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Bacteria & Nitrate Nitrogen Content of the Water of Barren River

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BACTERIA AND NITRATE NITROGEN CONTENT OF THE WATER OF BARREN RIVER

BY

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INTRODUCTION

A quantitative study of bacteria and nitrogenous compounds of Barren River in Warren County, Kentucky, was undertaken to determine the relation between the number of bacteria and the amount of nitrate nitrogen found.

Among the various elements which are essential for the growth of aquatic plant and animal life and which are present in minimum concentrations, nitrogen occupies a prominent place. Neither the plant nor the animal forms have the ability to use the gaseous nitrogen of the atmosphere (a function now known to be limited chiefly to certain specific groups of bacteria) but depend for their growth upon the combined nitrogen present in the water. Even the above form of nitrogen must be in a soluble, mineralized state before it can be assimilated by plants. Plants transform the soluble forms of nitrogen into complete organic substances and upon the death of the plants, as well as of the animals which feed upon them, the nitrogen is brought again into circulation in the form of ammonia through the action of various kinds of bacteria. However, before the nitrogen is again assimilated by the aquatic plants, the ammonia is usually oxidized first to nitrite and then to nitrate. It was not the purpose of the writer to determine the number of nitrifying forms, but to include all bacteria present.

The number of bacteria in water is affected by various

factors, namely: its composition, the amount and frequency of rainfall and the resulting drainage, the contamination with sewage, the depth of the water, the velocity of the current, and other factors. Because of natural variations thus introduced, the number of bacteria in fairly pure river water may range from a few hundred to several thousand per cubic centimeter.

This study may afford a good opportunity for a comparison of the abundance of nitrogen compounds and the number of bacteria at the two stations used in this study. These data may be of value in determining the efficiency of the sewage system now under construction in Bowling Green, Kentucky.

REVIEW OF LITERATURE

No literature dealing with the relation of the number of bacteria to nitrogenous compounds, in Kentucky rivers, has been found. This is probably due to the fact that there are many variable factors, and that literature on this subject would not be of general interest since it would apply only to a specific locality. However, numerous publications relative to this subject have been made on this subject in relation to water.

Boussingault in 1860, looked upon the ocean as an immense reservoir of nitrogen in a combined form. Schlosing demonstrated in 1875, that while land waters are rich in

nitrate, sea waters are richer in ammonia. It is recognized, however, that both ammonia and nitrate result from the decomposition of nitrogenous organic matter. Brandt stated emphatically that the cycle of nitrogen in the sea is essentially not very different from that on land.[1]

The first suggestion, that bacteria were responsible for the process of nitrification, was made in 1898 by Vernon. Gran and Nathanson were unable to demonstrate these nitrifying bacteria. Later, Gran found nitrifying bacteria in the Gulf of Naples; however, only near the shore. This led Nathanson to conclude that when bacteria are found in the sea not far from land it is due to their introduction by streams and rivers.

Brandt argued that, if nitrate nitrogen comes from the sea either from the atmosphere or from land, one would expect to find nitrate more abundant in the surface layers than in the lower depths. This has been shown to be contrary to actual facts.

Korinek[2] concluded in 1926 that ordinary bacteria continue to grow, with very little disturbance, in media containing sea water. Cultivation in sea water does not kill them or hasten autolysis. Since nitrifying bacteria

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[1] Selman A. Waksman, Margaret Hotchkiss, and Cornelia L. Carey, "Marine Bacteria and Their Role in the Cycle of Life in the Sea," The Biological Bulletin, Vol. LXV, No. 2 (October, 1933), pp 137-141.

[2] J. Korinek, "Fresh Water Bacteria in the Sea," Centralbl Bakt. 2 abt. 66 (1926) pp 500-505.

are not found at any great distance from the shore it is possible that all may have arisen from the soil and fresh water.[3]

Numerous experiments have shown that the bacterial content of sea water is comparatively low. Sea water also contains smaller quantities of nitrate and larger quantities of ammonical nitrogen than fresh water.

Thomsen, Issatchenko, Berkley, Lipman, and Harvey concluded that nitrifying bacteria are present on the sea bottom.

