coliphages most of the time. Coliphage densities are compared with the bacteriological indicators analyzed.

Standard Methods(2) includes formulae for the estimation of total and fecal coliform densities from coliphage results. For this study coefficients of empirical formulae to estimate TC and FC densities from coliphage occurrence are given and extended to E. coli densities. Coliphages have proven to be reliable indicators of the occurrence of TC and FC, though not Ec, in these samples and the ease, reliability and precision of the method suggest that it may with confidence be substituted for other methods for natural water monitoring.

Key Words: coliphage, natural water quality.

Methods & Materials

Samples were collected in clean, sterile polypropylene 0.5 or 1 L bottles. A dechlorinating agent was not used for samples from non-chlorinated sources. All samples were transported immediately to the laboratory and refrigerated. All analyses were completed within thirty hours of sample collection.

Analyses for coliphage, total coliform(TC), fecal coliform(FC) and fecal streptococci(FS) were in accordance with Standard Methods(2). Heterotrophic plate counts were made on R2A medium, by spread plate techniques and were incubated in the dark for 168 hours at ambient temperature. All coliphage determinations were made utilizing the host culture, Escherichia coli C, ATCC 13706 and following the technique in Standard Methods. Plaques were counted at 6 hours. Presumptive E. coli, (Ec) determinations were made utilizing MPN methods with media containing MUG. MUG-positive cultures (cultures which fluoresce when exposed to long-wave UV - approximately 340 nm) are presumed positive for the presence of E. coli.

Samples were collected from 4 sites on an irrigation canal system (canal samples), 5 sites on Río Guanajibo (river samples), a sewage treatment plant influent and effluent and a potable water treatment plant influent (collected at the plant, piped from a small reservoir). Two of the canal sample sites are the influent and effluent of a large wetland, consisting of approximately 350 acres with an average depth of water of 1.5 - 2 feet (1.8 billion gallons, 648,000 m^3)with an unknown residence time.

Statistical analyses were performed utilizing PC software, SPSS and SYSTAT, both from SPSS, Inc, Chicago, IL. Estimates of TC and FC identified as “calculated” (TCcalc, e.g.) were according to formulae 1 and 3 from Standard Methods.

Total Coliforms (TCcalc)

$$\log_{10}\left(\frac{\text{total coliforms}}{100\text{mL}}\right) = 0.627\left(\log_{10}\left(\frac{\text{coliphage}}{100\text{mL}}\right)\right) + 1.864 \quad (1)$$

$$\frac{\text{total coliforms}}{100\text{mL}} = e^{0.627\left(\ln\left(\frac{\text{coliphage}}{100\text{mL}}\right)\right) + 4.292} \quad (2)$$

Fecal Coliforms (FCcalc)

$$\log_{10}\left(\frac{\text{fecal coliforms}}{100\text{mL}}\right) = 0.805\left(\log_{10}\left(\frac{\text{coliphage}}{100\text{mL}}\right)\right) + 0.895 \quad (3)$$

$$\frac{\text{fecal coliforms}}{100\text{mL}} = e^{0.805\left(\ln\left(\frac{\text{coliphage}}{100\text{mL}}\right)\right) + 2.061} \quad (4)$$

Results

We use log-transformed ($\log_{10}$) data for all bacteriological analyses in these presentations. Means and standard deviations by source category are given at Table 1. Frequency of coliphage occurrence is shown at Table 2.

	Source Type	N	Mean	Std. Deviation		Source Type	N	Mean	Std. Deviation
LOGPFU	canal	80	2.0172	.6670	LOGECEc	canal	62	2.3912	.8017
	river	39	2.1720	.5375		river	21	2.7496	.6536
	STP Inf	30	4.2886	.4946		STP Inf	0	.	.
	STP Eff	34	3.0205	.5323		STP Eff	4	2.5828	.7729
	WTP Inf	8	1.1754	.3524		WTP Inf	0	.	.
	Total	191	2.5489	1.0388		Total	87	2.4865	.7746
LOGTC	canal	84	3.5439	.6094	LOGFS	canal	15	3.0075	.6393
	river	37	3.7110	.6265		river	21	2.7025	.6245
	STP Inf	28	6.7304	.5358		STP Inf	0	.	.
	STP Eff	30	4.1719	.8611		STP Eff	4	2.2669	.4629
	WTP Inf	0	.	.		WTP Inf	0	.	.
	Total	179	4.1821	1.2949		Total	40	2.7733	.6429
LOGFC	canal	78	2.5826	.9051	LOGHPC	canal	79	4.4936	.6364
	river	38	3.0669	.7966		river	14	4.3681	.4251
	STP Inf	26	6.2191	.3106		STP Inf	29	6.6454	.4508
	STP Eff	28	3.5181	.7334		STP Eff	30	5.7020	.7965
	WTP Inf	0	.	.		WTP Inf	0	.	.
	Total	170	3.4011	1.4733		Total	152	5.1311	1.0752

