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## Bacteriological Quality of Lake Red Rock and the Des Moines River Between Des Moines, Iowa, and Lake Red Rock<sup>1</sup>

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DEAN A. HOGANSON and STEPHEN C. ELLIOTT. Bacteriological Quality of Lake Red Rock and the Des Moines River Between Des Moines, Iowa, and Lake Red Rock. *Proc. Iowa Acad. Sci.*, 78(3-4): 50-56, 1972.

SYNOPSIS. The bacteriological quality of the Des Moines River from Des Moines, Iowa, to Lake Red Rock and the lake itself were studied on eleven sampling dates from July 1969 to May 1970. The examined river sites consistently had higher EMB plate counts than sites in the lake proper and counts from both river and lake were generally far above quoted standards of water to be

used for contact and non-contact water sports. The pH readings were all within quoted normal ranges. The expected effects of seasonal variation and runoff on bacteriological counts were found. Seven isolations of pathogenic, enteric bacteria were made during this study. These isolates were made at the two sampling points closest to the Des Moines sewage treatment plant. XLD medium proved to be more successful than DCC or BG in isolating these pathogens. These results were examined in view of the expected heavy use of Lake Red Rock for recreational purposes.

Many techniques have been developed to determine the causes and extent of pollution to bodies of water. Analysis of water for coliform bacteria is often used to determine the extent of pollution caused by fecal waste products. If fecal contamination is indicated, pathogenic organisms may be present. Bacteriological analysis techniques were used in this investigation to give information about fecal contamination of the Des Moines River below Des Moines, Iowa, and Red Rock Lake.

Lake Red Rock formed behind a dam on the Des Moines River about 45 miles southeast of Des Moines, Iowa. When the dam was closed in 1969 it was expected that several years would pass before the lake would completely fill. However, due to extremely heavy runoff, the reservoir reached the conservation pool elevation of 725 ft. within a few weeks. It eventually reached a flood control pool elevation of approximately 765 ft. during the summer of 1969.

The lake is primarily a flood control project. Secondarily it provides water of recreational value which should have few, or no, pathogenic bacteria. The prime obstacles to this are runoff from agricultural and wildlife areas and inadequate treatment of sewage by municipalities. The Des Moines River is fed by tributaries that drain land on which large numbers of cattle, hogs, and poultry are raised. Miner, et al. (1966) pointed out that cattle feedlot runoff, in many areas of the country, is a source of bacteria normally associated with pollution. Miner, Fina and Piatt (1967) made 26 isolations of salmonellae from the runoff of 14 cattle feedlots at Manhattan, Kansas. The 10 isolates that they serotyped were *S. infantis*. Newell, et al (1959) cultured rectal swabs from 162 pigs on five farms producing infected pigs. Nine per cent of them were infected with *Salmonella*. They also isolated *Salmonella* organisms from 23 out of 100 samples of cecal feces from pigs slaughtered in a bacon factory. Edwards (1958) believes domestic poultry constitute the

largest single reservoir of salmonellae among animals.

Braga (1966) studied survival of salmonellae in farm drainage and showed that these waters should be held 20 days to prevent possible contamination in the summer. Sixty days were required in winter. Mallman and Litsky (1951) found that *S. typhosa* could survive in Osktema sand for five days, 12 days in Miami loam, and 19 days in Brookston clay and muck.

Water temperature is quite important for the survival of bacteria in rivers and lakes. Wang, Dunlop and Munson (1966) recovered *Shigella* more consistently at 15°C than at 25°C from sewage and irrigation water. Hanes, Rohlic and Sarles (1966) made daily counts of coliform and enterococci organisms in samples of biological oxygen demand dilution water maintained at 10°C, 20°C, and 30°C. The sample held at 10°C showed a lower death rate than the other two samples of water.

There are many reports of pathogenic enteric organisms being recovered from rivers contaminated by municipalities. Spino (1966) found salmonellae as far as 73 miles and four days travel downstream from Fargo, North Dakota. Popp (1957) isolated *Salmonella* from the Ober River, Germany, as far as 21 miles below a sewage discharge point. Locally, DeMoss (1969) reported the isolation of three species of *Salmonella* and *Edwardsiella tarda* from the Des Moines River within the city limits of Des Moines, Iowa.

