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INVESTIGATION OF POLLUTION IN A KARST AQUIFER UTILIZING OPTICAL BRIGHTENER

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ABSTRACT

Optical brightener is an additive to laundry detergents and is found contaminating groundwater. Its concentration may rapidly and inexpensively be determined by fluorescence techniques, and because its source is human wastewater, its presence in groundwater serves as a direct indication of pollution from septic tanks, sewer leaks, and landfills.

A total of 105 wells and springs in an area within the Inner Bluegrass Karst Region near Lexington, Kentucky, were described and sampled. Analyses were made for optical brightener (430 samples), total coliform (91), fecal coliform (93), and fecal streptococci (90). As many as 20 optical brightener and 4 bacterial samples were analyzed from a single site during the period from May 20, 1984 to June 17, 1985. Data were also collected on spring discharges, well water levels, and other site characteristics.

Statistical analysis of the relationship between optical brightener and the bacterial indices showed low correlations for both springs and wells, in contrast to an earlier study. Although time constraints have precluded a thorough analysis of the data, the difference between the results of the two studies appear to be related to differing site populations and analytic and statistical procedures. The data further suggest that the low correlations between optical brightener and the bacterial indices may be a result of bacterial contamination being largely derived from animal waste and other non-human sources, and that optical brightener may be a more reliable indicator of human contamination.

ACKNOWLEDGEMENTS

We thank James Bibb, Dept. of Biology, Univ. of Kentucky; Barbara Porter, Lexington-Fayette County Health Department; and E.E. Geldreich, US EPA, for thier assistance with the conduct of the investigation. Appreciation is also expressed to the many landowners and tenants whose names appear in Table 1 for their cooperation in allowing access to their properties and their assistance in collecting data.

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CHAPTER I - INTRODUCTION

Numerous dye traces have been conducted during studies of the hydrogeology of the Inner Bluegrass Karst Region of central Kentucky (Thrailkill, et. al., 1982). Although the preferred dye for these water traces is optical brightener, its use is precluded in some areas by a "background" content of optical brightener in springs, a phenomenon which has also been noted in other karst areas (e.g., Quinlan and Rowe, 1977). Because optical brightener is a common additive to laundry detergents, its presence (when not introduced as a water tracing agent) suggests contamination of groundwater by septic tank effluent, sewer leaks, or landfill leachate. The concentration of optical brightener is rapidly and inexpensively determined by measuring its fluorescence, and it therefore promises to be an effective and useful index of human pollution of natural waters.

In order to investigate the utility of optical brightener as an index of pollution, its correlation with other measures of pollution should be determined. The most commonly used such measures are the bacterial indices: total coliform, fecal coliform, and fecal streptococci. A preliminary study showed relatively high correlations between these bacterial indices and optical brightener in stream and spring samples from sites in the Inner Bluegrass Karst Region. The present study was designed to confirm these correlations, extend the investigation to wells, and examine the relationship between patterns of pollution and cultural and hydrogeologic factors.

Project Objectives

1. To ascertain the degree to which optical brightener in groundwater correlates with bacteriologic indices of pollution and may thus be utilized as an indicator of human pollution.
2. To investigate relationships between such pollution and factors such as degree of urbanization, well and spring characteristics, degree of groundwater basin development, and flow directions in a portion of the Inner Bluegrass Karst Region.

CHAPTER II - RESEARCH PROCEDURES

Field Methods

An intensive reconnaissance was undertaken within a radius of about 25 km from the center of the city of Lexington, Kentucky, with the goal of locating wells from which water samples could be obtained. The area was chosen to minimize travel time and expense, and allow repeated sampling visits to at least some sites. The location of suitable wells was found to be controlled mainly by the distribution of municipal water service extensions, and no wells were found in the area south of Lexington which were judged suitable for sampling. The opportunity arose during the conduct of another project (Thrailkill, et al., 1985) to sample 2 wells west of the city of Frankfort, somewhat outside the original area. Springs were selected for sampling on the basis of their proximity to wells and other factors. One farm pond was also sampled.

The location of the 105 sites sampled is shown on Fig. 1. The study area lies near the center of the Inner Bluegrass Karst Region. The location of each site is also given in Table 1 in what is termed the LT coordinate system. This system has been found to be more convenient to use and less subject to error than the more familiar latitude and longitude system. LT coordinates consist of two letter groups followed by two numbers. The first letter group is a contraction of the name of the 7.5 minute quadrangle (CENV, Centerville; CLNV, Clintonville; FRFW, Frankfort West; GEOR, Georgetown; LEXW, Lexington West; MIDW, Midway; VERS, Versailles) and the second identifies one of nine 2.5 minute quadrangles within the larger quadrangle indicated by tick marks on the margin and within the map area (e.g., CC, center; NW, northwest; WC, west-center). The first number is the map distance in inches east of the west boundary, and the second the distance north of the south boundary, of the 2.5 minute quadrangle. Further explanation of the LT coordinate system, including formula to convert to latitude and longitude, is in Thrailkill, et al. (1982).

Other site or sample information collected includes the name of the owner or tenant (Table 1); the depth, well head elevation, pump type, and comments for wells (Table 2); and the date sampled (in day-month-year order), the estimated discharge of springs (using methods described in Thrailkill, et al., 1982), and the depth to water, measured with an electrical sonde, for wells (Table 3).

Spring samples were collected as near as possible to the spring rising, and well samples were taken from the nearest faucet to the well after pumping for at least 10 (and usually 30) minutes. Samples for bacterial analysis were

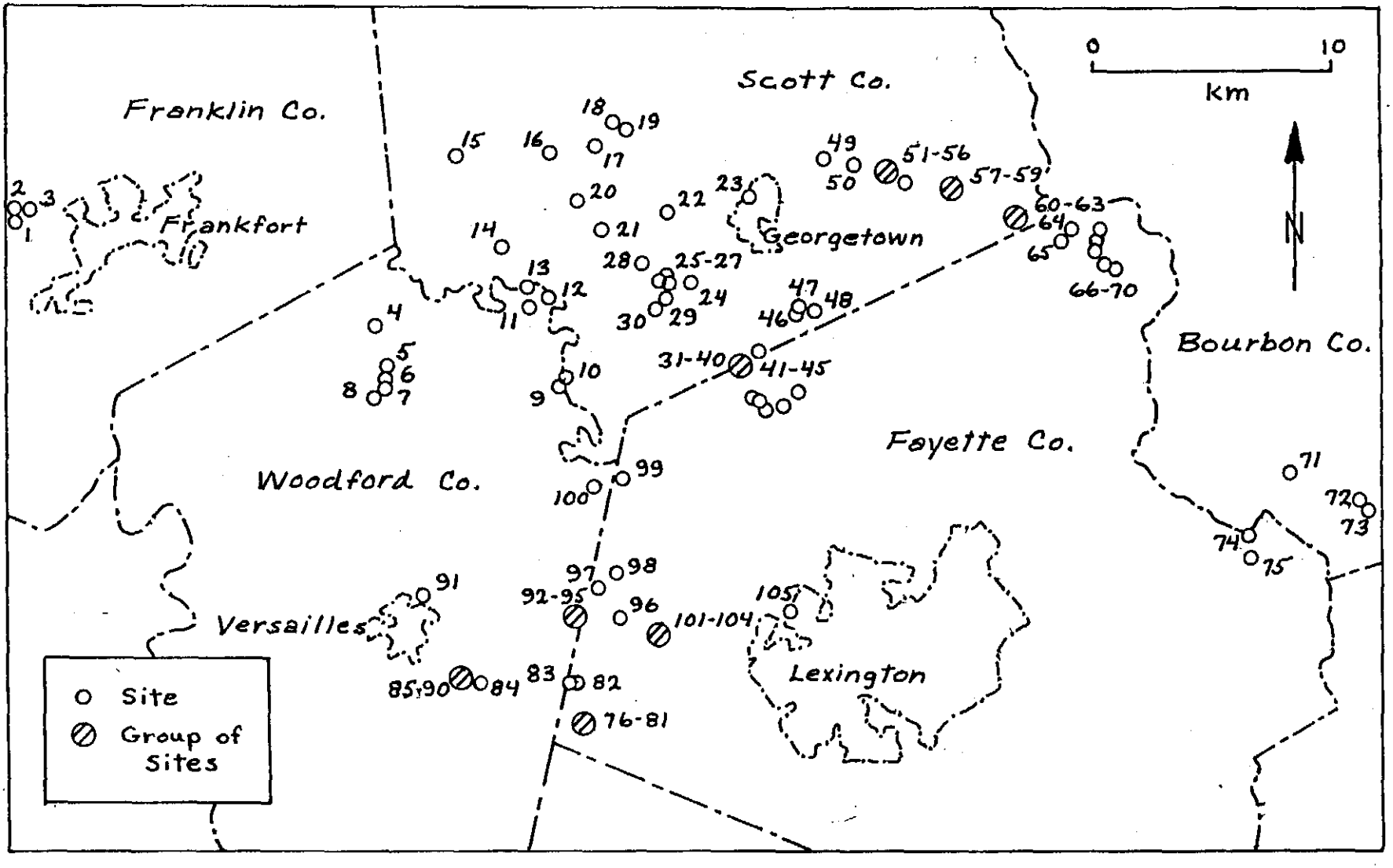

Fig. 1. Map showing location of sites sampled.

collected in sterile glass bottles and transported to the laboratory packed in ice. Samples for optical brightener were collected in 125 mL Nalgene bottles and transported in darkness.

