



Physicochemical and Microbiological Study of Thermal Springs Used for Recreation

¹Esperanza Robles V., Elizabeth Ramirez F., Reynaldo Ayala P., Blanca Martínez R., María de Guadalupe Sáinz M. y Ángel Durán D.

Environmental Conservation and Improvement Project. FES Iztacala, National Autonomous University of Mexico. Avenida de los Barrios 1, Los Reyes Iztacala, Tlalneantla, CP 54090, Mexico State, Mexico

¹Corresponding Author: erobles@unam.mx

Abstract:

Four thermal springs were sampled bimonthly for one year to determine their physicochemical and microbiological characteristics. Eleven physicochemical parameters, total coliforms, fecal coliforms and free-living amoeba were determined. In Mexico there is few works related with the quality of water from thermal springs, so this work helps to know the quality and some characteristics of this type of waters. The springs were classified by temperature as hypothermal and by the sulfates in the water as sulfate waters. From the dissolved salts content we determined that three were mineral waters and one was medium mineral water. The springs were hard water and carbonated or non-carbonated, predominantly the latter. Based on Ecological Water Quality Criteria, two springs exceed the recommended limit of 200 MPN/100 ml fecal coliforms in freshwater or seawater for recreational use with direct contact. Of the total, 83% of the samples tested positive for free-living amoebae and 7 genera were isolated; of these *Naegleria* was present in all the sampled sites. In general, the springs presented higher microbiological contamination in the pools than in the springs themselves due to the presence of bathers. The detection of total and fecal coliforms and the presence of thermophilic amoebae of the genera *Acanthamoeba* and *Naegleria* in the springs represent a health risk for users.

Keywords: Thermal waters, water quality, total and fecal coliforms, free-living amoebae.

1.0 Introduction:

Springs appear where water from underground flows to the surface. They are considered good quality natural water because before emerging at the ground surface the water has traveled through several kilometers of rock, sediment and soil which act as natural filters to remove all kinds of contaminants and, in many cases, enrich it with minerals and substances needed by humankind (IMTA, 2008). Springs are therefore widely used for bathing and curative purposes (Sukthana *et al.*, 2005). They are classified in different ways according to their physicochemical characteristics, such as temperature and salinity, among others. Springs with a temperature between 35° and 45°C are mesothermal; those with higher temperatures are hyperthermal and lower temperatures, hypothermal. Mineral waters (those with more than 1 g/L dissolved salts) may be sulfate, chloride or carbonated, among others types, depending on the predominant ion (Barrientos *et al.*, 2000). Spring water can be contaminated when it reaches the ground surface. Microorganisms that contaminate this kind of water come from the

environment and from the skin, mucus and clothes of bathers. The majority of these microorganisms are not pathogens, but some can cause infections in humans. There is epidemiological evidence to indicate that bathing or swimming in contaminated waters poses a potential health risk. Infections transmitted by swimming pool and spring water are probably the result of inadequate cleaning and/or disinfection (Vesaluoma *et al.*, 1995; Zbikowska *et al.*, 2013).

Given the difficulty in determining the presence of all the pathogenic microorganisms implicated in water contamination processes, and the need for a quick and reliable evaluation of the presence of pathogens, it has been suggested that certain groups of indicators be analyzed, such as total and fecal coliforms (Arcos-Pulido *et al.*, 2005). However, these indicator groups only represent the risk from pathogenic bacteria originating from fecal matter and so it is necessary to take into account microorganisms that represent other types of infection, like free-living amoebae, which can cause brain, eye and skin infections and can be

transmitted during swimming or by contact with naturally hot or artificially heated spring water that is contaminated with these protozoa (Visvesvara *et al.*, 2007).

In addition to recreational purposes, springs are also used to supply water for human consumption. However, the sources of contamination in surface water also affect groundwater and become a danger to health for users especially when they are used as a source of drinking water and/or for recreation. Such is the case in the work of Romeu-Álvarez *et al.*, who in their 2012 study concluded that the microbiological quality of the river Luyanó, which receives untreated urban and industrial wastewater, is unsuitable for recreation and agricultural irrigation. In 2010, Romero *et al.* studied 15 sites along the river Hardy finding that in the period of recreational activity only 5 sites met the standard while in the period from August to November all the sites were contaminated.

There are few works on the quality of spring water; among these is that of Cortés *et al.* (1989) who studied the isotopic and hydrochemical variation of 38 springs in the Valley of Mexico. In 2000, Barrientos *et al.* conducted a hydrochemical study of two springs in Venezuela. In the study conducted by Granel and Gales (2002), the authors concluded that population developments had affected the water quality, although the water quality index was acceptable for recreational use. González *et al.*, (2006) found that the water quality of 14 springs was acceptable even as drinking water with prior disinfection. Guimarães *et al.*

(2010) studied the water quality of 22 wells and 6 springs finding total coliforms in 93% and fecal coliforms in 82% of the study sites. Some studies have been conducted on free-living amoebae in hot springs in different countries, among them Mexico (Rivera *et al.*, 1989; Vesaluoma *et al.*, 1995; Sheehan *et al.*, 2003; Sukthana *et al.*, 2005; Gianinazzi *et al.*, 2010; Badirzadeh *et al.*, 2011; Kao *et al.*, 2012; Nazar *et al.*, 2012; Solgi *et al.*, 2012; Zbikowska *et al.*, 2013).

Due to the importance of the water quality of thermal springs with primary contact recreational use and the few works performed in Mexico, the aim of this study was to determine the physicochemical and microbiological characteristics of four thermal springs located in the state of Morelos, Mexico.

