International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

# Microbiological and Physico-chemical Study of Swimming Pool Water

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***Abstract***— *Swimming pools are places that must be constantly evaluated to avoid health problems for the users. The aim of this study was to evaluate the microbiological and physicochemical quality of swimming pools in the Metropolitan Area of Mexico City. Seven swimming pools were sampled during two periods; the pH and dissolved oxygen of each one was determined in situ. Two samples for microbiology were taken in sterilized bottles, one previously dechlorinated with sodium thiosulphate, the other for physicochemical determinations. In the laboratory, we determined free-living amoebae (FLA) by isolation in selective culture medium and morphological identification, Pseudomonas aeruginosa, total and fecal coliforms, residual free chlorine, turbidity, color, total alkalinity, total hardness, ammoniacal nitrogen, dissolved solids and conductivity according to the standard water analysis methods of the APHA, AWWA, WEF (2012). Bacteriologically, all the swimming pools were within the norm for fecal coliforms, however, Pseudomonas aeruginosa was found in three pools (AzA, At and GAM). Five species of FLA were isolated, of which Acanthamoeba polyphaga is the only one reported as a potential pathogen. The isolated strains of this amoeba presented different degrees of pathogenicity. This is important because Acanthamoeba is an opportunist amoeba that can cause disease in patients with low defenses, therefore swimming pools must undergo careful chlorination and cleaning. Turbidity was within the Official Mexican Standard NOM-245-SSA1-2010 in all pools. Levels of pH were within the limits of the Standard in all pools with the exception of At and TC, in the second period. However, residual free chlorine, only one swimming pool, TB, fell within limits in both periods and AzA, TC and GAM only in one period. In general, the swimming pools presented good microbiological quality, although some were outside the norm in physicochemical quality, some for having excess free chlorine and one for having very pH acid, which could compromise the health of the user.*

***Keywords***— *Fecal coliform, Free-living amoebae, Swimming pools.*

## I. Introduction

Recreational water, specifically swimming pool water, which not only provides recreation and improves health, can also be the means for the transmission of health risks, however, with improved management, modern water treatment technologies and careful water monitoring these facilities are safer (1).

On the other hand, without good controls, swimming pools can contain various contaminants that originate from other users (saliva, sweat, urine, contamination from creams, grease, cosmetic lotions and soap residue, among others) (2). Such contaminants may be microbiological or physicochemical and reach the user by involuntary water intake causing diarrhea ranging from mild to severe, primarily from *Cryptosporidium*, some enteropathogenic viruses and strains of *Escherichia coli* (1. 3, 4, 5).

Another possible source of contamination is the formation of films where potentially pathogenic and harmful bacteria grow (6). *Pseudomonas aeruginosa* is an opportunist pathogen that can be found in recreational water, often in the form of biofilm and therefore more resistant to disinfection procedures (7, 8).

*Pseudomonas* and *Staphylococcus aureus* are the main agents of skin infections and *Legionella* of respiratory infections. Furthermore, the presence of *Escherichia coli* (or thermotolerant coliforms) is an indication of recent fecal contamination, in which case additional measures must be considered, such as taking further samples and investigating possible sources of the contamination, which may include inadequate treatment or changes in the integrity of the distribution system (9, 10, 11).

Pathogenic free-living amoebae of the genera Acanthamoeba and Naegleria are also of interest in swimming pools. These amoebae can be found in the environment; some species can cause disease in man. Species with pathogenic potential cause serious infections of the central nervous system. Naegleria fowleri causes primary amoebic meningoencephalitis (PAM) and several species of Acanthamoeba cause granulomatous amoebic meningoencephalitis (GAM). In addition, Acanthamoeba can cause a serious eye infection, called Acanthamoeba keratitis (AK). A common factor in reported cases involving these amoebae has been bathing in pools, temperate water reservoirs and swimming pools. FLA pathogens are more frequent in natural tropical and sub-tropical bodies of water and in artificial bodies such as swimming pools with a temperature of 30ºC. Their main entry route is through the nostrils during aquatic activities (12, 13).

