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ORIGINAL RESEARCH

Microbiological and Physicochemical Quality of Well Water Used as a Source of Public Supply

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Abstract: The sources of microbial and chemical contamination of groundwater are numerous and have severe implications for public health. The objective was to determine the microbiological and physicochemical quality of wells in the west and east of the Cuautla-Yautepec aquifer in Mexico. Wells showed bacteriological contamination in at least one sampling. Coliform values were lower than the maximum permissible limit indicated in the Mexican Ecological Criteria of Water Quality (1000 colony forming unit (CFU)/100 ml of fecal coliforms) for supply sources. The number of isolated amoebae was low, but these were present all year round. Amoebae were found in 71.7% of the samples and belonged to 13 genera. The most frequent amoeba, *Hartmannella,* occurring in 44% of the samples, has been associated with eye and brain infection, but its role as a cause of infection has not been confirmed. A gradient was observed for dissolved solids according to altitude; the concentrations of dissolved solids increased in wells with lower altitudes. Total hardness values were above 180 mg/L $CaCO_3$, therefore the water is considered very hard, and both carbonate and non-carbonate hardness was detected. The average values of physicochemical parameters were below the maximum permissible limits indicated in the Mexican official norm.

Keywords: well water, groundwater, water quality, coliforms, free-living amoebae, physicochemical parameters

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Introduction

Natural groundwater is usually of good quality, but this can deteriorate due to inadequate source protection and poor resource management.[1] Mechanisms of groundwater recharge and the natural attenuation capacity, also depend on soil type and geomorphologic characteristics.[2,3]

Microbial and chemical contaminants have been detected in groundwater. The sources of contamination are numerous and include the land disposal of sewage effluents, sludge and solid waste, septic tank effluent, urban runoff, and agricultural, mining and industrial practices.[4–13]

The use of untreated or inadequately treated groundwater has been responsible for waterborne diseases including gastroenteritis, cholera, hepatitis, typhoid fever and giardiasis; the causative agents are bacterial and viral pathogens as well as protozoan parasites. In contrast to chemical hazards that may pollute groundwater, resulting in a long-range influence on public health in terms of time, microbiological pollution of groundwater sources has an immediate effect on large numbers of people.[1,6–9]

Chemical pollutants can cause a different type of intoxication. The effects of inorganic chemicals are better known than those of trace levels of organic chemicals detected in groundwater. The list of groundwater contaminants includes hydrocarbons, metals, cyanide, arsenic, various synthetic substances, soluble forms of nitrogen and phosphorus, and organic matter.[13–15]

Nitrate is one of the most common groundwater contaminants in rural areas; it can enter the system from a variety of natural and anthropogenic sources (mainly fertilizer usage).[13] Nitrate is regulated in drinking water because high levels may cause serious illness and sometimes death, and it also has the potential to cause shortness of breath, methemoglobinemia or "blue baby" disease, an increase in starchy deposits and hemorrhaging at the spleen.[5,14,15]

Dissolved organic matter (often referred to as dissolved organic carbon or DOC) can impart an undesirable taste and/or odor to water. High values of organic matter, methylene blue active substances (MBAS) and ammonia nitrogen comes from wastewater discharges, therefore their presence in drinking water is undesirable.[14,15]

There does not appear to be any convincing evidence that water hardness causes adverse health effects in humans. However, some studies have shown a weak inverse relationship between water hardness and cardiovascular disease in men, up to a level of 170 mg calcium carbonate ($CaCO_3$) per liter of water.[16–19] Hard water does not lather well with soap and therefore requires a higher consumption of soap; in industry, water hardness produces fouled hot water systems and must be constantly monitored to avoid costly breakdowns in boilers, cooling towers and any equipment that comes in contact with water.[14]

Chloride, which is responsible for the salty taste in water, is an indicator of water contamination caused by the residual chloride content of urine. Very acidic or very alkaline water is undesirable because it is corrosive or is difficult to treat. The health effects of pesticides depend on the type of pesticide. Some affect the nervous system, some may be carcinogens, and others may irritate the skin or eyes.[14,15]