2.0 Materials and Methods:

2.1 Sampling Site:

Samples were drawn from 4 spring resorts located in the state of Morelos: Atotonilco thermal baths in the municipality of Tepalcingo. (A) ($18^{\circ} 38' 42.95''$ N; $98^{\circ} 49' 53.85''$ W; elevation 1235 m), Agua Hedionda in Cuautla (B) ($18^{\circ} 48' 30.91''$ N; $98^{\circ} 55' 26.31''$ W; elevation 1362 m), Las Huertas Xicatlacotla, Tlaquiltenango (C) ($18^{\circ} 27' 49.69''$ N; $99^{\circ} 09' 12.12''$ W; elevation 958 m), and San Juan II in San Gabriel las Palmas (D) ($18^{\circ} 36' 19.13''$ N; $99^{\circ} 20' 48.85''$ W; elevation 1138 m). All the springs are thermal water and used to supply water to swimming pools (Figure 1).

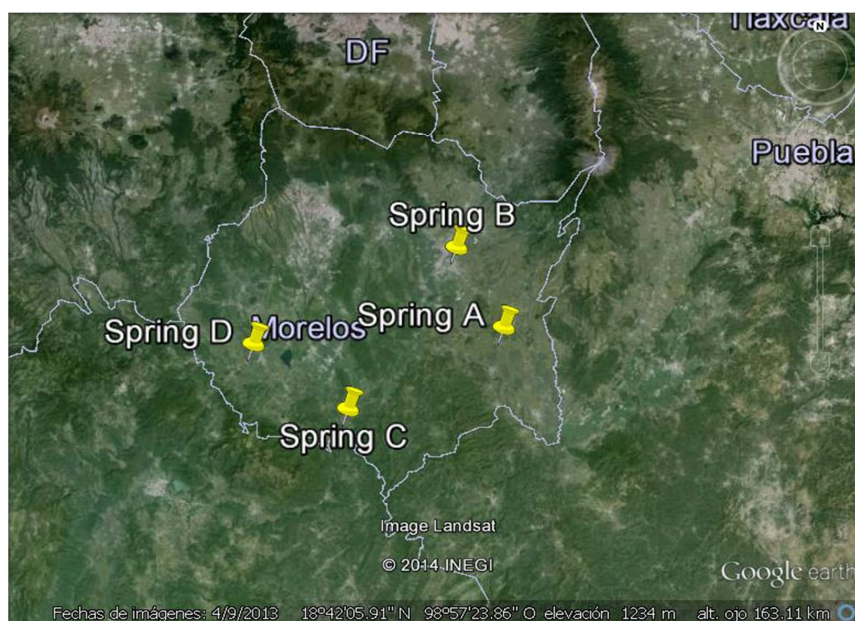


Figure 1: Localization of the springs in Morelos State, Mexico

2.2 Sampling Procedure:

The samples were drawn on a bimonthly basis for one year. Samples were collected from the sites where the spring water comes to the surface (BR) and from the pools (AL) which are fed from the spring water (Figures 2, 3, 4, 5, 6, 7, 8 and 9). Samples were taken in sterile containers for microbiological determinations (total coliforms,

fecal coliforms and free-living amoebae) and in 1500 mL container for the analysis of physicochemical parameters (total alkalinity, total calcium and magnesium hardness, chlorides, sulfates, nitrates, turbidity, conductivity and dissolved solids). Dissolved oxygen, pH and temperature were measured on-site.



Figure 2. Spring A-BR



Figure 3. Spring A-AL



Figure 4. Spring B-BR



Figure 5. Spring B-AL



Figure 6. Spring C-BR



Figure 7. Spring C-AL



Figure 8. Spring D-BR

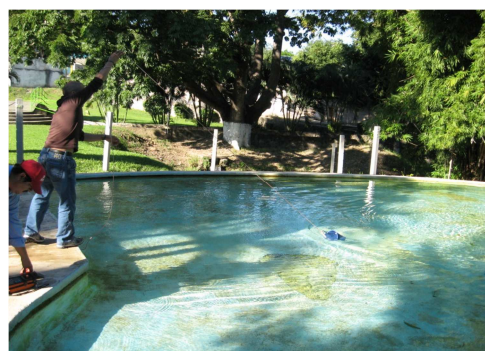


Figure 9. Spring D-AL

3.0 Results and Discussion:

A total of 352 analyses were performed. We used the results of the physiochemical parameters to calculate the average value, standard deviation and maximum and minimum values (Table 1).

Table 1. Values of the physicochemical parameters of the spring waters.

