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# Coliphages and Bacteria in Groundwater from Tehran, Iran

M. Shariatpanahi and A. C. Anderson

Tehran University, Tehran, Iran and Tulane University Medical Center, 1430 Tulane Avenue, New Orleans, Louisiana 70112

The purpose of this study was to examine the microbial quality of Tehran's groundwater and selected springs, using coliphages and selected bacteria as indicator organisms.

The water table in Tehran varies from approximately 160 meters in the north to approximately 5 meters in the south. Individual wells and subterranean man-made aqueducts (qanate) tap the groundwater.

Since Tehran lacks municipal sewage facilities, waste disposal is by means of seepage pits, privies and leaching cesspools. There is potential for waste from these sites to leach into the groundwater, particularly in the south where the water table is near the surface and the clay content of the soil holds moisture during periods of heavy rainfall.

Figure 1 illustrates the sampling regions ($R_1$ - $R_6$) in the study. Region 1 ($R_1$) consists of the affluent suburbs of the city at the foot of Mount Alborge. The water samples taken in this region are not part of the potable water supply of the neighborhood. Region 2 ($R_2$) is sparsely populated and consists of desert and areas planted in artificial forests. Region 3 ($R_3$) has deep wells (averaging 142 meters) and many are chlorinated. Only 2 wells were sampled in this region. In Region 4 ($R_4$), the southeast section of the city, the groundwater table is high and rainfall often results in localized flooding. Region 5 ($R_5$) is highly industrialized and was not sampled in this study. Region 6 ($R_6$) is a mix of farms, urban housing and industrial plants.

Send reprint requests to Dr. A.C. Anderson, Tulane University, School of Public Health and Tropical Medicine, Department of Environmental Health Sciences, 1501 Canal Street, New Orleans, Louisiana 70112

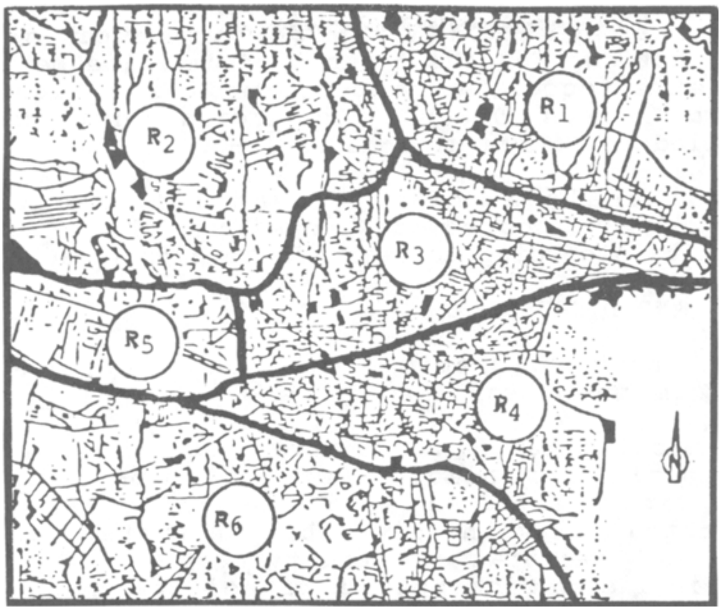


Figure 1. The City of Tehran Divided Into Six Sampling Regions.

MATERIALS AND METHODS

Water samples were collected from a total of 43 individual wells, springs and aqueducts in 2 liter sterile polyethylene containers according to Standard Methods (1980). Samples were refrigerated until analysis, and were run within 2 hours of sampling.

Pure stock cultures of *Escherichia coli*, *Citrobacter frundii* and *Enterobacter aerogenes* were isolated and identified from a raw water sample according to Standard Methods (1980).

Phage analyses consisted of an initial enrichment step. Fifty, ten and five ml water samples were distributed, respectively, to flasks containing 50 ml sterile peptone media (60 g/l peptone, 6 g/l NaCl, pH 7.5), and the appropriate bacterial pure culture. Flasks were then held at 56°C for 30 minutes.