Burm (1967) found that total coliform counts rose markedly in the Detroit River after moderate and heavy rainfall had caused overflows in combination storm and sanitary sewers that entered the river. He concluded that the coliform counts were proportional to the severity of the storm. If the storm was moderate the river was affected for about three days. A severe storm caused abnormally high coliform counts for up to six days. It was found that moderate rains would cause a thousandfold increase in coliform counts if the sampling site was near a discharge point. Total coliform counts exceeded 100,000 organisms/100 ml after a moderate rain and reached 1.6 million/100 ml after a severe storm.

A commonly used medium for isolating *Salmonella* is brilliant green agar. Edwards and Ewing (1962) felt that

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brilliant green agar should be used if one desires to isolate the largest possible number of *Salmonella*. They also point out that many workers use deoxycholate citrate agar if they are interested in isolating *Shigella* and *S. typhosa* with other salmonellae.

New media are constantly being developed to isolate enteric pathogens. Presently, one of the most promising is xylose-lysine-deoxycholate agar (XLD). Taylor and Schelhart (1967) reported that XLD gave more isolations of enteric pathogens from stools than eosin-methylene blue agar (EMB), brilliant green agar (BG), and *Salmonella-Shigella* agar (SS) combined. Taylor (1965) found that XLD was more sensitive than SS, BG or bismuth sulfite (BS) for fastidious organisms, such as *Shigella*. He also noted that XLD was more selective for enteric pathogens than EMB or MacConkey agar (MacC). According to Taylor and Schelhart (1968), XLD produced more *Salmonella* and *Shigella* isolates from stools than did MacC, deoxycholate agar (DC) or xylose-lysine-deoxycholate brilliant green agar (XLDBG). Isenberg, Kominos and Siegel (1969) found XLD to be less inhibitory to *Salmonella* and *Shigella* than SS, but XLD did not permit the growth of pathogenic bacteria in dilutions from which they were recovered with Hektoen (He). This medium, according to Taylor (1965), is designed to give smaller numbers of false-positives and, at the same time, allow better growth of the more fastidious pathogens than traditional media.

Most traditional media have been designed to separate the enteric bacteria into lactose fermenters and non-lactose fermenters. The enteric pathogens do not rapidly ferment lactose, but there are also many non-pathogens that do not ferment lactose rapidly. Xylose-lysine-deoxycholate agar was designed to eliminate this difficulty by using xylose in its formula. *Shigellae* and *Providencia* fail to ferment xylose and show an alkaline pH on XLD. *Salmonella* and *Arizona* ferment xylose but decarboxylate the lysine in XLD and revert to an alkaline pH. Lactose and sucrose are in the formula to cause organisms that ferment these two sugars to produce acid. The formula contains an H<sub>2</sub>S indicator and utilizes sodium deoxycholate as an inhibitor.

This investigation was designed to isolate and identify enteric pathogens present in Red Rock Lake and the Des Moines River. Brilliant green, xylose-lysine-deoxycholate and deoxycholate citrate agars were used as isolation media to determine whether the newer medium, XLD, allowed more isolates than the two more commonly used agars. This project was also conducted to determine how runoff and seasonal differences affect the total coliform count in the river and lake water.

#### METHODS AND MATERIALS

Sampling began July 21, 1969, and was concluded May 2, 1970. Ten sampling dates spaced throughout this period were chosen (Table 1). Another date (October 25th) was chosen to observe the effects of dumping sewage that had received only primary treatment into the Des Moines River. This was caused by a power outage at the Des Moines sewage treatment plant.

Six sampling sites were designated on the Des Moines River and Lake Red Rock. Three of the sites were located on the Des Moines River between Des Moines and Lake Red Rock. One sample was taken below the highway 46 bridge approximately three-fourths mile downstream from the dis-

TABLE 1. EOSIN-METHYLENE BLUE PLATE COUNTS (BACTERIA/100 ML X 10<sup>2</sup>) FROM DES MOINES RIVER AND RED ROCK LAKE WATER, 1969 AND 1970

