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Biothermalism: Daily bacteriological study of the thermal station Ain Allah Fez Morocco

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Abstract

We have tried in this work to study the daily and spatial variations of the microbiological characteristics of the waters of thermal station Aïn Allah in the region of Fez. Regarding bacteriology we found that the waters of the pool source (P_1) of Aïn Allah are free from any contamination by the indicator bacteria of the fecal contamination it means the good protection of the source against these contaminants. Regarding the pool water (P_2) always exists a contamination by the indicator bacteria of fecal pollution, and the presence of *Pseudomonas aeruginosa* coincides with the last days of the week, it could therefore be due to an over-frequentation of this station by the high number curists, accompanied by bad practices of the staff or contaminations brought by the users themselves. On the other hand at the level of P_2 and P_3 (Discharge water), are characterized by the presence of this bacterium, where the average value is recorded in Saturday and Sunday for P_3 where the use of this pool is important.

Keywords: Thermal station Aïn Allah, microbiological characteristics, pool source, pool water, Discharge water.

1. Introduction

Most studies have shown that thermal waters have been used as preventive and therapeutic remedies for many diseases: functional dyspepsia, respiratory tract, biliary tract, intestine, skin, genital tract, osteoarticular system, Otorhinolaryngology (Trautmann, 2005, Houtiand *al*., 2015). Several studies have shown that the therapeutic effect of thermal waters is associated with certain major elements present in water, such as magnesium and calcium, which play very important roles in lipid metabolism (Zichichiand*al*., 2006). In addition, other research has shown that salt-rich mineral waters have the ability to enhance the conversion of cholesterol to bile acids and their subsequent secretion (Gokgozand*al*., 2010). Studies conducted in Avène waters in France have revealed the beneficial effect of calcium in the treatment of

certain dermatological conditions and the regulation of intracellular calcium in keratinocytes (Athamena, 2006). Another study has shown the healing benefits of thermal waters in the treatment of Otorhinolaryngology pathologies (Ben Moussa and *al*., 2013). Some cations (Mg2 +, Fe2 +, Na +, K +) and anions (HCO3-, Cl-, SO42-, etc.) relieve diseases related to hypertension or heart palpitations, allergies, digestion, muscle fatigue, skin conditions, anemia, rheumatism and digestive, respiratory and gynecological disorders (Benmakhlouf, 2001).

2. Presentation of the study region

The station Aïn Allah is one of the three most famous and most visited spas in the region of Fez. It is frequently used by a large number of visitors and spa visitors for different uses.

The spa Aïn Allah located 15 km north-west of the city of Fez is located in the Douyet area (Fig. 1). The water from this spring, extracted from an artesian borehole at a depth of 1650 m, is used to feed the fountain, jet showers and two swimming pools that are open for public bathing.

The source of Aïn Allah is extracted by an artesian borehole at 1650 meters depth. The water from this source is without therapeutic indication. It is intended either for irrigation or to supply swimming pools open to popular swimming. But, what characterizes this source is the exfoliation and natural massage activities that take place in its pools and bathing.

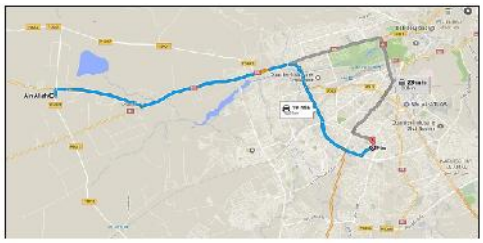

Figure 1: Location of the region of Aïn Allah extracted from Google Earth

Table 1: geographical location of Aïn Allah spa (Zizi, 1996, completed)

NAME	Map150000	Latitude	Longitude	Altitude (m)	Temp.(°C)
Aïn Allah	West Fez	34°02′24′′	05°08′10′′	383	45

3. Materials and Methods

The enumeration and research of the different germs were made in accordance with Moroccan standards. Except for *Pseudomonas aeruginosa* and *Legionella pneumophila*, where the research was done according to the protocols followed by the French standard (EN 162661) and the AFNOR standard (T90-431) respectively for the two bacteria.

Samples were taken from the study sites in sterile 500 ml ground glass stopper vials, leaving an air space in the vial; to facilitate the resuspension of microorganisms, by stirring before seeding in the appropriate culture media.

The samples are stored in a cooler of about 4 ± 2 ° C for transport to the Regional Laboratory for Epidemiological Diagnosis and Environmental Health (LRDEHM) (Rodier, 2009).

Following a pre-test we decided to prepare a dilution of 10-1 for only the water samples P2 and P3 sampling points (from the center and the point of evacuation of the pool). We apply the filtration technique which consists of filtering through a membrane (porosity 0.45µm for Coliforms, enterococci, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and a porosity of 0.22µm for anaerobic sulphateducting agents) a volume of 100ml of the sample and then incubate this membrane in a dish containing appropriate culture medium (Table 1). The bacteria that may be present on the filter will thus be able to develop during the incubation period and give rise to easily identifiable colonies. Counting CFUs (Colony Forming Units) makes it possible to evaluate the microbiological quality of water. This is an advantageous method because it is fast and inexpensive.