Optical Brightener Analysis

Analytic procedures were generally as given in Thrailkill (1983). Samples were examined with an Aminco SPF - 125S scanning spectrofluorometer (xenon lamp) in standard 12.5 x 12.5 mm (OD) cuvettes which were rinsed twice with distilled water and 3 X with the sample. All measured intensities were corrected by reference to the fluorescent intensity of a uranium-doped glass black to correct for variations in lamp output.

Buffered (1 mM $NaHcO_3$) standard solutions were made up from a commercial preparation (CIBA - Geigy Tinopal LPW) of Fluorescent Brightener 28 (Constitution Number 40622, Society of Dyers and Colourists, 1971) with the laboratory designation Dye 14. This preparation was earlier thought to be Fluorescent Brightener 351 (Thrailkill, 1983) but is now known to be FB 28. Optical brightener concentrations in samples are reported as Dye 14 equivalents in µg/L (parts per billion) relative to the weight of the dye as received (which includes inert ingredients). Various sets of standards were used throughout the study which showed variations in intensity (probably due to instrumental effects) of about 15%. A typical corrected intensity for a concentration of 10 µg/L was 0.00169.

Fluorescent intensities were measured at an excitation wavelength of 360 nm and an emission wavelength of 430 nm. Fluorescence of organic compounds other than optical brighteners has been reported from surface waters by Smart, et al. (1976). The possibility exists, therefore, that the measured sample fluorescence may be due to compounds other than optical brightener. A comparison of emission scans of Dye 14, commercial laundry detergents, and water samples (Fig. 2), together with the fact that some of the spring waters are known to produce the characteristic optical brightener fluorescence when adsorbed on cotton, has led to the provisional conclusion that the measured fluorescence of samples (at least above a certain threshold) is produced by optical brightener. Further investigation of this subject is needed, however.

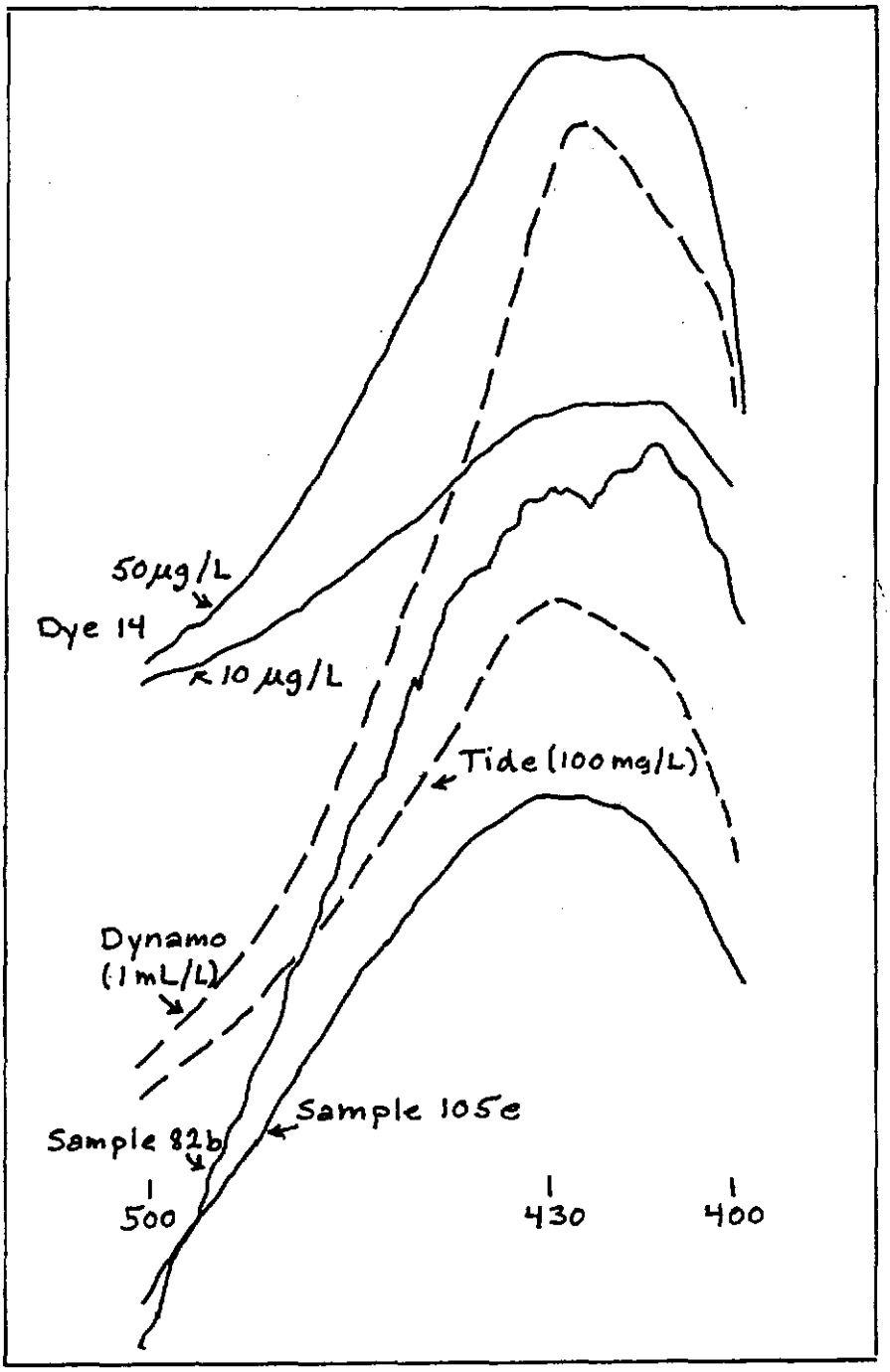

Fig. 2. Emission spectra of Dye 14 used as optical brightener standard (upper solid lines), commercial detergents (dashed lines), and field samples (lower solid lines). All spectra at 430 nm emission, 2 mm excitation slit, 1 mm emission slit. Horizontal scale emission wavelength in nm, fluorescent intensity (vertical scale) varies for different spectra due to scale factor and uncorrected instrument response.

Bacterial Analysis

Total coliforum, fecal coliform, and fecal streptococci were analyzed according to Standard Methods 909A, 909C, and 910B (American Public Health Assoc., 1980), respectively. Difco media mEndo, total coliform; mFc, fecal coliform; and KF Strep Broth, fecal coliform; were rehydrated with distilled deionized water, stored in darkness at 40^0C, and used within 15 hours of preparation (following Bordner, et al., 1978). In the earlier portion of the study, rosolic acid, added to mFc broth to inhibit nonfecal coliforms, was kept for approximately 4 weeks. Although the recommended storage period is 2 weeks (Bordner, et al., 1978), addition of rosolic acid is optional and growth of nonfecal coliforms was not significant.

A vacuum pump connected to 4 membrane filtration units was used. Prior to March, 1985, Millipore HA filters were used, and Gelman GN6 filters subsequently. Although studies have indicated that the Gelman filters recover more fecal coliforms than the Millipore filters (Presswood and Brown, 1973; Green, et. al., 1975), more recent investigations conducted by the EPA indicate that both filters are equivalent and can be used interchangeably (A. Dufour, personal communication). Sterile phosphate-buffered diluent and rinse water was prepared from distilled deionized water and kept on ice during use to avoid stressing the microorganisms (McFeters, et al., 1984).

Up to 4 sample increments, in order of increasing volume, were filtered to obtain colony counts within the recommended ranges (20-80 for total coliform, 20-60 for fecal coliform, and 30-300 for fecal streptococci). Individually wrapped sterile disposable borosilioate glass pipettes were used for sample volumes of 10 mL or less. Membrane filtration units were sterilized between samples by autoclaving for 10 min. at 121^0C and 15 psi.