Among the conditions that can arise from chemical contaminants is the formation of chloramines, which are a product of the reaction of the chlorine and ammonium with urea from sweat and urine, hence chlorination must be very well controlled. However, in recent years, researchers such as Teo *et al*., 2015, Cheema *et al*., 2017., Young *et al*., 2018., Hu *et al*., 2018 (14, 15, 16, 17), among others, have found a variety of trace chemical contaminants which occur in swimming pools. The aim of water quality practices and monitoring is to prevent the propagation of microbial infections and diseases. Nevertheless, disinfection by-products (DBP) are formed when the disinfectants used react with



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organic and inorganic materials in the swimming pool. Other chemicals may also be present in swimming pools from anthropogenic sources (body excretions, lotions, cosmetics, etc.) or from the water source used, where trace chemical products may already be present.

In light of the above and since the user is in direct contact with the water, good control over water quality in swimming pools must be maintained in order to ensure they do not become foci of infection. The aim of this investigation was to evaluate the microbiological and physicochemical quality of water in 7 swimming pools in the Metropolitan Area of Mexico City.

## II. Methodology

Seven swimming pools were selected and sampled in two periods (from September 2015 to September 2016), in the Metropolitan Area of Mexico City. The swimming pools were located as follows: two in the Azcapotzalco district (AzA and AzB), one in the Gustavo A. Madero district (GAM), one in the municipality of Atizapán de Zaragoza (At) and three in the municipality of Tlalnepantla (TA, TB and TC) (Figure 1).

Fig. 1. Sampled Pools in the Metropolitan Area of Mexico City

Samples were taken in sterile containers from two separate places in the swimming pools for the microbiological samples (bacteria and free-living amoeba). The bacteriological containers were pre-treated with sodium thiosulphate to counteract the bactericidal effects of the chlorine and transported to the laboratory under refrigeration. Pathogenic free-living amoebae were determined from a one-liter sample taken from each site and kept at room temperature until analyzed. The samples for physicochemical analysis were taken in 1.5-L bottles from a single site in each swimming pool and kept under refrigeration until analyzed.

The Membrane Filter technique was used to determine total and fecal coliforms, seeding onto mEndo agar and incubating at 35ºC for 24-48 h for total coliforms and onto mFC agar and incubating at 44.5ºC for 24h for fecal coliforms, according to Standard methods of water and waste water analysis (18).

Pseudomonas aeruginosa was identified by seeding samples onto asparagine broth and incubating for 24 to 48 h at 37ºC. Positive tubes were reseeded onto cetrimide agar and incubated at 37ºC for a further 24 to 48 h. Positive cultures were then reseeded onto a nutrient agar slant and incubated at 37ºC for 24 h. API 20E tests were performed according to Standard methods of water and waste water analysis (18).

To determine free-living amoebae, the sample was filtered through a 1.2 µ membrane placed face down on non-nutrient agar with *Enterobacter aerogenes* (NNE), which is a selective medium for the isolation of this group of amoebae. The cultures were incubated at 30 and 37ºC and inspected daily with an inverted microscope to detect amoebic growth. The identification of the amoebae was carried out by observing fresh preparations under the optical contrast microscope at 40x and 100x phases. The morphological characteristics of the vegetative or trophozoite phase and the cyst were taken into account (19).

Isolated amoebae belonging to potentially pathogenic genera (Naegleria and Acanthamoeba) were axenized in Chang medium to obtain massive amoebae cultures and perform the pathogenicity test. The test was performed on five three-week-old male mice which were inoculated with axenic cultures of the amoebae at a concentration of $1 \times 10^5$ to $1 \times 10^6$ trophozoites per ml by taking 0.02 ml of the concentrate and applying it through the nostrils of the mouse. The animals were observed for a period of 21 days. The mice who survived the observation period were sacrificed. The brain, lungs, kidneys and liver of these mice and those who died during the observation period were extracted and seeded onto NNE agar to recover the amoeba.

The physicochemical parameters determined were pH, temperature, dissolved oxygen, residual free chlorine, turbidity, total alkalinity, total hardness, ammoniacal nitrogen and dissolved solids, according to the Standard methods of water and waste water analysis (18).

The results were compared to the maximum allowable limits in Official Mexican Standard NOM 245-SSA1-2010 Sanitary and Water Quality Requirements for Swimming Pools (20) and Competence Standards (EC 0207) (21).

