

EFFECTS OF BACTERIA ON  
NITRATE AND NITRITE CONCENTRATIONS  
IN GROUNDWATER OF THE OGALLALA AQUIFER

by

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## ABSTRACT

The Ogallala Aquifer of the eastern High Plains of New Mexico has been examined for groundwater contamination. Previous studies have indicated the presence of numerous bacteria and many insidious chemical species. The origin of some contaminants were obvious (e.g. coliforms and fecal coliforms). High levels of nitrate concentration were resolved with the probable origin being land application of fertilizer or leaching from septic sewage systems. The life span of the nitrate contamination in the Ogallala, however, has always been short-lived, and quite variable between sampling intervals. Determining cause of the observed variation was an objective of this investigation. Nitrate ions can be converted to nitrite ions through microbial action. An inverse variation between these two chemical contaminants would indicate a microbial conversion, while directly proportional variation would be indicative of a chemical cause.

The potential microbial responsibility for the fluctuations in nitrate and nitrite ions was examined in this project. Fluctuations were observed to be inversely proportional. Coliforms were not sufficiently numerous to explain the rapidity of the fluctuations. Consequently, the presence and quantitation of other nitrate-reducing organisms were determined.

Large numbers of bacteria were found which were capable of reducing nitrate. Their numbers fluctuated in direct proportion to the observed nitrate concentrations. Nitrate concentrations, regardless of source, were short-lived, highly variable, and were apparently reduced as a result of microbial populations, other than coliforms, occurring in the Ogallala Aquifer.

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## PROBLEM DEFINITION

### Introduction

The Ogallala formation is one of the Nation's major aquifers, furnishing agricultural, domestic, and municipal water for an area of approximately one-quarter million square miles. The Ogallala formation extends from southern South Dakota to central western Texas and eastern New Mexico. The Ogallala formation was deposited during the late Miocene and Pliocene times in pre-existing valley systems across the Great Plains by streams flowing eastward to southeastward from sources in the existing mountains. Late Tertiary or early Pleistocene warping placed the alluvial deposits in a position vulnerable to Pleistocene erosion. Erosion modified the landscape in such a manner that the area covered by the Ogallala now forms the plateau between the eroded mountains, the foreland, and the central interior plains (Frye, 1970).

The portion of the Ogallala formation in this study includes almost the entire eastern border of New Mexico (Figure 1). The formation in this entire region is either exposed at the surface or is covered with relatively thin deposits of aeolian sands and silts or by shallow pond deposits. The Ogallala contains significant quantities of sand and gravel and is generally underlain by older rocks of much lower permeability making it the most extensive and useable aquifer of this portion of the Great Plains Province (Counselman, 1970). The Ogallala Aquifer in the study area is



