

A SURVEY OF POLLUTION INDICATOR BACTERIA IN WATER WELLS OF RUTHERFORD COUNTY, TENNESSEE

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ABSTRACT

Water samples were collected from 100 unchlorinated wells within the upper Stones River drainage basin in Middle Tennessee. These samples were analyzed by the membrane filter technique for the presence of total coliform, fecal coliform and fecal streptococcus bacterial groups. Total coliform bacteria were detected in 87% of the samples, fecal coliform bacteria in 62%, and fecal streptococcus bacteria in 79%. Nine of the wells sampled were negative for the presence of all three groups. Wells were checked for proper sanitary protection, and readings were made of the water turbidity, temperature, pH and approximate depth.

INTRODUCTION

There has been growing interest in recent years concerning the bacteriological quality of wells in Rutherford County, Tennessee. Increase in development of suburban and rural areas of the county has led to widespread use of septic tanks and absorption fields for domestic sewage disposal. Municipal sanitary sewer is often not available. This condition, in addition to the continuation of livestock production and unmanaged surface runoff over much of the county, has increased the probability of pollution of the subsurface water supplies.

Three bacterial groups (total coliform, fecal coliform and fecal streptococcus) have been established as indicators of pollution in surface and ground waters (Environmental Protection Agency 1978 and American Public Health Association et al. 1976). Total coliforms (all bacteria of the coliform group) may be detected in soils and on plants and insects (Reneau and Pettry 1975) and may be indicative of a fecal source of pollution or a non-fecal source such as surface runoff. Fecal coliforms and fecal streptococcal bacteria are inhabitants of the intestinal tract of warm-blooded animals and, therefore, suggest recent fecal and potentially dangerous pollution of ground water (Gehm and Bregman 1976).

The purpose of this paper was to survey the occurrence of three pollution indicator bacteria (indicated above) in 100 wells within the study area and to determine physical characteristics of well sites which may influence the quality of the underground water supply.

METHODS

Water samples were collected from 100 privately owned wells within the study area (Fig. 1). Ninety-eight of the wells were unchlorinated. Collections were made from April 3, 1979 to

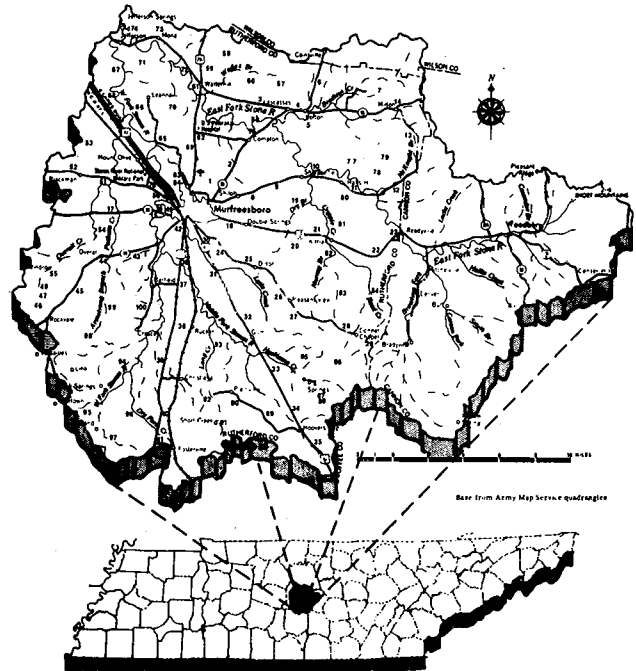


FIG. 1. Location of upper Stones River drainage basin (from Burchett and Moore 1971) and 100 wells in Rutherford County, Tennessee.

April 20, 1979. Three samples were collected at each well and were analyzed by the membrane filter technique for the presence of total coliform, fecal coliform and fecal streptococcus groups. Sample collection, preservation and storage methods, and all bacteriological procedures used were according to the American Public Health Association et al. (1976).

The degree of well protection was recorded with respect to the presence of a sanitary well casing seal and a concrete slab around the well casing and recorded as proper or improper protection. Well protection is described by Tennessee Department of Public Health (1963).

At each site a sample was collected for pH and turbidity measurements. Readings for pH were made in the laboratory with a pH/electrometer in accordance with American Public Health Association (1976). Turbidity measurements were made with a Hellige turbidimeter as described by Welch (1948).

Temperature (C°) was measured on-site by means of a mercurial thermometer. Approximate well depths were obtained from the owners, and the major use of the water supply (primary drinking water or farmyard use) was recorded.