## III. Results and Discussion

Bacteriologically, all the swimming pools were within the limits of NOM-245-SSA1-2010 (20) for fecal coliforms, however *Pseudomonas aeruginosa* was found in three pools (AzA, At and GAM), all in the sample from the second period (Table 1).

*Pseudomonas aeruginosa* and *Aeromonas* sp., may be indicators of a health risk for users (2, 3, 10), since these are known to cause different diseases in swimming pool users, such as otitis externa, conjunctivitis, dermatitis, septicemia, bacteremia, wound infection and folliculitis (11). Other studies have also reported strains of Pseudomonas aeruginosa isolated from swimming pools that are multi-resistant to antimicrobials (8).



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TABLE I. Presence of Coliform and Pseudomonas bacteria in the pools

| | Period | Sample | Total Coliforms *cfu/100 ml | Fecal Coliforms *cfu/100 ml | *Pseudomonas Spp.* |
|---|---|---|---|---|---|
| Norm 245 SSA1 | | | | < 40 | |
| AzA | P2 | M2 | - | - | **Presence** |
| TA | P2 | M2 | 25 | - | - |
| At | P2 | M1 | 35 | - | - |
| | | M2 | - | - | **Presence** |
| GAM | P2 | M2 | - | - | **Presence** |

*Cfu= Colony forming units

Of the seven swimming pools sampled, free-living amoebae were isolated from two pools in both sampling periods (At and GAM) and from two pools in only one period (AzB and TC) (Table II). Swimming pool At presented the highest number of amoeba species: *Acanthamoeba polyphaga*, *Vahlkampfia aberdonica*, *Vahlkampfia enterica*, *Vannella platypodia* and *Vexillifera bacillipedes.* With the exception of *A. polyphaga*, the isolated species have not been reported as pathogens, although *Vannella* may host human pathogenic bacteria (22). Only *Acanthamoeba polyphaga* was isolated from the other swimming pools and was the most frequently found in all the pools.

TABLE II. Pathogenic free-living amoebae isolated from swimming pool.

| Swimming pool | Incubation temperature (°C) | |
|---|---|---|
| | **30** | **37** |
| AzA 1 | Negative | Negative |
| AzA 2 | Negative | Negative |
| TA 1 | Negative | Negative |
| TA 2 | Negative | Negative |
| AzB 1 | *Acanthamoeba polyphaga* | *Acanthamoeba polyphaga* |
| AzB 2 | Negative | Negative |
| TB 1 | Negative | Negative |
| TB 2 | Negative | Negative |
| At 1 | *Acanthamoeba polyphaga* *Vahlkampfia aberdonica* *Vahlkampfia enterica* | Negative |
| At 2 | *Acanthamoeba polyphaga* *Vannella platypodia* *Vexillifera bacillipedes* | Negative |
| TC 1 | Negative | Negative |
| TC 2 | *Acanthamoeba polyphaga* | *Acanthamoeba polyphaga* |
| GAM | *Acanthamoeba polyphaga* | Negative |
| GAM | *Acanthamoeba polyphaga* | Negative |

The isolated strains of *Acanthamoeba polyphaga* presented different degrees of virulence (Table III). The strain isolated from AzB was pathogenic, killing all the mice during the test period and amoebae were recovered from all organs. The strain isolated from TC was invasive, did not kill the mice, but amoebae were recovered from the brain and lung of mice sacrificed after the test period. This fact is important because *Acanthamoeba* is an opportunist amoeba that can cause brain infection in people with low defenses (23, 12). Both strains were isolated at 37ºC and the water temperature in the pools was 30 and 33ºC, respectively. The strains of *A. polyphaga* isolated from At and GAM were not pathogenic and they only grow at 30ºC; the water temperature in the swimming pools was below 30ºC. This behavior coincides with the relationship between water temperature and pathogenicity of the amoebae, at higher water temperature, greater the likelihood that the amoebae are pathogenic (24).