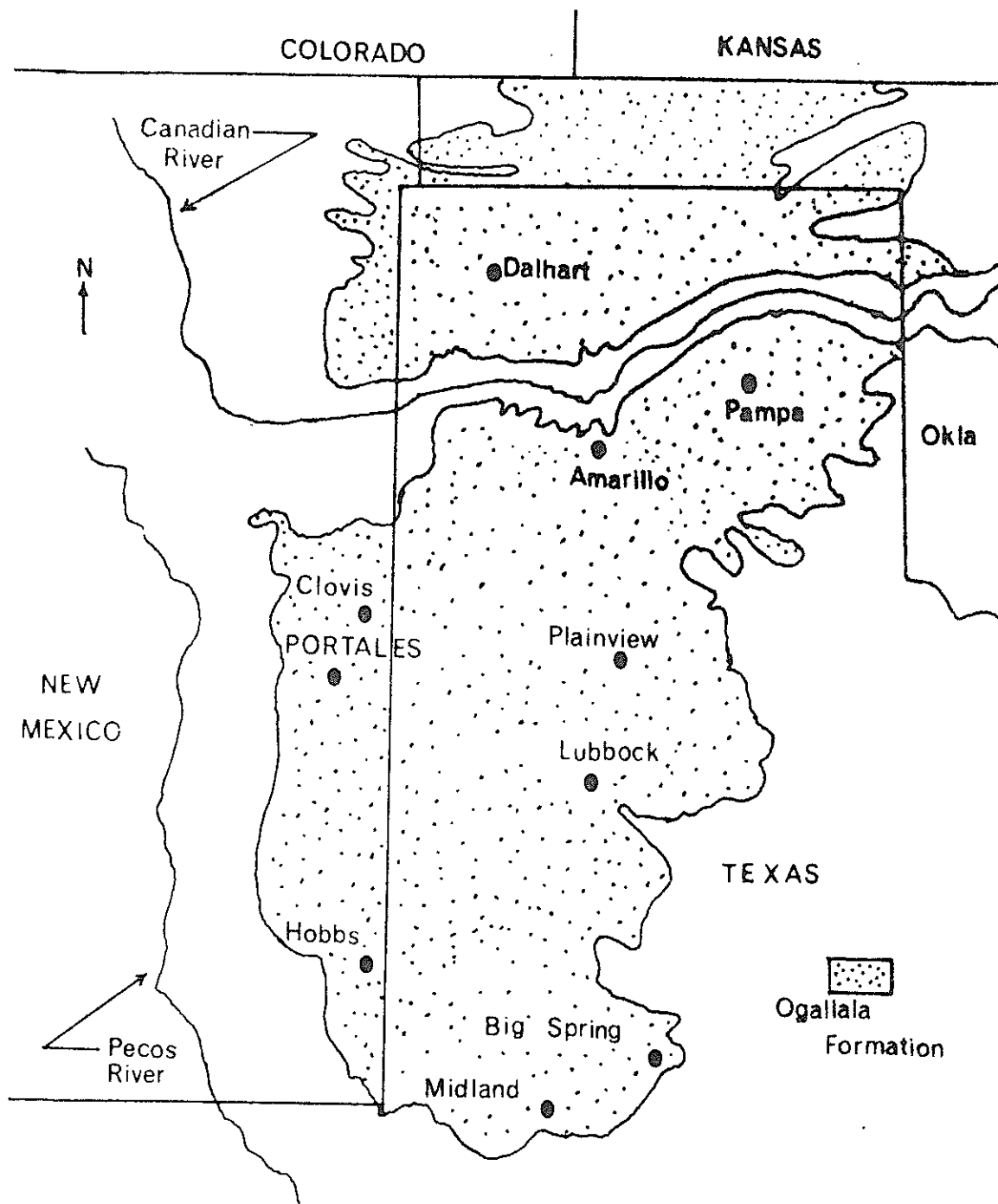


Figure 1  
Southern Ogallala Formation

isolated from any appreciable recharge water source. Natural recharge of the aquifer from meteoric waters is negligible while return flow from irrigation waters is appreciable.

The quantity of contaminants that can be "leached" into the water table is directly related to the degree of permeability of the soil through which these pollutants must pass. According to the USDA survey (1967), all the soil types in the study area are permeable from the surface down to the aquifer. There is no aquaclude between the surface soil and the water table in the study area.

The effects of downward percolation of contaminants within the study region have been previously documented (Bigbee, 1972; Taylor and Bigbee, 1973; Taylor and Pitt, 1973; Wills, 1972). The effect of bacteria in the water table has not been examined relative to the occurrence, fluctuation, and disappearance of nitrate and nitrite levels in the water.

Nitrate salts are the most highly oxidized form of nitrogen in the nitrogen cycle of bacteria and normally reach important concentrations in the final stages of biological oxidation (A.P.H.A., 1971). Manure used in fertilization or manure on feed lot surfaces is converted from organic nitrogen to nitrate through ammonification and nitrification (Thimann, 1963). Large numbers of the agricultural fields in the study area are fertilized with ammonia, which likewise can be oxidized by soil bacteria to form nitrate salts. In addition, some agriculturalists apply nitrate fertilizers directly to the fields. Nitrate is water soluble and is not complexed by soil; therefore, it is easily leached from the soil

and transported by water (Stanier, et. al., 1970). These characteristics of nitrate often result in its attaining excessively high levels in groundwaters where return flow turnover is high and entrance of new nitrate-free water is low. In excessive amounts, nitrate contributes to a disease of humans, infant methemoglobinemia (Oser, 1965). The disease is due to bacterial conversion of nitrate to nitrite, in the intestine, and its rapid absorption by the blood.