Contamination of groundwater has severe implications for public health, particularly in small communities and developing countries where groundwater is often the preferred source of drinking water.[6] In Mexico, although nearly 50% of the water used for domestic, industrial and agricultural activities comes from groundwater sources, in many cases, little is known about its geology, the volumes of water available and its quality.[20] In addition, the overexploitation of aquifers and reduced natural recharge due to high urbanization and anthropogenic activity have caused a decrease in groundwater quality in many areas.[21] The decrease in microbiological and chemical quality has been reported in several aquifers around the country.[22–29] The objective of this research was to determine the microbiological and physicochemical quality of well water in the Cuautla-Yautepec aquifer in Morelos, Mexico.

Materials and Methods

Study area and sampling procedure

The Cuautla-Yautepec aquifer is one of the four aquifers in the state of Morelos; it is located in the portion center north of the state, south of Mexico City (Fig. 1), between longitudes 98° 42' 46"W and 99° 08' 13"W, and latitudes 18° 56' 31"N and 19° 04' 48"N. It has an area of 2.231 km^2, 1.451 km^2 of

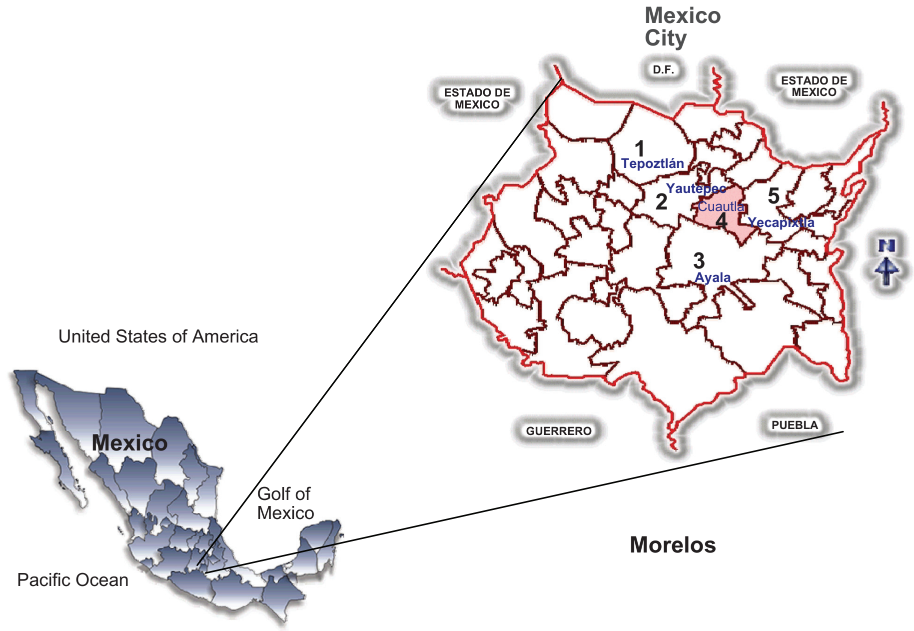

Figure 1. Location of study area and sampling wells in Morelos, Mexico.

which are the catchment (recharge) area, with slopes that vary from steep to moderate, and 780.07 km^2 of which is the aquifer area. The prevailing weather is warm and sub-humid with rains in summer, an annual average temperature of 20.0 °C and an annual average precipitation of 1003 mm.