Table 1. Descriptive Statistics for Source Categories

		Source Type					
		canal	river	STP Inf	STP Eff	WTP Inf	Total
pfu/100mL	<5	7	2			10	19
	5-35	20	6			7	33
	36-228	29	16		7	1	53
	229-1,168	30	15		8		53
	>1,168	1	2	30	19		52
Total		87	41	30	34	18	210

Table 2. Frequency of coliphage occurrence.

"<5" is no plaques on the four five mL portions plated in the technique; other categories are quartiles.

To illustrate the agreement between the bacteriological densities in this study predicted by eqs 1 and 3, a single source is given, FC from the sewage treatment plant effluent (treating largely domestic (household) waste. As both Standard Methods and common sense suggest, coefficients can and should be calculated for each individual source. The plot and empirical coefficients for the data are also shown at Figure 1. As may be seen there is good agreement between the regression slopes; r^2 for the test slope is not significant.

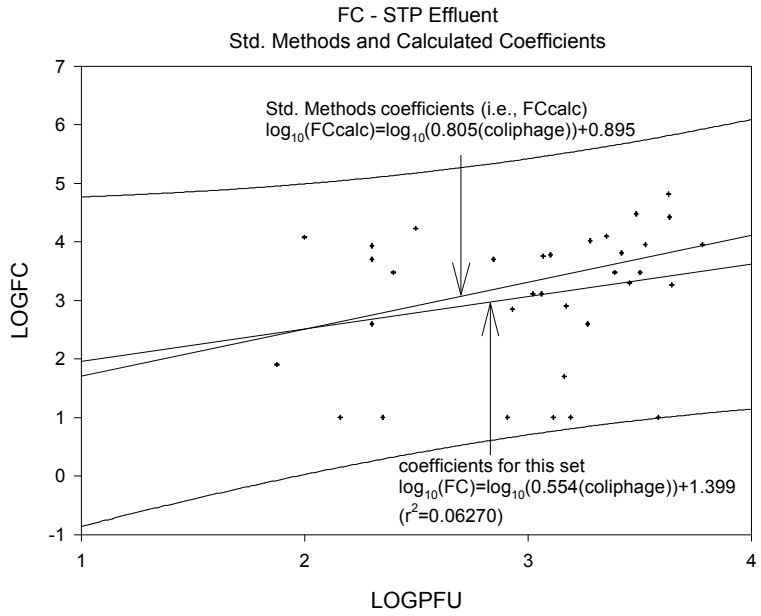

Figure 1. Fecal coliform in STP effluent; outer lines are prediction limits.

Scatter plots of all bacteriological species analyzed are presented at Figure 2. It is apparent from Figure 2 that only FS, of the species analyzed, is not likely to have a linear correlation with the other indicators. Figure 2 also suggests that coliphage, TC and FC, at least, are bimodal. This is related to the much higher densities and ranges for these indicators in the STP influent, see Table 3.

	Values for wetland effluent, other canal, STP Effluent and river samples				Values for wetland influent and STP Influent samples			
	N	Min	Max	Mean	N	Min	Max	Mean
LOGPFU	161	70	3.78	2.22	30	3.29	4.93	4.29
LOGTC	151	2.23	5.89	3.71	28	4.65	7.38	6.73
LOGFC	144	1.02	5.13	2.89	26	5.59	6.78	6.22

Table 3. Comparative statistics for groups of sample sources.