Chlorinators on two chlorinated wells were disconnected, and water allowed to run waste until no chlorine residual remained. Chlorine residual was measured with a Penwalt-Wallace field comparator, using orthotolidine as the indicator agent as described in the *Sanitarians Handbook* (Freeman 1957).

RESULTS AND DISCUSSION

Of the surveyed wells, 83 were used as the primary,

source for drinking water, and 72 of these (86.7%) were positive for one or more of the three bacterial groups. Percent occurrence of indicator bacterial groups is listed in Table 1.

A high number of wells (91%) were positive for one or more of the three pollution indicator bacterial groups, a potentially dangerous condition in the underground water sources of the study area. Although the results of the study were obtained from a single sampling of each well, the fact remains that if any of the indicator bacteria are detected, a distinct possibility exists of further contamination to the water supplies by other enteric and possibly pathogenic bacteria. These findings are even more important since 72% of the 91 positive wells are used as the primary source for drinking water.

It should be noted that the occurrence of fecal coliform and fecal streptococcus bacteria (62% and 79%, respectively) suggest recent, and possibly dangerous, contamination since these bacteria die rapidly in natural waters (Thresh et al. 1944, and Mitchell and Starzyk 1975). The higher percent occurrence of fecal streptococcus over fecal coliform bacteria demonstrates that, although the two groups die rapidly, the fecal streptococcus bacteria have a higher survival capacity as stated by Mitchell and Starzyk (1975). Total coliform bacteria in the water supply indicate the possible presence of fecal bacteria, thus presenting the possibility of pathogenic enteric bacteria. This is supported by the present study, since 62 of the 87 (71%) total coliform positive wells were positive for fecal coliform bacteria and 75 of the 87 (86%) wells were also positive for fecal streptococcus bacteria.

TABLE 1. Percent occurrence of pollution indicator bacteria (total coliform, fecal coliform, and fecal streptococcus) by group in 100 wells in Rutherford County, Tennessee.

Individual and Combined Groups	Percent Of Total
Total coliform detected	87.0
Total coliform and no other group	12.0
Fecal coliform detected	62.0
Fecal coliform and no other group	0.0
Fecal streptococcus detected	79.0
Fecal streptococcus and no other group	4.0
Total coliform and fecal coliform only in same sample	0.0
Total coliform and fecal streptococcus only in same sample	13.0
Percent positive for all three groups in same sample	63.0
Percent negative for all three groups	9.0
Percent positive for any one or more of the three groups	91.0

Data from this study indicate a high percentage of total coliform positive wells (60.9%) and fecal coliform positive wells (50%) having plate counts too

numerous to count (TNTC), which is more than the countable limits of 80 colonies per ml and 60 colonies per ml, respectively (American Public Health Association et al. 1976). Since these wells showed high plate counts, it may be assumed that a high rate and quantity of contamination was entering the water supplies. Fecal streptococcus positive wells had a slightly lower TNTC plate count percentage of 39.2%. Table 2 shows the colony numbers by group and general numbers per plate.

A review of the locations of the well sites and the wells which were positive indicates no trend by county location for positive or negative wells. This possibly indicates a uniformly polluted condition of wells in the study area.

Well protection appears to have little effect upon the positive and negative well percentages. Of the nine negative wells, eight were not properly protected. Of 74 improperly protected wells, 66 (89.2%) were positive.

Wells of 300 feet or less in depth had a high occurrence of the pollution indicator bacteria (94.2%). Although the sample size was small (N=5), wells deeper than 300 feet were less frequently contaminated (40%). This agrees with the general view that deeper wells may be less contaminated (Freedman 1957). Of the wells sampled, 49 were 100 feet deep or less, 29 were 200 to 101 feet deep, six were 300 to 201 feet deep, and five were deeper than 300 feet. Eleven wells were of unknown depth. The deepest well sampled was 966 feet and the shallowest well 10 feet in depth. Totally negative wells ranged in depth from 30 feet to 966 feet with an average depth of 127 feet.

TABLE 2. Colony counts for total coliform, fecal coliform, and fecal streptococcus bacterial groups in 100 wells in Rutherford County, Tennessee.

