use, and it grows readily at temperatures usually employed for incubation of sterility tests. This culture has been used in a manner comparable to that employed by Brewer in control of powders and oils (Brewer 1949, Long 1942).

SUMMARY

A heat-resistant, spore-forming microorganism identified as *Clostridium sporogenes* has been isolated from cotton. The microorganism is suggested for the deliberate contamination of equipment to serve as an indicator of the efficiency of sterilization techniques.

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Bacterial Indicators of Pollution in Surface Waters[1]

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The recognition of the coliform group of bacteria as a reliable indicator of pollution in municipal water supplies and the introduction of adequate tests for their detection marked the beginning of a new era in the sanitation of drinking water. It was recognized from the beginning, however, that the coliform test was not a perfect indicator, and early in this century serious efforts were made to distinguish between the coliforms of man and those of the lower animals. Also, numerous investigations have been directed toward the finding of additional, supplementary, bacterial indicators of dangerous contamination. In this connection the fecal streptococci have received considerable attention over the years, particularly in England.

After more than fifty years of research on bacteriological methods for determining the sanitary quality of drinking water the procedure is still imperfect. As a criterion of the potability of treated waters the coliform test has proved to be quite satisfactory; their presence in such waters in significant numbers is indicative of failure in the treatment process or of contamination subsequent to treatment. The ubiquity of this group of organisms in surface waters apparently free of dangerous pollution almost nullifies the value of the test when it is applied to untreated waters. This difficulty long has been recognized and has led to a voluminous literature dealing with the sanitary significance of various members of the group when found in water. Much of this literature has been reviewed by Prescott, Winslow, and McCrady (1946) and it need not be considered here.

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Evidence that some coliforms may multiply in water has been reported by a number of workers, including Caldwell and Parr (1933), Leahy (1932) and Mallmann (1928). In each instance the increase occurred in the presence of organic matter such as cotton string, rope, leather gasket, or material collected on a filter. An organism capable of multiplication in water is obviously an imperfect indicator of pollution in water supplies.

Houston (1899) long ago recognized the shortcomings of the coliform group as an indicator of pollution, and considered the desirability of employing the streptococci as indicators of recent and dangerous pollution. He believed that the presence of this group of intestinal bacteria in water was indicative of recent pollution with sewage; but that their absence, however, did not prove the absence of pollution. The early work of Houston was followed by investigations of this group in India by Clemesha (1912), in England by Savage and Read (1917), and in this country by Winslow and Hunnewell (1902), Prescott (1902), Prescott and Baker (1904), Mallmann (1928, 1940), Hajna and Perry (1943) and many others. Much of the earlier work has been reviewed by Calvert (1937).

Since the fecal streptococci apparently never multiply in water (Savage and Wood, 1918), as some of the coliforms have been found to do, but on the contrary disappear rather rapidly, they seem to possess an advantage over the coliform group as an indicator of recent and, therefore, dangerous pollution.

The warm temperatures of Louisiana waters and their high content of organic matter should provide suitable conditions for growth of any organisms capable of multiplication in natural water. The purpose of this investigation was to determine the incidence of coliforms and enterococci in the surface waters of southern Louisiana, and whether these organisms multiply in stored samples of these waters.

Experimental Methods

The water sources selected for study varied widely in degree of fecal pollution as indicated by sanitary inspection. Eleven sources were studied and bacteriological examinations were made on each at intervals of about one month throughout the year. Records were kept of rainfall and temperature. A brief description of seven of the sources follows:

Drainage canal. A ditch which at the time of this study carried surface drainage and some sewage from the southern part of Baton Rouge. During dry weather, when the volume of flow was low, fecal particles were observed in the water. This source was classed as heavily polluted.

University Lake. This is an artificial lake of about 0.35 square miles in area in the southern outskirts of Baton Rouge. It is filled by storm drainage in its immediate vicinity; sources of pollution included effluents from a few septic tank seepage areas at private homes, and the wild fowl which were numerous during the winter months.