DATE	SAMPLE SITES					
	A	B	C	D	E	F
JULY 21	7,800	1,400	160	240	310	24
SEPT. 20	2,100	3,100	1,700	160	180	140
OCT. 25	2,800	750	1,900	1,200	230	190
NOV. 1	850	60,000	51,000	15,000	120	66
MEAN COUNT	3,400	16,000	14,000	6,700	210	110
NOV. 30	1,300	220		1,200		30
JAN. 3	680	130		77		8
JAN. 30	8,800	17,000		22,000		200
FEB. 28	1,200	4,500		6,500		700
APR. 4	340	1,200		300		540
MAY 2	670	2,700		270		83
MEAN OF ALL SAMPLES	2,700	9,100	14,000	5,900	210	200

charge point of the Des Moines sewage treatment plant (site A). Sample (B) was taken below the highway 316 bridge at Runnells, Iowa, and (C) was recovered from the river, approximately three miles upstream from the entrance of the lake. Three samples were taken from the lake. They came from under the highway 14 bridge (D), the approximate center of the lake (E) and one was taken near the dam (F). These six sites are shown in Figure 1. Sites C and E could not be sampled for six of the dates because of unsafe ice and difficulty experienced in reaching the sites by boat.

Samples were collected from the surface waters of the river and lake sites in sterile glass bottles. At each sampling site the water temperature, pH and time were recorded. The river level, precipitation and air temperature records for the week preceding each sampling date were obtained from the U.S. Weather Bureau. The samples were kept in an ice chest filled with water of the same temperature as the first collections site until they could be processed in the laboratory. In the laboratory, each of the water samples was first tested for turbidity with a Hellige turbidimeter (Hellige Co., Garden City, N.Y.). The turbidity readings were recorded as equivalent to ppm of SiO<sub>2</sub>.

One hundred ml of each water sample was added to 100 ml of double strength selenite enrichment broth. The samples were then incubated at 37°C for 24 hr, after which each was streaked on two plates each of XLD, BG and DCC agar. These plates were then incubated for 24 hrs. at 37°C. Colonies on the various media showing reactions indicative of being possible pathogens were selected from the plates and inoculated into triple sugar iron agar slants. All cultures showing an alkaline over acid reaction were then further tested by biochemical and serological reactions. These included urease and indole production and lysine decarboxylation. Hydrogen sulfide production was detected from the triple sugar iron agar. Possible pathogens were typed with *Salmonella*, *Arizona* and *Shigella* antisera. A tentative identification of any pathogen or potential pathogen was made and then it was sent to the State Hygienic Laboratories at Iowa City, Iowa, for confirmation.

Due to the failure to isolate any pathogens during the first two sampling dates, a different sampling technique was used. Moore sampling swabs were placed at sites A and B