Nutrient agar plates were inoculated with 24 hour broth cultures of each host microbe. After allowing the plates to dry, several drops of the enrichment cultures were superimposed on the respective host cell plates using Pasteur pipettes. Plates were incubated at 37°C. Following 6-8 hours incubation, plates were examined for plaques, indicating the presence of the respective phage (Harrigan and McCance, 1976).

Standard plate counts, the 5 tube MPN method for coliforms, the IMViC procedure for species identification, and the 5 tube sodium azide method for fecal streptococci were performed as indicated in Standard Methods (1980). Clostridium perfringens was identified in litmus milk media (WHO, 1963).

Chemical analyses of water samples included pH, conductivity, total dry residue, hardness and alkalinity according to Standard Methods (1980).

RESULTS AND DISCUSSION

Table 1 shows the chemical analyses of water samples taken from the 43 sampling sites.

The chemical parameters indicate that Tehran's groundwater is slightly acidic, with a mean pH of $6.0 \pm 0.2$. Acidic waters are corrosive and tend to accumulate trace metals from the distribution systems and from utensils. The total alkalinity ($\bar{x} = 215 \pm 38$ mg/l as $CaCO_3$) is due to bicarbonate. Specific conductivity, indicative of the total concentration of dissolved ions, was $894 \pm 388$ u mhos/cm. Total dried residue,

Table 1. Chemical Analyses of Water Sample

| Parameter | Range | Mean $\pm$ Standard Deviation of 43 samples |
|---|---|---|
| pH | 5.5-7.0 | $6.0 \pm 0.2$ |
| Conductivity umhos/cm | 229-2550 | $894 \pm 388$ |
| Total Dry Residue (at 180°C), mg/l | 203-1821 | $630 \pm 342$ |
| Total Hardness (as $CaCO_3$), mg/l | 64-557 | $220 \pm 108$ |
| Total Alkalinity (as $CaCO_3$), mg/l | 68-450 | $215 \pm 38$ |

refering to solid matter suspended or dissolved in the water, ranged from 203 to 1821 mg/l. Highly mineralized water, as indicated by the last two parameters, is considered undesirable since it causes transient gastrointestinal distress, especially in consumers unaccustomed to the water. Additionally, waters with high mineral content are unsuitable for many industrial applications. Tehran's groundwater is hard, since total hardness values exceed 75 mg/l as $CaCO_3$. Many studies indicate an inverse correlation between incidents of cardiovascular disease and the amount of hardness (NAS, 1979).

Table 2 presents the results of the bacteria and coliphage analyses. The depth of the water table and well depth are noted. Samples drawn from springs and aqueducts are also indicated.

Of the 43 sites tested, only 3 were totally indicator free. Ten sites were positive for heterotrophs only as monitored by plate counts. The remaining 30 sites were positive for one or more additional indicators, particularly coliforms (26 sites). Regarding coliphages, 11, 3 and 16 sites were positive for the phages of E. coli, C. frundii and E. aerogenes respectively. Twenty of the 26 sites were positive for both coliforms and coliphages.

All regions tested showed microbial/coliphage contamination of the water supplies tested. Only three sites in an industrial area of $R_6$ were totally indicator free. All springs and aqueducts were heavily contaminated, with the exception of one spring in $R_1$ which is in a sparsely populated area near the foot of Mount Alborze.

Generally, the number of bacteria and phage positive sites increased from north to south and roughly parallels the movement of the groundwater toward the surface as it flows south toward Region 4.

In addition, southern sites are more easily contaminated due to breakthrough of sewage from seepage pits, privies and leaching cesspools to the groundwater. Well contamination may also be due to infiltration of microbes through defective well casings (Keswick and Gerba, 1980) Further, in the south of Tehran Plain, the clay content of the soil increases dramatically, which allows the soil to retain moisture which may favor the survival of microorganisms (Young and Greenfield, 1923).