		A-BR	A-AL	B-BR	B-AL	C-BR	C-AL	D-BR	D-AL
pH	Mean	6.8 ± 0.08	7.2 ± 0.05	6.2 ± 0.14	6.5 ± 0.13	6.8± 0.05	7.6 ± 0.08	7.1 ± 0.13	7.4 ± 0.08
	Max.	7.2	7.3	6.4	6.6	7	7.9	7.3	7.6
	Min.	6.7	7.1	6.1	6.3	6.7	7.4	6.9	7.1
Total hardness in mg/L as CaCO ₃	Mean	732 ± 35	753 ± 12.5	1679 ± 310	1648 ± 158	1277 ± 123	1250 ± 64	559 ± 175	637 ± 29
	Max.	758	764	1919	1843	1412	1404	594	657
	Min.	672	734	1428	1423	1140	1111	535	603
Calcium hardness in mg/L as CaCO ₃	Mean	503 ± 60.6	495 ± 69.6	594 ± 103	547 ± 76.7	826 ± 83	815 ± 119	205 ± 91	285 ± 91
	Max.	566	549	700	631	936	968	360	424
	Min.	374	400	384	435	681	671	128	133
Magnesium hardness in mg/L as CaCO ₃	Mean	189 ± 75.7	210 ± 74.1	1069 ± 136	1090 ± 116	417 ± 118	348 ± 181	321 ± 101	628 ± 104
	Max.	384	364	1323	1252	586	619	466	657
	Min.	157	200	905	940	310	143	196	603
Total alkalinity in mg/L as CaCO ₃	Mean	215 ± 38	203 ± 32	625 ± 264	609 ± 265	213 ± 37	212 ± 36	250 ± 43	237 ± 40
	Max.	234	215	733	748	228	225	269	256
	Min.	188	184	477	479	191	191	216	203
Nitrates in mg/L	Mean	1.52 ± 0.66	1.33 ± 0.42	0.035 ± 0.01	0.05 ± 0.04	0.097 ± 0.02	0.085 ± 0.03	0.086 ± 0.04	0.031 ± 0.05
	Max.	2.32	1.9	0.149	0.169	0.121	0.146	0.137	0.105
	Min.	0.819	0.863	0	0	0.064	0.045	0.038	0
Chlorides in mg/L	Mean	25.1 ± 3.7	26.6 ± 0.93	74.7 ± 18.9	74.4 ± 19.5	15.5 ± 3.1	16.6 ± 2.9	6.9 ± 1.76	5.9 ± 1.71
	Max.	42	44	85	84	24	25	9.8	11.6
	Min.	14.9	21.8	43.9	42.5	9.6	11.2	3.7	2.3
Sulfates in mg/L	Mean	780 ± 41	888 ± 35	1410 ± 60	1466 ± 87	1539 ± 74	1530 ± 88	391 ± 28	569 ± 53
	Max.	888	959	1605	1597	1642	1616	442	613
	Min.	534	777	786	1127	1278	1410	342	501
Dissolved solids in mg/	Mean	1166 ± 35	1231 ± 37	2454 ± 160	2441 ± 43	1947 ± 58	1918 ± 32	744 ± 60	855 ± 64
	Max.	1226	1289	2581	2553	2011	1998	823	918
	Min.	1136	1188	2247	2301	1824	1869	687	816
Temperature in °C	Mean	32.4 ± 1.9	31.2 ± 1.7	26 ± 1	25.6 ± 0.96	30.3 ± 1.4	30.6 ± 2.4	29.8 ± 1.5	29.9 ± 1.75
	Max.	35	35	29	28	33	34	32	34
	Min.	31	29	24	24	29	28	27.5	29
Dissolved oxygen in mg/L	Mean	1.97 ± 0.27	3.45 ± 0.8	1.23 ± 0.1	2.8 ± 0.19	1.25 ± 0.05	5.82 ± 0.57	2.72 ± 0.26	4.2 ± 0.22
	Max.	3.8	4.2	2	4.2	2.6	6.8	3	5
	Min.	1.6	3	0.5	2.2	0.8	4.8	2.1	3

A, B, C and D = springs; BR = surface source; AL = swimming pool

The most acidic pH values were found in spring B-BR with 6.2 and B-AL with 6.5, while the most alkaline values were found in spring D-BR with 7.1 and D-AL with 7.4, however, it can be seen that the pH values measured at the surface source of the springs were slightly lower than those measured where swimming occurs or there are more bathers. According to the standard for swimming pool water, NOM-245-SSA1-2010 (SS, 2012), pH should be between 6.5 and 8.5, and although this standard does not apply to springs, given their recreational use the values found are, in general, within the recommendations, with the exception of spring B-BR which had an average pH of 6.2 and B-AL which reached the limit of 6.5. Although primary skin irritation appears to be related to high pH, the mechanism remains uncertain and while high or low pH is unlikely to be the direct cause of irritation or dermatitis, these conditions can be aggravated, especially in sensitive subjects. The eye can also be affected and high or low pH may favor and aggravate eye irritation by chemical substances (Díaz-Solano, 2011; OMS, 2000). The springs had pH lower than the reported by Romero *et al.* (2010), they reported average values between 8.0 and 8.4 in recreation sites; while Diaz-Solano *et al.* (2011) were 7 and 8.18 in the effluent from pool

The conductivity of the springs varied between 840 and 2535 $\mu\text{s}/\text{cm}$ and this values were lower than the reported by Romero *et al.* (2010). The conductivity depended on the concentrations of dissolved solids, which were highest in spring B in both surface source and swimming pool (2,454 and 2,441 mg/L), and lowest in spring D (744 and 855 mg/L). Based on the dissolved salt content, springs A, B and C are considered mineral waters since their content is above 1 g/L (1.17, 2.48 and 1.97 g/L respectively) while spring D is considered medium mineral since its content is between 0.2 and 1 (0.744 g/L) (Fagundo, 2007). The standard allows turbidity up to 5 NTU and in all cases the values were between 0.23 and 0.82, therefore meeting the criterion. Low concentrations of dissolved oxygen were found in the springs where they emerged at the surface, increasing where bathers swim due to the water's exposure to air during its trajectory and the aeration caused by the bathers. The highest value at surface source was in spring D-BR with 2.72 mg/L and in pools C-AL with 5.82 mg/L. Dissolved oxygen is important to avoid the formation of undesirable amounts of hydrogen sulfide. A concentration of more than 80% saturation is sufficient to obtain well

oxygenated water. The highest nitrate values were found in A-BR with 1.52 mg/L and the lowest in B-BR with 0.035 mg/L.