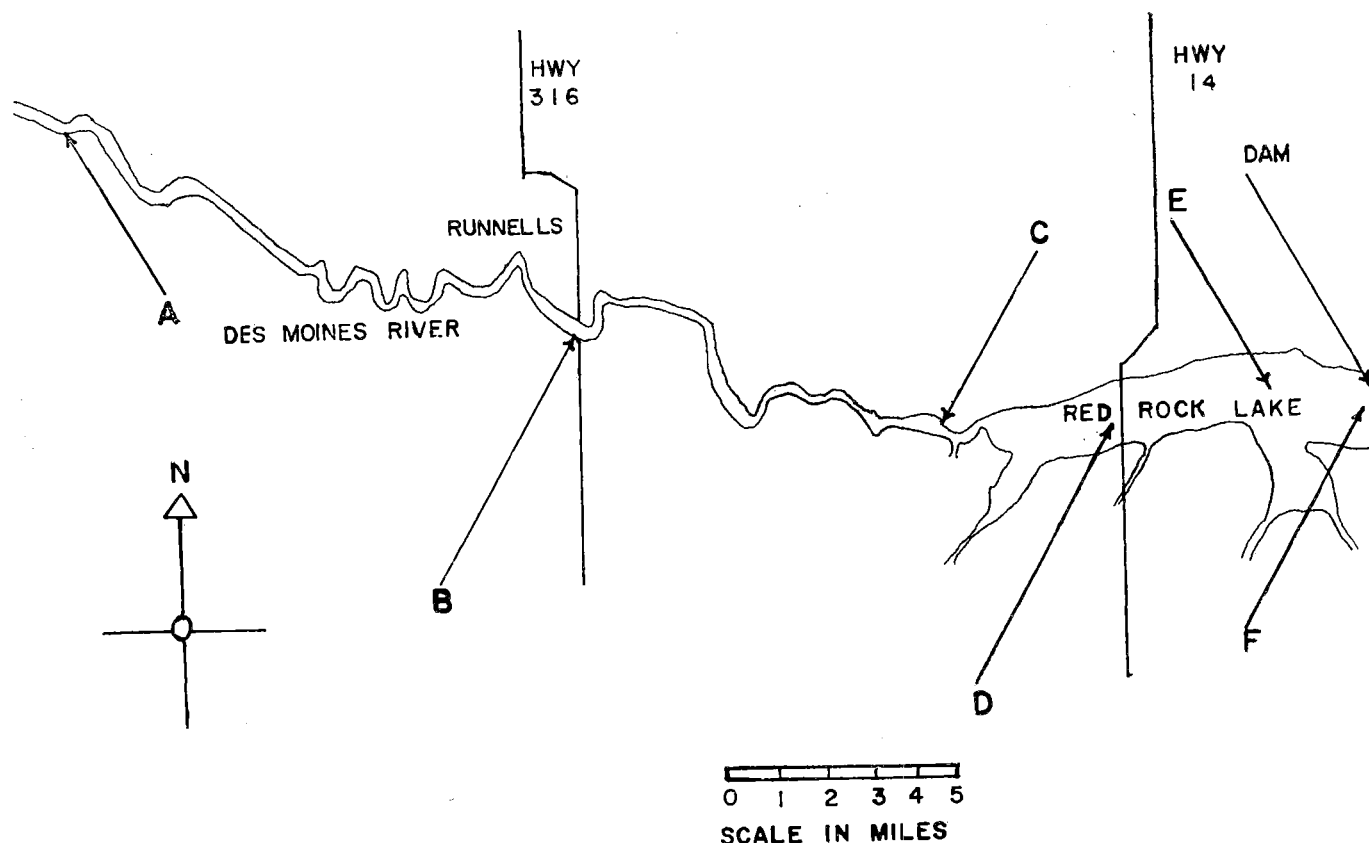


Fig. 1. Sampling sites on the Des Moines River and Red Rock Lake.

for the September 21st and October 28th sampling dates to determine whether this method of sampling would yield more pathogen isolates than the surface sampling technique. The swabs were placed just under the water surface and left for five days. They were recovered, placed in plastic bags and brought to the laboratory. The swabs were then placed in 300 ml of selenite broth for 24 hrs. at 37°C. The primary plating media and additional testing was the same as that used for surface samples.

Eosin-methylene blue plate counts were made from the water taken from each site. Due to the difficulties with EMB agar in determining whether subsurface colonies were lactose fermenters or non-fermenters, all colonies were counted.

#### DATA

The EMB plate counts were higher for sites A, B, C and D than for sites E and F. The mean counts for the first four sampling dates from sites A, B, C and D were 340 thousand, 1.6 million, 1.4 million, and 670 thousand bacteria per 100 ml of water. The mean counts for the first four sampling dates from sites E and F were 21 thousand and 11 thousand. Site B had the highest mean EMB plate count for all ten sampling dates and site F the lowest. These mean counts were 910 thousand and 20 thousand bacteria per 100 ml of water. The highest EMB plate counts were recorded from samples taken on November 1st and January 30th. The lowest counts were from samples taken on January 3rd. The

EMB plate count data is shown in Table 1.

The October 28th sampling date was chosen to note the effect of the city of Des Moines dumping sewage that had received only primary treatment into the Des Moines River. Site A had an EMB count of 1.1 million organisms per 100 ml of water. This represents the highest count recorded for this sampling point. The site B EMB count was 27 thousand bacteria per 100 ml of water.

Like the bacteria counts, the turbidity readings were higher in water samples taken from sites A, B, C and D than in samples from sites E and F. For the first four sampling dates the mean turbidity readings in ppm SiO<sub>2</sub> equivalent for sites A, B, C and D were 72, 235, 127, and 18. The highest turbidity readings were recorded from the November 1st samples while the January 3rd samples gave the lowest. Turbidity data is given in Table 2. The correlation between EMB counts and turbidity readings is shown in Figure 2.