On the basis of these results Brandt stated in 1926, with much justification, that the results so far obtained are sufficient to establish definitely that bacteria capable of oxidizing ammonium salts are completely absent in surface water, but are present in marine bottoms. Waksman, Hotchkiss, and Carey[4] concluded in 1933 that few if any of the nitrifying bacteria were present in surface water, but were found in abundance on the sea bottom.

We may then conclude that the same condition might exist in fresh water streams if it were not for certain specific factors. The velocity of the river under consideration in this study is relatively rapid and thus tends to prevent

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[3] C. B. Lipruan, "The Concentration of Sea-Water as affecting its bacterial population," Journal of Bacteriology, Vol. XII No. 5, (1926) pp 311-315.

[4] Selman A. Waksman, Margaret Hotchkiss, and Cornelia L. Carey, "Marine Bacteria and Their Role in the Cycle of Life in the Sea," The Biological Bulletin, Vol. LXV, No. 2 (October, 1933) pp. 137-141.

sedimentation. Much organic matter remains in suspension for a considerable length of time. It therefore seems probable that there may be nitrifying bacteria acting on these particles in suspension, as well as on organic matter which has settled to the bottom.

The greatest variation in number of bacteria exists in river water. In the Chicago drainage canal Jordan[5] found 1,245,000 bacteria per c. c. at Bridgeport, and 650,000 per c. c. at Lockport, twenty-nine miles below. The Rhone River, above Lyons, contains an average of 75 bacteria per c. c. while the bacterial content of the river below Lyons was 800 per c. c. The Dee contains 88 bacteria per c. c. above Braemar and 2,829 per c. c. below Braemar. The Rhine River contains 4,786 bacteria per c. c. above Cologne while the bacterial content six miles below is 30,432 per c. c.

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5 C. E. Renn, "Studies on the Biology and Chemistry of the Gulf of Maine."

George E. Marshall, Microbiology (Philadelphia, P. Blakeston's Son and Co., 1917), p. 262.

TABLE I

BACTERIA AND NITROGENOUS CONTENT

Date	Sample Number	Stage of River	Water Temperature	Milligrams per Liter			Number of Bacteria
				Ammonia	Nitrite	Nitrate	
April	1	6.3	13.	1.7	0.1	3.	700
7	2	6.3	13.5	4.5	0.1	5.	1,500
April	1	5.8	14.5	3.		3.	400
14	2	5.8	15.	5.	0.1	5.	1,250
April	1	5.4	16.	2.5	0.1	3.	900
19	2	5.4	16.	4.5	0.2	5.	2,800
April	1	5.2	17.	4.	0.1	5.	1,100
28	2	5.2	18.	6.	0.2	8.	4,600
May	1	4.9	20.5	4.5	0.4	5.	2,600
5	2	4.9	21.	6.5	0.7	10.	18,000
May	1	4.9	21.5		0.2	3.	1,200
12	2	4.9	22.		0.4	8.	9,000
May	1	4.9	22.		0.2	3.	900
19	2	4.9	23.		0.4	5.	4,500

TABLE I (Continued)

BACTERIA AND NITROGENOUS CONTENT

Date	Sample Number	Stage of River	Water Temperature	Milligrams per Liter			Number of Bacteria
				Ammonia	Nitrite	Nitrate	
May	1	4.8	24.		0.2	3.	*
26	2	4.8	24.5		0.4	5.	
May	1	4.9	24.		0.2	3.	1,100
31	2	4.9	24.5		0.4	5.	5,400
June	1	5.5	27.	8.6	0.4	10.	11,000
22	2	3.	28.	9.8	0.7	15.	34,000
June	1	4.5	28.5	7.5	0.7	10.	18,000
29	2	3.5	29.	10.7	1.0	20.	41,000
July	1	4.9	28.5	6.3	0.4	8.	*
5	2	4.9	29.5	8.3	0.7	10.	
July	1	4.5	29.	5.9	0.4	8.	6,000
13	2	4.4	29.5	6.3	0.7	10.	14,000

*Control contaminated.