Based on the temperature, the springs can be classed as hypothermal (20 to 35°C). The spring with the highest values was A-BR (32.4°C) and the lowest, B-AL (25.6°C). Total alkalinity was highest in B-BR (625 mg/L as CaCO_3) and lowest in A-AL (203 in mg/L as CaCO_3). Water alkalinity is necessary for the pH to remain stable over time (buffer capacity) and with the water use. However, high alkalinity values cause problems in water since they lead to the formation of small particles that cause turbidity (Fagundo, 2007). Due high sulfate values mean, the spring water is classified as sulfate mineral water. The highest values were in springs C and B both at surface source and in swimming pools (1539 and 1530 the first, and 1410 and 1466 mg/L the second) (Table 1). Spring B had the highest values of chlorides in both surface source and pool (74.7 and 74.4 mg/L). The lowest values were in spring D in surface source and pool (6.9 and 5.9 mg/L). Thus, chlorides are not the predominant anions and would be classed as low chloride (less than 10 g/L) (Fagundo, 2007). The highest total hardness presented in B-BR (1679 mg/L as CaCO_3) and the lowest in D-BR (559 mg/L as CaCO_3). All the springs had very hard water and based on the total alkalinity to total hardness ratio, the springs are both hard carbonated and hard non-carbonated, non-carbonated predominating (Table 2) (Romero, 1999). The prevailing hardness in springs A and C was calcium and in B and D magnesium (Table 2).

Regarding bacteriological contamination, the six samples from spring A-BR contained total and fecal coliforms, four of which had values above 1000 cfu/100 ml, followed by C-AL which also had contamination in all six samples, but only one was above 1000 cfu/100 ml. The spring with the lowest incidence of total and fecal coliforms was A-AL with one sample containing fecal coliforms and two with total coliforms (Table 3). In general, the springs were less contaminated at surface source than in the pools, with the exception of spring A-BR (with the geometric mean of 2370 cfu/100 ml for total coliforms and 1316 cfu/100 ml for fecal coliforms) (Table 4). However, the pool at this resort (A-AL) was the least bacteriologically contaminated, perhaps due to the fact that the pool was fed by another source in addition to the spring, allowing the water to be diluted.

According to the World Health Organization (WHO) and the Federal Commission for Protection against Health Risks (*Comisión Federal para la Protección contra Riesgos Sanitarios, México, or COFEPRIS*), the increased coliforms in the pools may be caused by fecal contamination which may be transmitted by the bathers themselves, or to the water being contaminated as a result of an accidental fecal release, or the result of direct contamination by animals such as birds or rodents.

Other potential sources of contamination by pathogenic organisms could be the vomit, mucus, saliva and skin of the bathers. Moreover, certain bacteria and free-living amoebae can grow in pools, including pathogenic bacteria that may cause respiratory, dermal or central nervous system infections (OMS, 2000; COFEPRIS, 2013; Salas, 2000).

Table 2. Classification of the water hardness of the springs.

Spring	Total Alkalinity in mg/L as CaCO ₃	Total Hardness in mg/L as CaCO ₃	Type of Water Source: Romero, 1999	Carbonated Hardness	Non-carbonated Hardness
A-BR	215	732	Very hard	215	517
A-AL	203	753	Very hard	203	550
B-BR	625	1679	Very hard	625	1054
B-AL	609	1648	Very hard	609	1039
C-BR	213	1277	Very hard	213	1064
C-AL	212	1250	Very hard	212	1038
D-BR	250	559	Very hard	250	309
D-AL	237	637	Very hard	237	400

Table 3. Frequency of Total and Fecal Coliforms

CFU/100 ml	A-BR TC/TF	A-AL TC/TF	B-BR TC/TF	B-AL TC/TF	C-BR TC/TF	C-AL TC/TF	D-BR TC/TF	D-AL TC/TF
0		4 / 5	0 / 5	0/1	0/1			0 / 1
1 -100	0/1	1 / 0	6 / 1	4/ 3	5 / 4	0/ 2	4 / 4	0 / 1
101 -200	1/ 0					2 / 0	1 / 2	2 / 2
201 – 399		1 / 1		1 / 1	1 / 1	1/ 2	1/0	2 / 1
400 – 1000	1/1			1/1		2 / 1		2/1
> 1000	4/4					1/1		

Table 4. Geometric Means of Total and Fecal Coliforms

	A-BR	A-AL	B-BR	B-AL	C-BR	C-AL	D-BR	D-AL
Total C.	2370	0.48	10	65.5	11.03	481.2	66.4	272.6
Fecal C.	1316	0.25	0	13.35	4.1	263.5	25.13	60.4

Table 5. FLA Genera Present in Springs

FAL Genera	A-BR	A-AL	B-BR	B-AL	C-BR	C-AL	D-BR	D-AL
<i>Acanthamoeba</i>	-	-	-	+	-	-	-	-
<i>Korotnevella</i>	+	-	-	-	-	-	-	-
<i>Naegleria</i>	+	+	+	+	+	+	+	+
<i>Thecamoeba</i>	+		-	+	-	+	+	-
<i>Vannella</i>	-	+	-	-	-	-	+	+
<i>Vermamoeba</i>	+	+	+	+	-	+	+	+
<i>Vexillifera</i>	+	+	+	+	+	-	+	+

+Presence - Absence

In accordance with the Official Mexican Standard NOM-245-SSA1-2010 (SS, 2012), fecal coliforms must be in concentrations lower than 40 MPN/100 ml; thus, springs A-BR, C-AL and D-AL do not meet the standard. However, according to the Ecological Water Quality Criteria, fecal coliforms must not exceed 200 MPN/100 ml in fresh water or seawater for recreational use with direct contact and no more than 10% of the monthly samples should exceed 400 MPN/100 ml (SEDUE, 1989), consequently, only springs A-BR and C-AL do not comply with the recommendations. Romero *et al.* (2010) found that in two of the sampled sites, used for recreation, fecal coliform were above the USEPA criteria (geometric mean of 126 MPN / 100 ml) and in this work sites A-BR and C-AL also were above the USEPA criteria, due to possible contamination of the aquifer and pollution caused by the users of the pools, as reported by Diaz-Solano (2011) who found no fecal coliforms in the influent of the pool and pollution in the effluent.