Figure 2 : Membrane filtration technique

For the waters thermal of Aïn Allah, three sampling points were chosen:

o Site P1: Shower water;
o Site P2: Pool water;
o Site P3: Discharge water;
The analyzes were completed at the LRDEHM and involved.

4. Results and Discussion

4-1- Daily and spatial variations of total sprouts at 22 ° C

The total germs at 22 °C are undetectable at the water level of the shower P1 while at the other two points its microorganisms are more abundant not exceeding in the first two days an average of 250 CFU / ml for P3 and 200 CFU / ml for P2, then its values decrease the days of Wednesday and Thursday as the care and support of the station after, starting at maximum values on Saturday and Sunday from 300 to 400 CFU / ml for P2 and 450 to 500 CFU / ml for P3; which shows the great impact of these two days related to the large attendance of bathers (Fig. 3).

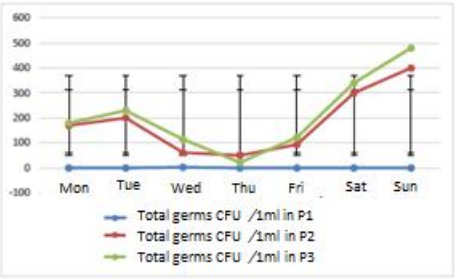

Figure 3 : Daily variation of total germs at 22 ° C in CFU / ml (P1 source point, P2 pool center, P3 evacuation point)

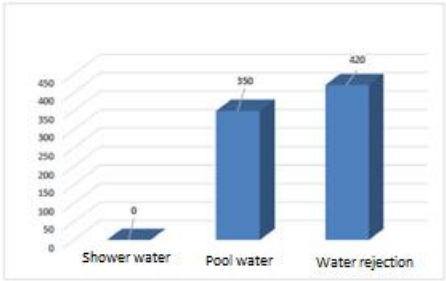

Figure 4 : Spatial variation of total sprouts (GT) at 22 ° C as a function of sampling points

According to the position of the sampling sites, there is a large difference between the averages recorded for the sites with a total absence of P1 shower water, which shows its purity and protection against these germs, which meets the Moroccan standard mineral waters consistent with Houtiand*al*. (2015), against a high rate of 350 CFU / ml for P2 and 400 CFU /ml for P3, which shows the contamination of the areas surrounding its last points and which undoubtedly results from the inflow of swimmers (Fig. 4) .

4-2- Daily and spatial variations of total sprouts at 37 °C

The daily variation during the week of the GT at 37 ° C also shows a complete absence of shower water. But on the other hand for P2 and P3 their values differ and vary taking almost the same curvature starting with stable values the first three days of the week and then decrease the day of support of swimming pool after this diurnition there is that the increase to maximum values on Sunday of 400 CFU / ml for P2 and 480 CFU / ml for P3 (Fig.5).

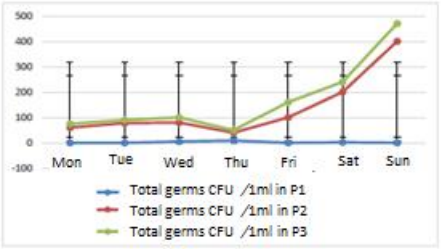

Figure 5, Daily variation of total sprouts at 37 ° C.

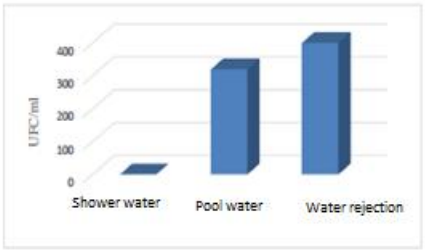

Figure 6, Spatial variation of total sprouts at 37 ° C

Les eaux des sites P2 et P3, correspondant aux eaux de la piscine et les eaux de rejet, présentent des teneurs maximales de 320 UFC/ml et 400 UFC/ml, respectivement (Fig.6). Cette forte charge pourrait être d'origine exogène et semble être liée essentiellement à la fréquentation de la piscine par un nombre important des baigneurs durant ces périodes. Par contre, les eaux du site P1 sont exemptes par ces germes, ce qui montre la protection de la source contre ces germes, (Fig. 6).

The waters of the P2 and P3 sites, corresponding to the pool water and the discharge water, have maximum levels of 320 CFU / ml and 400 CFU / ml, respectively (Fig. 6). This heavy load could be of exogenous origin and seems to be mainly related to the frequentation of the swimming pool by a large number of swimmers during these periods. On the other hand, the waters of the site P1 are exempt by these germs, which shows the protection of the source against these seeds, (Fig. 6).

