

## Health risks of water wells in the Moorish baths: correlation between pathogenic bacteria and determination of gradients of contamination

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**Abstract:** The emergence of pathogens in water cold of baths Moors served by wells have been chlorinated or not, is a major concern in terms of the public health industry and the public authorities concerned. Among these pathogens, some are of fecal origin (*Escherichia coli*, intestinal enterococci, anaerobic sulphite-reducing or *Clostridia*), while others live in the natural environment (*Staphylococcus aureus*, and *Pseudomonas aeruginosa*). In order to establish a risk analysis related to the presence of these pathogens, it is important to increase our knowledge on the ecology of these microorganisms and to develop analytical tools to achieve better health monitoring. In total 568 samples sanitary cold water during the four seasons, to evaluate by PCA (Principal Component Analysis) the bacteriological quality of pathogens and to define spatiotemporal and seasonal gradients of contamination. Thus, could be determined three groups of bacteriological contamination (i) Gr1 well Moorish baths which is moving towards a contamination by fecal indicators (*E. coli*, intestinal enterococci, anaerobic sulphite-reducing); (ii) Gr 2 wells Moorish baths which is moving towards a contamination with *P. aeruginosa*; (iii) Gr 3 wells Moorish baths which is moving towards a contamination by *S. aureus*. Also, a seasonal gradient of correlation between these pathogens increases slightly in the winter, spring and summer to autumn.

**Keywords:** bath Moor, pathogens, PCA, sanitary cold water, wells

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### I. Introduction

The exploitation of groundwater has grown steadily under the combined effects of demographic pressure, the search for satisfactory food self-sufficiency, industrialization and of the political will to balanced regional development [1]. These waters constitute an important resource for the community and ecosystems from regions including quality, in a perspective of sustainable development, must be protected. To this end, the legal and regulatory framing of risk activities consist increasingly the obligation to realize monitoring of the groundwater quality [2]. Thus, Microbiological characterization of sanitary water Moorish baths (Hamams) served by wells can appreciate bacteriological quality and evaluate the risks related to the presence of bacteria pathogen and emergent. These facilities operate in a closed system and the same water is used simultaneously or consecutively to several bathers or consumers.

The water distribution systems in most of these baths are very defective and provide ecological niches favorable for the proliferation of bacteria difficult to eradicate by ordinary treatments [3]. The frequentation of these collectives' baths has not ceased to increase despite the associated risks.

Considering the increasing popularity of these collective baths, the incidence of these infections is likely to increase in the coming years [4]. In fact ridership is approximately 40 to 200 bathers per day, despite the evolution of the means of modernization and health programs to fight against these communal baths and advice to install bathrooms at home.

Certain bacterial populations are able to adapt, temporarily or not, to the oligotrophic conditions in the water systems. In the pipes of cold water, by forming the biofilms of *Pseudomonas aeruginosa* [5, 6]; can escape to severe bactericidal treatments [7] and adapting to extreme environmental conditions (the action of disinfectants, the water temperature, flow rate, stagnation, pipe materials, the degree of corrosion of the pipes, the high shear stress of water rinsing and ...) [8, 9]. Also, the continuous release of microorganisms in these systems, and in particular of fecal indicator bacteria (*Escherichia coli*, intestinal enterococci, anaerobic sulphite-reducing or *Clostridia*) opportunistic pathogens or non (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) entailing the non respect of the criteria of potability [10, 11]. The control by bacterial indicators and monitoring of pathogens, have reduced drastically the prevalence of infectious waterborne diseases.

In addition, the apparition of viable but non-culturable bacteria [12] and multidrug even the emergence of strains totorésistantes [13], must be taken into consideration by reason of the therapeutic impasses and the severity of situations they generate [14].

These recreational waters are followed to protect the health of bathers against infection with these pathogens. In addition, the microbial quality of the water can change quickly within 24 hours [15]. However, absence of regulation and monitoring of the water quality in these collective baths, the risk of microbiological contamination has become a problem to solve [16].

In the present work our aim was to answer some important uncertainties related to the quality of waters sanitary from Moorish baths and to determine correlation between bacteria pathogens

## **II. Materials And Methods**

### **1. Study site and sampling**

The city of Rabat, capital of Morocco, in which there are several Moorish baths (121 baths) especially in the popular districts. These collectives baths do not have disinfection procedure or at a distance from any disinfection. In the region, groundwaters (99% of wells) are used for bathing, served into waters hot or cold sanitary are treated with chlorine dioxide.