The maximum values obtained were pH 8.53 in At2, residual free chlorine 11 mg/L in AzA2, dissolved solids 3490 mg/L in TB1, alkalinity 384 mg/L in TB2, total hardness 497 mg/L in AzB2, ammoniacal nitrogen 3.3 mg/L in GAM2 and turbidity 2.1 utn in At2 (Table IV and V).

With regard to NOM 245-SSA1-2010, pH was within the limits in all swimming pools except in the second period of At and TC (8.53 and 6.22, respectively). Free residual chlorine was below the standard limit in 6 samplings: pool At and AzB in periods 1 and 2, TA and GAM in second period; and above the limit in pool TC and TA in period 1, AzA in the period 2. Only pool TB met the standard in both periods, AzA and GAM only in period 1, TC in the period 2 (Table IV and V).

TABLE III. Pathogenicity of the *Acanthamoeba polyphaga* strains

| Strain | Pathogenicity |
|---|---|
| AzB[1] | Positive[2] |
| TC[1] | Invasive[3] |
| At | Negative |
| GAM | Negative |

1. The amoeba strain isolated at 37°C was used to do the pathogenicity test.
2. The amoeba killed all the mice and were recovered from all organs.
3. The amoeba did not kill the mice, but it was recovered from the brain and lung of mice sacrificed after the test period.

Swimming pool TC stood out by having a very high concentration of residual free chlorine of 9.0 mg/L in the first period and no isolated amoebae, then dropping to 3.3 mg/L in the second period, and even though is not a low concentration, the invasive strain of *Acanthamoeba polyphaga* was isolated from the swimming pool. It has been reported that species of *Acanthamoeba* can resist high concentrations of chlorine due to the cellulose in the wall of its cyst making them very resistant to environmental conditions (24).

Swimming pool At had the lowest concentration of residual free chlorine (0.4 and 0.2 mg/L) and the highest number of isolated amoeba species. This may be due to the pool being in the open air and exposed to dust and vegetable matter. Several studies indicate that amoeba cysts can reach bodies of water by air (24).

Turbidity in all the swimming pools met the standard in both periods.

Alkalinity, hardness and dissolved solids were compared to the reference of the Competence Standard (21) which suggests values less than or equal to 120 mg/L, 250 mg/L and 1500 mg/L, respectively. Therefore, pool TA in periods 1 and 2, TB in 1 and At in 1 are below the limit of this criteria for alkalinity and AzA in 1, AzB in 1 and 2, TB in 2, At in 2 and GAM in 1 and 2 are above the limit (Table IV and V).

With regard to hardness, pool AzA in periods 1 and 2, and At in 2 are below the limit; AzB, TB and GAM in periods 1 and 2, and TC in 2 are above the recommended value of 250, although the maximum acceptable value is 800 mg/L, which is never exceeded.



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For dissolved solids, TA, TB and TC in both periods and AzB in 1 are above the suggested 1500 mg/L (Table IV and V).

The water temperature of the pools was in a range from 25 to 33ºC, with the exception of At, which was below these values (18 and 20.5ºC) (Table IV and V). This parameter is not contemplated in the standard, but it is common for swimming pool temperatures to be around 30ºC; it has been reported that temperatures above 30ºC are conducive to the presence of pathogenic amoebae (24). This was the case with pools AzB and TC with temperatures of 30 and 33ºC, respectively, and where pathogenic and invasive strains of *Acanthamoeba polyphaga* were found.

Dissolved oxygen was in the range of 5.5 to 10.2 mg/L. These concentrations are suitable for the presence of free-living amoebae, since they are aerobic organisms (24).

Physicochemically, only one swimming pool, TB, complied with the standard in both periods, while the remaining pools had problems, primarily with the excess or reduction of chlorine in one or both periods. Based on the Competence Standards (EC 0207) (21), the majority exceeded the limits of alkalinity and dissolved solids; very low levels of alkalinity may have an impact on the presence of stains and corrosion of the metallic parts of the pools, while high levels affect irritation in the nose and throat of users.

According to the Pearson correlation coefficient, the only pool that showed significant differences from the others was TC2, the greatest difference being with TA2. Only pool TC presented significant differences between the two periods (0.45). The differences between pool TC2 and the others are due to this pool belonging to a housing development which shows signs of lack of maintenance and a control program, coinciding with Masoumbeigi et al (5), who states that poor water quality even in the best swimming pools can cause health problems and the transmission of infectious diseases to swimmers. Those authors propose the operation of monthly programs to teach the principles of operation and water quality maintenance to those responsible and to beneficiaries, and to ensure effective water treatment in the pools through a regular monitoring program.