4-3- Daily and spatial variations of total coliforms (TC)

Aïn Allah water analysis results show that the two sites P2 and P3 follow a similar pace during the week translates to an increase at the beginning and in order of the week which is closely related to the contribution of the bathers, but there is a remarkable decrease the day of Thursday seen the care and support of the station so Wednesday. In addition, these bacteria are detected in P2 and P3 site waters where the highest numbers are recorded during the study months with a maximum of 400 CFU/100ml for P2 and 500 CFU/100ml for P3 (Fig.6). The presence of total coliforms in the latter site is due to bather contamination. On the other hand, the absence of coliforms at the site P1 indicates a total protection against these bacteria indicating the fecal contamination.

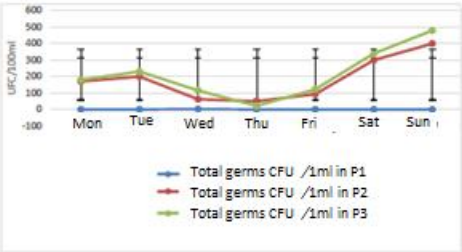

Figure 7 : Daily variation of total coliforms in CFU / 100ml

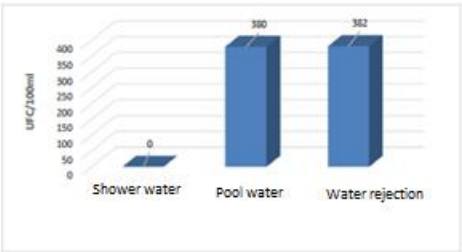

Figure 8 : Spatial variation of total coliforms

The presence of this type of bacteria at the two sites P2 and P3, indicates a fecal contamination of these studied waters of the spa of Aïn Allah and a total absence of these bacteria at the site P1, since the protection of these waters against all anthropogenic contaminants. So we can say that the variation between these three sampling sites is too significant with mean values close to them, for the site P2 380 CFU/100ml and 382 CFU/100ml for P3 (Fig. 8).

4-4- Daily and spatial variations of fecal coliform (CF):

In shower water, *E. coli* is undetectable throughout the week. On the other hand for the other points the presence is detected and takes values starting from the beginning of the week increasing relatively after stabilizes with values which does not exceed 20 UFC/100ml for P2 and 50 UFC / 100ml for P3, then the number increases to higher mean values on Saturdays, and extremes on Sunday for P2 and P3 respectively of 30 and 70 CFU/100ml, which does not exceed the bathing water guideline value set for *E. coli* (100 CFU/100ml) (Fig.9).

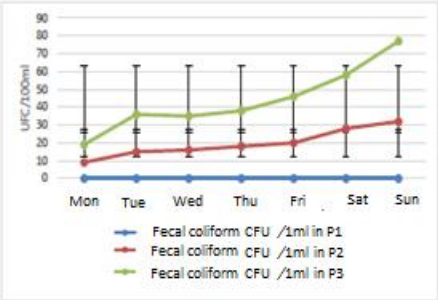

Figure 9: Daily variation of fecal coliforms in CFU / 100ml

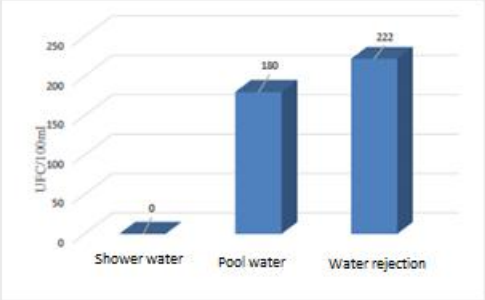

Figure 10: Spatial variation of fecal coliforms

Monitoring the spatial evolution of fecal coliforms in the studied water reveals an absence of these bacteria in the shower water and the presence of a very large number of 180 CFU/100ml in the pool water and 222 CFU/100ml in the waters of rejection (Fig. 10), the presence of this type of bacteria indicates fecal contamination and as total coliforms, the existence of these bacteria could be of anthropogenic origin.

4-5- Daily and spatial variations of intestinal enterococci:

Enterococci at the point P1 are absent. However for points P2 and P3 there is a detection with a variation of the same pace in the first days of the week until Friday when we notice a decrease of 20 CFU/100ml for P3 close to zero for P2, the value for P3 so the week exceeds the guide value set for these bacteria in the pool water and wastewater (guide value = 100UFC / 100ml), (Fig.11).