For quantification of pathogenic bacteria (*Escherichia coli*, intestinal enterococci, anaerobic sulphite-reducing or Clostridia, *Pseudomonas aeruginosa* and *Staphylococcus aureus*), during the four seasons of the year 2011, two missions per season in total, 568 cold waters samples were collected from 71 baths of the popular districts. All water samples are collected at the tap of Hamams served by wells. Samples of cold water were collected, according to the technical manual sampling [17, 18], from taps in a sterile polypropylene bottle of 500 mL after buckling tip tap and a brief flow. These water wells are generally treated, and to neutralize the free residual chlorine, sodium thiosulfate was added to the vials. Immediately after collection, the sample was stored between 2 and 5 ° C. The microbiological analysis was performed within 8 hours of collection. A strict protocol was established to standardize procedures for sample collection, transport, handling and storage until analysis according to NM ISO 5667-3-14 standard [19, 20].

### **2. Detection and enumeration of *E. coli***

*E. coli* was isolated according to official Morocco method [21]: 100 mL of water wells to be analyzed were filtered aseptically on sterile cellulose nitrate membrane nominal porosity of 0.45 µm (Sotrorius). The membrane was then transferred to a medium agar TTC (2, 3, 5-triphényltérazoliumchloride) -Tergitol (Biokar: BK038HA). Incubation time was 48 h at 44 ± 2 ° C. Yellow colonies surrounded by a yellow halo are the Gram-negative bacilli, confirmed *E. coli* by the middle EMB (EMB, Oxoid CM: 0069) with a metallic sheen.

### **3. Detection and enumeration of intestinal enterococci**

Intestinal *enterococci* were isolated according to official Morocco method [22]: 100 mL of water to be tested were filtered through a cellulose nitrate membrane with a nominal porosity of 0.45 µm (Sortorius). The membrane was then transferred to Slanetz and Bartley agar added TTC (Bio-Rad 356-4934). Incubation time was 48 h at 37 ± 2 ° C. The filter membrane was transferred to the confirmatory BEA agar (Bile Esculin Azide, Bio-Rad: 64184).

### **4. Detection and enumeration of anaerobic sulphite-reducing**

Anaerobic sulphite-reducing were isolated according to official Morocco method [23]: 50 mL of water were heated in a water bath at 75 ± 1 ° C for 15 min, to allow vegetative bacteria are killed. The cooled sample was filtered through a sterile membrane (nominal porosity 0.2 µm, Sortorius). The membrane was placed on the SPS agar (Polymyxin-Sulfidiazine-Sulfite, Pronadisa: 1082.00). The plate was incubated under anaerobic conditions a 37 ± 2 ° C for 48h. Colonies surrounded by a black halo corresponding to sulfite-reducing anaerobes. Confirmation is completed by determining the biochemical profile using the API anaerobic (API 20 A®, REF 20300, bioMérieux).

### **5. Detection and enumeration of *S. aureus***

*S. aureus* were isolated according to the official Québec method [24]: 100 mL of water to be tested were filtered through a cellulose nitrate membrane with a nominal porosity of 0.45 µm (Sortorius). The membrane was then transferred to Baird Parker medium supplemented the egg yolk and potassium tellurite, colonies of *Staphylococcus* appear black convex, shiny, with a diameter between 0.5 and 2 mm surrounded by a clear halo after incubation for 48h at 37 ° C. Suspect colonies were identified to the species *S. aureus* by the detection of free coagulase (using fresh rabbit plasma), DNase and biochemical API Staph gallery.

## 6. Isolation and identification of *P. aeruginosa* strain

*P. aeruginosa* was isolated and characterized from water specimens according to the ISO and official Morocco method [25]: aliquots of 100 ml were filtered through a 0.45 µm gridded cellulose nitrate membrane (Sortorius) and the membrane was placed on Cetrimid Agar (*Pseudomonas* CFC agar, Oxoid CM559) with 10 ml Glycerol/l, incubated at 42 °C for 48 h. Colonies that clearly showed pyocyanin production (blue green colonies and fluorescence at 360 nm) were considered positive for *P. aeruginosa*. All colonies were confirmed using King P Agar (Scharleau) and King F Agar (Scharleau) incubated at 37 °C for 24 h and on testing were found to be oxidase positive (Merck-testswobs).