FIGURE 1.--AMMONIA CONTENT AS AFFECTED BY TEMPERATURE.
STATION ONE ---------

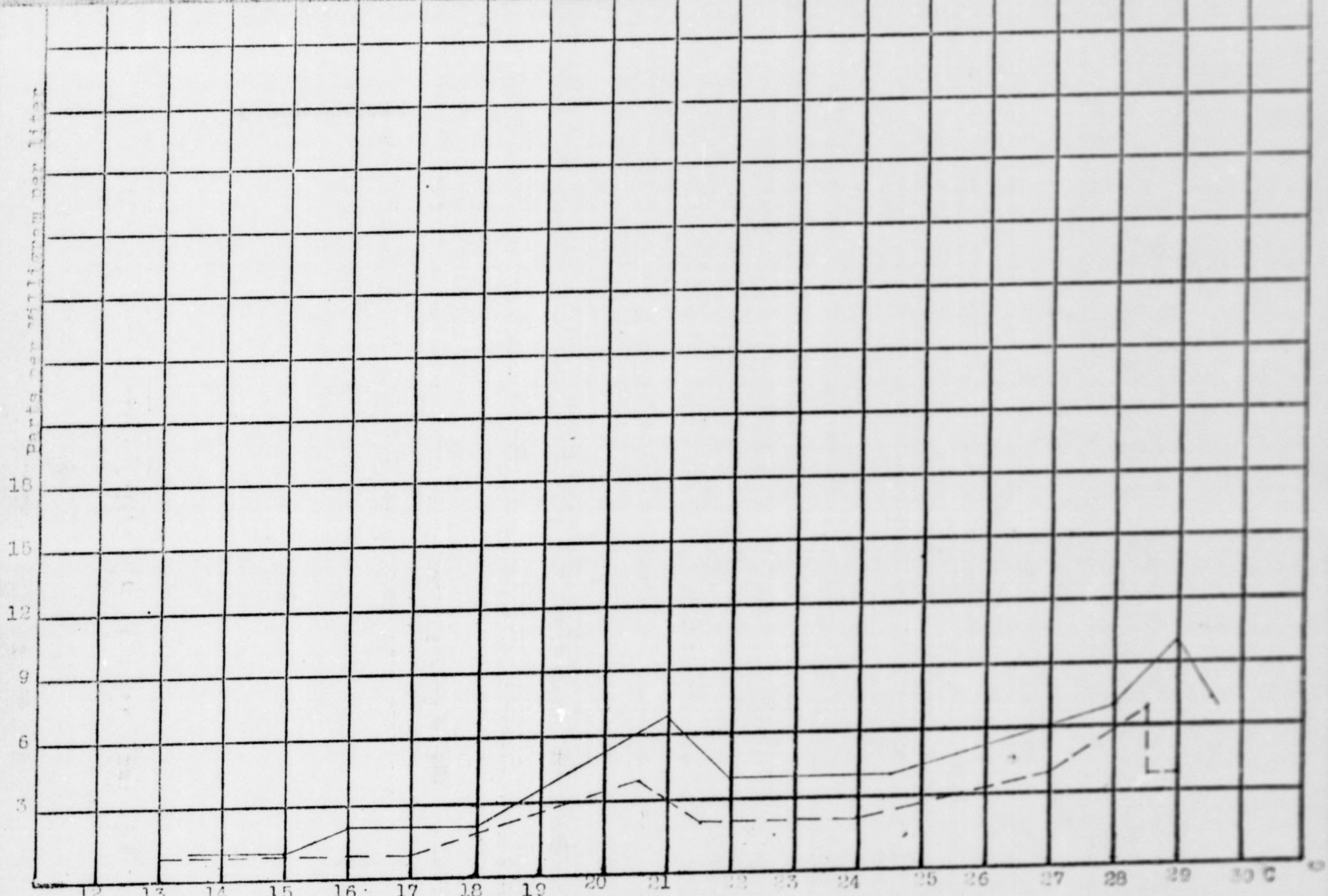

FIGURE 2.--RELATION OF NITRITE TO TEMPERATURE.
STATION ONE --------

PROCEDURE

The sewage disposal system of Bowling Green empties into Barren River just below the city. Along the course of this river through Warren County there are numerous springs and under-ground seeps, drainage taking place through crevices between the large beds of limestone. East of Bowling Green along the course of Barren river there are numerous solution sinks which possibly drain into the river. Thus through these sources there is a constant supply of mineral compounds all of which may affect the results of this study.

To facilitate the study two sampling stations were established. Sampling station number one was located near the end of Chestnut Street, about fifty feet below the source of the city water supply, and number two was located near the end of Church Street, about one hundred yards below the sewage outlet. By water route these stations are about ten miles apart; however, the distance by land is only two miles. The width of the river is about the same at both stations but the depth at the lower station, number two, is much greater.