#### Problem and Objectives

Relatively high levels of nitrite have been observed previously in the groundwater of the study region. Nitrite in water supplies can be readily absorbed by the blood.

Seasonal fluctuations in nitrate concentrations have also been observed previously in the study region. Coliform bacteria, naturally occurring in the aquifer of the study region, were examined for their role in the conversion of nitrate to nitrite (denitrification). Correlations of land use and soil permeabilities were demonstrated previously to affect quantitatively the presence of certain bacteria in the aquifer; and nitrate conversion to nitrite had been demonstrated qualitatively, however, quantitative relationships by these bacteria had not been ascertained (Bigbee, 1972; Taylor and Bigbee, 1973; Wills, 1972).

The purpose of this study was to investigate the total numbers of bacteria that were present relative to those which could reduce nitrate, and to correlate these numbers with observed fluctuations of nitrate and nitrite concentrations. Numbers of bacteria and nitrate concentrations present in the aquifer water were also correlated to the permeability of soil types. Such correlations

would permit analyses of whether land use was, by itself, a primary cause of aquifer water deterioration; or whether bacterial populations in water would, through biological processes, lower certain chemical contaminants to within safe limits. Enough repetition of samples for statistical validity was required because populations and chemical parameters had been observed to fluctuate.

This study has far reaching implications when sewage effluent disposal on land surface and solid waste sanitary land fill disposal sites are considered. The implications of nitrate-nitrite and agricultural effects on groundwater quality either as point source or non-point source pollutants, relative to P.L. 500, Section 208, were considered in this investigation. The results of this study are applicable to many regions where highly permeable aquifers exist at or near the land surface which are not overlain by aquacludes. Many such aquifers exist in the western and southwestern United States.

## METHODS AND MATERIALS

### Sampling Grid

A sampling grid was established in the middle of the aquifer using Portales, New Mexico, the county seat of Roosevelt County, as the center. Samples were collected weekly from wells covering a ten-mile radius on the four compass points. Samples were collected at approximately two-mile intervals at each of the compass directions (Figure 2). Approximately 500 ml of aquifer water were collected at each sampling point in sterile glass containers and kept cold until returned to the laboratory for analysis.

Water temperatures were recorded at each sampling site to ensure fresh aquifer water at a constant temperature was collected each time.

### Microbiological Analysis

Bacteriological analyses included the use of the following media and/or procedures: Nitrate Agar (BBL), membrane filtration (Millipore, Inc.), M-endo broth (BBL), anaerobic incubation under 1 atmosphere nitrogen, and aerobic incubation. Aerobic cultivation procedures for each sample included the transfer of 0.5 ml of water to a sterile petrie dish employing the pour plate technique using nitrate agar and incubation at 24<sup>0</sup> C. Each sample was plated in triplicate for statistical analysis. Replicates were counted after five days of incubation. Anaerobic cultivation procedures for each sample were also performed by the pour plate technique using nitrate agar with a 0.5 ml water sample. Incubation was under one