The pH readings ranged from 6.7 at site B on February 28th to 8.9 at site D November 30th. The pH readings did not vary greatly at the six sites.

Water temperature readings were highest on the July 21st sampling date when the range was from 26.0 C at site A to 28.5 C at sites D, E, and F. The lowest readings were recorded on January 3rd and ranged from 1.0 C at sites A and B to 0.5 C at sites D and F. There appeared to be some correlation between water temperature and bacteria counts. The EMB counts at site A are plotted against the water temperature at site A in Figure 3.

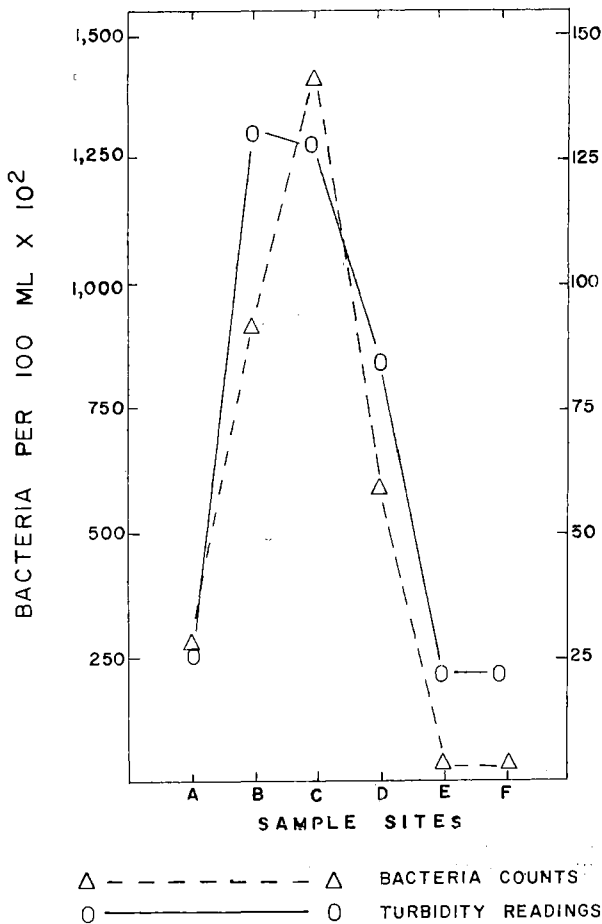


Fig. 2. A comparison of mean turbidity readings and mean bacterial counts on EMB agar at sampling sites on the Des Moines River and Red Rock Lake.

TABLE 2. TURBIDITY READINGS (EQUIVALENT TO PPM SiO<sub>2</sub>) FROM DES MOINES RIVER AND RED ROCK LAKE WATER, 1969 AND 1970

DATE	SAMPLE SITES					
	A	B	C	D	E	F
JULY 21	170	75	19	15	15	11
SEPT. 20	48	70	59	33	23	23
OCT. 25	40	46	46	55	30	18
NOV. 1	30	750	385	215	20	20
MEAN						
TURBIDITY	72	235	127	80	22	18
NOV. 30	26	23		32		23
JAN. 3	6	20		12		8
JAN. 30	23	74		120		8
FEB. 28	75	125		300		48
APR. 4	32	40		25		24
MAY 2	45	69		40		30
MEAN						
TURBIDITY	50	129	127	85	22	21
OF ALL						
SAMPLES						

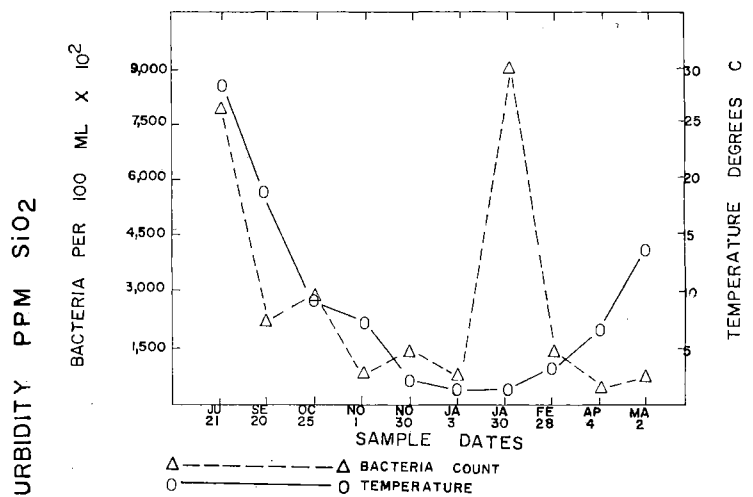


Fig. 3. A comparison of mean bacteria counts on EMB agar and water temperatures at site A.