Of the 48 samples analyzed, 83% tested positive for the presence of free-living amoebae. Seven genera of amoebae were isolated, *Naegleria* being present in all the sampled sites both at surface source and in the pools. Genera *Vermamoeba* and *Vexillifera* were present in 7 of the 8 sites (Table 5). Of the isolated amoebae, *Naegleria* and *Vermamoeba* (*Hartmannella*) have been reported frequently in hot springs and swimming-pools

(Nazar *et al.*, 2012; Solgi *et al.*, 2012; Zbikowska *et al.*, 2013).

Of the amoebae isolated, genera *Naegleria* and *Acanthamoeba* have been reported as potential pathogens and presented in 39% and 2% of the analyzed samples, respectively. *Vermamoeba* has been reported in association with a brain infection and eye infections, but its causative role has not been proven. However, the high percentage (39%) reported here is a reason for caution. Amoebae of genera *Korotnevela*, *Thecamoeba*, *Vexillifera* and *Vannella* have not been reported as pathogens, but have been frequently isolated from domestic wastewater (Ramirez *et al.*, 1993, 2005). The amoebic genera that grew at 42°C were *Acanthamoeba*, *Naegleria* and *Vermamoeba*. The presence of the first two in the water may represent a health risk since it has been reported that all pathogenic amoebae are thermophiles, although not all thermophiles are pathogenic (Visvesvara *et al.*, 2007). In general, the number of amoebic isolates was low, but the mere presence of genera *Acanthamoeba* and *Naegleria* in water used for swimming is cause for concern given the pathogenic potential of these amoebae (SS, 2012). Boxplots were drawn to determine the variation of the physicochemical and microbiological parameters between the two sampling zones: swimming pool (AL) and surface source (BR) (Figure 10).

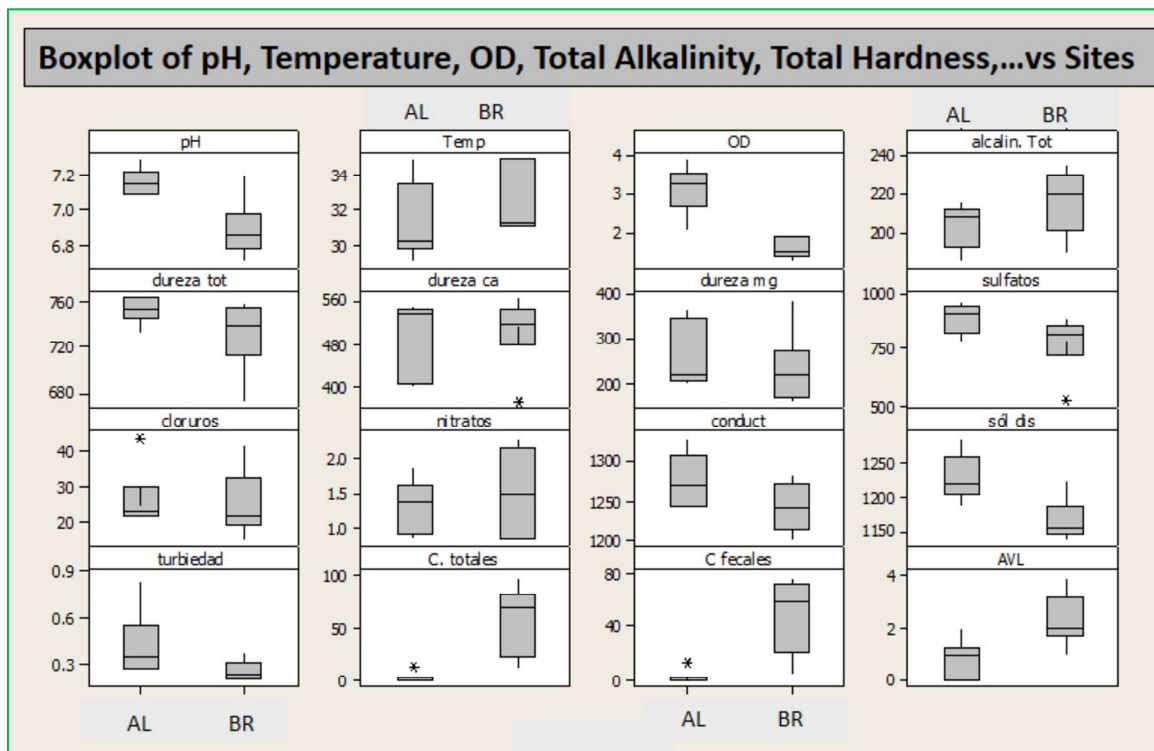


Figure 10. Boxplots by sampling site (surface source and pool)

The parameters with the highest concentrations in the pools were pH, DO, sulfates, magnesium hardness, total hardness, conductivity, dissolved solids and turbidity; while the highest concentrations at surface source were temperature, total alkalinity, chlorides, nitrates, total coliforms, fecal coliforms and FLA. The parameters that had the highest dispersion above the median were pH, DO and FLA at surface source; total hardness, magnesium hardness and conductivity in pools; and temperature, chlorides, dissolved solids and turbidity at both sites. The values that had the highest dispersion under the median were DO, nitrates and FLA in pools; total hardness, total coliforms and fecal coliforms at surface source; and total alkalinity, calcium hardness and sulfates at both sites.

The distribution of data was more similar for pH, total hardness, total coliforms and fecal coliforms in pools, and magnesium hardness, turbidity, DO and conductivity in surface sources. There was greatest variability in DO, calcium hardness and magnesium hardness in pools; and in nitrates, total coliforms and fecal coliforms in surface sources; and in temperature in both pools and surface sources. The number of free-living amoebae isolations was higher in surface sources than in the pools (Figure 10); this may be due to contamination by people who swim in those sites in addition to the amoebae that are naturally found in thermal springs (Vesaluoma *et al.*, 1995) as was the case of springs A-BR and D-BR. Boxplots were used to find the seasonal variation of the parameters (Figure 11). In general the variation was too great and no difference could be observed between the months studied.