Nitrogen determinations were made, with few exceptions, by the methods outlined in the Standard Methods of Water Analysis.[6] The distillation method using Nessler's reagent

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[6] Standard Methods of Water Analysis (7th ed., New York: American Public Health Association, 1933), pp. 15-22,122-124.

and standard ammonium chloride solution was used in determining ammonium nitrogen. Because of difficulty encountered in reading ammonia content of the first few samples a titration method was substituted for the later determinations. Nitrite nitrogen was determined by the use of the sulfanilic acid method. Nitrate determinations were made by the use of the phenoldisulfonic acid method. Two one-liter flasks and two 500 c. c. flasks were used as collecting bottles. The flasks were made on the principle of Russell's[7] collecting bottles. A cork was attached to a string about three inches long which in turn was tied around the neck of a flask. The flask having a weight attached to the bottom was lowered to the required depth, and at this time a sharp jerk of cord attached to the three-inch string released the cork. When the bottle reached the surface the cork was immediately replaced.

Bacterial counts were made immediately after collection. It was found necessary to resort to dilution before plating. The medium was made up as recommended by the Standard Methods of Water Analysis. Standard petri dishes were also employed.

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7 H. W. Con, Agricultural Bacteriology (Philadelphia, P. Blakeston's Son and Co., 1909) pp. 128-136.

Edwin O. Jordan and I. S. Falk, The Newer Knowledge of Bacteriology and Immunity, (Chicago, The University of Chicago Press, 1928), pp. 562-570.