## IV. CONCLUSIONS

In general, the swimming pools were of good microbiological quality, but the presence of pathogenic strains of *Acanthamoeba polyphaga* must serve as a wake-up call so that water chlorination and cleaning are not neglected. Although coliforms were within the standard, the presence of *Pseudomona aeruginosa* in some samples, due to its high tolerance to chlorine, is a health risk to bathers, and therefore the control of water chlorination and cleaning is also important in such cases.

With regard to physicochemical quality, some swimming pools fell outside the standard by not meeting the values of free chlorine and one for having very acidic pH levels, conditions which may be harmful to users' health.

Alkalinity, total hardness and dissolved solids were also outside the recommended values in some swimming pools, which may be problematical for users' health and pool maintenance.

The lack of control over swimming pool maintenance was reflected in the chlorination problems they presented.

TABLE IV. Results of the pysicochemical parameters in its two sampling periods

| Parameters | AzA 1 | AzA 2 | TA 1 | TA 2 | AzB 1 | AzB 2 | TB 1 | TB 2 |
|---|---|---|---|---|---|---|---|---|
| pH | 7.1 | 7.46 | 7.27 | 6.84 | 7.65 | 7.47 | 8.3 | 8.11 |
| Mexican Standard: 245 SSA1 (pH) | 6.5 a 8.5, every 4 hours | | | | | | | |
| Temperature | 29 | 30 | 30 | 31 | 30 | 30 | 30 | 29 |
| Dissolved oxygen mg/L | 5.9 | 10.2 | 6.7 | 5.56 | 5.5 | 5.86 | 6.3 | 6.76 |
| Residual free chlorine mg/L | 2.8 | **11** | 8.25 | **0.3** | **0.9** | **0.48** | 2.7 | 4 |
| Mexican Standard 245 SSA1 Residual free chlorine | 1 a 5 mg/L, every 4 hours | | | | | | | |
| Turbidity *UTN | 0.565 | 0.513 | 0.92 | 0.453 | 1.17 | 0.317 | 0.53 | 0.44 |
| Mexican Standard 245 SSA1 Turbidity | Maximun 5 UTN, once a day (noon) | | | | | | | |
| Conductivity µs/cm | 1127 | 507 | 3320 | 4255 | 3850 | 2170 | 6300 | 5200 |
| Dissolved solids mg/L | 630 | 303 | **1645** | **1820** | **2608** | 1270 | **3490** | **3027** |
| EC 0207 Dissolved solids | ≤1500 mg/L | | | | | | | |
| Ammonia nitrogen mg/L | 0 | 0 | 1.2 | 2.89 | 0.2 | 0.18 | 1.98 | 0.69 |
| Total Alcalinity mg/L $CaCO_3$ | 155 | 105 | 33.04 | 22.55 | 202 | 356 | 22.4 | 384 |
| EC 0207 Total Alcalinity | Total Alcalininity 80 – 120 up to 500 acceptable | | | | | | | |
| Total Hardness mg/L $CaCO_3$ | 108 | 88.67 | 202 | 241.25 | 739 | 497 | 341 | 425 |
| EC 0207 Total Hardness | Total Hardness 150 - 250 up to 800 acceptable | | | | | | | |