## III. Results

### 1. Evaluation of non-conformity by parameter and season

Seen that contamination occurs by ingestion or inhalation, and most of the bathers consumed this water (100% of children), we considered thresholds potability of water from Moroccan standard [26], therefore, total absence of these pathogens searched in this work.

Table 1 shows high levels of non-conformity of 61%, 48%, 44%, 32% and 30% respectively for parameters: *E. coli* (EC), *P. aeruginosa* (PA), intestinal *enterococci* (EI), sulphite-reducing anaerobes (ASR) and *S. aureus* (SA); resulting an overall percentage of non-conformity of 74%. Therefore, only 26% (149/568) of cold water sanitary analyzed was being conform and five water wells of these Moorish baths were potable during the eight missions of 2011. These Moorish baths are newly refurbished with PVC conduit. Also it was found that non-conformity by number parameter is 74%, 87%, 85%, 83%, 85% and 86%, respectively, 0, 1, 2, 3, 4 and 5 parameters.

### 2. Coefficient correlation matrix

In statistics, correlation matrix comprises correlations between them of several variables, the coefficients of the variables indicating the influence on each other. Table 2 shows the analysis of the correlation matrix between the five variables EC, EI, ASR, PA, and SA during the eight missions of 2011. We found a high correlation between pathogenic indicators of fecal contamination EC, EI and ASR (correlation close to 1). Contamination of these waters by PA is not correlated with EC and EI, then there's a correlation between SA and other parameters (correlation close to 0).

### 3. Analysis and typological structure (PCA) of bacteriological contamination by microbiological five variables (EI, EC, ASR, PA and SA)

The projection plane F1xF2 (Fig.1) shows that five variables: EI, EC, ASR, PA and SA are shown on the mapping. The factorial F1 axis is well correlated with the EC, AR and ASR. The inertia of the variable SA is not absorbed by the first two axes F1 and F2 but by the third F3 axis.

Fig.2 concerning the representation of wells according to their contamination by pathogens studied, it was possible to identify three gradients of bacterial contamination: the first F1 along the axis defined by the indicators of fecal contamination (EC, EI and ASR) the second gradient in the F2 axis defined by the PA and the third as the F3 axis defined by SA.

### 4. Seasonal statistics treatment

We found during the summer and fall that there's a high correlation between EC, EI, ASR and SA, especially between ASR, EC and EI in summer with a correlation between two axes F1 and F2: 79.09 in summer and 84.39% in autumn (Fig.3, 4). However, firstly in the winter and spring it there's a high correlation between ASR, EC, EI and especially in winter, and secondly between SA and PA with a correlation between the two axes: 66.49 % in winter and spring in 71.16% (Fig.5, 6). The inertia force of axis F1 increased the 44.16%, 46.51% and 57.60% to 63.36% respectively of the winter, spring and summer to autumn. The approximation of correlations between variables in the factorial F1 and F2, is as better the winter, spring, summer to autumn, respectively the 60.49%, 71.17%, 79.09 to 84.39 (Fig. 3, 4, 5, 6).

## IV. Discussion

The systems of water distribution in the Moorish baths are supplied continuously by flows of organic and inorganic materials not eliminated by the potabilisation treatments, as well as microorganisms from wells but also punctual incidents on the system itself same (rupture of integrity, overruns treatments, repairs) [10]. The main populations are represented bacteria, viruses, protists and macroinvertebrates [12, 27]. Also present fungi and yeasts well as microalgae [28]. All these organisms are found in varying proportions in the systems, usually fixed to the surface of materials in the form of microcolonies mixed with corrosion products and inorganic precipitates, this assembly forming the biofilm [7]. Among the microorganisms get into the distribution systems,

heterotrophic bacteria can be easily adapted to oligotrophic conditions even in the presence of a residual disinfectant and can represent up to 91% of the total biomass [29].

### **Evaluation of non-conformity by parameter and season**

Our work shows a 74% of non-conformity. Thereby the presence of these bacteria may have an impact on the health of bathers and consumers because some have a pathogenic power or opportunistic pathogen, can induce infections [6]. However, the exhaustive search for undesirable bacteria could not be envisaged considering the number of species in search of their low concentrations and often lengthy analytical techniques and limited efficacy, the microbiological quality of cold waters sanitary is generally appreciated by monitoring of fecal indicator bacteria and pathogenic bacteria in the waters of collective baths. Their presence in these waters poses so problems as in terms of public health that in terms of non conformities. Exposure to contaminated tap water occurs primarily by ingestion. Contact with skin or inhalation (especially during showers) are also possible routes of penetration.