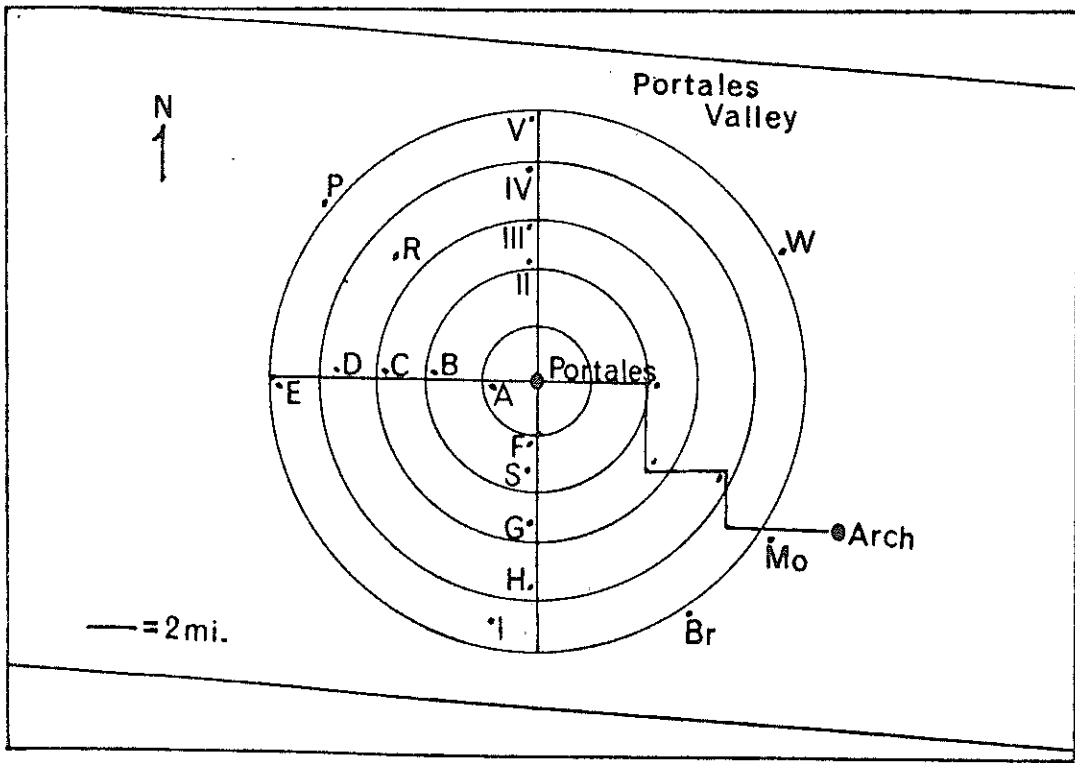


Figure 2  
 Portales Valley Bounded Water Basin  
 Sampling Grid and Aquifer in the Portales Valley

atmosphere of nitrogen at 24<sup>0</sup> C for five days. Total counts were recorded. Anaerobic cultivation counts, when compared to aerobic counts, permitted the establishment of the numbers of facultative organisms present in each sample.

Membrane filtration was performed on a 100 ml aliquot of each sample. After filtration, the membrane filter was incubated on a M-endo broth impregnated filter disk at 37<sup>0</sup> C for 24 hours. Total numbers of organisms and total coliforms were differentiated and recorded.

The nitrate agar pour plates were flooded, after counting, with equal volumes of 1-naphthylamine and sulfanilic acid (Taylor, 1974). These reagents give a qualitative indication of nitrate reduction to nitrite by production of a pink to red color in the media surrounding the colony. Numbers of colonies reducing nitrate to nitrite both aerobically and anaerobically were recorded. Additionally, the organisms were differentiated based on discrete colony morphology and the numbers recorded for each replicate.

#### Nitrate Determination

Nitrate concentrations were determined on each water sample by the Brucine method (STORET NO. 00620) of the Environmental Protection Agency (1976). The method is based upon the reaction of the nitrate ion with brucine sulfate in a sulfuric acid solution. The color of the resulting complex was measured at 410 nm. Briefly, 10 ml of sample were added to 10 ml of concentrated sulfuric acid and the mixtures permitted to come to thermal equilibrium in an ice bath. A 0.5 ml aliquot of brucine-sulfanilic acid reagent was

added; and the samples were placed in a 100° C water bath until thermal equilibrium was reached at 20-25° C. Absorbance of standards and samples were determined at 410 nm against a reagent blank.

#### Nitrite Determination

Nitrite was analyzed by the method reported by Strickland and Parsons (1972). The method is based on the classical Griss reaction where the nitrous acid is converted to a highly colored "azo" dye. One ml of naphthylethylenediamine (Marshall's reagent) was added to 50 ml of sample and mixed thoroughly. After standing two hours, the absorbance was determined at 543 nm. The nitrite nitrogen concentration in microgram-atoms of nitrogen per liter was determined from the expression:

$$\text{microgram-atoms N/L} = \text{corrected absorbance} \times F$$

The factor F was determined from the expression:

$$F = \frac{2.00}{E_s - E_b}$$

where  $E_s$  was the mean absorbance of four standards and  $E_b$  was the mean absorbance of two blanks.