In recent years, coliphages (initially, bacteriophage infecting Escherichia coli) have been proposed as indicators of fecal pollution. Several convincing arguments have been propounded in support of coliphage indicators:

They occur in significant numbers in water and wastewater with mean concentrations on the order of 5 X $10^5$ plaque forming units (PFU)/100 ml. Their numbers in wastewater exceed animal virus counts. Coliphage appear relative resistant to water and wastewater treatment processes as expressed by coliform:coliphage Table 2.

Table 2. Bacteria and Phage Analyses of Tehran Groundwater

| Sample No. | Well Depth | Water Table | Bacteriaphage E.coli | Bacteriaphage E.frundii | Bacteriaphage E.aerogenes | Plate Count 24 hrs. at 37oC | Plate Count 72 hrs. at 22oC | MPN | Confirmed Test | Completed | IMViC | Cl.perfringens | S.faecalis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | | | | | | | | | | | | | |
| 1 | x | | + | - | - | 8 | 94 | 74 | + | + | E.c.,S. | - | + |
| 2 | * | | + | - | - | 1750 | TNTC | 1609 | + | + | E.c.,S. | - | + |
| 3 | * | | + | - | - | 5 | 43 | 345 | + | + | E.c.,S. | + | + |
| 4 | 36 | 8 | + | - | - | 4 | 40 | - | - | - | - | - | - |
| 5 | 100 | 60 | - | - | - | 16 | 8 | - | - | - | - | - | - |
| 6 | x | | - | - | - | 2 | 34 | - | - | - | - | - | - |
| 7 | 80 | 35 | - | - | - | 2 | 24 | - | - | - | - | - | - |
| 8 | 75 | 40 | - | - | - | 53 | 16 | - | - | - | - | - | - |
| R2 | | | | | | | | | | | | | |
| 9 | 160 | 100 | - | - | - | 7 | 6 | - | - | - | - | - | - |
| 10 | * | | - | - | + | 288 | 528 | 8 | + | + | C.d. | - | - |
| 11 | 40 | 28 | - | - | + | 5 | 189 | 11 | + | + | C.f. | - | - |
| 12 | * | | - | + | + | 6 | 52 | 8 | + | + | K-E,C.d. | - | - |
| 13 | 75 | 40 | - | - | - | 6 | 12 | - | - | - | - | - | - |
| 14 | * | | + | - | + | 16 | 27 | 17 | + | + | E.c. | - | - |
| 15 | * | | - | + | - | 6 | 20 | 5 | + | + | E.f. | - | - |
| 16 | * | | + | - | + | 63 | 1080 | 34 | + | + | E.c.,K-E | + | + |