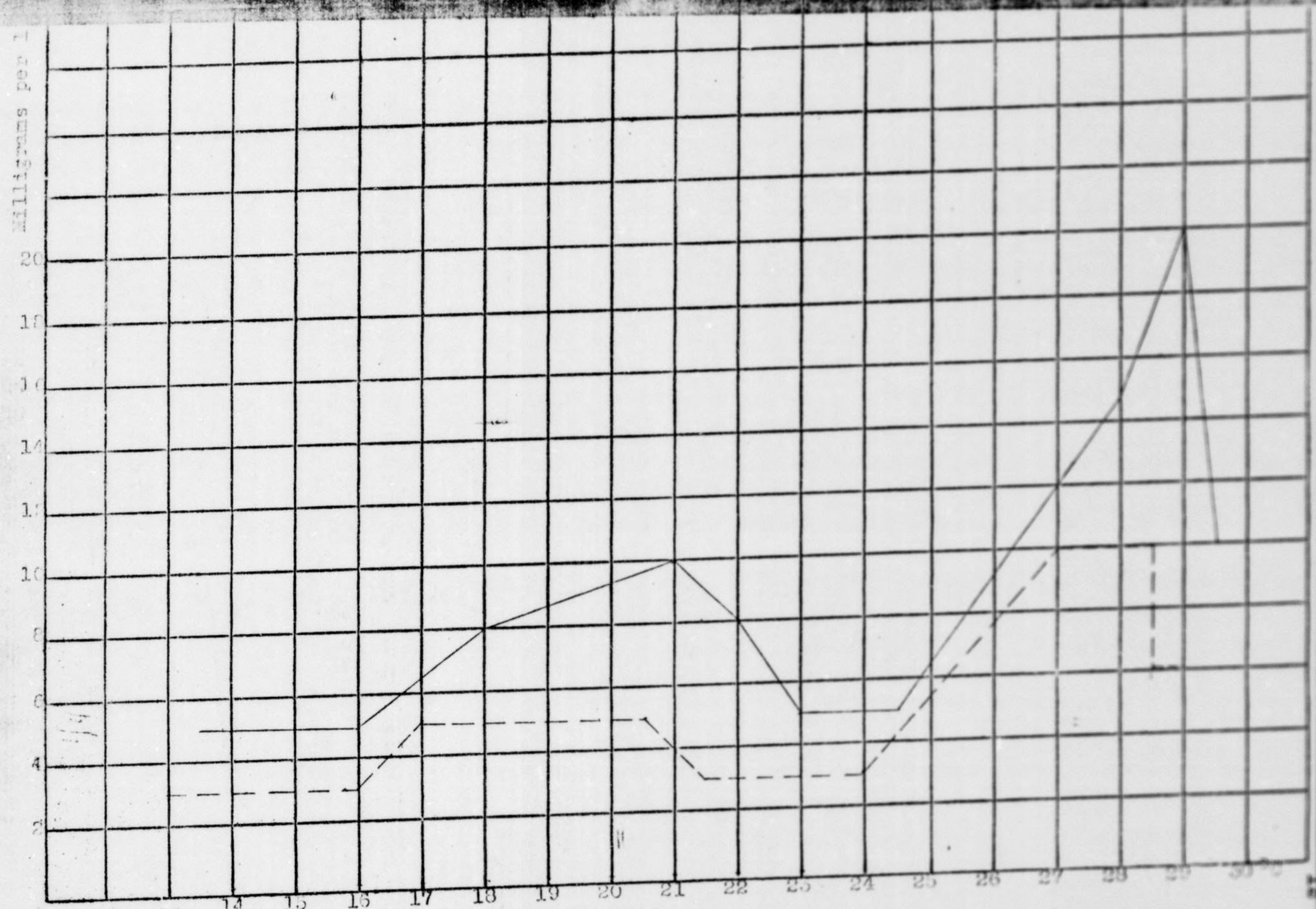

FIGURE 3.--RELATION OF NITRATE TO TEMPERATURE.

STATION ONE --------

The study was started late enough in the spring to avoid the increased bacterial count caused by winter precipitation and early spring rains. Collections were made approximately every seventh day from April 7 to July 13. No radical weather changes occurred during this period except occasional rains, with a gradual rise in temperature.

Samples were collected at a depth of six feet. The amount taken at each station was one liter collected in two 500 c. c. portions, and later mixed. All collecting apparatus was sterilized at fifteen pounds pressure for thirty minutes. At the time of sampling, the temperature of the water was taken near the surface. The samples were then brought to the laboratory and the bacteriological examination made. The sample bottle was shaken vigorously twenty-five times and 1 c. c. withdrawn and diluted to 100 c. c. After shaking the dilution bottle vigorously twenty-five times 1 c. c. was withdrawn and diluted to 10 c. c. From each of these dilutions three plates were inoculated. The inoculations, in most cases, were made before pouring the agar-agar, because it afforded a more uniform distribution of the colonies. The inoculated plates with the controls were then incubated for twenty-four hours at thirty-seven degrees Centigrade. Whenever possible, plates showing between 30 and 300 colonies were the only ones considered in recording results. In each case the average of the three plates was recorded. To facilitate counting, a lens having a magnification of approximately five diameters was used.