Amite River. This river drains a farm and timberland area. Numerous summer camps are located along the river, and many people swim in the stream during the summer months. Samples were taken at Port Vincent.

Colyell Bay. This is a wide bayou with ill-defined banks merging with the adjacent swamp. It receives the flow of Colyell Creek which drains a lightly populated farm and timberland area. The water of Colyell Bay is dark with leachings from the swamp.

Old River. This is an oxbow lake formed by an old channel of the Mississippi River and is contained within the Mississippi River levee system. It receives no water other than rainfall within the levee, except when the Mississippi River is high. This usually occurs during the spring or early summer. Except for a few fishermen this lake has no known pollution from human sources save that brought in periodically from the Mississippi River.

Mississippi River. The Mississippi River was selected for study because of its common use as an outlet for sewage, both raw and treated, throughout its length, and its frequent use also as a source of water for municipal supplies. To avoid local contamination, the samples were collected at midstream and upriver from the Baton Rouge—Port Allen ferry route.

Well No. 1. (A shallow well in current use on a farm in East Baton Rouge Parish.) The well was about 25 feet deep and was cased with 1 x 6 pine planks extending into the water. Water was drawn from the well with a rope and a tubular metal bucket with a valve in the bottom. Pollution of the well was possible from the hands on the rope and bucket, and from poultry droppings on the cover.

Samples were also taken from the Amite River at a bridge near Denham Springs, from the Tickfaw River, from the False River and from a second open well. Results of bacteriological examinations were similar to those obtained from other sources; therefore they are not reported in detail.

The bacteriological examination included the determination of the plate count, coliforms and enterococci. The plate count was made with tryptone glucose extract agar as described in "Standard Methods for the Examination of Water and Sewage" (1946). Most probable numbers of coliforms were determined in lactose broth, using five replicate tubes of each dilution in the presumptive test. Confirmation was made in brilliant green bile (2 per cent) broth. The completed test was run on all samples, using EMB as the plating medium. In some of the samples *Escherichia coli* was determined with the Hajna and Perry (1943) modification of the Eijkman test.

The enterococci were determined by the method of Winter and Sandholzer (1946) using 5 replicate tubes of each dilution. Confirmation was made with the slant-broth method of Winter and Sandholzer (1946), followed by determination of gram reaction and cell morphology.

Results and Discussion

The total numbers of bacteria as shown by the plate count, and the most probable numbers (MPN) of enterococci and coliforms found at each examination of the various water sources are presented in figures 1 to 7. In some instances rainfall records were available for only part of the month; in these cases the number of days included in the rainfall report is shown on the graph in the figures.

The effect of rainfall on the total count and on the two groups of indicator organisms was usually, but not always, in accord with expectations. In University Lake (figure 1) the coliforms and enterococci increased in periods of heavy rainfall; the total count was less affected by rainfall. The correlation between coliforms and enterococci was quite close, with the coliforms present in considerably larger numbers. In the heavily polluted drainage canal the numbers of coliforms decreased in periods of heavy rainfall and were higher in periods of dry weather. The enterococci did not fluctuate significantly in response to rainfall (figure 2).

In Well 1 (figure 3) a situation was revealed which is probably typical of most shallow wells in this section of the country. The total count of organisms was consistently high, suggestive of gross pollution, and the coliforms far exceeded the tolerance allowed by the Treasury Department Standard (1943) for water to be used on interstate common carriers. There was a general, but inexact relationship, between the numbers of coliforms and the enterococci.

In the Mississippi River there was a very close agreement among the three measures of contaminations (figure 4); increases or decreases in one group were accompanied by the same changes in the other groups. In each group the highest counts occurred in November, at the time of the first rise in the river, not at the time of highest water, which was about March 1, 1949. Lest the figures indicating the river stage be misunderstood, it should be pointed out that when the river stage is zero feet, ocean-going ships ply the river.