TABLE 3. DES MOINES RIVER LEVELS AT DES MOINES, IOWA, AND WEATHER INFORMATION FOR ONE WEEK PREVIOUS TO SAMPLE DATES, 1969 AND 1970

DATE	RIVER LEVEL	WEATHER INFORMATION
JULY 21	17.7'	The Des Moines Weather Bureau reported 1.10" of rain on July 17th and .46" on the 18th. Pella, Iowa, reported 5.78" July 17th and .28" on the 18th.
SEPT. 20	15.2'	Pella reported .18" Sept. 14th and .01" Sept. 16th.
OCT. 25	14.8'	Des Moines reported .25" Oct. 19th. Pella reported .25" on Oct. 19th and .60" on the 20th.
OCT. 28	14.8'	No precipitation reported.
NOV. 1	14.9'	Des Moines had 1.18" of rain on the 30th and 31st of Oct. Pella reported .87" on the 30th and 31st.
NOV. 30	12.8'	No precip. or snow melt runoff.
JAN. 3	12.8'	No precip. or snow melt runoff.
JAN. 30	12.7'	Des Moines reported a trace of precip. over the period. The snow cover decreased at D.M. from 5" on Jan. 23rd to 0" on Jan. 30th.
FEB. 28	14.4'	No precip. or snow melt runoff.
APR. 4	13.7'	Des Moines reported 3.9" of snow April 1st. High temperatures April 2, 3, and 4 were in the middle forties.
MAY 2	14.1'	Des Moines reported .1" rain April 30th.

The river level at Des Moines, Iowa, ranged from 12.7 ft. on January 30th to 17.7 ft. on July 21st. Significant rainfall was reported at Des Moines, Iowa, and Pella, Iowa, within one week prior to the July 21st, October 25th and November 1st sampling dates. Snow melting was reported within one week previous to the January 30th and April 4th dates. Des Moines River levels and weather information collected during the investigation are shown in Table 3.

Seven isolations of pathogenic, enteric bacteria were made during the study. These included two isolations each of *Salmonella thompson* and Bethesda-Ballerup, and one isolation of *Salmonella oranienburg*, *Providencia alcalifaciens*, and a

TABLE 4. PATHOGENIC ISOLATES FROM DES MOINES RIVER AND RED ROCK LAKE WATER, 1969 AND 1970

ORGANISM	SITE	DATE	SAMPLING METHOD	ISOLATION MEDIUM
<i>Salmonella thompson</i>	A	9/21	SWAB	XLD
<i>Salmonella thompson</i>	A	9/21	SWAB	XLD
<i>Salmonella oranienburg</i>	A	11/30	SURFACE	XLD
<i>Providencia alcalifaciens</i>	A	11/30	SURFACE	DCC
<i>Salmonella</i> group B (non-motile)	A	5/2	SURFACE	XLD
Bethesda-Ballerup	A	5/2	SURFACE	XLD
Bethesda-Ballerup	A	5/2	SURFACE	XLD

*Salmonella* species, group B (non-motile). All seven of these isolates came from water at site A. *Providencia alcalifaciens* was isolated on DCC agar. The other isolates were isolated on XLD agar. The two *Salmonella thompson* cultures were recovered using the Moore swab recovery technique. The other pathogens were recovered using the surface sampling method. This data is shown in Table 4.

Thirteen isolations were made of bacteria that could be considered weakly or potentially pathogenic. These included five isolations of *Aeromonas hydrophilia*, one of *Aeromonas shigelloides*, four of *Enterobacter hafniae*, and three of *Enterobacter cloacae*. Five of these isolations were made with XLD agar, six were recovered from DCC agar and two were isolated with BG agar. Two of these isolates came from site A, seven were from site B, one from site C, one from site D, two from site E and two were isolated from site F. All of the above isolates were recovered using the surface recovery method.