#### Counting

Graphing of all data was accomplished using a Tektronix desk-top computer employing a digital plotter. All graph lines (aerobic numbers, anaerobic numbers, nitrate concentrations in ppm, and nitrite concentrations in ppb) were determined by use of this computer to connect data points. All bacterial counts were expressed as organisms per ml of sample. The too-numerous-to-count



(TNTC) expression was employed when the numbers of bacteria exceeded 10,000 per ml. Estimates for numbers below 10,000 per ml were determined using illuminated table counter by grid averaging. The number of organisms reported represents a minimum number which was established by the grid averaging technique.

#### Statistical Analysis

Statistical analysis of the replicated counts employed the Q test. Sample replicates ( $x_1$ ;  $x_2$ ;  $x_3$ ) were arranged in order of increasing magnitude. For the smallest value,  $x_1$ , the Q was determined as follows:

$$Q_{x_1} = \frac{x_1 - x_2}{x_n - x_1}$$

For the largest value,  $x_n$ , the Q was determined as follows:

$$Q_{x_n} = \frac{x_n - x_{n-1}}{x_n - x_1}$$

The replicates were three in number ( $n=3$ ) and  $Q_{.90} = 0.94$ , while  $Q_{.96} = 0.98$ .

## RESULTS

Eighteen sampling sites were examined on a weekly basis for thirty-four weeks. Three replicates of each sample at each site were obtained; and five tests were performed on each sample. The quantity of raw data generated was voluminous. It was condensed, analyzed, and graphically represented. The complete tabular and graphic data are found in the Appendix (available separately - copy charge \$3.90).

The data ranges are found in Table 1 for each of the sampling sites. Taxonomic identification was not attempted on the microorganisms. Gross colony morphology was utilized to determine potential variation in types of organisms present. A representative listing of colony types is found in Table 3. The numbers of different organisms are those shown to reduce nitrate to nitrite. Total membrane filter count was reported since numerous organisms that grew were capable of reducing nitrate. Media employed for this count was an all-purpose nutrient media to which nitrate salt had been added.

Figure 3 demonstrates typical results in many of the sampling locations when aerobic and anaerobic populations of microorganisms were low, nitrate and nitrite concentrations changed inversely, i.e. when nitrate decreased, nitrite was increasing. Sample values for Figure 3 are shown in Table 2. A lag time between parameters being measured was noted. When aerobic or anaerobic populations were

high, nitrate and nitrite concentrations were low (note November of Figure 3). When nitrate concentrations were high, organism population counts were low (note February-March of Figure 3).

The microbial populations were not as rapid in recovery during the February-March period as they were during the October-November period. It was noted that water temperatures were also different during this time period. It is well known that temperatures affect growth rates.

Data found in the Appendix support the findings represented in Figures 2 and 3. A few exceptions were noted in a few sample locations. The variables already reported on water movement (greater than six miles per year, Taylor and Bigbee, 1973) could be responsible for the exceptions. A more permeable aquifer at a particular sampling site would allow a larger time span between measured values of nitrate-nitrite and population blooms.

The results indicate the responsibility of organisms other than coliforms and their effect upon nitrate and nitrite ion concentrations. Nitrate has been shown to be affected by land use activities (Taylor and Bigbee, 1973). The transient behavior of nitrate can now be explained by the blooms of naturally-occurring, non-coliform, bacteria in the aquifer. The blooms appear seasonal and data indicate the aquifer temperature is probably responsible.