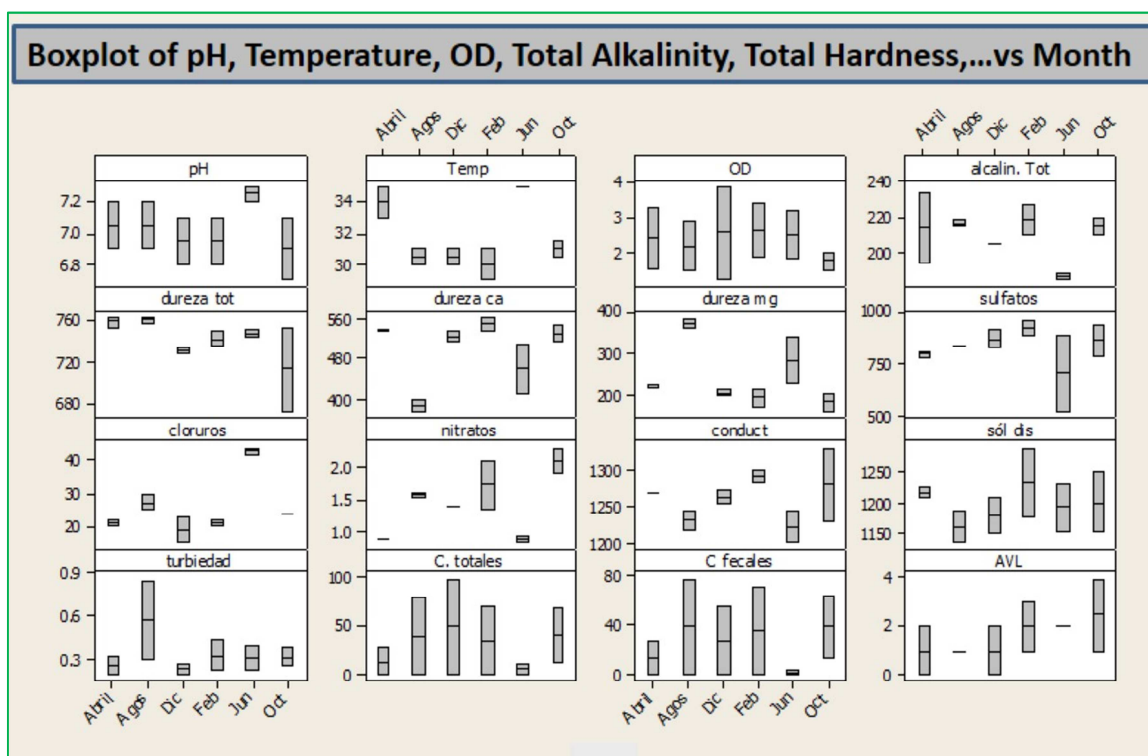


Figure 11 Seasonal variation of the parameters analyzed

The ANOVA showed that only pH, DO, total coliforms and fecal coliforms presented significant differences ($p \leq 0.005$). The other parameters showed no significant variation throughout the year (Table 6). One function was obtained from the discriminant analysis that represented 92.9% of the variation and was comprised of the parameters conductivity, dissolved solids, total hardness, total alkalinity, chlorides and sulfates (Table 7). This information was used to calculate the Mahalanobis distances (Table 8) and a

scatterplot was obtained (Figure 12), where the greatest distances can be observed between **B** and **D** with a value of 2237 and the lowest between springs **A** and **D** with a value of 254. Thus, springs **B** and **D** presented the highest differences according to the parameters that formed function one, and springs **A** and **D** were the most similar. The parameters comprising function one were conductivity, dissolved solids, total hardness, total alkalinity, chlorides and sulfates which constituted 92.9% of the total variation.

Table 6. Results of the ANOVA of Physicochemical and microbiological parameters

Parameter	F	P
pH	13.2	0.005
Temperature	0.89	0.369
DO	31.6	0.000
Total alkalinity	2.22	0.167
Total hardness	2.45	0.149
Calcium hardness	0.04	0.843
Magnesium hardness	0.42	0.531
Sulfates	3.21	0.103
Chlorides	0.08	0.782
Nitrates	0.40	0.543
Conductivity	3.27	0.101
Dissolved solids	10.83	0.008
Turbidity	3.15	0.107
Total coliforms	17.41	0.002
Fecal coliforms	15.78	0.003
FLA	8.27	0.017

Table 7. Results of the Discriminant Analysis between the Springs.

Function	Characteristic value	X ²	Observed significance level (P)	Cumulative variance percentage	Variables
1	353.9	425.1	0.000	92.9	Conductivity, dissolved solids, total hardness, total alkalinity, chlorides and sulfates.

Table 8. Mahalanobis Distances between Sampling Springs. (p-level = 0.000)