Total coliform and fecal streptococci plates were inverted, enclosed in tight-fitting plastic containers with wet towels to maintain 100% humidity, and incubated at 35^0C $\pm$ 0.5. Fecal coliform plates were placed in a water bath (44.5^0C $\pm$ 0.2) for incubation within 30 min. of filtration. Temperatures were determined with a mercury thermometer (0.1^0C graduations) calibrated against an NBS - certified thermometer.

Total and fecal coliform colonies were counted after 24 hours of incubation, and fecal streptococci after 48 hours, using a wide-field binocular microscope (10X or 20X magnification) with daylight-type fluorescent illumination. Total coliforms showed a green sheen on mEndo media while noncoliforms were medium to dark red. Fecal coliforms were blue and noncoliforms green or

red depending on the age of the rosolic acid. Fecal streptococci were pink or red and nonfecal streptococci white to cream.

Verification of total coliform colonies was done according to Bordner, et al. (1978), and in all cases green-sheen colonies confirmed positive and medium to dark red colonies confirmed negative. Gram stains showed the green-sheen colonies to be gram-negative rods.

Precision of the values for the 3 bacterial indices were estimated by replicate analyses of 5 samples and less than 5% variation was found in all cases. Some samples showed no bacteria, indicating that no contamination was introduced during sample collection or analysis.

Problems were encountered on June 22, 1985 with one lot of KF Strep media, which produced many white colonies and some pink colonies. Gram stains showed some of the white and all of the pink colonies to be gram-positive cocci while other white colonies were gram-positive rods. In confirmation tests (Bordner, et. al., 1978), some white and all pink colonies confirmed positive and other white colonies confirmed negative indicating a nonfecal streptococcal origin. No explanation for the abundant white colonies can be offered, and the data were discarded.

Difficulties may arise with membrane filter techniques when analyzing turbid waters and recovering stressed microorganisms (Bordner, et. al., 1978). Turbidity was significantly high in only 4 samples (67, 69, 75, and 84). Counts from the highest dilutions were used to alleviate the turbidity problem in these samples. None of the waters sampled were chlorinated. Although stressed microorganisms may result from pollution of natural waters (Geldreich, personal communication) and preenrichment on nonselective media is recommended, no attempt was made to assess the possible effects of such stress.

CHAPTER III - DATA AND RESULTS

A total of 430 water samples from 105 sites were analyzed for optical brightener. More than half (59) of the sites were sampled twice to 20 times. The first sample was collected on May 26, 1984 and the last on June 17, 1985. Of the 105 sites, 23 are springs, 81 are wells, and 1 a pond. The optical brightener results are given in Table 3.

Determination of bacterial indices were made on up to 4 samples from each of 58 sites (13 springs and 45 wells). Analyses were made for total coliform (91 samples), fecal coliform (93 samples,), and fecal streptococci (90 samples). These analyses are also given in Table 3.

The principal objective of the project was:

> To ascertain the degree to which optical brightener in groundwater correlates with bacteriologic indices of contamination and may thus be utilized as an indicator of human pollution.

Plots of log optical brightener versus log bacterial indices are shown in Fig. 3-8 for those samples analyzed for both constituents. On these plots, and in the following statistical analyses, optical brightener data were combined into tenth log-cycle groups and bacterial indices into half log-cycle groups. Zero values of the bacterial indices were considered to be located below the lowest group (1-3 colonies/100 ml).

The slope of the regression line of optical brightener (considered the dependent variable) on the 3 bacterial indices (and the correlation coefficient) are all positive. The correlation coefficients and slopes of the optical brightener - total coliform and optical-brightener-fecal streptococci relationships for both springs and wells (Fig. 3, 4, 7, 8) were very low. A test of the null hypothesis Ho: = 0 (Snedecor and Cochran, 1967) and Ho: = 0 (Krumbein and Graybill, 1965) showed none of these values of r or b to be significantly non-zero at the 95% confidence interval (Table 4). The correlation coefficients and slopes of the regression lines for the optical brightener-fecal coliform relationship in springs and wells were, however, significantly non-zero (Fig. 5, 6; Table 4).

These results raise two questions. The first regards the discrepancy with the preliminary study and the second, and more fundamental, deals with the assessment of the value of optical brightener as an index of human pollution.

In the preliminary study conducted by M.H. Elliott, analyses for optical brightener and bacterial indices were done on 23 samples from surface streams (6 samples) and springs (17 samples). Correlation coefficients between optical

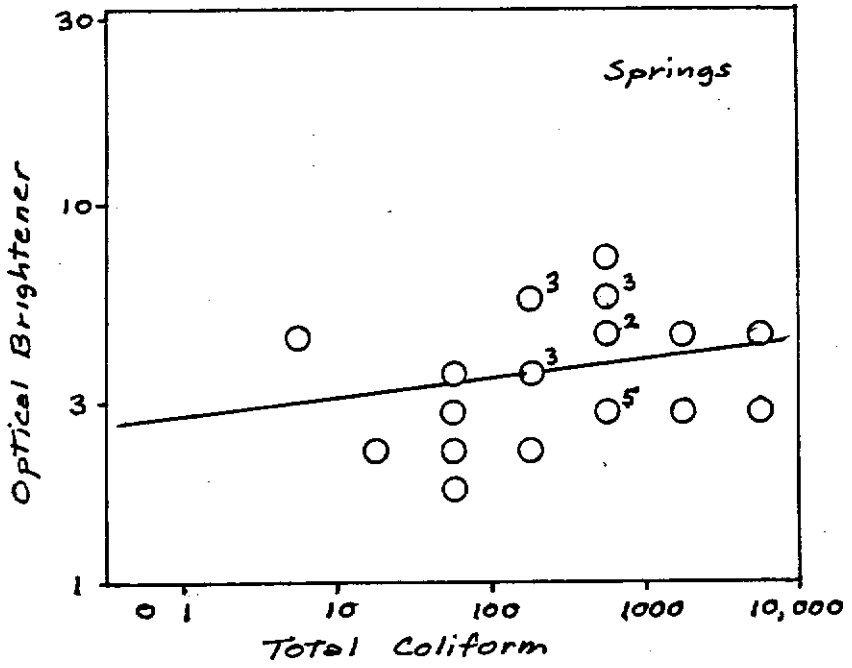

Fig. 3. Optical brightener (µg/L Dye 14 equivalent) vs. total coliform (colonies/100 mL) in springs. More than one sample shown by numeral. Line is regression of y on x.

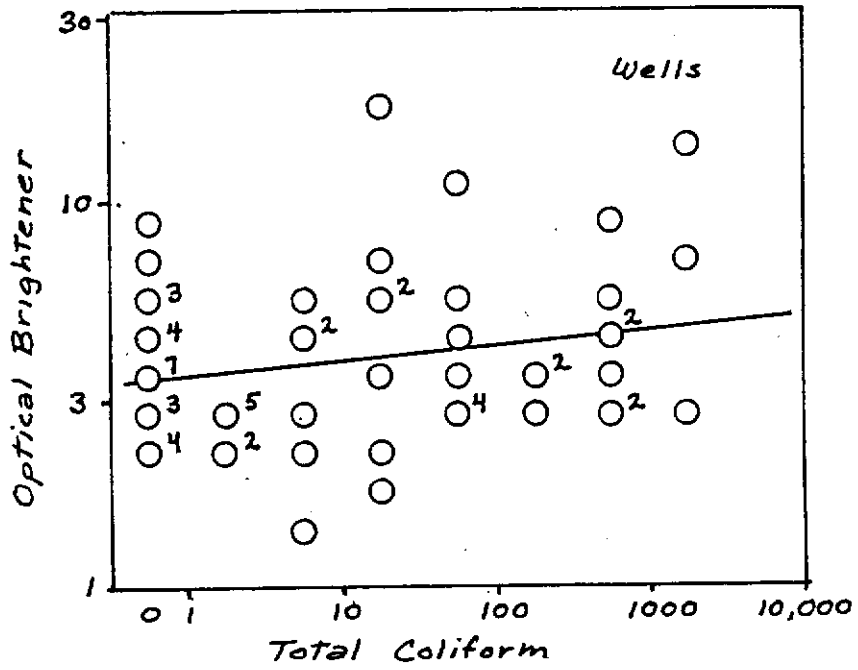

Fig. 4. Optical brightener vs. total coliform in wells. See Fig. 3 for additional explanation.