Table 4 gives Pearson's correlation coefficient for these data. For all these data, TC, FC and HPC are significantly correlated with coliphage. Note also, as suggested by Figure 2, that FS is significantly correlated only with Ec and TC. Ec is significantly correlated to all other indicators except coliphage. The bimodality is largely due to the STP influent source, which has significantly higher densities of TC, FC and HPC than other sources. Table 5 shows correlations for all sources except the STP influent. All the correlations are the same as for all sample sources (Table 4). FS results are based on only 38 samples, and their importance should not be overestimated. For the STP influent alone, only HPC is significantly correlated with coliphage.

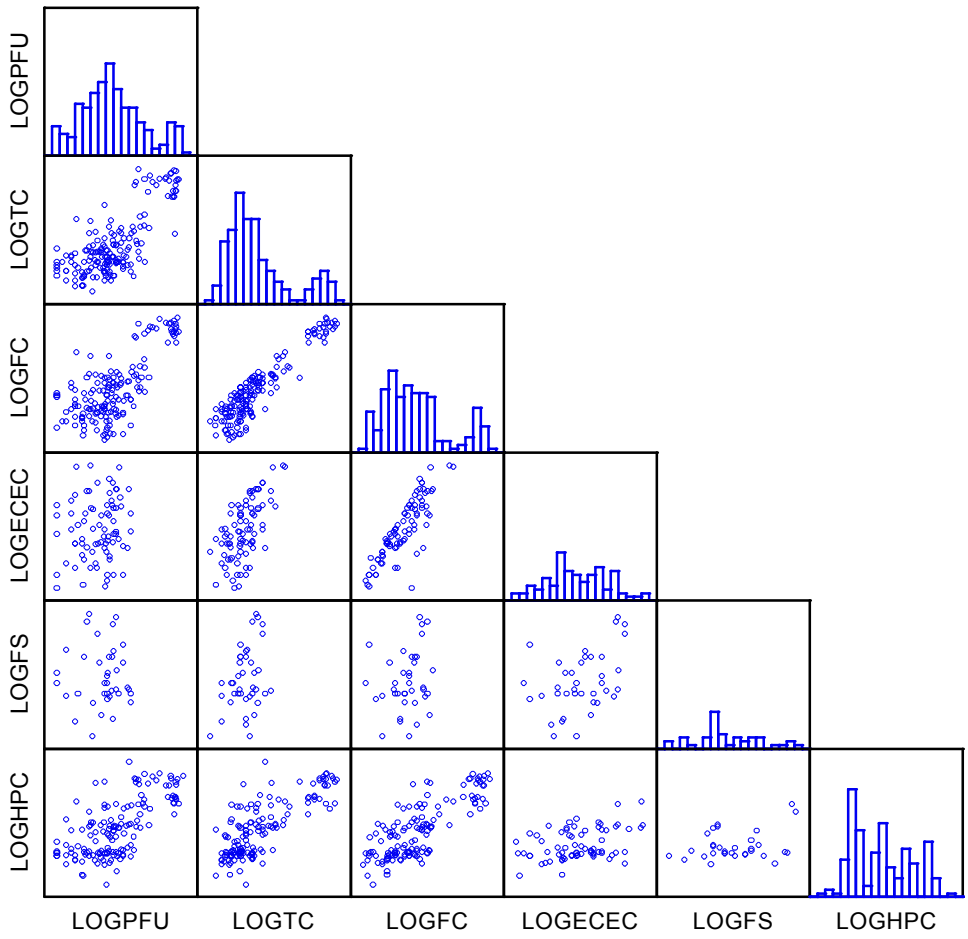

Figure 2. Scatter plots of logged (log_{10}) data. LogECEc is E. coli from EC medium plus MUG.

		LOGPFU	LOGTC	LOGFC	LOGECEC	LOGFS	LOGHPC
Pearson Correlation	LOGPFU	1.000	.755**	.718**	.143	-.006	.674**
	LOGTC	.755**	1.000	.928**	.635**	.373*	.785**
	LOGFC	.718**	.928**	1.000	.851**	.210	.796**
	LOGECEC	.143	.635**	.851**	1.000	.420**	.382**
	LOGFS	-.006	.373*	.210	.420**	1.000	.308
	LOGHPC	.674**	.785**	.796**	.382**	.308	1.000
N	LOGPFU	191	170	161	79	38	146
	LOGTC	170	179	165	85	40	144
	LOGFC	161	165	170	87	38	136
	LOGECEC	79	85	87	87	38	71
	LOGFS	38	40	38	38	40	32
	LOGHPC	146	144	136	71	32	152

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4. Correlation on all sources.