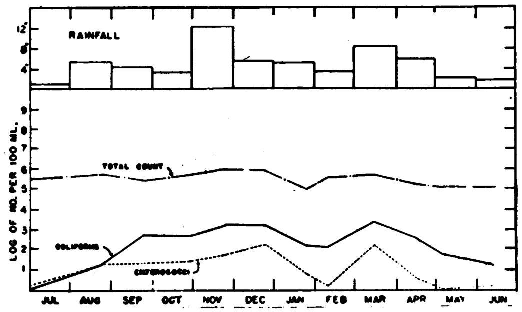

FIG. 1. Bacteria, enterococci and coliforms in University Lake.

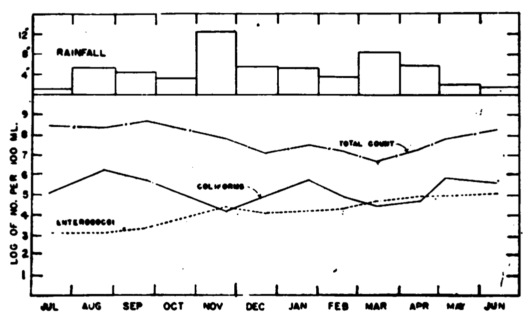

FIG. 2. Bacteria, enterococci and coliforms in Drainage Canal

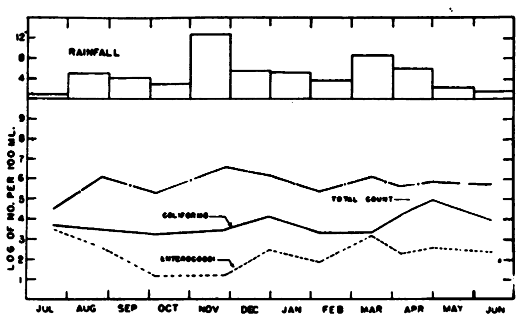

FIG. 3. Bacteria, enterococci and coliforms in Well 1

Samples from the Amite River (figure 5) taken during flood stage shortly after a heavy rainfall showed higher counts than samples taken during high water due to several days of moderate to heavy precipitation.

The results obtained with samples from Colyell Bay and Old River (figures 6 and 7) indicate fewer bacteria of each group to be present than in the streams and wells studied. The enterococci were not detected in most of the samples from Old River, and were present in very small numbers in Colyell Bay.

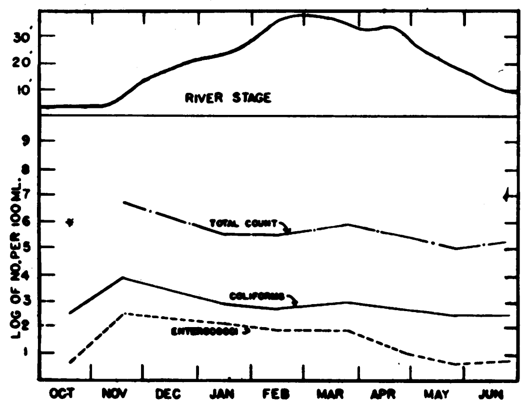

FIG. 4. Bacteria, enterococci and coliforms in Mississippi River.

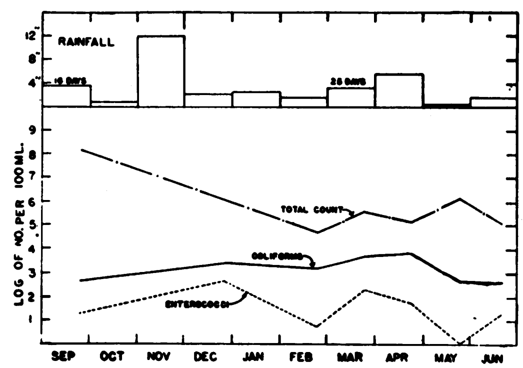

FIG. 5. Bacteria, enterococci and coliforms in Amite River at Port Vincent.