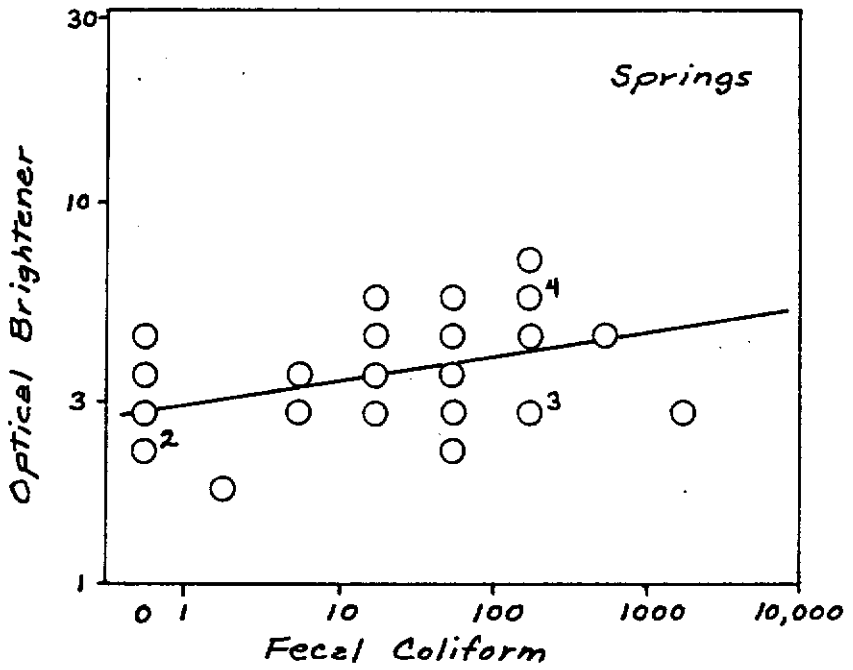

Fig. 5. Optical brightener vs. fecal coliform in springs. See Fig. 3 for additional explanation.

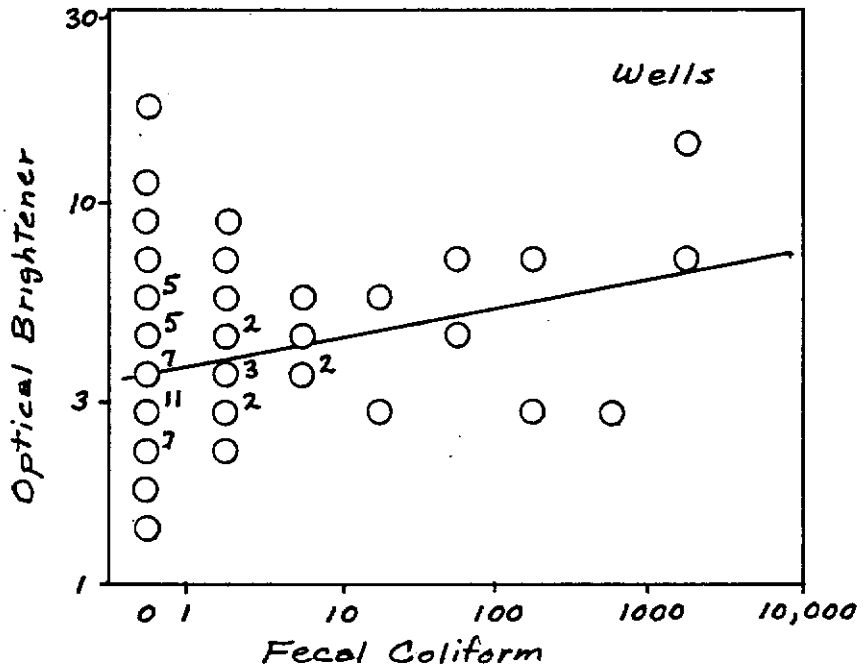

Fig. 6. Optical brightener vs. fecal coliform in wells. See Fig. 3 for additional explanation.

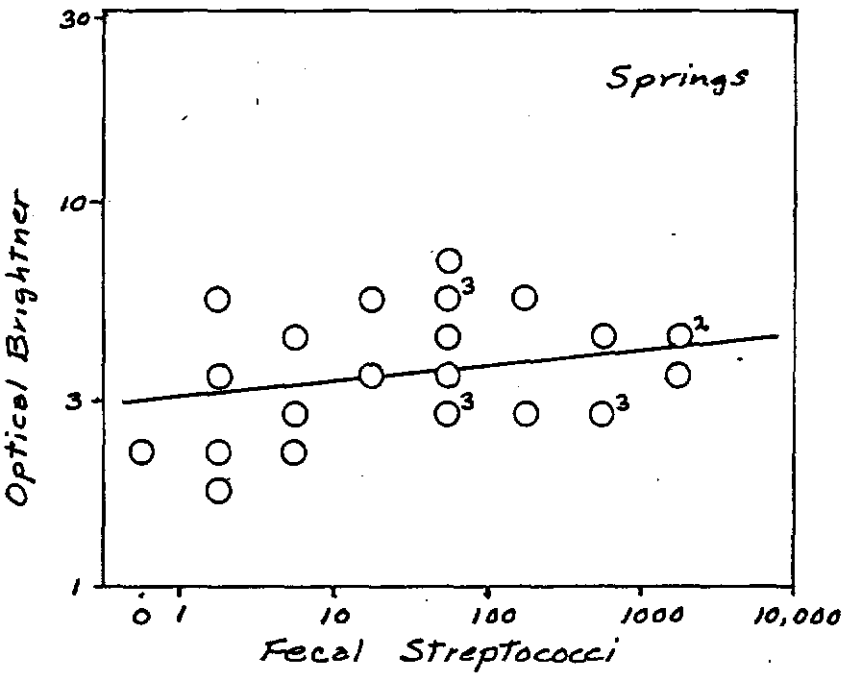

Fig. 7. Optical brightener vs. fecal streptococci in springs. See Fig. 3 for additional explanation.

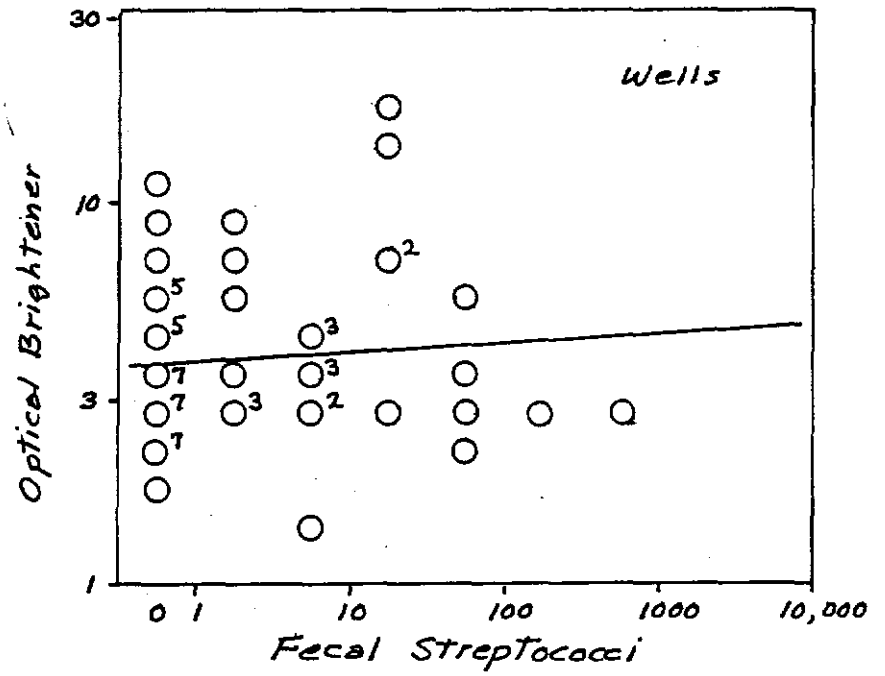

Fig. 8. Optical brightener vs. fecal streptococci in wells. See Fig. 3 for additional explanation.

Table 1. Location, ownership or tenancy, and type of sampled sites. See text for discussion of LT system used for locations.