Study area is located on the southern side of the mountains Sierra de Chichinautzin (3690 m) and Sierra Nevada (volcano Popocatépetl, 5452 m), made up of volcanic materials (alternating basalt and tezontle), which gives it very distinctive geomorphologic features. The recharge area corresponds to the high areas or mountainous areas, while the aquifer area corresponds to the lower parts or valleys. The water passes from the upper to the lower parts, ie, from the Nevada Sierra to the southern portions of the Cuautla and Yautepec ravines. The aquifer is located in two units of rock: the first unit is made up of fractured basalt igneous rocks of Chichinautzin formation, presenting high permeability and irregular distribution. The second unit contains sedimentary volcanic rocks of the so-called alluvial clastic material, which show a medium permeability and an irregular distribution; these cover the lower parts of the Cuautla and Yautepec valleys. In the valleys, the groundwater circulates in granular materials, giving rise to the aquifer.

Productive activities that predominate in the region are processing industries, agriculture (mainly cultivation of rice and sugar cane) and livestock. Tourism deserves special attention in this context, since it has grown over recent decades because of the pleasant climate, the beautiful landscapes, the presence of numerous springs that have led to several spas being established in the zone, and the proximity of the State of Morelos to Mexico City. Groundwater is the main source of water in the Cuautla-Yautepec Valley, supplying 70%–80% of the population. The water quality deteriorated, mainly by the use of fertilizers, septic tank effluent, and disposal of sewage effluent, and solid waste.[30]

Five wells in the west and east of the aquifer were sampled (Fig. 1). The wells' altitudes are in the range of 1227 to 1643 m above sea level (a.s.l). The wells have a depth of 150 m, except Well 5, which is 220 m deep. Wells 1 and 2 were in urban areas with tourism and small commerce activities, Well 1 near a cemetery; Wells 3, 4, 5 were near areas with agricultural activity throughout the year. Monthly sampling was carried out during one year. Twelve samples per sampling point were collected to carry out each type of analysis. The samples were taken before the chlorine dispenser to determine the natural conditions of the aquifer.

Laboratory analysis

The microbiological parameters analyzed were: total and fecal coliforms and free-living amoebae. Total and fecal coliforms were analyzed by the membrane filter technique according to standard methods for the examination of water and wastewater.[31]

Free-living amoeba samples were concentrated by filtration through 1.2-µm membranes, and were then placed face down on non-nutritive agar with *Enterobacter aerogenes* (NNE). The plates were incubated at 30 °C and were observed daily under an inverted microscope in order to detect amoeba growth. Amoebae were identified on the basis of their morphological characteristics, their growth rates at different temperatures and their capacity to produce flagella.[32,33]

The physicochemical parameters were: pH, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammonia nitrogen, total alkalinity, phenolphthalein alkalinity, total hardness, calcium hardness, chlorides, sulfates, dissolved solids, nitrates, nitrites, MBAS and turbidity, according to standard methods for the examination of water and wastewater.[31] Statistical methods (ANOVA and correlation coefficient) were performed in order to compare the concentration of the bacteriological and physicochemical parameters in the wells.

Results and Discussion

Well 1 had the highest concentration of total and fecal coliforms, while Well 4 had the lowest. Wells 2, 3 and 5 had low concentrations, and coliforms were absent in some samplings (Table 1). Differences among wells may be due to the medium and high vulnerability of the soil materials in some zones and inadequate construction or deficient protection of the wells. The high bacterial contamination presented in Well 1 may be due partly due to its location near a cemetery in an area where there is no drainage system but uses septic tanks to dispose of their waste. Moreover, this well is in the aquifer zone that is set on basalt, which has a high degree of permeability product of intense fracturing and volcanic scoria (volcanic rock), which allows the infiltration of water.[30]

ANOVA test showed significant differences ($P < 0.05$) for the total and fecal coliforms (Table 2), and the correlation coefficient showed significant differences ($P < 0.05$) among the wells.

Fecal coliform values were lower than the maximum permissible limit indicated in the Mexican Ecological Criteria of Water Quality (1000 colony forming unit (CFU)/100 ml of fecal coliforms) for sources of public supply.[34] If we consider that the water is chlorinated after its extraction, it meets the Mexican regulations for drinking water, which indicates that total and fecal coliforms must be absent.[35]

The number of isolated amoebae was low, but they were present all the year, showing two peaks, one in December–March and another in August–September (Fig. 2). As far as the number of isolated

Table 1. Well concentration of total and fecal coliforms.