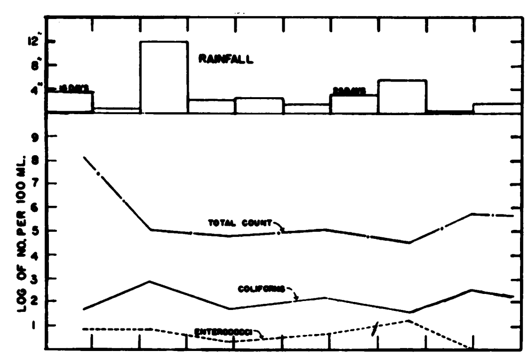

FIG. 6. Bacteria, enterococci and coliforms in Colyell Bay.

Owing to the interest in the fecal member of the coliform group, the numbers of *Escherichia coli* were determined in the samples taken during May and June, 1949. Since the non-fecal member of the group, *Aerobacter*, is generally considered to be a normal inhabitant of soil, much less significance can be given to it as an indicator of dangerous pollution than to the fecal *E. coli*. The results of the May and June determinations are shown in table 1. The waters are listed in the decreasing order of pollution, using *E. coli* as the index. The three groups of indicator organisms agree reasonably well in revealing the probable sanitary quality of the water. It will be noted, however, that the contrast between the heavily polluted drainage canal and the relatively clean waters is shown more clearly by the enterococci and *E. coli* than by the coliform group as a whole. This tendency of the enterococci to magnify the difference between clean and polluted waters is shown by the coliform/enterococci ratios (table 2). The more polluted waters have the lower ratios, and in water free of enterococci the ratio would be infinity.

A problem which recurs from time to time, and about which there is still some disagreement in the literature, is whether the indicator organisms may grow in water supplies during processing and distribution. Ellison, Hackler and Buice (1929) concluded that it was not necessary to ice samples of water to be sent to the laboratory for analysis. Cox and Claiborne (1949) reported steady decreases in numbers of coliforms in uniced samples of water. Noble (1928) also reported marked decreases in coliforms in stored water, but considerable numbers survived after 37 days' storage.

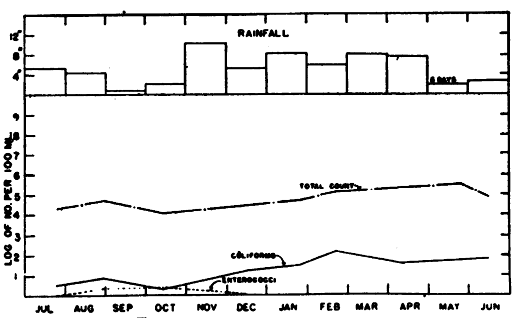

FIG. 7. Bacteria, enterococci and coliforms in Old River

To determine the ability of the indicator organisms to multiply in surface waters, samples were stored in 2-liter flasks at room temperature and in the light near a north window. The flasks were not exposed to the direct rays of the sun. Two samples from each water source were stored and examined at intervals until the indicator organisms disappeared. At each examination the samples were shaken to prevent erroneous results due to sedimentation of the bacteria. The results are shown in table 3. Since both samples from a given source showed the same death curve, only one sample from each source is presented in the table. The steady decline in numbers of indicator organisms in the stored samples, which resulted in their disappearance within a few days, suggests that these organisms probably do not multiply in the warm organically rich surface waters of Louisiana. There remains the possibility, however, of growth of some types on decaying vegetation along the banks and at the bottoms of these waters.

The ubiquity of coliform organisms in surface waters which apparently are not polluted with human excreta is generally recognized. It is necessary, therefore, to interpret the bacteriological results in a quantitative manner in order to arrive at the sanitary quality of water.