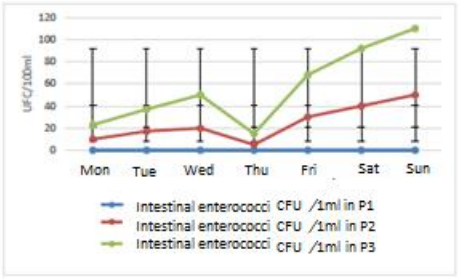

Figure 11: Daily variation of intestinal enterococci in CFU / 100ml

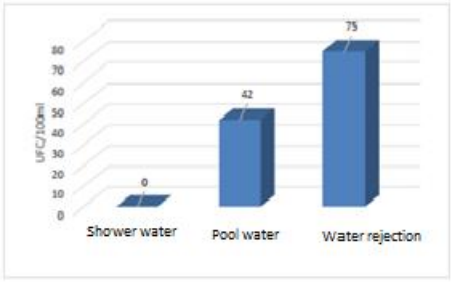

Figure 12: Spatial variation of intestinal enterococci in CFU / 100ml

The spatial variations in the number of intestinal enterococci in the studied waters reveal that the waters of the P2 and P3 sites are loaded by this bacterium with an average of 42 CFU/100ml recorded in P2 and 75 CFU/100ml recorded in P3 (Fig. 12).

4-6- Daily and spatial variations of *Pseudomonas aeruginosa*:

The daily variation of *Pseudomonas aeruginosa* very remarkable at the two sites P2 and P3 during the week, a significant decrease on the days of Thursday and Friday as the support and the swimming pool by detergent and disinfectant, after the day of Friday the number of this pathogenic bacterium increases at weekends with maximum values of 80 CFU/100ml P3 and 35 CFU/100ml P2 (Fig. 13). The presence of this bacterium is in direct relationship with the attendance of curists . On the other hand, the shower water characterized by an absence of this pathogenic bacterium indicates total protection of the source against the pathogenic bacteria.

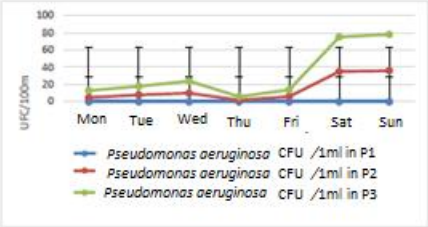

Figure 13, Daily variation of *Pseudomonas aeruginosa* in UFC / 100ml

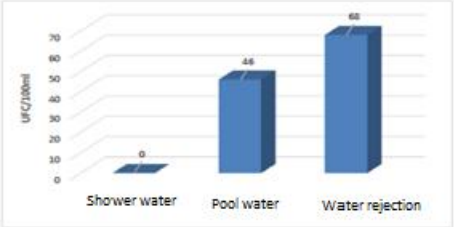

Figure 14, Spatial variation of *Pseudomonas aeruginosa* in UFC / 100ml

Throughout the study period, the shower water (P1) was characterized by the absence of *Pseudomonas aeruginosa*. On the other hand at the level of P2 and P3, are characterized by the presence of this bacterium, where the average value is of the order of 60 UFC / 100ml recorded in Saturday and Sunday for P3 and the value of 40 UFC / 100ml in P2 (Fig. 14), where the use of this pool is important. This contamination is due to the presence of this bacteria in the immediate environment or it comes from infected bathers, as indicated by World Health Organization in 2006. Our results are consistent with the results of the work of Morin and Graber-Duvernay (2002) conducted on the thermal waters of Aix-Les-Bains in France, which revealed the presence of *Pseudomoas aeruginosa* at the point of use and no contamination with this bacteria was detected at the entrance of these baths. The presence of *Pseudomoas aeruginosa* in these waters therefore represents a health risk for fragile, immunocompromised people (Trabelsi and *al*., 2007. As an opportunistic pathogen, it causes a wide variety of infections: atrial, ocular, urinary, skin or respiratory tract infections (Trautmannand*al*., 2005).

4-7- Variation of other types

During the three-month study period, 3 types of microorganisms were not detected: sulphite-reducing anaerobes (ASR), *Staphylococcus aureus* and yeasts and molds.

It is noted that the absence of (ASR) is consistent with the results of Houti et *al*. (2015). However, the absence of these three types in general indicates that the composition of the Aïn Allah thermal water matrix constitutes an unfavorable environment for their proliferation.

Conclusion

By the present work, we have tried, to present the results of daily and spatial variations study of the microbiological characteristics of the waters of the spa Ain Allah in the region of Fes

From the temperature of the waters of Aïn Allah, we can classify these waters as meso-thermal waters.

Bacteriologically we found that the waters of the pool source of Aïn Allah are free from any contamination by the indicator bacteria of the fecal contamination it means the good protection of the source against these contaminants. Regarding the pool waters, there is still contamination by the indicator bacteria of fecal pollution, and *Pseudomonas aeruginosa* coincides with the last days of the week, it could therefore be due to an over-frequentation of this station by the high number of patients, accompanied by poor staff practices or contamination caused by the users themselves.

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