		LOGPFU	LOGTC	LOGFC	LOGECEC	LOGFS	LOGHPC
Pearson Correlation	LOGPFU	1.000	.405**	.305**	.143	-.006	.400**
	LOGTC	.405**	1.000	.770**	.635**	.373*	.553**
	LOGFC	.305**	.770**	1.000	.851**	.210	.542**
	LOGECEC	.143	.635**	.851**	1.000	.420**	.382**
	LOGFS	-.006	.373*	.210	.420**	1.000	.308
	LOGHPC	.400**	.553**	.542**	.382**	.308	1.000
N	LOGPFU	161	142	135	79	38	117
	LOGTC	142	151	139	85	40	117
	LOGFC	135	139	144	87	38	110
	LOGECEC	79	85	87	87	38	71
	LOGFS	38	40	38	38	40	32
	LOGHPC	117	117	110	71	32	123

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 5. Correlation on all samples except STP Influent.

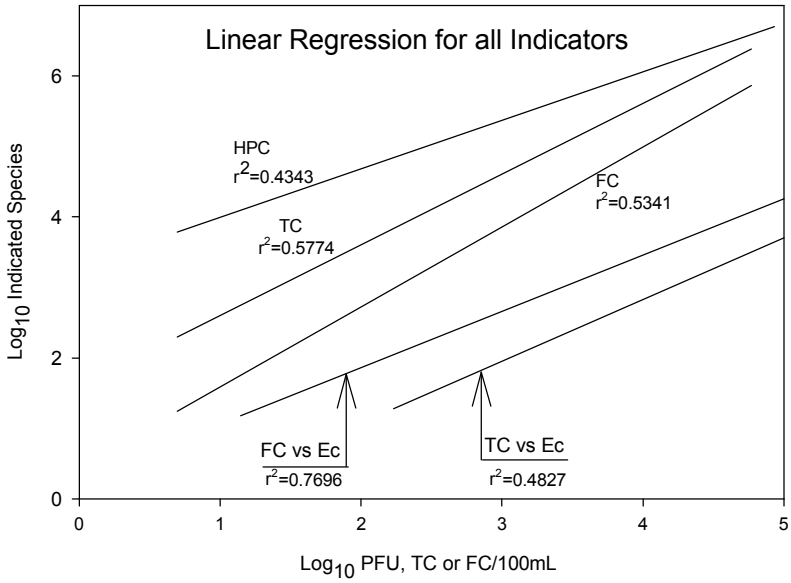

Figure 3. Linear regression lines and coefficients of determination (r^2).

Discussion

Figure 3 shows regression lines and r^2 for coliphage versus TC, FC and HPC. All of these are significant (TC F=169.6, p<0.000; FC F=222.7, p<0.000 and HPC F=120.0, p<0.000). Coefficients of the linear approximation for $\log_{10}$ data are: TC = 1.135(PFU)+ 0.4477; FC = 1.004(PFU) + 1.592 and HPC = 0.688(PFU) + 3.302. In addition, Figure 3 shows, for comparison, regressions of Ec versus TC (0.875(TC) + 0.674, F=56.03, p<0.000) and Ec versus FC (0.797(FC) + 0.269, F=223.6, p<0.000). There is thus, excellent correlation in our data set between coliphage and the typical indicators of natural water quality, TC and FC as well as HPC.

Historically, various empirical estimates of water quality indicators or of relationships between indicators have been used for the purpose of ascribing a source to those indicators (to assist in evaluating the risk of pathogen occurrence from those indicators). For example, the fecal coliform/fecal strep ratio enjoyed a brief period of fashion in assigning organisms from a specific sample to a source, nominally to estimate likelihood of occurrence for microbial pathogens, or at least those transmitted by human wastes. In addition, somatic coliphage has been

used for the purpose of estimating the densities of other, longer-used indicators. For example, Standard Methods has used proposed empirical formulae to estimate TC and FC densities (eqs 1 & 3) from coliphage counts for some time. The question of interest at this juncture is whether coliphage results may be used to estimate water quality, or at least the presence and number of other indicators.