Note: x Spring
* Aqueduct
\- Not detected

E.c. - *E.coli*
c.f. - *Citrobacter frundii*
C.d. - *Citrobacter diversus*

S - *Shigella*
K-E - *Enterobacter* group

Table 2, cont. Bacteria and Phage Analyses of Tehran Groundwater

| Sample No. | Well Depth | Water Table | Bacteriaphage E.coli | Bacteriaphage E.frundii | Bacteriaphage E.aerogenes | Plate Count 24 hrs. at 37oC | Plate Count 72 hrs. at 22oC | MPN | Confirmed Test | Completed | IMViC | Cl.perfringens | S.faecalis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R3 | | | | | | | | | | | | | |
| 17 | 220 | 80 | - | - | - | 2 | 10 | - | - | - | - | + | + |
| 18 | 200 | 75 | - | - | - | 7 | 2 | 141 | + | + | K-E | - | - |
| R4 | | | | | | | | | | | | | |
| 19 | 110 | 60 | + | + | - | 5 | 27 | 4 | + | - | E.c.,C.f.<br>K-E | - | + |
| 20 | 120 | 70 | - | - | + | 16 | Uncount able | 23 | + | + | K-E | - | + |
| 21 | 75 | 30 | - | - | - | 6 | 26 | 49 | + | + | K-E | + | + |
| 22 | 75 | 35 | - | - | - | - | 2 | - | - | - | - | - | - |
| 23 | 80 | 40 | - | - | - | - | 16 | 5 | + | + | K-E | - | + |
| 24 | 55 | 25 | - | - | + | - | 16 | 5 | + | + | K-E | - | + |
| 25 | 80 | 40 | + | - | - | - | 4 | 13 | + | + | E.c. | - | - |
| 26 | 75 | 35 | + | - | + | 8 | 140 | 8 | + | + | E.c. | - | - |
| 27 | 110 | 50 | - | - | - | 8 | 130 | 5 | + | + | K-E | - | + |
| 28 | 100 | 50 | - | - | - | 9 | 27 | - | - | - | - | - | - |
| 29 | 16 | 10 | + | - | + | 88 | 155 | 130 | + | + | E.c.,K-E | + | + |

Note: x Spring
* Aqueduct
\- Not detected

E.c. - E.coli
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Table 2, cont. Bacteria and Phage Analyses of Tehran Groundwater

| Sample No. | Well Depth | Water Table | Bacteriaphage E.coli | Bacteriaphage E.frundii | Bacteriaphage E.aerogenes | Plate Count 24 hrs. at 37oC | Plate Count 72 hrs. at 22oC | MPN | Confirmed Test | Completed | IMViC | Cl.per fringens | S.fae calis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R6 | | | | | | 69 | 95 | 11 | + | + | K-E | - | - |
| 30 | 100 | 55 | - | - | + | - | 200 | 11 | + | + | K-E | - | - |
| 31 | 160 | 80 | - | - | + | - | 20 | 8 | + | + | K-E | - | - |
| 32 | 150 | 60 | - | - | - | - | 116 | 7 | + | + | K-E | - | |
| 33 | 200 | 80 | - | - | - | - | 5 | - | - | - | - | - | - |
| 34 | 150 | 60 | - | - | - | - | 220 | 22 | + | + | K-E | - | - |
| 35 | 142 | 65 | - | - | + | - | - | - | - | - | - | - | - |
| 36 | 100 | 50 | - | - | - | - | - | - | - | - | - | - | - |
| 37 | 80 | 45 | - | - | - | - | - | - | - | - | - | - | - |
| 38 | 85 | 50 | - | - | - | - | 8 | - | - | - | - | - | - |
| 39 | 100 | 60 | - | - | - | - | 64 | 2 | + | + | S | - | - |
| 40 | 120 | 60 | - | - | + | - | 460 | - | - | - | - | - | - |
| 41 | 120 | 65 | - | - | - | - | 31 | - | - | - | - | - | - |
| 42 | 120 | 60 | - | - | - | 680 | Uncount able | 896 | + | + | S | + | + |
| 43 | * | | + | - | + | | | | | | K-E | | |

Note: x Spring
* Aqueduct
\- Not detected

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ratios of 360 for raw waste and 10 for treated effluents. Similarly, coliphage: virus ratios are on the order of $10^3$ to $10^4$ in both trickling filters and oxidation ponds, thus offering an option for monitoring virus removal in wastewater treatment plants. The coliphage assay is rapid, accurate and inexpensive, and offers an opportunity to indicate both bacterial and viral contamination. (Bitton, 1980)

As with all indicators, coliphages have drawbacks. A major concern is the fate of coliphages in the environment, particularly their ability to multiply in receiving waters. Nevertheless, coliphages are effective measures of the efficiency of water treatment processes, as well as processing, disinfection and land disposal of wastewaters. (Bitton, 1980). In this study coliphages proved to be a reasonable indicator of pollution; parelleling, for the most part, coliform levels. In addition, coliphages are secondary indicators of the potential presence of pathogens, bacteria, and viruses (Bitton, 1980).

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