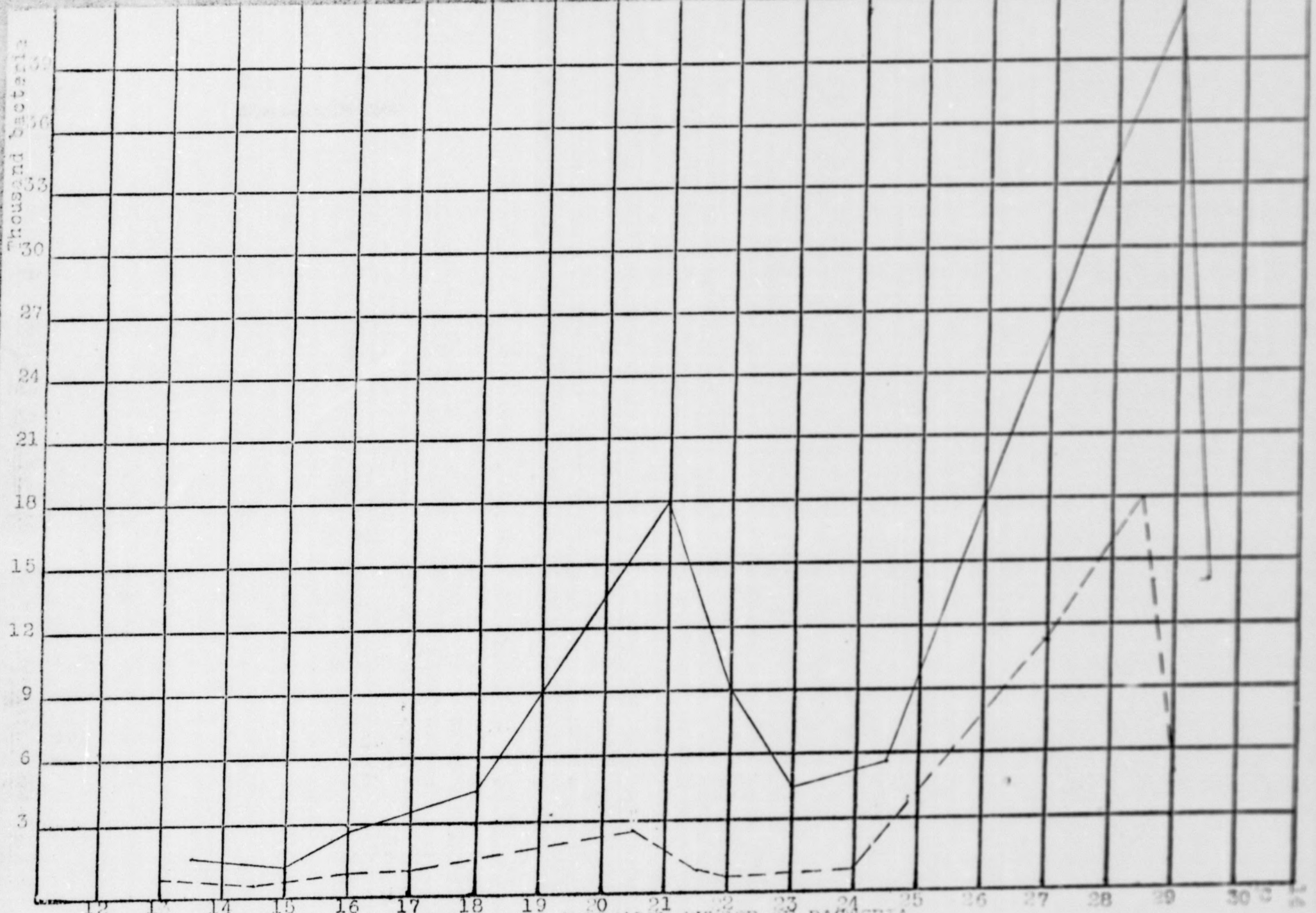

FIGURE 4.--RELATION OF TEMPERATURE TO INCREASED NUMBER OF BACTERIA
STATION ONE ---------

Hydrogen ion determinations were made by adding 2 c. c. of the tested sample to 8 c. c. of distilled water to which 5 c. c. of brome thymol blue had been added. This was then placed in a colormeter for color comparison.

A series of sixteen Nessler tubes containing the following volumes of standard ammonium chloride solution were prepared and diluted to fifty cubic centimeters with ammonia-free water; namely, 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 6.0 c. c. These solutions contained 0.01 milligram of nitrogen for each cubic centimeter of the standard. Determinations of ammonical nitrogen were made by using 500 c. c. of the sample, containing 10 c. c. of phosphate buffer solution. This distillate was placed in a distilling flask and distilled at a rate of 6 to 10 c. c. per minute. The distillate was collected in 50 c. c. portions in four Nessler tubes. The four portions of the distillate and the standards were then Nesslerized by adding 1 c. c. of Nessler reagent to each tube. After addition of the reagent the tubes were allowed to stand ten minutes before comparing the sample with the standards.

Nitrate nitrogen was determined by evaporating to dryness 100 c. c. of the sample. To this 2 c. c. of phenoldisulfonic acid were added. The acid was diluted with distilled water, and potassium hydroxide was added until the maximum color appeared. The solution was then filtered and compared with

standards made by adding 2 c. c. of strong potassium hydroxide with various volumes of standard nitrate solution and diluting them to 50 c. c. in Nessler tubes. Comparisons were made by use of the following volumes; namely, 1, 3, 5, 8, 10, 15, 20, 30, and 40 c. c.

RESULTS AND DISCUSSION

Table I shows clearly that there is no correlation between the stage of the river and the bacterial content. This is due to two factors: namely, temperature and amount of surface water. The increase in bacterial content on May 5 and from June 22 through July 13, is due to large amounts of surface water flowing into the river. The stage of the river on April 7 is the highest recorded; yet, the bacterial content with one exception is the lowest. The water table is higher in the spring than in midsummer; thus the stage of the river is high because of natural conditions. Because of the low temperature of the water on April 7 the bacterial content was comparatively low.