Site	Location	Owner or Tenant	Well (w), Spring (s), or Pond (p)
1	FRKW EC 0.10 6.89	Lukjan, Mr.	s
2	FRKW NE 0.61 0.10	Florinen Mr.	w
3	FRKW EC 1.23 7.40	Palmer A.	w
4	MIDW SW 1.00 5.83	Alexander Family	s
5	MIDW SW 1.63 3.88	Winchester L.	w
6	MIDW SW 2.93 2.55	Brewer B.	w
7	MIDW SW 2.70 2.20	Brewer B.	w
8	MIDW SW 2.10 2.10	Brewer B.	s
9	MIDW SE 2.20 3.49	Phillips, Mr.	w
10	MIDW SE 2.20 3.60	Phillips, Mr.	s
11	MIDW CC 5.71 0.80	Rauss, I.	w
12	MIDW EC 1.40 1.70	Rauss, I.	w
13	MIDW CC 5.69 1.78	Mobearl, Mr.	s
14	MIDW CC 4.19 4.95	Sewell, T.	w
15	MIDW NC 1.32 3.15	Davis, H.	w
16	MIDW NE 1.39 3.72	Wells, Mr.	w
17	MIDW NE 4.52 4.26	unknown	s
18	MIDW NE 5.55 5.73	Shephard, Mr.	w
19	MIDW NE 5.93 5.57	unknown	w
20	MIDW NE 3.07 0.15	Sloane, G.	w
21	MIDW EC 4.78 6.50	Robinson, E.	w
22	GEOR NW 3.45 0.25	Kittering, P.	w
23	GEOR NC 3.03 0.00	Georgetown	s
24	GEOR WC 4.41 2.32	Conner, D.	w

(Table 1 continued)

Site	Location	Owner or Tenant	Well (w), Spring (s), or Pond (p)
25	GEOR WC 3.62 2.57	Long, G.	w
26	GEOR WC 3.47 2.30	Sloane, Mr.	s
27	GEOR WC 3.00 2.38	Sloane, Mr.	s
28	GEOR WC 2.25 3.78	Bryan, R.	s
29	GEOR WC 3.38 1.08	Whitlock, Mr.	s
30	GEOR WC 3.08 0.80	Baker, S.	s
31	GEOR SC 4.10 5.48	Hughe, B.	s
32	GEOR SC 3.02 4.93	Barber, J.	w
33	GEOR SC 3.05 5.05	Willoughby, Mr.	w
34	GEOR SC 2.87 4.78	Stone, R.	w
35	GEOR SC 2.48 5.35	Crimson King Farm	w
36	GEOR SC 2.40 4.88	Crimson King Farm	p
37	GEOR SC 1.87 5.02	Crimson King Farm	w
38	GEOR SC 1.92 4.15	Newtown, N.	w
39	GEOR SC 2.78 4.20	Crimson King Farm	w
40	GEOR SC 1.45 3.15	Mereworth Farm	w
41	GEOR SC 3.32 1.70	Roeckel, I.	s
42	GEOR SC 3.80 1.60	Roeckel, I.	w
43	GEOR SC 4.22 0.88	Arnold, G.	w
44	GEOR SC 4.55 0.60	Wagner, R.	w
45	GEOR SE 0.49 1.95	Petar, Mr.	s
46	GEOR EC 0.45 0.40	Brumback, H.	w
47	GEOR EC 0.61 0.82	Marshall, H.	w
48	GEOR EC 1.40 1.10	Palmer, Mr.	s
49	GEOR NE 2.00 3.32	Courtney, D.	w

(Table 1 continued)

Site	Location	Owner or Tenant	Well (w), Spring (s), or Pond (p)
50	GEOR NE 1.90 1.85	Smith, L.	w
51	GEOR NE 3.55 2.28	Sills, Mr.	w
52	GEOR NE 4.21 3.06	Bell, C.	w
53	GEOR NE 4.94 2.65	Johnson, C.	w
54	GEOR NE 5.30 2.40	Blackburn, Mrs.	w
55	GEOR NE 5.40 2.07	Mulhall, Mr.	w
56	GEOR NE 4.91 2.25	Marshall, Mr.	s
57	CENT NW 5.02 1.38	Varellas, Mr.	w
58	CENT NW 5.28 1.35	Varellas, Mr.	w
59	CENT NW 4.80 0.71	Varellas, Mr.	w
60	CENT CC 3.40 7.07	Cregall, J.	w
61	CENT CC 4.36 6.92	Cregall, R.	w
62	CENT CC 4.19 6.48	Stakelin, Mrs.	w
63	CENT CC 4.21 6.28	Murphy, J.	w
64	CENT EC 2.02 5.78	Wall, N.	w
65	CENT EC 1.18 5.28	Carmine, C.	w
66	CENT EC 3.08 5.69	Biddle, Mr.	w
67	CENT EC 2.93 5.43	Evanoff, Mr.	w
68	CENT EC 2.65 4.48	Ferguson, Mr.	w
69	CENT EC 3.28 3.80	Estes, J.	w
70	CENT EC 3.52 3.32	Carr, Mr.	w
71	CLIN NC 4.15 4.35	Aulick, F.	w
72	CLIN NE 3.34 3.21	Wells, Mr.	w
73	CLIN NE 3.46 1.38	King, F.	w
74	CLIN NC 1.55 0.19	Marshall, Mr.	w

(Table 1 continued)

Site	Location	Owner or Tenant	Well (w), Spring (s), or Pond (p)
75	CLIN CC 1.41 6.02	Bluegrass Army	w
76	VERS SE 3.58 2.65	Wagner, R.	w
77	VERS SE 3.54 2.50	Muddiman, Mr.	w
78	VERS SE 3.40 2.50	Kirkland, A.	s
79	VERS SE 3.39 2.52	Barron, J.	w
80	VERS SE 3.31 2.62	Bowers, G.	w
81	VERS SE 3.20 2.43	McDaniel, Mr.	w
82	VERS SE 2.37 5.93	Jackson, Mr.	w
83	VERS SE 2.31 5.99	Brown, G.	w
84	VERS SC 1.50 5.45	Brown, F.	w
85	VERS SC 1.60 5.83	Walker, Mr.	w
86	VERS SC 1.59 5.92	Woodley, Mrs.	w
87	VERS SC 1.52 6.12	Bottoms, R.	w
88	VERS SC 1.47 6.19	Bottoms, R.	w
89	VERS SC 1.17 6.19	Russell, Mr.	w
90	VERS SC 1.08 6.08	Miles, Mr.	w
91	VERS WC 4.45 3.9	Curtis, H.	w
92	VERS EC 1.66 2.65	Merryman, R.	w
93	VERS EC 1.72 2.69	Stone, D.	s
94	VERS EC 1.80 2.68	Marshall, R.	w
95	VERS EC 2.15 2.80	Hutcherson, J.	w
96	VERS EC 5.18 2.70	Zeller, Mr.	w
97	VERS EC 4.90 3.87	Taulbee, Mr.	w
98	VERS EC 5.80;4.93	Risner, Mr.	w
99	VERS NE 5.78 3.50	Marsh, Dr.	w

(Table 1 continued)

Site	Location	Owner or Tenant	Well (w), Spring (s), or Pond (p)
100	VERS NE 3.95 3.97	Davis, I.	w
101	LEXW WC 2.89 1.89	Keenland Assoc.	w
102	LEXW WC 3.02 1.96	Keenland Assoc.	s
103	LEXW WC 2.05 0.49	Keenland Assoc.	w
104	LEXW WC 1.98 0.40	Keenland Assoc.	s
105	LEXW CC 5.88 2.82	unknown	s

Table 2. Additional data on wells sampled. Well depth in meters is measured or reported (r). Elevation in meters to ground level. Pump type is submersible (sub), jet, or hand.

Site	Well Depth		Elevation	Pump Type	Comments
2	12	r	152	jet	rarely used
3	24	r	152	jet	never dry
5	148		258	sub	dom. and stock use
6	36		255	sub	rarely used, goes dry
7	37		266	sub	rarely used, goes dry
9	17	r	242	jet	H2S, dom. use, never dry, bacterial pollution (July, 1983)
11	27	r	257	sub	H2S, stock use
12	24	r	251	sub	H2S, dom. use, goes dry
14	12	r	254	jet	H2S, dom. and stock use, often dry
15	58	r	255	--	black, H2S, goes dry
16	18	r	230	jet	dom. use, goes dry
18	37	r	258	jet	dom. and stock use, goes dry
19	--		257	jet	H2S, stock use
20	--		263	sub	H2S
21	8 12	r	233	sub	H2S, never dry
22	--		258	jet	dom. use
24	--		254	jet	dom. use
25	30 26	r	260	sub	H2S, stock use, never dry
32	44 46	r	271	sub	black, H2S, dom. use, goes dry

(Table 2 continued)

Site	Well Depth		Elevation	Pump Type	Comments
33	33		271	sub	H2S, never dry
34	36 56	 r	277	sub	goes dry
35	--		278	sub	dom. and stock use, never dry, gasoline leak reported, bacterial pollution Jan. (1984)
37	17 41	 r	271	sub	stock use, never dry
38	--		278	--	
39	--		280	sub	black, H2S, corrosive, use discontinued (Nov., 1984)
40	82		277	sub	dom. and stock use
42	26	r	277	jet	H2S, dom. and stock use
43	23	r	280	jet	dom. and stock use, very good supply
44	--		287	--	H2S, dom. use, goes dry
46	18		255	jet	dom. use, never dry
47	16	r	256	jet	garden irrigation, goes dry
49	63	r	265	sub	dom. use
50	29		265	sub	dom. and stock use
51	24	r	265	sub	dom. use
52	37	r	268	jet	dom. and stock use, poor quality after snow melt
53	20	r	261	jet	dom. and stock use, muddy and bacterial pollution after rain (July, 1982)
54	18	r	262		dom. use, sewage pollution (1980)
55	30		260	jet	H2S, dom. use, never dry