S A M P L E	WATER TEMPERATURE RANGE		CRGS./1 ML. AEROBIC RANGE				CRGS./1 ML. ANAEROBIC RANGE				CRGS./100 MLS. MILLIPORE RANGE			PFM WATER SAMPLE NO <sub>3</sub> RANGE		PFB WATER SAMPLE NO <sub>2</sub> RANGE		
			Different Numbers to		Total Count to		Different Numbers to		Total Count to		Total Count to	Coliform Count to		to		to		
	to																	
A	7.0	22.5	0	7	0	1425	0	5	0	2700	0	TNTC 5000+	0	13	3.75	27.80	*	78.40
B	8.0	22.5	1	5	0	TNTC 10000	1	5	0	TNTC 10000	0	TNTC 5000+	0	22	4.65	48.80	*	48.80
C	6.0	27.0	0	4	0	TNTC 10000	0	5	0	TNTC 10000	0	TNTC 5000+	0	37	5.70	40.00	*	90.20
D	7.0	26.0	0	5	0	TNTC 10000	0	5	0	TNTC 10000	0	TNTC 5000+	0	164	4.30	40.00	*	43.20
E	13.0	24.0	2	4	2	1000	2	5	22	1500	1	2000+	0	5	4.50	23.00	*	136.00
F	11.0	30.0	0	4	0	3000	0	4	0	TNTC 10000	0	TNTC 5000+	0	9	4.35	22.40	*	608.00
G	9.0	27.0	0	4	0	TNTC 10000	0	4	0	TNTC 10000	0	TNTC 5000+	0	210	4.20	29.80	*	*
H	10.0	30.0	1	6	2	TNTC 10000	0	4	0	TNTC 10000	0	TNTC 5000+	0	6	4.30	19.40	*	*
I	7.0	23.0	0	4	0	2091	0	3	0	TNTC 10000	0	TNTC 5000+	0	0	4.30	27.20	*	*
J	9.0	26.0	0	4	0	TNTC 10000	0	4	0	TNTC 10000	0	TNTC 5000+	0	2	3.75	34.00	*	32.80
K	8.0	25.0	0	5	0	TNTC 10000	0	5	0	TNTC 10000	0	TNTC 5000+	0	1	0.50	25.00	*	3.20
L	7.0	28.0	0	4	0	5000	0	5	0	5056	0	TNTC 5000+	0	0	4.35	40.00	*	48.00
M	7.0	26.0	0	5	0	TNTC 10000	0	4	0	3000	0	TNTC 5000+	0	1	3.60	31.50	*	*
N	9.0	27.0	0	5	0	2000	0	6	0	3000	0	TNTC 5000+	0	0	2.75	17.00	*	*
O	8.0	26.0	0	4	0	1200	0	4	0	1000	0	TNTC 5000+	0	8	3.00	27.40	*	14.20
P	8.0	22.0	0	4	0	TNTC 10000	0	5	0	TNTC 10000	0	TNTC 5000+	0	4	4.70	28.20	*	*
Q	8.0	29.00	0	7	0	TNTC 10000	0	5	0	3000	0	TNTC 5000+	0	4	4.35	24.80	*	*
R	9.0	26.0	0	4	0	2000	0	5	0	2000	0	TNTC 5000+	0	10	4.00	22.80	*	*

Table 1  
Composite Data Ranges

DATE	3 REPLICATES AEROBIC		ANABROBIC		HILLPOD		WATER SAMPLE NITRATE (NO <sub>3</sub> ) ppm	WATER SAMPLE NITRITE (NO <sub>2</sub> ) ppb	WATER TEMP °C
	Mean Total Count	Total Diff. Nos.	Total Count	Diff. Nos.	Total Count	Total Coliforms			
6 Sept. 77	18	2	0	0	667	0	PRCDR. NOT ESTAB	*	22.5
13 Sept. 77	0	0	0	0	0	0	14.70	*	22.0
20 Sept. 77	286	4	612	2	TNTC	13	6.25	*	22.0
27 Sept. 77	99	3	172	3	0	0	6.60	*	22.0
4 Oct. 77	---S A M P L E N O T A V A I L A B L E---								
11 Oct. 77	283	3	600	3	1000+	0	8.80	*	19.0
18 Oct. 77	454	2	432	3	4000+	0	7.85	*	19.0
25 Oct. 77	102	4	260	3	144	0	5.95	*	17.0
1 Nov. 77	---S A M P L E N O T A V A I L A B L E---								
8 Nov. 77	105	3	208	3	279	0	3.75	78.40	15.0
15 Nov. 77	119	3	152	3	1000+	1	9.05	*	14.0
22 Nov. 77	582	3	1812	3	738	0	4.35	13.80	12.0
29 Nov. 77	1425	3	2700	3	TNTC	0	6.05	17.60	14.0
6 Dec. 77	---S A M P L E N O T A V A I L A B L E---								
13 Dec. 77	---S A M P L E N O T A V A I L A B L E---								
20 Dec. 77	---S A M P L E N O T A V A I L A B L E---								
27 Dec. 77	---S A M P L E N O T A V A I L A B L E---								
3 Jan. 78	---S A M P L E N O T A V A I L A B L E---								
10 Jan. 78	---S A M P L E N O T A V A I L A B L E---								
17 Jan. 78	---S A M P L E N O T A V A I L A B L E---								
24 Jan. 78	79	7	68	5	TNTC	0	N.A.	N.A.	9.0
31 Jan. 78	8	2	10	2	0	0	3.25	*	8.0
7 Feb. 78	3	2	0	0	3	0	4.25	*	10.0
14 Feb. 78	0	1	2	1	1	0	N.A.	N.A.	8.0
21 Feb. 78	16	2	28	3	5	0	11.80	*	7.0
28 Feb. 78	---S A M P L E N O T A V A I L A B L E---								
7 Mar. 78	21	4	48	3	24	1	N.A.8	N.A.	9.0
14 Mar. 78	1027	4	666	4	18	0	27.80	*	10.0
21 Mar. 78	---S A M P L E N O T A V A I L A B L E---								
28 Mar. 78	449	4	100	3	2	0	N.A.	N.A.	10.0
4 Apr. 78	109	3	64	3	1	0	N.A.	N.A.	11.0
11 Apr. 78	114	3	180	2	3	0	5.30	*	10.0
18 Apr. 78	75	3	24	3	200	0	N.A.	N.A.	10.0
25 Apr. 78	7	2	4	1	N.A.	N.A.	N.A.	N.A.	10.0