TABLE 1. *The relationship between total coliforms,* Escherichia coli *and enterococci*

SOURCE	ENTEROCOCCI*	COLIFORMS*	*E. coli**
May, 1949			
Drainage Canal	92,000	920,000	13,000
Well No. 2	350	92,000	3,300
Well No. 1	640	54,000	2,300
Mississippi River	4	350	110
False River	0	280	49
Amite River	4.5	240	11
Tickfaw River	0	350	7.8
Colyell Bay	0	350	7.8
University Lake	0	79	4.5
Old River	0	49	0
June, 1949			
Drainage Canal	130,000	540,000	130,000
Amite River	7.9	540	240
Mississippi River	49	350	130
Tickfaw River	79	130	23
False River	4	33	13
Well No. 2	230	7,800	7.8
Colyell Bay	0	130	6.8
University Lake	2	23	2
Well No. 1	7.8	45	0
Old River	0	17	0

* M.P.N./100 ml.

TABLE 2. *Relationship between the coliform bacteria and enterococci in Louisiana surface waters*

SOURCE	SAMPLES EXAMINED	AVERAGE M.P.N. PER 100 ML.		RATIO C/E*
		Coliforms	Enterococci	
Old River	9	30	0.4	75
False River	10	110	8	14
Colyell Bay	7	252	5	50
Tickfaw River	11	567	46	12
University Lake	12	900	58	15
Amite River	10	1,225	203	6
Mississippi River	8	1,650	90	18
Well No. 2	10	13,130	2,350	6
Well No. 1	10	14,600	682	21
Drainage Canal	11	366,000	40,550	9

* Coliforms/enterococci.

Based on experience with ground water and treated water supplies, the presence of coliforms in excess of one per 100 ml is considered to be indicative of dangerous pollution. Whether the same criterion of safety should be applied to untreated supplies is open to serious question. The cleanest surface waters in this section of Louisiana are, by this standard, highly polluted and dangerous. Old River, the least polluted source of water in this study, contained from 1.8 to 110 coliforms per 100 ml, while Colyell Bay, another source which inspection indicated was only slightly polluted, contained from 46 to 920 coliforms per 100 ml.

TABLE 3. *Survival of organisms in stored samples*

SOURCE	DAYS STORAGE	NUMBER OF ORGANISMS (M.P.N./100 ML.)			PLATE COUNT/ML.
		Coliforms	*E. coli*	Enterococci	
Well No. 1	0	54,000	2,300	640	5,600
	2	240	230	49	8,000
	7	7.8	0	0	4,240
	10	0	0	0	33,000
Well No. 2	0	92,000	3,300	350	7,200
	2	23	2	4	9,000
	7	1.8	2	0	92,400
	10	0	0	0	150,000
Drainage Canal	0	540,000	110,000	130,000	2,240,000
	3	39,000	54,000	13,000	2,560,000
	6	1,600	240	1,600	2,400,000
	9	240	78	0	2,580,000
	12	0	0	0	2,420,000
City Lake	0	23	2	2	1,800
	3	1.8	0	0	1,950
	6	0	0	0	1,900
Tickfaw River	0	350	7.8	0	500
	3	79	0	0	540
	6	0	0	0	700
Amite River at Denham Springs	0	350	7.8	0	500
	3	79	0	0	540
	6	0	0	0	70
Mississippi River	0	350	110	4	1,100
	3	240	0	0	125
	6	0	0	0	1,500
Old River	0	49	0	0	3,000
	3	0	0	0	4,000
False River	0	240	49	0	300,000
	3	78	1.8	0	221,000
	6	0	0	—	210,000
Amite River at Port Vincent	0	490	240	23	9,000
	3	240	78	0	11,000
	6	0	0	0	20,000
Colyell Bay	0	130	6.8	0	5,100
	3	7.8	1.8	0	6,000
	6	1.8	0	—	6,250
	9	0	—	—	7,300

In certain foods the test for the coliform group as a criterion of sanitary quality has been abandoned in favor of the *E. coli* test. This organism is also quite widely disseminated in nature, but it is far less abundant in "clean" waters and soil than the *Aerobacter* section of the coliform group. Consequently, its presence in waters seems more significant than the presence of other coliforms. An improved qualitative test for determining the potability of untreated water is obviously desirable. There is no need, however, to relax the present standards for treated water supplies.