(Table 2 continued)

Site	Well Depth		Elevation	Pump Type	Comments
57	21		268	jet	good quality
58	21		268	jet	stock use
59	12	r	274	jet	H2S, dom. use, went dry summer, 1983
60	33	r	275	jet	dom. and stock use, never dry
61	35	r	287	jet	H2S, dom. and stock use, never dry
62	27	r	281	sub	dom. use, never dry
63	17	r	277	jet	dom. use, never dry
64	--		296	jet	H2S, stock use, never dry
65	--		288	jet	stock use, never dry
66	--		287	jet	dom. and stock use, never dry
67	18	r	283	jet	dom. use, never dry
68	24	r	282	jet	dom. use, never dry
69	20 21	r	287	sub	stock use, very muddy, never dry
70	--		283	sub	dom. use, never dry
71	--		287	jet	H2S, stock use, never dry
72	--		293	jet	H2S, dom. use, never dry
73	--		289	--	dom. and irrigation use, never dry, H2S in beginning but no longer problem
74	24	r	300	jet	dom. and stock use, never dry
75	46	r	286	--	muddy
76	21 15	r	260	jet	H2S, garden irrigation, never dry
77	20		254	jet	dom. use, never dry

(Table 2 continued)

Site	Well Depth		Elevation	Pump Type	Comments
79	6	r	268	jet	dom. use, never dry
80	25		277	jet	dom. use, goes dry
81	11		276	jet	dom. use, goes dry
82	--		271	hand	not used, sewage contamination reported
83	18	r	271	hand	not used, H2S at beginning, sewage contamination reported
84	21		299	jet	dom. use, goes dry
85	18		295	jet	H2S, never dry, dom. use, bacterial pollution (Dec. 1982 and June, 1983)
86	12 13	r	294	jet	dom. use
87	18 11	r	290	none	dom. use
88	--		290	jet	H2S in summer, never dry
89	--		290	--	dom. use, never dry
90	--		291	none	never dry
91	--		280	sub	dom. use, stains baths
92	4		267	jet	dom. use, never dry
94	11 10	r	264	jet	iron from pipes, never dry
95	14	r	261	jet	dom. use, never dry
96	66	r	268	sub	H2S, goes dry, no bacterial pollution (Aug. 1983)
97	20	r	258	jet	dom. use, never dry, no bacterial pollution (Nov. 1981)

(Table 2 continued)

Site	Well Depth	Elevation	Pump Type	Comments
98	--	260	--	H2S, dom. and irrigation use, never dry
99	--	251	jet	H2S, dom. use, low supply
100	21	274	jet	irrigation use, never dry
101	12	274	jet	muddy
103	25	276	sub	H2S, turf track irrigation

Table 3. Optical brightener content (g/l Dye 14 equivalents); Total Coliform (TC), Fecal Coliform (FC), and Fecal Streptococci (FS) bacteria content in colonies/100 milliliters; Spring discharge (s) in liters/sec; and static water level in well (w). Date is in day-month-year order.

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
1	a	070385	6.94	640	150	50		
2	a	070385	4.59	8	1	8		
3	a	070385	3.80	0	0	0		
4	a	140684	7.36				57	
	b	021084	6.65				9	
	c	311084	7.18				130	
	d	241184	6.70				540	
	e	200285	7.25				2300	
	f	230285	5.93				2700	
	g	280285	5.25	110	20	31	1800	
	h	270385	4.45				720	
	i	180485	5.14				680	
	j	230485	5.05	590	128	65	600	
	k	040585	2.52				250	
	l	190585	5.12	600	131	155	200	
	m	010685	6.77				160	
	n	170685	8.50				140	
5	a	130684	2.08					27.08
	b	021084	1.72					
	c	311084	1.60					
	d	241184	1.90					27.52
	e	230285	2.27					
	f	190385	2.35	0	0	0		
	g	270385	2.18					26.75
	h	180485	2.34					
	i	040585	2.06					26.62
	j	010685	1.85					26.67
6	a	140684	3.86					20.40
	b	021084	3.20					
	c	311084	2.67					29.95
	d	241184	3.92					27.70
	e	200285	4.90					16.19
	f	230285	4.34					13.35
	g	190385	3.93	270	5	3		14.27
	h	270385	3.52					15.00
	i	180485	3.60					16.10
	j	230485	3.34	90	1	6		16.63
	k	040585	3.48					17.64
	l	190585	3.08	67	2	27		19.40

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
7	a	130684	6.29					
	b	230285	6.27					
	c	280285	6.42	20	1	2		
	d	270385	5.45					
	e	180485	5.50					
	f	230485	5.28	0	0	0		
	g	050585	4.88					
	h	190585	4.83	0	0	0		
8	a	130684	2.43					
	b	021084	1.79					
	c	311084	1.90				1	
	d	241184	7.95				3	
	e	200285	3.36				18	
	h	230285	2.67				20	
	i	280285	2.47	20	0	2	7	
	j	270385	2.52				4	
	k	180485	2.38				1	
	l	230485	2.21	38	0	0	0.5	
	m	040585	2.37				0.3	
	n	190585	1.91	40	1	3	0.1	
	o	010685	1.60				2.0	
	p	170685	2.38					
9	a	200285	2.13					
10	a	200285	7.03				170	
11	a	171084	1.90					23.72
12	a	171084	3.20					
13	a	171084	5.87					
14	a	171084	3.44					
15	a	171084	7.97					
16	a	171084	2.31					10.21
17	a	171084	5.99				4	
18	a	171084	2.31					
19	a	171084	3.20					
20	a	171084	4.98					
21	a	171084	5.22					51.44

(Table 3 contnued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
22	a	171084	3.09					
	b	311084	3.32					
23	a	171084	9.37				11	
	b	311084	12.40				317	
24	a	300684	2.80					
	b	021084	3.68					
	c	311084	2.37					
25	a	020684	4.81					
	b	300684	2.29					
	c	021084	2.43					15.83
	d	051084	2.97					15.84
	e	311084	2.49					15.77
	f	161184	2.73					15.62
	g	200285	3.51					
	h	210285	3.47					14.71
	i	190385	3.36	0	0	0		15.05
	j	030485	2.92					15.00
	k	290485	2.60					15.61
	l	260585	2.74					15.67
26	a	310584	5.87				9	
	b	300684	6.45				3	
	c	021084	7.30					
	d	051084	6.70					
	e	311084	9.85					
	f	161184	6.29				13	
	g	200285	6.72				1200	
	h	210285	5.78				1200	
	i	190385	4.08	350	74	66	800	
	j	030485	4.30				1100	
	k	180485	4.16				120	
	l	220485	3.88	220	30	47	40	
	m	290485	4.07				0.1	
	n	040585	4.40					
	o	190585	4.74	500	30	790		
	p	260585	4.69					
	q	170685	4.45				85	
27	a	300684	6.72				0.2	
	b	200285	6.86				19	
	c	170685	6.24				2	
28	a	170685	4.80					
29	a	310584	7.83				2	
	b	300684	4.78				0.3	

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
29	c	021084	5.87				< 0.1	
	d	311084	2.97				< 0.1	
	e	161184	5.46				0.7	
	f	200285	4.82				9	
	g	210285	4.86				9	
	h	190385	4.35	6	0	6	8	
	i	030485	4.30				13	
30	a	220485	3.16	120	37	2640	3	
	b	290485	2.97				5	
	c	040585	3.33				11	
	d	190585	3.08	4800	1500	970	4	
	e	260585	3.40				3	
	f	170685	3.05				7	
31	a	020684	4.92				2	
	b	010784	4.43				0.2	
	c	100784	4.75				0.3	
	d	021084	6.71				0.1	
	e	131084	5.70				0.1	
	f	311084	6.23				< 0.1	
	g	171184	5.70				3	
	h	190185	4.75				6	
	i	200285	6.05				15	
	j	210285	8.23				16	
	k	260385	3.38				3	
	l	020485	4.54				20	
	m	030485	4.56				20	
	n	180485	4.24				8	
	o	220485	4.49	6600	750	2520	3	
	p	300485	3.83				3	
	q	040585	4.05				3	
	r	190585	3.99	1000	310	1700	1	
	s	270585	4.33				3	
	t	170685	3.78				7	
32	a	260584	4.81					
	b	120784	2.73					
	c	021084	2.73					
	d	131084	2.91					
	e	311084	2.73					
	f	171184	4.21					
	g	200285	3.28					
	h	220285	3.24					
	i	190385	2.72	2	0	0		
	j	260385	3.20					
	k	180485	2.74					
	l	300485	2.34					
	m	270585	2.80					