↑ orgs./1ml      ↑ orgs./1ml      ↑ orgs./100mls      ↑  
 TNTC=10,000+      TNTC=10,000+      TNTC=5,000+      \* - Trace or less  
 N.A. - Data Not Available

Table 2  
Sample Values for Figure 3

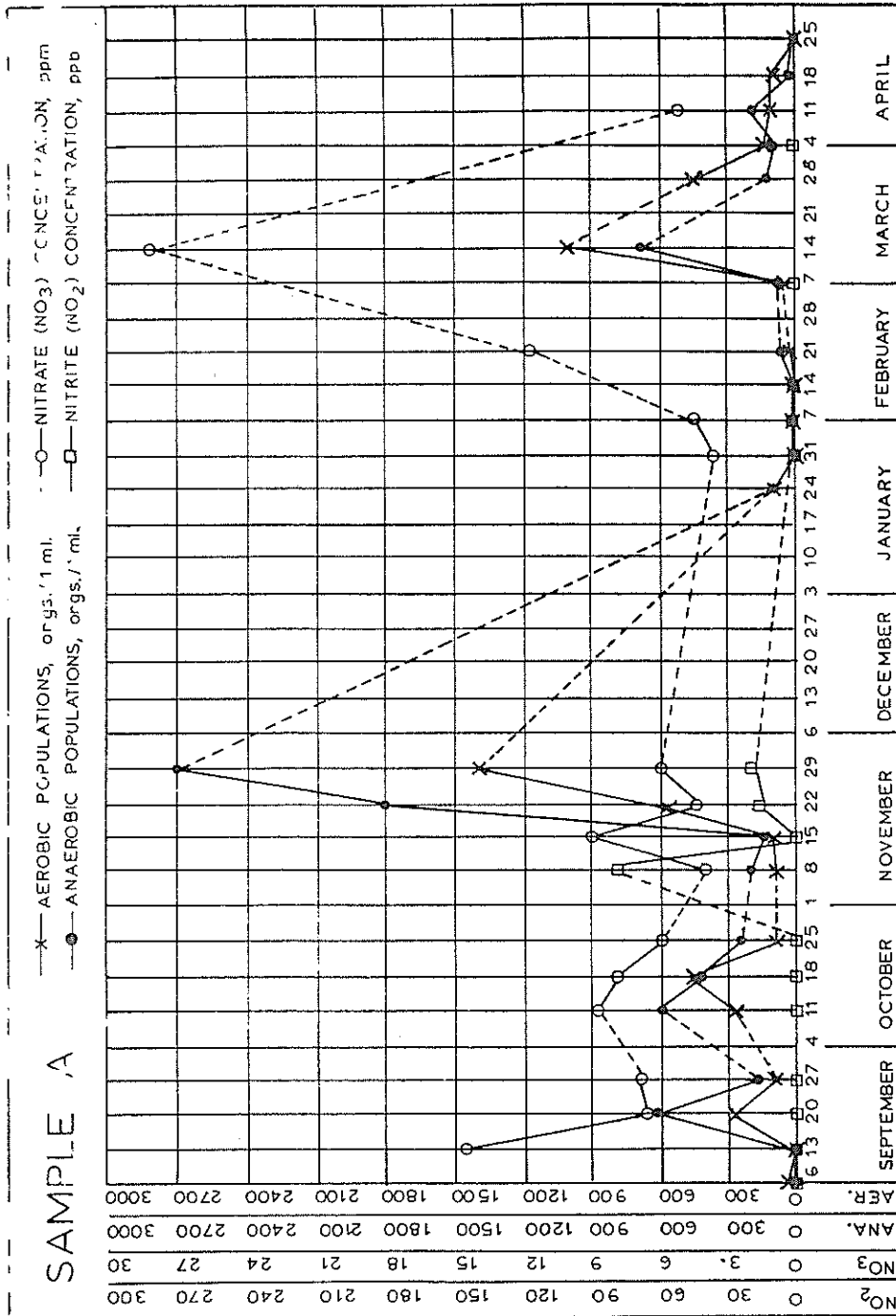


Figure 3

Graph of Analyzed Parameters

TABLE 3  
COLONY TYPES - SAMPLE A

AEROBIC

smooth, white-tans; surface and spindles\*  
milky-white opaques; surface and spindles  
smooth, milky-whites\*  
milky-white mottled colonies  
pale white, transparent; bottom and surface  
white, mucoid, motile  
white, smooth; surface and bottom\*  
white spindles\*  
white, rough-center colonies  
rough; surface  
yellows; surface and spindles\*  
pale reds\*

ANAEROBIC

white-tans; surface and spindles\*  
pale whites, extremely motile\*  
white, smooth; surface  
white spindles\*  
white, mucoid, motile; surface  
white, mottled and rough; mid and bottom  
yellow; surface and spindles\*  
pale reds\*

\* suspected for nitrate reduction

## CONCLUSIONS

An examination of the data indicates the presence of numerous bacteria in the groundwater which are capable of interacting with the various chemical species of nitrogen. Additionally, patterns in nitrogen ion concentrations were established which correlate with bacterial blooms.

One of the problems in establishing whether the bacteria observed in any particular sample are an accurate estimate, or whether they are an artifact of sampling, had to be addressed. If a high percentage of organisms capable of removing nitrate or nitrite from the water was established, the percentage would be only as valid as the accuracy of the counting. Replicate sampling and Q test statistics were employed. From the data, it can be seen that the observed numbers were indeed significant, highly significant, or at least often in acceptable ranges.