The enterococci are abundant in sewage and in heavily polluted waters, and usually they are absent in water free from obvious sources of fecal pollution. In this study the difference between the most polluted water (drainage canal) and the cleanest water (Old River) is shown more strikingly by the enterococci test than by the usual coliform test. Only twice were we able to demonstrate the presence of enterococci in Old River, and then they were present in very small numbers. The enterococci offer certain advantages over the coliforms as indicator organisms in that they apparently never multiply in waters (as some coliforms have been reported to do) but disappear rather rapidly when added to streams or lakes (Savage and Wood, 1918). In this study they showed good correlation with sanitary quality as estimated by inspection.

The results obtained with the limited number of samples which were examined for *E. coli* by the Hajna and Perry modification of the Eijkman procedure indicated that the numbers of *E. coli* usually corresponded much more closely with the numbers of enterococci than with the coliforms. When the enterococci were not found, *E. coli* was either absent or present in small numbers.

SUMMARY

In all waters studied, high total counts were associated with relatively high counts of coliforms, *Escherichia coli*, and enterococci.

The difference between relatively clean and recently polluted water was more strikingly shown by the enterococci than by the coliform test.

The two shallow wells included in this investigation were found to be of poorer bacteriological quality than most of the surface waters. As measured by the Treasury Department standard, both wells were heavily polluted.

The results obtained in this study indicate that the cleanest surface waters in this area fall far below the standard for drinking water. The value of the coliform test in the examination of surface waters is limited; more reliance must be placed on sanitary inspection.

No evidence that the coliforms and enterococci multiply in any of these waters was obtained; on the contrary, they were found to die at a fairly rapid rate in stored samples.

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Studies on Vitamin B_{12} Production with *Streptomyces Olivaceus*[1]

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Since the discovery by Rickes *et al.* (1948) that microorganisms may synthesize vitamin B_{12}, several fermentation processes have been reported for the production of this vitamin on a commercial scale. The fermentative organisms used are principally species of *Streptomyces*, *Bacillus*, and *Flavobacterium*, although some other microorganisms have been employed. Vitamin B_{12} may be the primary fermentation product (Lewis *et al.*, 1949; Petty and Matrishin, 1949; Ansbacher and Hill, 1949; Gary *et al.*, 1951; Hall *et al.*, 1951; Hodge *et al.*, 1952), or it may represent a secondary product in the manufacture of certain antibiotics (Rickes *et al.*, 1948; Stockstad *et al.*, 1949; Pierce *et al.*, 1949; Fricke *et al.*, 1950; Jackson *et al.*, 1951). The enrichment of fish products by fermentation with vitamin B_{12}-producing organisms has been described (Tarr *et al.*, 1950). Surveys of vitamin B_{12}-producing microorganisms have been reported by Hall *et al.*, 1950; Burton and Lochhead, 1951; Saunders *et al.*, 1951; Shull and Routien, 1951.

During the survey at this laboratory of classified cultures in our culture collection and of soil isolates, preliminary studies with an organism which proved to be *Streptomyces olivaceus* (Waksman) (Breed *et al.*, 1948) indicated that it offered promise as a producer of vitamin B_{12}. Detailed studies of the fermentation product, including chick feeding trials with whole dried culture, showed this organism to be valuable for the production of vitamin B_{12}, especially for the enrichment of animal feeds.

A preliminary account describing the general aspects of the fermentation was given earlier (Hall, *et al.*, 1951). The present paper reports a study of factors which

[1] Presented in part before the 119th National Meeting, American Chemical Society, Cleveland, Ohio, April 8–12, 1951.

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