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
33	a	120785	3.20					
	b	021084	4.27					
	c	141084	2.73					
	d	311084	7.83					
	e	181184	2.25					
	f	200285	2.68					
	g	220285	2.56					18.80
	h	190385	2.59	1	0	0		
	i	260385	2.66					
	j	180485	2.74					
	k	300485	2.34					19.96
	l	270585	2.38					
34	a	120784	2.97					27.50
	b	260385	3.11					
	c	020485	2.78					
	d	180485	2.74					21.91
	e	220485	2.82	980	540	0		
	f	170685	2.64					
35	a	110784	7.18					
	b	021084	6.88					
	c	131084	7.24					
	d	311084	22.60					
	e	171184	22.84					
	f	190185	9.49					
	g	200285	11.14					
	h	220285	11.19					
	i	260385	10.66					
	j	180485	9.40					
	k	220485	9.12	590	2	1		
	l	220485	10.02					
	m	270585	10.44					
	n	170685	11.30					
36	a	020684	12.30					
37	a	260584	4.39					6.71
	b	110784	2.73					8.09
	c	131084	2.55					
	d	311084	2.61					8.58
	e	181184	2.67					6.95
	f	200285	2.89					6.90
	g	220285	2.59					3.72
	h	190385	2.45	0	0	0		5.32
	i	260385	2.40					6.28
	j	180485	2.50					6.68
	k	300485	2.70					7.21
	l	260585	2.61					7.38

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
38	a	200285	4.54					
39	a	120784	2.25					26.47
	b	131084	2.61					28.77
	c	310885	3.03					29.89
40	a	270584	6.35					
41	a	120684	5.93				< 0.1	
	b	021084	6.88				< 0.1	
	c	030385	6.02				2	
	d	070385	5.88	160	1	3	2	
42	a	120684	6.47					
	b	021084	6.11					
	c	021084	5.28					
	d	030385	5.68					
	e	070385	5.12	12	1	0		
43	a	120684	4.21					
	b	100385	3.99					
	c	180385	3.75	157	5	5		
44	a	120684	2.79					
	b	100385	4.05					
	c	180385	4.22	0	0	0		
45	a	140685	6.05				3	
	b	100385	6.78				64	
	c	180385	6.07	240	69	87	102	
46	a	270584	4.63					2.64
	b	100784	4.04					3.03
	c	021084	3.50					5.11
	d	051084	3.26					5.13
	e	311084	3.50					3.33
	f	171184	3.62					2.90
	g	190185	3.80	0	0	0		2.88
	h	200285	3.72					2.58
	i	030385	3.81					2.79
	j	090385	3.23					
	k	180385	3.57	0	0	0		2.56
	l	020485	3.28					2.56
	m	180485	3.77					3.03
	n	220485	3.62	0	0	0		3.20
	o	050585	3.34					3.17
	p	200585	3.16	0	0	0		4.19
	q	020685	2.98					3.90
	r	170685	3.38					2.83

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
47	a	270584	7.71					
	b	100784	4.51					
	c	021084	4.51					
	d	051084	3.56					
	e	311084	4.39					
	f	161184	4.57					
	g	200285	6.22					
	h	030385	8.82					
	i	090385	9.27					
	j	180385	5.33	20	0	0		
	k	020485	4.29					
	l	020485	6.31					
	m	050585	4.03					
48	a	020684	3.62					
	b	100784	4.15					
	c	021084	3.38					
	d	051084	2.85					
	e	311084	3.44					
	g	161184	6.05					
	h	190185	4.03					
	i	200285	6.22				20	
	j	030385	4.18				34	
	k	090385	4.20				16	
	l	180385	3.62	70	6	11	75	
	m	020485	4.04				73	
	n	180485	3.42				16	
	o	220485	3.28	70	0	1	13	
	p	050585	3.38					
	q	200585	2.80	90	6	38		
	r	020685	2.68					
	s	170685	4.16					
49	a	311084	2.08					
50	a	171084	3.09					20.63
	b	311084	2.49					21.02
51	a	171084	2.43					
	b	311084	2.43					
52	a	171084	5.28					
	b	311084	6.88					17.94
53	a	171084	4.81					
	b	311084	4.33					
	c	180385	5.24	360	28	57		
54	a	171084	2.08					

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
54	b	11084	2.79					
	c	180385	3.18	0	0	0		
55	a	171084	1.66					12.92
	b	100385	1.70					12.71
	c	180385	1.73	11	0	0		12.53
56	a	171084	6.76				1	
	b	311084	6.34				5	
	c	100385	6.40				910	
	d	180385	6.23	620	77	80	890	
57	a	171084	2.91					12.75
58	a	171084	4.92					
59	a	171084	3.62					
60	a	171084	3.03					
61	a	171084	2.61					
62	a	171084	2.55					
63	a	171084	2.85					10.9
	b	230385	3.32	19	1	7		
	c	210585	4.10	80	3	--		
64	a	230385	4.81	0	0	0		
65	a	230385	2.49	0	0	0		
	b	210585	2.25	2	0	0		
66	a	230385	2.66	3	0	2		
	b	210585	2.49	4	1	0		
67	a	230385	5.61	6	0	0		
68	a	230385	5.60	0	0	2		
69	a	230385	14.30	1300	1300	22		11.94
70	a	230385	2.57	0	0	8		
71	a	200385	2.18	0	0	0		
72	a	200385	9.01	0	0	0		
73	a	200385	4.36	8	0	8		

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
73	b	210585	5.92	71	8	--		
74	a	200385	4.36	0	0	0		
75	a	200385	6.50	--	1700	29		
	b	210585	7.60	1000	90	--		
76	a	021084	3.32					4.89
	b	311084	3.74					5.02
	c	020385	3.09					4.58
	d	220385	2.62	77	0	1		4.10
	e	180485	2.95					4.78
	f	200585	2.50	15	0	0		4.87
	g	170685	2.31					4.51
77	a	021084	3.50					4.94
	b	311084	2.61					
	c	020385	2.82					4.44
	d	220385	2.93	133	0	69		
	e	180485	2.72					
	f	230485	2.74	2	0	0		3.77
	g	200585	2.54	3	0	0		
	h	170685	2.31					
78	a	021084	3.92					
	b	311084	6.29				2	
	c	240185	2.20	300	95	5	6	
	d	020385	3.53				8	
	e	180485	2.45				8	
	f	230485	2.86	420	29	4	8	
	g	200585	2.77	700	48	46	6	
	h	170685	3.46					
79	a	240185	5.16	0	0	0		
80	a	020984	3.50					
81	a	220385	1.39	5	0	7		
82	a	280285	18.45	30	0	14		
	b	170685	12.07					
83	a	280285	11.93	50	0	0		
	b	170685	8.66					
84	a	210385	2.54					9.33
	b	220385	7.17	--	170	22		
	c	230485	4.29	560	53	0		10.49
	d	200585	3.12	80	1	1		

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
84	e	170685	3.12					
85	a	240285	1.37					
86	a	210385	2.40					6.34
	b	220385	2.14	1	0	66		
87	a	210385	4.25					
	b	220385	4.19					
88	a	280285	4.45	330	4	4		
	b	220385	3.40	490	3	35		
	c	170685	2.43					
89	a	210385	3.60					
	b	220385	3.01	0	0	0		
90	a	210385	4.89					
91	a	021084	1.96					
92	a	021084	2.73					2.84
	b	311084	4.57					2.75
	c	061184	4.33					2.70
	d	251184	3.68					2.68
	e	240185	2.97	61	12	5		
	f	240285	4.17					2.59
	g	020385	3.36					2.68
	h	220385	3.08	690	0	104		2.70
	i	030485	5.25					2.64
	j	180485	3.11					2.71
	k	230485	2.86	2200	150	420		2.71
	l	290485	7.81					2.71
	m	170685	8.41					2.71
93	a	021084	3.68				0.5	
	b	311084	4.09				0.9	
	c	251184	3.86				19	
	d	240185	2.91	470	300	400	10	
	e	240284	4.17				60	
	f	020385	3.57				20	
	g	220385	3.08	620	0	161	13	
	h	030485	3.61				45	
	i	180485	3.07				9	
	j	230485	2.82	1800	130	335	8	
	k	290485	7.98				7	
	l	050585	4.31				5	
	m	200585	2.96	350	120	78	4	
	n	020685	2.98				5	

(Table 3 continued)