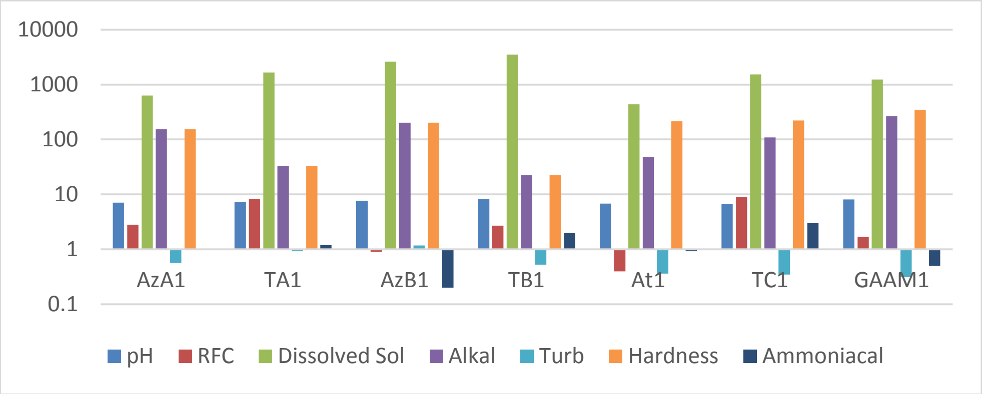


Fig. 2. Results of the physicochemical parameters in the first period

TABLE V. Results of the pysicochemical parameters in its two sampling period

| Parameters | At 1 | At 2 | TC 1 | TC 2 | GAM 1 | GAM 2 |
|---|---|---|---|---|---|---|
| pH | 6.8 | **8.53** | 6.6 | **6.22** | 8.11 | 8.25 |
| Mexican Standard: 245 SSA1 (pH) | 6.5 a 8.5, every 4 hours | | | | | |
| Temperature | 20.5 | 18 | 25 | 33 | 20 | 27 |
| Dissolved oxygen mg/L | 7.64 | 7.67 | 7.45 | 5.95 | 9.7 | 6.2 |
| Residual free chlorine mg/L | **0.4** | **0.2** | **9** | 3.3 | 1.7 | **0.01** |
| Mexican Standard 245 SSA1 Residual free chlorine | 1 a 5 mg/L, every 4 hours | | | | | |
| Turbidity *UTN | 0.36 | 2.1 | 0.35 | 0.44 | 0.313 | 0.563 |
| Mexican Standard 245 SSA1 Turbidity | Máximo 5 UTN, once a day (noon) | | | | | |
| Conductivity µs/cm | 804 | 1764 | 2440 | 0 | 1783 | 1431 |
| Dissolved solids mg/L | 440 | 1120 | **1533** | **2240** | 1225 | 968 |
| EC 0207 Dissolved solids | ≤ 1500 | | | | | |
| Ammonia nitrogen mg/L | 0.93 | 0.15 | 3 | 1.05 | 0.6 | 3.3 |
| Total Alcalinity mg/L $CaCO_3$ | 48.38 | 142 | 108.5 | 92.3 | 269 | 265 |
| EC 0207 Total Alcalinity | Total Alcalininity 80 – 120 up to 500 acceptable | | | | | |
| Total Hardness mg/L $CaCO_3$ | 215.3 | 144 | 222 | 270 | 343 | 291 |
| EC 0207 Total Hardness | Total Hardness 150 - 250 up to 800 acceptable | | | | | |

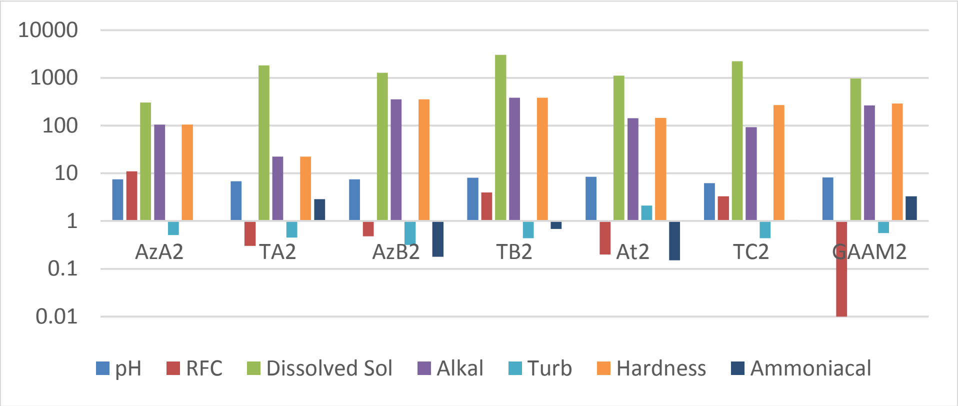


Fig. 3. Results of the physicochemical parameters in the second period

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