The data demonstrate that nitrate ion concentrations fluctuated. Their fluctuations were observed to increase as a result of irrigation return flow or rainfall (Taylor and Bigbee, 1973). Observed decreases following nitrate surges were correlated to increases in nitrate reducing coliforms. The number of coliforms, however, were not sufficient to account for the total decline in nitrate. At that time other bacterial organisms were not examined as to numbers or ability to reduce nitrate. Nitrate reduction is not unique to the coliforms (Taylor and Bigbee, 1973).



The results of this study showed the presence of numerous other microorganisms, both aerobic and anaerobic, which were capable of reducing nitrate to nitrite. Nitrite was quantitatively examined and was also shown to fluctuate relative to the nitrate cycles. It is apparent from this study that the peak appearances of nitrate which occur in the Ogallala formation were transient in nature. The disappearance of nitrate was caused by the bacteria which are found in the aquifer water. Nitrite followed similar cyclic patterns which also appeared to be a result of the microbial populations in the groundwater.

Point source contaminators in an agricultural community would be difficult to determine with respect to nitrate. The decline of nitrate is usually followed by a quantitative increase in the more insidious nitrite ion. Nitrite is rarely looked for as a contaminant in potable water testing. Microbial populations have been shown to be directly responsible for the fluctuations in nitrate. The populations were shown to be non-coliforms and belonged to numerous genera. The presence of these organisms in groundwater have been statistically substantiated as responsible for nitrate disappearance. Their growth and populations appear to be a function of the presence of nitrate concentrations and aquifer temperature.

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