It has been shown that no current indicator used in a routine monitoring program will serve reliably to elucidate a fortuitous or acute incident of contamination of a water distribution system through the use of any indicator which is present in very low densities (Minnigh, et.al.(1986), Pipes & Dempsey(1986), Pipes(1986), Pipes & Minnigh(1987, 1990)). Gerba (1987) felt that coliphages were not reliable indicators of enteric pathogens, but might be acceptable indicators of, or substitutes for, TC and FC in natural waters. In evaluating coliphages as indicators of viral removal efficiency (i.e., of viral presence in treated potable water) Payment & Franco(1993) suggested that coliphages might be acceptable in that role, but suggested Clostridium perfringens for specificity and sensitivity of method and they felt that the spores of C. perfringens provided an additional safety factor (in that they are more resistant) and might also serve as an indicator of cyst removal. Jofre, et.al.(1995), again looking for indicators of viral removals in potable water treatment, found that phages infecting Bacteriodes fragilis were better indicators, being present in densities in raw water much higher than enteroviruses and occurring at densities high enough in treated water to allow their detection. The authors do note that coliphages occurred at higher densities than the B. fragilis phages, and did not require concentration. Toranzos & Alvarez(1992) give a PCR techniques would serve to reduce the detection limit for enteric pathogens to a single bacterium. While this would obviate the need for indicators altogether, the techniques are not simple, and do not lend themselves to routine, rapid monitoring of source waters at this time.

Toranzos(1991) found no coliphages in water uncontaminated with sewage and he and Alvarez (1992) note that coliphages may be absent when pathogens are present, and present when pathogens are absent. The sources known not to be downstream from sewage treatment plant effluents here may be effected by overland flow from areas in pasture used for beef production or horse grazing. For the sources which are least likely to be influenced by STP outfalls, the irrigation canal, the water treatment plant influent and Río Guanajibo above the STP discharge, 37.5% (12 of 32) of the samples had fewer than 5 coliphage/100 mL. For these samples, only HPC was significantly correlated with coliphage when we use untransformed data (not logged, to include zero counts). The WTP Influent is from a small reservoir, and Gerba has noted that settled sources may have fewer coliphages, and that the quantity of suspended or particulate material may effect counts. Even for this source only 53% (10 of 19) of samples were negative (Table 2).

Count

		FC Quartiles				
		FC<200	FC > 199 < 1350	FC > 1349 < 9375	FC > 9374	Total
pfu quartiles	<35	14	11	9	1	35
	36 - 228	17	19	9	4	49
	229-1,168	13	17	17	4	51
	>1,168	1	7	8	33	49
Total		45	54	43	42	184

Table 6. FC quartiles vs. coliphage quartiles.

Finally, in Table 6, we look at FC and coliphage results, by quartile, for all sources. While the categories of these two species, their quartile counts in this example, do differ significantly (χ^2=83.34, df 9, p<0.000) the general trend is higher coliphage densities with higher FC densities. As we have seen, correlation analyses bear out the reliability of this relationship. At the same time, low coliphage counts do occur with high FC counts and low FC counts do occur with high coliphage counts. Results for TC and HPC are similar.

While bacteriological monitoring which elucidates the efficiency of a specific process or plant in removing various pathogens (or indicators) is crucial, much of the bacteriological monitoring or analyses of water has to do with whether a specific source water is or could be made acceptable for treatment. In addition, much routine monitoring is done to ascertain when or whether a specific source water has changed or is changing, and the degree of that change. The point is, for this sort of monitoring we are not trying to fulfill all the requirements of an indicator for pathogens. For this purpose, for the routine monitoring of natural waters for indications of change or general bacteriological water quality, somatic coliphage analyses are a valuable extension of the more conventional TC or FC analyses, and may be an acceptable substitute for at least the largest portion of conventional analyses. This is even more the case with recent, even simpler techniques (Ijzerman, M.M. and C. Hagedorn(1992), Ijzerman, M.M., et.al. and (1993), Ijzerman, M. Marian, et.al., 1994). In addition, the ease with which the sensitivity of the Standard Methods technique may be extended, simply by plating more sample aliquots, is an important consideration. For treated water, the use of the colorimetric techniques of Ijzerman, et.al., in conjunction with analyses of long-term presence-absence data should enhance the utility of coliphage in the evaluation of treated waters.

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