Site and Sample		Date	Optical Brightener	Bacteria TC	FC	FS	s	w
93	o	170685	8.28				9	
94	a	021084	2.90					2.38
	b	311084	3.62					2.23
	c	240185	2.85	0	0	0		
	d	180485	3.88					1.91
	e	290485	3.74					
95	a	240285	4.80					
96	a	240285	13.55					
97	a	240285	4.06					
98	a	200285	3.20					
99	a	200285	1.46					
100	a	200285	6.71					
	b	190385	7.39	0	0	0		
101	a	021084	5.40					1.94
	b	311084	5.34					1.62
	c	061184	6.59					
	d	090385	3.16					1.52
102	a	021084	3.80				2	
	b	311084	4.98				3	
	c	061184	5.04				5	
	d	090385	3.65				40	
103	a	021084	3.62					
	b	311084	2.73					
	c	061184	2.31					
104	a	021084	4.81				0.7	
	b	311084	5.70				0.9	
	c	090385	5.13					
105	a	090984	14.33					
	b	021084	14.48				18	
	c	311084	9.73				85	
	d	061184	9.14				106	
	e	170685	6.59					

Table 4. Correlation and regression statistics and hypothesis testing: optical brightener versus bacterial indices for springs and wells. N, sample size; t_{cr}, critical value of t (= .05, d.f. = n-2, 2 - tailed); r, sample correlation coefficient; t_1, calculated t (Snedecor and Cochran, 1967, p. 184); ρ, population correlation coefficient; b, slope of sample regression line (optical brightener on bacterial index); t_2, calculated t (Krumbein and Graybill, 1965, p. 231); β, slope of population regression line.

	Total Coliform	Fecal Coliform	Fecal Streptococci
Springs			
n	28	28	28
t_{cr}	2.06	2.06	2.06
r	.2052	.4017	.2630
t_2	1.07	2.24	1.39
Ho: =0	accept	reject	accept
b	.0480	.0618	.0409
t_2	1.09	2.43	0.78
Ho: β=0	accept	reject	accept
Wells			
n	63	65	62
t_{cr}	2.00	2.00	2.00
r	.2195	.3026	.0822
t_2	1.76	2.52	0.64
Ho: ρ=0	accept	reject	accept
b	.0393	.0729	.0232
t_2	1.73	2.43	0.64
Ho: β=0	accept	reject	accept

brightener and the bacterial indices were calculated for the ungrouped combined data. The value of r for optical brightener - total coliform was .67, optical brightener-fecal coliform was .64, and optical brightener-fecal streptococci was .38. Possible reasons for the much lower values calculated in the present study (Fig. 3-8; Table 4) include differences between the two investigations in the site population, the optical brightener determinations, the bacterial determinations, and the statistical treatment.

The preliminary study sampled surface streams and springs while the present study sampled springs and wells. In addition, the springs sampled in the preliminary study were generally larger than those of the present study. It is possible, therefore, that the differences between the populations sampled in the two studies contributed to the different results. Because of the considerable differences found in both studies of both optical brightener and bacterial indices at a single site with time, no attempt was made to resample the sites of the preliminary study.

Although both studies used similar procedures for the optical brightener analyses, different standards were used. In the earlier study a liquid product (Dye 12) was used that is no longer using manufactured. Although the active ingredient in both Dye 12 and Dye 14 (solid used in the present study) is Fluorescent Brightener 28, each contains inactive ingredients of unknown concentration (and composition). A small amount of Dye 12 was available, and a comparison showed Dye 14 (in µg/L) to produce substantially higher intensities than Dye 12 (in nL/L). The intensity ratio between the two dyes varied with concentration, however, being highest at 50 ppb (15.7) and lowest at 2 ppb (7.0). The small lot of Dye 12 showed signs of deterioration and may have changed in fluorescent intensity. The minimum Dye 12 equivalent concentration found in samples in the earlier study was 20 nL/L and the minimum Dye 14 equivalent concentration in samples in the present study was 1.46 µg/L (Table 3). This ratio of 13.3 at low concentrations suggests that the Dye 12 intensity may have increased with age.

The methods used for the determination of bacterial indices were somewhat different in the two studies. In the earlier study, distilled (but not sterilized or buffered) rinse water was used; total coliform and fecal streptococci were incubated at 37°C (rather than 35°); fecal coliform were incubated in an air incubator (instead of a water bath); incubation was begun between 30 minutes and 6 hours after filtration (less than 30 minutes in the present study); and filtration units were rinsed with distilled (but not sterilized) water between samples. It is possible that these different

procedures, especially the last described, may have contributed to higher values for bacterial indices found in the preliminary study.

Lastly, a possible, but unlikely, factor contributing to the different correlation coefficients found in the two studies may be the statistical treatment of the two data sets. In the earlier study, a single value of r was calculated for each bacterial index, without separating springs and surface streams, and the data were not grouped. In the present study, wells and springs were considered separately (because of the larger data set available) and the data were grouped as described earlier. This last was done because of the limited time available to prepare this report following the completion of analytic work. It is intended to further analyze the data from the two studies using consistent techniques.

The larger question, however, deals with the implications of the low values of r found in the present study relative to the value of optical brightener as an index of human pollution. One possibility is that the bacterial indices are a good measure of human pollution but that the optical brightener concentrations are, in fact, not optical brightener but some other fluorescing substance unrelated to human pollution. As discussed earlier (under Research Procedures), other organic compounds in surface waters have been found to fluorescence at the same wavelength as optical brightener (Smart, et. al., 1976). Although it is believed, for reasons discussed, that optical brightener is producing the fluorescence in at least some of the samples measured, it is quite possible that other compounds are contributing some of the fluorescent intensity. This is especially likely at low intensities, since it does not seem reasonable that all of the sites sampled contained optical brightener. A complete examination of the fluorescence data set (over 400 samples) is needed before further conclusions on this question can be reached.

The alternate explanation for the low correlation between optical brightener and bacterial indices is that the bacterial indices are unrelated to human pollution. This is almost certainly true in many cases. The lowest correlation found was between optical brightener and fecal streptococci, which would be expected inasmuch as fecal streptococci are known to be more abundantly derived from animal than human sources. It has been proposed that a fecal/coliform fecal streptococci ratio equal to or greater than 4 indicates a human source, and a ratio less than or equal to 0.7 an animal source (Geldreich, 1966). Although other work has suggested the rapid die-off of certain bacteria may render these ratio invalid after 24 hours (Geldreich and Kenner, 1969; McFeters, et. al.,

1974), it is obvious that this and other aspects of the bacteria data set should be examined. One such aspect that must be investigated is the local conditions at the spring or well. During the study, there were indications that bacterial contamination may be related more to such local conditions than to conditions in the aquifer.

The time available since the conclusion of analytic work and the deadline for this report has not been sufficient for the analysis both of the fluorescent data and the bacterial indices needed to arrive at further conclusions regarding the primary objective. It is intended that such data analyses will be undertaken prior to publication of the findings of the study.

The second objective of the project was:

> To investigate relationships between... pollution and factors such as degree of urbanization, well and spring characteristics, degree of groundwater basin development, and flow directions in a portion of the Inner Bluegrass Karst Region.

Sites were selected for sampling during the study in order to pursue this objective, and substantial amounts of data (e.g., spring discharges, well characteristics) were collected to evaluate the factors which may influence groundwater pollution.

It is felt that it is necessary to further analyze the data relating to the first objective before attempting to reach conclusions on the second objective. As discussed above, time constraints have precluded this analysis. Once it is completed, a further analysis of the data to evaluate factors influencing groundwater pollution is planned.

CHAPTER IV - CONCLUSIONS

A total of 105 wells and springs in an area within the Inner Bluegrass Karst Region near Lexington, Kentucky were described and sampled. Analyses were made for optical brightener (430 samples), total coliform (91), fecal coliform (93), and fecal strepticocci (90). As many as 20 optical brightener and 4 bacterial samples were analyzed from a single site between May 26, 1984 and June 17, 1985.

Statistical analyses of the relationship between optical brightener and the bacterial indices showed low correlations for both springs and wells, in contrast to a preliminary study. Only the correlation between optical brightener and fecal coliform was significant. There has not been sufficient time since the completion of analytic work for a complete analysis of the data, and only tentative conclusions can be offered. Reasons for the substantially lower correlations than those found in the preliminary study are probably related to the different site population, differences in analytic techniques and standards used for the optical brightener and bacterial indices determinations, and/or different statistical treatment between the two studies.

It appears that at least the higher levels of optical brightener found in the samples may be a more satisfactory measure of the degree of contamination of the aquifer, and that the bacterial indices are more influenced by animal pollution and local conditions at the well or spring. The large data set available will permit an investigation of the importance of cultural and hydrogeologic factors, but this must await further data analysis.

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