Month	Well 1		Well 2		Well 3		Well 4		Well 5	
	TC	FC	TC	FC	TC	FC	TC	FC	TC	FC
March	133	25	0	0	–	–	0	0	0	0
April	115	55	0	0	0	0	200	120	–	–
May	200	150	0	0	18	6	1	0	1	0
June	400	40	90	0	2	0	0	0	24	0
July	200	200	0	0	66	8	1	1	1	1
August	103	39	1	0	38	15	0	0	2	0
September	200	200	60	55	7	7	0	0	0	0
October	125	118	10	10	200	200	1	1	200	200
November	200	160	65	25	10	10	5	5	60	44
December	40	26	16	10	10	6	15	4	1	1
January	50	25	0	0	0	0	0	0	–	–
February	50	25	26	16	5	0	4	2	2	1
Geometric mean	123	63	2.2	0.88	6.3	2.2	0.95	0.64	2.54	0.78

Note: –, no value recorded.
Abbreviations: TC, total coliform; FC, fecal coliform.

Table 2. Results of ANOVA test of microbiological and physicochemical parameters.

Parameter	Fo	Ft	Probability
FLA	3.138	2.546	0.0217283
Total coliforms	8.70	2.549	1.869E-05
Fecal coliforms	4.64	2.549	0.0027
Dissolved solids	73.1	2.553	1.721E-20
Total hardness	80.3	2.549	1.29E-21
Total alkalinity	61.8	2.548	3.89E-19
Chloride	55.9	2.549	3.168E-18
Sulfates	37.01	2.549	1.238E-14
Turbidity	3.89	2.549	0.0077
pH	41.7	2.549	1.184E-15

Abbreviation: FLA, free-living amoebae.

amoebae, three wells were in the range of 19 to 24 isolates, and two were in a lower range of 7 to 8 isolates (Fig. 3).

Amoebae were found in 43 (71.7%) of the 60 analyzed samples. They belonged to the genera *Cochliopodium, Echinamoeba, Filamoeba, Guttulinopsis, Hartmannella, Mayorella, Naegleria, Rosculus, Thecamoeba, Platyamoeba, Vahlkampfia, Vannella,* and *Vexillifera*. The most frequent amoeba was *Hartmannella*, occurring in 44% (Fig. 4). The amoebae found are free-living and play an important role in the natural biological process by feeding on bacteria and helping nutrient recirculation. However, *Hartmannella, Vahlkampfia* and *Vannella* have been isolated from corneal tissue and contact lenses of keratitis patients, and *Hartmannella* from the cerebrospinal fluid of a patient with meningoencephalitis and bronchopneumonia. In none of these cases, however, was the role of the amoebae confirmed as a causative agent of the diseases.[36–43]

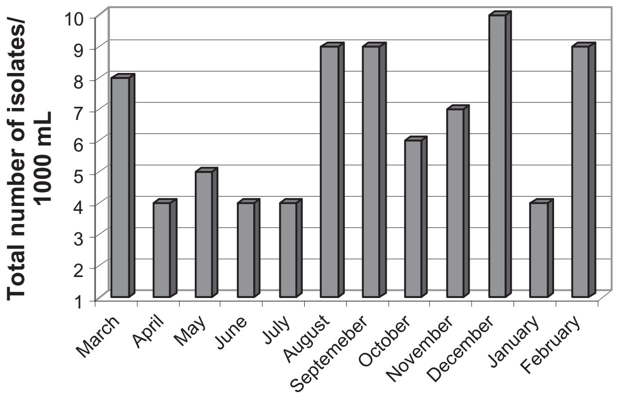

Figure 2. Seasonal variation of free-living amoebae.