	A	B	C
A			
B	1239		
C	326	437	
D	254	2237	1095

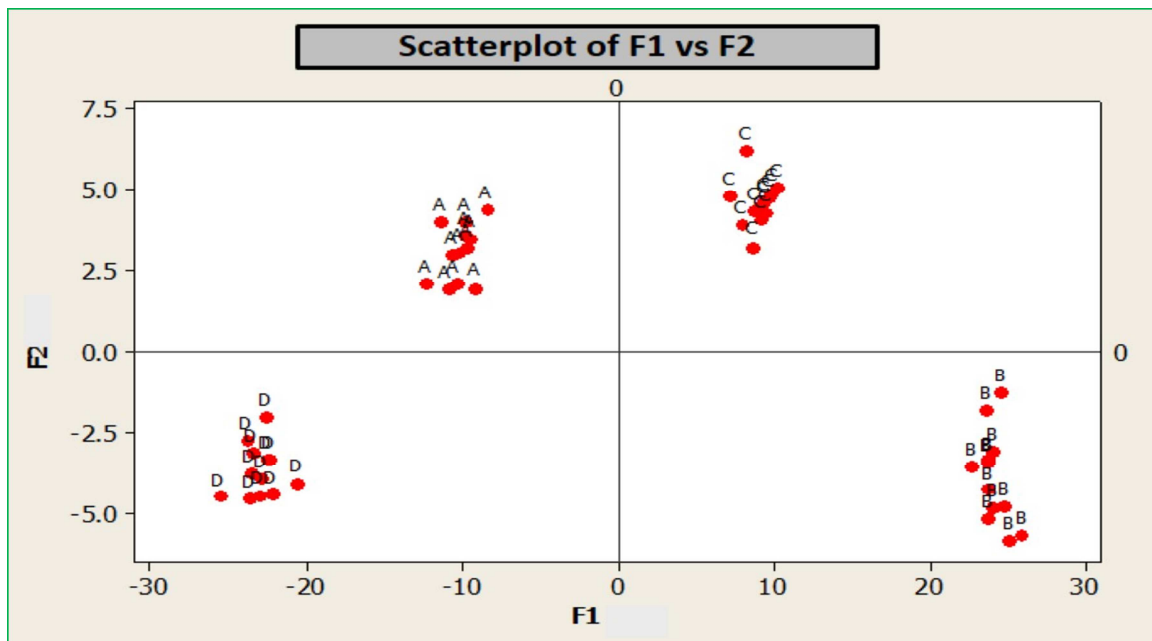


Figure 12 Scatterplot

4.0 Conclusions:

The four springs are hard water and predominantly non-carbonated, but in springs A and C the hardness is due to calcium and in B and D to magnesium. The temperatures observed and high values of dissolved salts and sulfates indicate that the springs can be considered hypothermal with sulfate mineral water. Spring B had lower-than-recommended pH and the highest total alkalinity values which may cause problems in the skin, eyes and nose mucus of bathers. Springs A and D were the most similar and spring B the most different in relation to conductivity, dissolved solids, total hardness, total alkalinity, chlorides and sulfates. In general, the springs presented higher microbiological contamination in the pools than in the surface source due to the presence of bathers. The detection of total and fecal coliforms and the presence of thermophilic amoebae of genera *Acanthamoeba* and *Naegleria* in the springs represent a health risk to users.

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References:

- 1) Arcos-Pulido, M. P., Avila-Navia, S. L., Estupiñán-Torres., S. M. and Gómez-Prieto, A. C. (2005): Indicadores Microbiológicos de Contaminación de las Fuentes de Agua. *Nova-Publicación Científica*, 3: 69-79.
- 2) APHA-AWWA-WEF, (1998): Standard Methods for the Examination of Water and Wastewater. 20th Ed., Washington, D. C. 1325 p.
- 3) Badirzadeh, A., Niyyati, M., Babaei, Z., Amini, H., Badirzadeh, H. and Rezaeian, M. (2011): Isolation of Free-Living Amoebae from Sarein Hot Springs in Ardebil Province, Iran. *Iranian J. Parasitol.*, 6:1-8.
- 4) Barrientos, Y., González, D. and Urban, F. (2000): Hydrochemical Study of Two Springs:

Cumbotico y Cumbote, Colonia Tovar, Aragua State, Venezuela. *Ecotropicos*, 13: 81-89.

- 5) Cortés, A., Arizalbo R. D., Rocha R. (1989): Estudio Hidrogeoquímico Isotópico de Manantiales en la Cuenca de México. *Geof. Int.*, 28 (2): 265-282.
- 6) COFEPRIS (Comisión Federal para la Protección contra Riesgos Sanitarios, México). (2013): Lineamientos para Determinar la Calidad de Agua de Mar para Uso Recreativo con Contacto Primario.
- 7) <http://www.cofepris.gob.mx/Paginas/Playas%20Limpias/Programa%20Integral%20de%20Playas%20Limpias/LineamientosCalidadAgua.aspx> (Accessed 25th august 2014).
- 8) Dallas, E. J. (2000): Métodos Estadísticos Multivariados Aplicados al Análisis de Datos. Ed. International Thomson Editores. México, D.F. 566 p.
- 9) Díaz-Solano, B. H., Esteller M. B., Garrido H. S. F. (2011): Calidad Físico-Química y Microbiológica del Agua en Parques Acuáticos. *Hidrobiología*, 21(1): 49-63.
- 10) Fagundo, J. R. (2007): Contribuciones al Desarrollo de la Hidrogeoquímica. Cap. 11 Aguas Naturales, Minerals y Mineromedicinales. <http://www.fagundojr.com/index.html> (Accessed 20th august 2014).
- 11) Gianinazzi, C., Schild, M., Zumkehr, B., Wüthrich, F., Nüesch, I., Ryter, R., Schürch, N., Gottstein, B. and Müller, N. (2010): Screening of Swiss Hot Spring Resorts for Potentially Pathogenic Free-Living Amoebae. *Exp. Parasitol.*, 126: 45-53.
- 12) González, C. J. C., Cabrera G. A., Ayala G. J. M. (2006): Flujo, Calidad de Agua y Uso Potencial de los Manantiales de la Microcuenca Atécuaro, Morelia, Michoacán, México. *Biológicas*. Universidad Michoacana de San Nicolás de Hidalgo. 8: 31-46.
- 13) Guimarães, R. R., De Souza L. C. Costa D. R. (2010): Water Quality of Springs and Wells in Properties Located in Rural and Peri-Urban Areas from Botucatu Region SP: Brazil. *Vet. e Zootec.*, 17(2): 275-287.
- 14) Granel, E. C. y Gales H. L. (2002): Deterioro de la Calidad de Agua Subterránea por el Desarrollo Poblacional: Cancún Q. Roo. *Ingeniería*, 6(3):41-53.
- 15) IMTA, (Instituto Mexicano de Tecnología del Agua). (2008): Los Manantiales, la Fuente de Agua más Pura del Planeta. <http://www.atl.org.mx> (Accessed 16th september 2014).