#### DISCUSSION

Sites B, C, and D consistently showed higher EMB plate counts than did site A. This could have been, in part, due to multiplication of the coliform organisms in the river after leaving Des Moines sewage treatment plants. Kittrell and Furfari (1963) pointed out that coliforms increase in number until a maximum density is reached 10-15 hr after discharge into a stream. At the point of maximum density they stated that the total number of coliforms is four to eight times the number released into the stream. The total mean EMB counts for sites A and B were 270,000 and 910,000 organisms/100 ml respectively. It is also possible that the increase downriver from Des Moines could have been due to organisms being carried into the river by smaller streams emptying into the river below Des Moines.

The EMB plate counts from lake sites E and F were substantially lower than from the river sites A, B, C and D. The same pattern was shown by the turbidity readings. Streeter (1934) reported that the bacterial counts in the Ohio River below Cincinnati, Ohio, were significantly lower when the stream was high and flowing fast. He concluded that the bacteria were carried to the stream bottom by sediment as it settled out. In our studies, this explanation could be the reason the counts in Lake Red Rock were lower than the ones in the Des Moines River.

We found some correlation between water temperature and EMB counts at site A. In general, the counts were lower during the winter months when water temperatures were low. The primary exception to this was the count made on January 30th. The abnormally high count on this date was

probably due to runoff resulting when the snow of five inches melted between January 23rd and January 30th. The generally lower counts during the winter at site A were probably due to slower multiplication rates of the bacteria than the rates during the summer and autumn months.

The EMB counts from samples collected on the November 1st collecting date were also high. Runoff from 1.18 inches of rain on October 30th and 31st was probably the main reason for this. There is evidence to show that runoff causes higher coliform counts than when there is no runoff. Recently, the U.S. Public Health Service (unpublished data) conducted a study on the Missouri River below Kansas City to determine the effects of runoff on the coliform count of the river. They found that the average Most Probable Number of coliforms during a dry three day period was 110,000/100 ml compared to 302,000/100 ml for four days in which there was runoff from rain.

The U.S. Public Health Service has used a standard of 5000 organisms per 100 ml for drinking water sources requiring treatment. This has also been used as a standard for non-contact water sports, such as boating. According to the results of a survey of the states taken by the Public Health Activities Committee of the A. S. C. E. (1963), the most common standard for water contact sports, such as swimming, is a total coliform count of 1000/100 ml. They pointed out that some states have levels as low as 50/100 ml and some are as high as 2400/100 ml. One basis for these levels is the work done by Stevenson (1953). One of his objectives was to determine what differences in illness incidence might be expected from swimming in waters containing various degrees of bacterial pollution. In a Chicago study he made a comparison between swimmers using water with high coliform counts and those using water with low coliform counts. He reported swimmers that used waters averaging 2300 coliforms/100 ml had a significantly higher illness incidence than those swimmers that used water with an average coliform count of 43/100 ml. He pointed out that this evidence was not conclusive, but did provide a warning signal.

If one applies any of the standards mentioned above, the Des Moines River, between Des Moines, Iowa, and Lake Red Rock, is not suitable for either contact or non-contact water-sports. The EMB plate count method used in this study is not a standard method for determining total coliform counts because gram negative organisms which fail to ferment lactose will grow in this medium. However, it was estimated that 90 per cent of the organisms on these showed coliform colony characteristics. The counts obtained from sites A, B, C, and D were not close to any of the accepted standards. Recently, the same sites were examined by Messley (1971) by the currently accepted standard method of millipore filter technique and counting only typical coliform colonies. Her results were extremely close to ours for sites A and B during similar months.

Counts made from site F on Lake Red Rock show that the water was suitable for contact water sports two of the 10 sampling dates if the standard of 2400/100 ml is used. However, to get a more accurate idea of the suitability of the lake water for recreational use, a standard method of getting a total coliform count should be used.