NO. COLONIES/ PLATE	INDICATOR BACTERIA GROUP					
	TOTAL COLIFORM ON 87 PLATES		FECAL COLIFORM ON 62 PLATES		FECAL STREPTOCOCCUS ON 79 PLATES	
	No.	Percent for Group	No.	Percent for Group	No.	Percent for Group
1 to 80*	34	39.0	31	50.0	48	60.7
TNTC**	53	60.9	31	50.0	31	39.2

*1 to 80/plate maximum countable range for total coliform

1 to 60/plate maximum countable range for fecal coliform

1 to 100/plate maximum countable range for fecal streptococcus

**TNTC—Too numerous to count, totals ranged from 81, 61, and 101, respectively, up to 500 plus.

Positive wells exhibited a wide range of turbidity, pH and temperature. Turbidity in Jackson Turbidity Units ranged from 0.0 to 47.0, temperature from 12°C to 18°C and pH from 6.5 to 8.2. Rainfall appeared to have little effect since the number of positive wells was

almost constant during the study period. The minimum, average and maximum daily rainfall was trace, 0.23 and 2.21 inches, respectively.

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SPORULATION AND ZONATION IN *STEMPHYLIUM*

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ABSTRACT

On certain solid media, the imperfect fungus *Stemphylium botryosum* Wallr. exhibits a growth pattern of concentric rings, composed of alternating zones of sparse and profuse sporulation. This growth pattern is induced in response to alternation of light and darkness and also in response to fluctuations of temperature.

INTRODUCTION

In August 1979 there appeared as a contaminant in a petri dish of corn meal agar a fungus which exhibited the phenomenon of zonation. This is characterized by a growth pattern of concentric rings, consisting of zones in which few or no spores are produced, alternating with others in which sporulation is profuse. The macroscopic appearance of a typical culture is shown in Figure 1.

By reference to the keys and description of Neergaard (1945), the organism was identified as *Stemphylium botryosum* Wallr. This is an imperfect fungus which has as its perfect stage the ascomycete *Pleospora herbarum* (Pers.) Rabenh. Because perithecia were not formed by our isolate, it will be referred to as *S. botryosum* throughout this report.

ZONATION AND SPORULATION

Zonation is known to occur in a wide variety of fungi when grown upon solid media, and there exists a large literature on the subject. It may be induced either in response to alternation of light and darkness or to fluctuations in temperature, or to both of these environmental factors. Further, there is a nutritional factor, since some fungi produce zonate growth upon certain media but not on others, under conditions

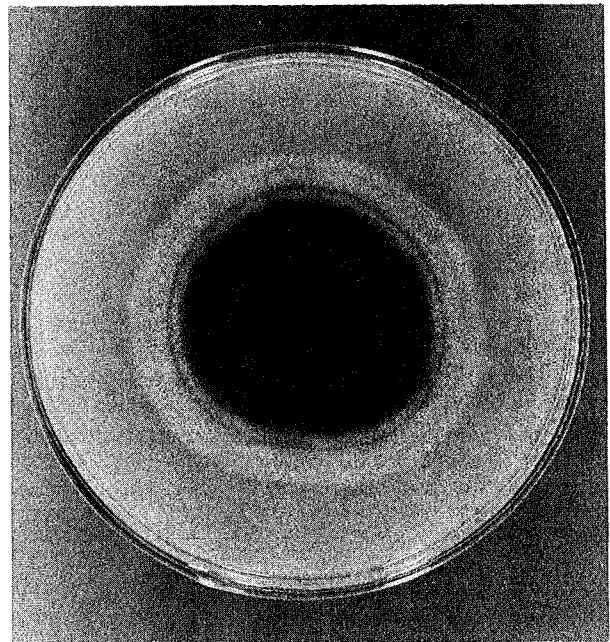


FIGURE 1. Culture of *Stemphylium botryosum* on corn meal agar, showing zonation.

identical except for the composition of the medium.

Ellis (1931) observed no zonation in cultures of *P. herbarum* grown under continuous darkness in combination with constant temperature. When, however, cultures either in constant darkness or in alternating light and darkness were subjected to alternations of temperature, perithecial primordia were produced in zones.

It has long been known that sporulation can be induced in many fungi by exposure to near ultraviolet or blue light. And since periodicity of sporulation is prerequisite for zonation, instances of the control of zonation by light are common. Leach and colleagues have performed extensive studies on the relation of ultraviolet light to sporulation of *S. botryosum*. This organism did not grow in a zonate fashion under continuous 310-400 nm illumination, or in continuous darkness, but zonation was induced by a 12:12 hr. alternation of ultraviolet light and darkness (Leach, 1962). Conidial production was induced by monochromatic ultraviolet irradiation throughout the range 238-366 nm (Leach and Trione, 1966). The range inducing perithecia was essentially similar, but extended to 405 nm in the blue end of the visible spectrum (Leach, 1963).