- 16) Kao, P. M., Hsu, B. M., Chen, N. H., Huang, K. H., Huang, S. W., King, K. L. and Chiu, Y. Ch. (2012): Isolation and Identification of *Acanthamoeba* Species from Thermal Spring Environments in Southern Taiwan. *Exp. Parasitol.*, 130: 354-358.
- 17) OMS, (Organización Mundial de la Salud). (2000): Guidelines for Safe Recreational-Water Environments Vol 2 Swimming Pools, Spas and Similar Recreational-Water Environments. http://www.who.int/water_sanitation_health/bathing/bathing2/es/ (Accessed 29th august 2014).
- 18) Nazar, M., Haghghi, A., Taghipour, N., Ortega-Rivas, A., Tahvildar-Biderouni, F., Nazemalhosseini-Mojarad, E. and Eftekhar, M. (2012): Molecular Identification of *Hartmannella vermiformis* and *Vannella persisten* from Man-Made Recreational Water Environments, Theran, Iran. *Parasitol. Res.*, 111: 835-839.
- 19) Page, F. C. (1988): A New key to Freshwater and Soil Gymnamoebae with Instructions for Culture. Culture Collection of Algae and Protozoa. Cumbria. 5-110.
- 20) Ramirez, E., Warren, A., Rivera, F., Bonilla, P., Rodriguez, S., Calderon, A., Ortiz, R. and Gallegos, E. (1993): An Investigation of the Pathogenic and Non-Pathogenic Free-Living Amoebae in an Activated Sludge Plant. *Water, Air and Soil Poll.*, 69: 135-139.
- 21) Ramirez, E., Robles, E., Bonilla, P., Sainz, G., Lopez, M., De La Cerda, J. M. and Warren, A. (2005): Occurrence of Pathogenic Free-Living Amoebae and the Indicators in a Constructed Wetland Treating Domestic Wastewater. *Eng. Life Sci.*, 5: 1-6.
- 22) Rivera, F., Gallegos, E., Ramirez, E., Bonilla, P., Calderon, A., Martinez, J. J., Rodriguez, S. and Alcocer, J. (1989): Pathogenic Amoebae in Natural Thermal Waters of Three Resorts of Hidalgo, México. *Environ. Res.*, 50: 289-295.
- 23) Romero, R. J. A, (1999). Calidad del agua. Alfaomega, México. 82-87
- 24) Romero, S., García J., Valdez B. y Vega M. (2010): Calidad del Agua para Actividades Recreativas del Río Hardy en la Región Fronteriza México-Estados Unidos. *Información Tecnológica*, 21(5): 69-78.
- 25) Romeu-Álvarez, B., Larrea-Murrell, J., Lugo-Moya D., Rojas-Hernández N., Heydrich-Pérez M. (2012): Calidad Microbiológica de las Aguas del Río Luyanó, La Habana, Cuba. *Revista CENIC, Ciencias Biológicas*. 43 (3): 1-9.
- 26) Salas, H. J. (2000): Historia y Aplicación de Normas Microbiológicas de Calidad de Agua en el Medio Marino. Hojas de Divulgación Técnica HDT 29. Centro Panamericano de Ingeniería Sanitaria y Ciencias del Ambiente (CEPIS). <http://cdam.minam.gob.pe:8080/bitstream/123456789/302/1/CDAM0000162.pdf>. (Accessed 27th august 2014).
- 27) SEDUE, (Secretaría de Desarrollo Urbano y Ecología). (1989): Criterios Ecológicos de Calidad del Agua. CE-CCA-001/89. Diario Oficial de la Federación, Mexico.
- 28) Sheehan, K. B., Fagg, J. A., Ferris, M. J. and Henson, J. M. (2003): PCR Detection and Analysis of the Free-Living Amoeba *Naegleria* in Hot Springs in Yellowstone and Grand Teton National Parks. *Appl. Environ. Microbiol.*, 69: 5914-5918.
- 29) Solgi, R, Niyiyati, M, Haghghi, A and Nazemalhosseini Mojarad, E (2012): Occurrence of Thermotolerant *Hartmannella vermiformis* and *Naegleria* spp. In Hot Springs of Ardebil Province, Northwest Iran. *Iranian J. Parasitol.*, 7: 42-52.
- 30) SS, (Secretaria de Salud), 2012: Norma Oficial Mexicana NOM-245-SSA1-2010. Requisitos Sanitarios y Calidad del Agua que Deben Cumplir las Albercas.
- 31) Sukthana, Y., Lekkla, A., Sutthikornchai, C., Wanapongse, P., Veijajiva, A. and Bovornkitti, S. (2005): Spa, Springs and Safety. *Southeast Asian. J. Trop. Med. Public Health*, 36: 10-16.
- 32) Vesaluoma, M., Kalso, S., Jokipii, L., Warhurst, D., Ponka, A. and Tervo, T. (1995): Microbiological Quality in Finnish Public Swimming Pools and Whirlpools with Special Reference to Free Living Amoebae: a Risk Factor for Contact Lens Wearers? *British J. Ophthalmol.*, 79:178-181.
- 33) Visvesvara, G. S., Moura, H., Schuster, F. L., (2007): Pathogenic and Opportunistic Free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunol. Med. Microbiol.*, 50: 1-26.
- 34) Zbikowska, E, Walczak, M and Krawiec, A (2013): Distribution of *Legionella pneumophila* Bacteria and *Naegleria* and *Hartmannella* Amoebae in Thermal Saline Baths Used in Balneotherapy. *Parasitol. Res.*, 112: 77-83.