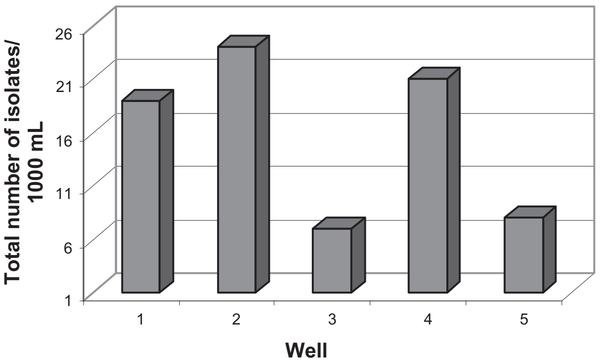

Figure 3. Spatial variation of free-living amoebae.

Biochemical oxygen demand, COD, ammonia nitrogen and MBAS were below the detection limit of the techniques, suggesting that organic matter is absent or is present in very low amounts in the water.[14,44]

ANOVA showed significant differences ($P < 0.05$) for physicochemical parameters (Table 2) and the correlation coefficient showed significant differences ($P < 0.05$) among the wells.

A gradient of dissolved solids was observed according to altitude, where the concentrations of dissolved solids increased in wells with lower altitudes, due to the groundwater dissolving soil salts as it flows downwards: Well 3 (1227 m a.s.l) > Well 2 (1261 m a.s.l) > Well 4 (1369 m a.s.l) > Well 1(1643 m a.s.l). Well 5 (1564 m a.s.l), however, did not fit in the gradient; it had a lower concentration than Well 1, probably because Well 5 (220 m deep) is deeper than Well 1 (150 m deep).

According to the correlation coefficient, Wells 3 and 4 showed a very similar seasonal pattern of dissolved solids (0.84); while the others showed significant differences.

The well water can be considered very hard: total hardness values were above 180 mg/L $CaCO_3$ (ASTM, 1976, cited by Robles et al 2004).[14] Carbonate and non-carbonate hardness was detected (Table 3). Carbonate hardness can be precipitated by prolonged boiling but non-carbonate removal is more difficult; consequently, it is known as "permanent hardness". It is usually caused by the presence of calcium and magnesium sulfates, chlorides and/or nitrates in the water.[14,44] Alkalinity was also due to bicarbonates.

The average values of the physicochemical parameters were below the maximum permissible

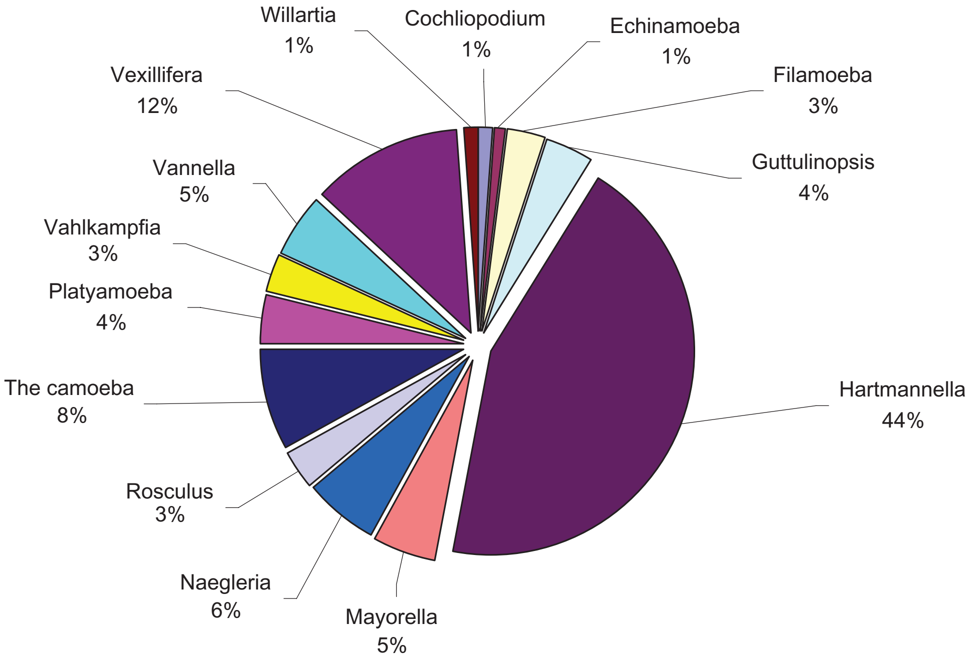

Figure 4. Frequency of free-living amoebae isolated from well water.