Figures 1, 2, and 3 demonstrate the relation of the nitrogenous compounds to the increase in temperature. Figure 1 indicates a gradual increase in ammonia content of the water at both stations; however, after temperatures of 27° C. at station one and 29.5° C. at station two had been reached there was a gradual decline in ammonia content. The downward trend in ammonia was probably due to the fact that the river

was replenished with fresh water, since at this time it had just receded from a rise. These data show that factors other than temperature were operating to influence the number of bacteria present.

Figures 2 and 3 show a gradual increase in nitrite and nitrate nitrogen, interrupted by abnormal advances in content. These high concentrations may be explained by the rise of the river at these periods. As the river lowered, the quantity of nitrite and nitrate nitrogen decreased in the same proportion as ammonia. Surface water and swollen streams bring into the river considerable quantities of ammonia, nitrite, and nitrate accompanied by a large number of nitrifying bacteria, which have washed from the soil. Thus the graphs showing the amount of ammonia, nitrite, and nitrate and that showing bacterial content are found to be practically parallel. From the evidence shown by comparison of these tables, we may conclude that ammonia, nitrite, and nitrate are in some way related to the number of colonies of bacteria found.

In Figure 4 we have a gradual increase in the number of bacteria due to an increasing temperature. Here again, the zenith of this graph is marked by the introduction of large amounts of water by surface drainage, showing clearly that many organisms are brought in from the land.

Thus from Figures 1, 2, 3, and 4 we may conclude: (1) that the amount of surface water greatly affects the quantity of ammonia, nitrite, and nitrate nitrogen present; the

presence of these compounds being directly correlated with the number of bacteria; (2) that temperature is only a minor factor in controlling the number of bacteria.

The variation in the number of colonies of bacteria was exceedingly great even when the quantity of nitrate remained constant. Figure 5 is a comparison of the average number of bacterial colonies with the presence of a definite quantity of nitrate nitrogen. The amount of nitrate varies directly with the number of bacteria. The lowest nitrate content of sampling station number one was three milligrams per liter, with an average bacterial content of 850 per c. c. The highest nitrate content of the same station was ten milligrams per liter with a bacterial count of 15,000 per c. c. From the lowest to the highest bacterial count there was a uniform increase in nitrate content. The number of bacteria per unit of nitrate is greater at station number two and also reaches a higher level in nitrate content. However, since there are more bacteria per unit of nitrate, we may conclude that the increase in bacterial content is due to large quantities of sewage bacteria which do not function in nitrification.

Although hydrogen ion determinations were made on each sample, the reaction of the water apparently is not a significant factor in this study. The hydrogen ion concentration is undoubtedly affected by the introduction of carbonic acid and other materials. Hence, the variation in hydrogen

ion concentration cannot be attributed solely to nitrification and other organic reactions.

SUMMARY

A quantitative study of bacteria and the nitrogenous compounds of Barren River in Warren County, Kentucky, was undertaken to determine the relation between the number of bacteria and the amount of nitrate nitrogen found.

Nitrogen determinations and bacteriological examinations, with few exceptions, were made by the methods outlined in the Standard Methods of Water Analysis.

1. Table I shows clearly that there is no correlation between the stage of the river and bacterial content. This is due to two factors: namely, temperature and amount of surface water.

2. Figures 1, 2, and 3 show a gradual increase in ammonia, nitrite, and nitrate nitrogen interrupted occasionly by abnormal advances. The quantity of all decreased in about the same proportion, as the river lowered.

3. Figure 4 shows a gradual increase in the number of bacteria due to an increasing temperature.

4. Figures 1, 2, 3, and 4 show: (a) that the amount of surface water greatly affects the quantity of ammonia, nitrite, and nitrate nitrogen present, the presence of these compounds being directly correlated with the number of bacteria; (b) that temperature is only a minor factor in

controlling the number of bacteria.

5. The variation in the number of colonies of bacteria was exceedingly great, even when constant quantities of nitrate were found. The increase in number of bacteria is uniform with an increase in nitrate. The number of bacteria per unit of nitrate is greater at the lower station. It is also noted that at this station the nitrate content reaches a higher level. Since more bacteria per unit of nitrate were present we may conclude that the increase in bacterial content is due to large quantities of sewage bacteria which do not function in nitrification.