The samples taken October 28th were obtained during a period in which the city of Des Moines was dumping sewage that had received only primary treatment. The EMB count for site A was 1.1 million organisms per 100 ml. This was the

highest colony count for any of the sampling dates at this site. This would indicate that secondary sewage treatment does result in a lower coliform count. However, the count at site B was only 27,000 organisms per ml. Only two counts (Nov. 30th and Jan. 3rd) were lower than this count. No pathogens were isolated from either site on this date.

The pH readings obtained from water samples taken from the Des Moines River and Red Rock Lake were within the limits given by Streeter (1949) for recreational water. The desirable range given by him was 5.8 to 9.0. The pH range in this study was 6.7 to 8.9.

All of the pathogenic organisms isolated in this study were found in water taken from site A even though this site showed lower mean coliform counts than sites B, C and D. According to traditional concepts about coliform counts, water showing the highest coliform counts should yield the highest number of animal pathogens. These concepts are being questioned by many because much of the coliform contamination in water sources results from causes other than fecal pollution. Geldreich (1967) pointed out that some strains of the coliform group have a wide distribution in the environment and are not necessarily of fecal origin. He noted that many of the coliforms found on vegetation and insects are not fecal coliforms. It is also true that a low number of coliforms does not necessarily mean the water is of high bacteriological quality. Gallagher and Spino (1967) isolated salmonellae from river water with total coliform counts of less than 1000/100 ml. Greenberg and Ongerth (1966) isolated *Salmonella typhimurium* from city wells in Riverside, California, that met all U. S. P. H. S. coliform standards for drinking water.

Three species of *Salmonella* were isolated during this investigation. These included *Salmonella thompson*, *Salmonella oranienburg* and a non-motile species in group B. The *Salmonella* group, in general, is responsible for a wide range of gastro-intestinal illnesses. The most pathogenic organism of the three species isolated is *Salmonella oranienburg*. Dubos (1958) pointed out that this organism may produce gastroenteritis, enteric fever or a septicemia.

*Providencia alcalifaciens* and two isolates of the group Bethesda-Ballerup were made during this study. According to Burrows (1968), members of the genus *Providencia* have been associated with institutional diarrhea and may be considered as possible pathogens. He notes that members of the Bethesda-Ballerup group are isolated with some frequency from patients with enteric disease and some strains are possible pathogens.

*Enterobacter* and *Aeromonas* species were isolated with regularity during the study. They should not be taken lightly when considering harmful aspects of polluted water. Hermann (personal communication) pointed out that *Enterobacter* strains are not uncommonly associated with urinary tract infections. Nygard, Bissett, and Wood (1970) believe that because of the number of aeromonads being submitted to their laboratories for identification and confirmation, infections caused by this group are more frequent than is currently recognized. Von Graevenitz and Mensch (1968) reported a case history of a 15 year-old boy that had received a scalp laceration while swimming in a pool. He was hospitalized because of infection with *Aeromonas hydrophila*. The infection spread and caused bilateral periorbital edema.

The use of the Moore swab resulted in the isolation of *Salmonella thompson* on September 21st. No pathogens were

recovered the second time the swab method was used. The primary value of using the swab is that it permits the detection of large numbers of pathogens that are released at one time from a source. All species of pathogens, with the exception of *Salmonella thompson*, were isolated from "grab" samples of surface water. The isolation of four species of pathogens using this method would indicate that a large number of pathogens were present in the water. The chance of picking up pathogens with a single "grab" sample would be small if there were only a few pathogens present.

All species of pathogens, with exception of *Providencia alcalifaciens*, were isolated from XLD agar. There are two reasons for this. The medium may have been more suitable for the growth of the pathogens than DCC or BG agar. It is also possible that pathogens growing on DCC and BG agar were not recognized as non-lactose fermenters. Brilliant green agar was not inhibitory enough to permit easy recognition of possible pathogens. DCC agar had large numbers of colonies from all sites that appeared to be non-lactose fermenters. However, when these were inoculated into triple sugar iron agar and incubated, it was found that most of them gave acid over acid reactions. Pathogens were easily distinguished on XLD agar. They appeared as a bright red color on this medium as compared to the yellow non-pathogen colonies. The H<sub>2</sub>S positive pathogens had glossy black colony centers on XLD agar. Xylose-lysine deoxycholate agar was the most efficient isolation medium used in this study for the recovery of pathogens.

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