S. botryosum sporulates poorly or not at all in continuous darkness, and forms only sterile conidiophores in continuous light (Leach, 1968). Leach (1967) distinguished an "inductive phase" leading to the formation of conidiophores, which is stimulated by the near ultraviolet and is operative at relatively high temperatures, and a "terminal phase" in which conidia are produced, which is strongly or completely inhibited by near ultraviolet or blue light, and which has a lower temperature optimum. Thus, at 27°C sporulation of *S. botryosum* was completely inhibited by light, but at 21°C or below light inhibition of conidial production did not occur (Leach, 1967, 1968).

When sporulation of *P. herbarum* was induced by near ultraviolet irradiation, the mycelia produced substances which were absent from non-sporulating cultures grown in darkness (Leach, 1965). These compounds, designated P-310's, exhibited a characteristic absorption spectrum with maxima at 240 and 310 nm, but showed no absorption in the visible range. When a droplet of P-310's was applied to cultures of *P. herbarum* grown in darkness on malt extract agar and subsequently incubated in darkness, sporulation occurred near the point of application 3 days later. Trione *et al.* (1966) established that there were 3 different P-310's, that their molecular weights were below 1000, and that the compounds were stable at 100°C.

Trione and Leach (1969) presented the results of their studies of P-310's from a number of fungi including *P. herbarum* and *S. sarcinaeforme* Cav. The three compounds were designated P-310A, P-310B and P-310C, the latter compound being the most abundant. In addition to data concerning the solubility of P-310C, infra red and nuclear magnetic resonance studies indicated the presence of OH, C=O, ethyl or ethoxy, very few CH, CH₂ or CH₃ groups, with most of the protons attached to O-bearing C atoms, and no aromatic H's.

The accuracy of these findings was amply confirmed by Favre-Bonvin *et al.* (1976), who isolated P-310C from *Stereum hirsutum* (Willd, ex. Fr.) Fr., and, by a combination of methods including ultraviolet, infra red and nuclear magnetic resonance using tritium

and ¹³C, established the structure as 2-methoxy-3-bis (hydroxymethyl) methylamino-5-hydroxy-5-hydroxymethyl-2-cyclohexen-1-one. This compound was designated as mycosporine (Favre-Bonvin *et al.*, 1976) or mycochrome (Kumagai, 1978).

Trione *et al.* (1966) speculated that the differential effects of certain media upon sporulation may, in some instances, be due to the necessity of ultraviolet irradiation in order to convert a precursor into P-310. In *Stemphylium solani* Weber, which sporulates poorly on the usual media. Diener (1955) found that sporulation was abundant when the organism was grown on media containing V-8 juice and irradiated with ultraviolet. Sproston and Setlow (1968) found that ergosterol could substitute for the ultraviolet irradiation requirement for conidial production in this species.

The Nashville isolate of *S. botryosum* formed very few conidia in continuous darkness, but sporulated much more profusely on corn meal agar in white light. Zonation was marked under a regime of 12 hours of light followed by 12 hours of darkness. Further, zonation occurred in constant darkness when the ambient temperature was allowed to fluctuate from day to night. The fungus failed to grow on Mycosel agar, and zonation did not occur on Sabouraud dextrose agar, but did occur on Czapek agar.

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When water-bearing rock transmits water upward to wells and springs, it is called an aquifer. A common belief is that lake water contains significant concentrations of bacteria because lakes are functioning ecosystems. Also, it is believed that groundwater usually has fewer microorganisms than lake water because of its long travel time in the subsurface environment. To check the validity of these beliefs, I gathered my samples from the northeast tip of Lake Sheen in Orange County, FL. In order to ensure the quality of the experimental results, it was necessary to have an experimental control. I decided to use distilled water because it is the purest form of water I could procure. Bacteria as an indicator can predict the possibility of the pathogens in water, but cannot accurately predict the level of contamination. There are several types of indicator plants of pollution in the environment such as *Typha* sp. as an indicator for Cadmium (Cd) and Nickel (Ni) pollutant. Moreover, *Juncus* sp. could be used as indicators of Zinc (Zn) pollution in urban water runoff [44]. *Clostridia* sp. were typically found in soil, sewage, and marine sediments, as well as the intestines of animals and humans. Some *Clostridia* species are used in commercial production of alcohol and solvent industry such as *C. butyricum* and *C. pasteurianum* in producing nitrogen.