limits indicated in the Mexican official norm (NOM-127-SSA1-1994)[35] for drinking water, with the exception of the pH of Well 1, which was slightly lower (6.3) (Table 4).

Conclusions

The differences found among wells were created by the direction of water flow (from the upper to the lower parts), which is determined by the geomorphologic features. However, in some cases, this behavior was modified by special characteristics of the wells: the depth in the case of Well 5, the environment where the well was located in the case of Well 1 (near a cemetery) and the rock permeability where the well is set, also in the case of Well 1, which is located in an area of fractured basalt igneous rocks.

Coliform concentrations were below than the maximum permissible limit and the physicochemical parameters fully meet the sanitary requirements; therefore, the well water is suitable to be used as source of public supply.

The presence of free-living amoebae in the wells is not a risk to health, since the pathogenicity of the amoebae found in our samples has not been confirmed. However, it is suggested as precautionary measure that contact lens users should not use this water for washing lenses, due to the amoebae having been detected in eye infections.

The occurrence of total and fecal coliforms in some samples is an indication that contamination is beginning to reach the aquifer. For this reason, we recommended avoiding discharges of wastewater without treatment, mainly from septic tanks, which are extensively used in the area.

Table 3. Well average concentration of hardness and alkalinity.

Well	Total alkalinity	Total hardness	Carbonated hardness	Non-carbonated hardness
1	111	211	111	100
2	134	300	134	166
3	279	468	279	189
4	276	380	276	104
5	112	247	112	135

Note: Data in mg/L as $CaCO_3$.

Table 4. Comparison of the well average concentration of physicochemical parameters with the Mexican norm for drinking water.[35]

Parameter	Well					Maximum permissible limits
	1	2	3	4	5	
pH	6.3 ± 0.16	6.6 ± 0.12	7 ± 0.358	7.3 ± 0.09	6.7 ± 0.19	6.5–8.5
*Total hardness	211 ± 20.2	300 ± 60.8	380 ± 23.6	247 ± 37.9	111 ± 17.8	500
Nitrites (mg/L)	bdl	bdl	bdl	bdl	bdl	1.0
Nitrates (mg/L)	3.4 ± 1.16	2.6 ± 0.691	2.6 ± 0.624	1 ± 0.166	0.95 ± 0.272	10
Ammonia nitrogen (mg/L)	bdl	bdl	bdl	bdl	bdl	0.5
Chloride (mg/L)	30.5 ± 8.3	11.7 ± 4.1	16.2 ± 1.9	6.1 ± 2.7	4.8 ± 3.1	250
Sulfates (mg/L)	36.4 ± 10.8	139 ± 52.4	111 ± 29.2	60.3 ± 19.4	8.9 ± 7.4	400
MBAS (mg/L)	bdl	bdl	bdl	bdl	bdl	0.5
Dissolved solids(mg/L)	381 ± 49.1	447 ± 74.8	535 ± 29.9	400 ± 39	184 ± 23.4	1000
Turbidity (NTU)	0.52 ± 0.29	0.25 ± 0.2	0.38 ± 0.2	0.27 ± 0.15	0.23 ± 0.11	5
Chemical oxygen demand (mg/L)	bdl	bdl	bdl	bdl	bdl	
Biochemical oxygen demand (mg/L)	bdl	bdl	bdl	bdl	bdl	

Note: *, Data in mg/L as $CaCO_3$.
Abbreviations: NTU, Nephelometric turbidity units; bdl, below the detection limit of the technique.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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