6. Hydrogen ion concentration is not a significant factor in this study.

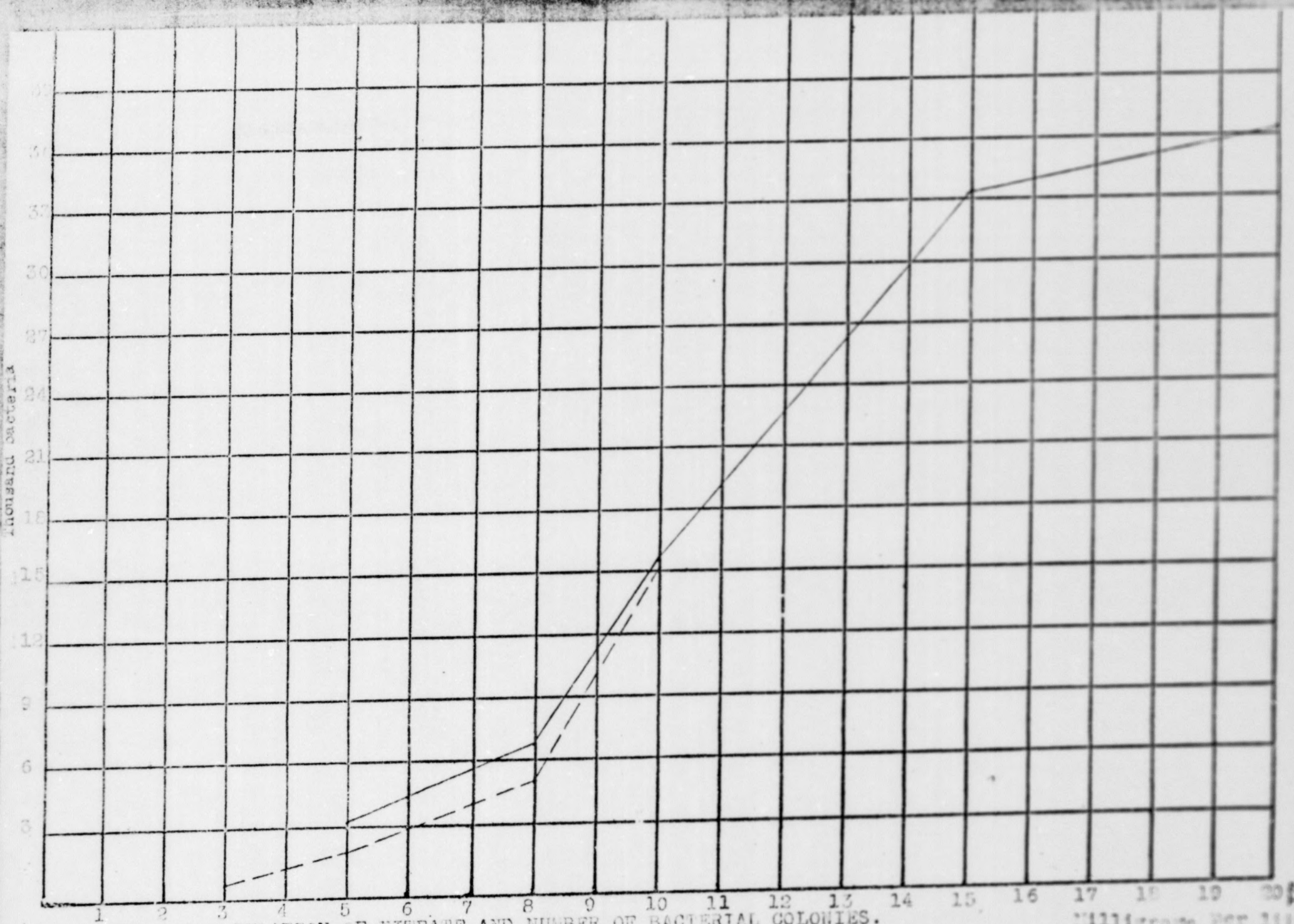

FIGURE 8.--RELATION OF NITRATE AND NUMBER OF BACTERIAL COLONIES.
- STATION C-8 ----------

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