Non-conformity by number parameter is 74%, 87%, 85%, 83%, 85% and 86%, respectively, 0, 1, 2, 3, 4 and 5 parameters. These species form a bacterial community multi-species, the biofilms. The colonization of these waters adduction conduits by one to five shows the scale of pathogenic contamination. The bacterial reviviscence in these systems is the result of the interaction of different factors such as: the nature of the materials of the pipelines, temperature, nutrients present in the systems, the residual rate of oxidant, the interactions between these organisms and the spatial and temporal fluctuations in the number of pathogens in water [30].

### **Coefficient correlation matrix**

The correlations between these pathogens are well studied, but the mechanisms of bacterial interaction are yet to be developed [31]. From observations in mapping (Fig.1), we conclude that the F1 axis corresponds instead to contamination by pathogenic indicators of fecal contamination including ASR, EC and EC while the axis instead F2 corresponds to a contamination by PA.

### **Analysis and typological structure (PCA) of bacteriological contamination by microbiological five variables (EI, EC, ASR, PA and SA)**

By synthesizing the Fig.2 of PCA, it is concluded that there exist three groups of bacteriological contamination (i) Gr1: wells of Moorish baths which is moving towards a contamination by fecal indicators (EC, EC and ASR); (ii) Gr 2 wells Moorish baths which is moving towards a contamination by *P. aeruginosa*; (iii) Gr 3 wells Moorish baths which is moving towards a contamination by *S. aureus*.

These results could be used in the choice of means of biological treatment, chemical or physical against the proliferation of bacteria in question [32].

### **Seasonal statistics treatment**

Depending on the season, in general, there is no change in percentage of non conformity; it oscillates between 70% (fall and spring) and 77% (winter and summer). This could be explained by the large frequentation of the Moorish baths in winter and summer.

The angle between a variable PA and the F2 axis closes the spring, winter, fall to summer, while the angle between a variable SA and the F2 axis opens more and more toward F3 axis to F1 axis, whether the spring, winter, summer to autumn. For indicators of fecal contamination, the angle that they form with the F1 axis, opens and move away the axis, and this, the summer, winter, autumn to spring. We conclude therefore that the contamination of wells by PA is increasing the spring, winter, fall to summer, whereas SA is growing the spring, winter, summer to autumn, while than EC, EI and ASR is increasing the spring, fall, winter to summer (Figure 3, 4, 5, 6). One could say that would usually seasonal gradient of bacteriological contamination from spring to summer [33].

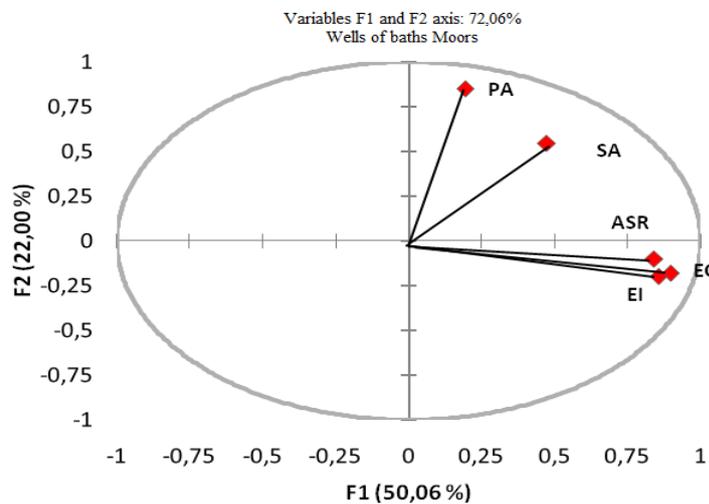
We conclude that there is a gradient correlation between these pathogens increases slightly the winter, spring, summer to fall and that SA played a role in the orientation of the gradient and the structure of group correlated bacteria. Thus, the means of mastery, control and treatment of these wells must respect this gradient, for example, by increasing the procedures in summer and especially in autumn.

**Table 1:** Distribution of non conformity based on contamination by parameter and season

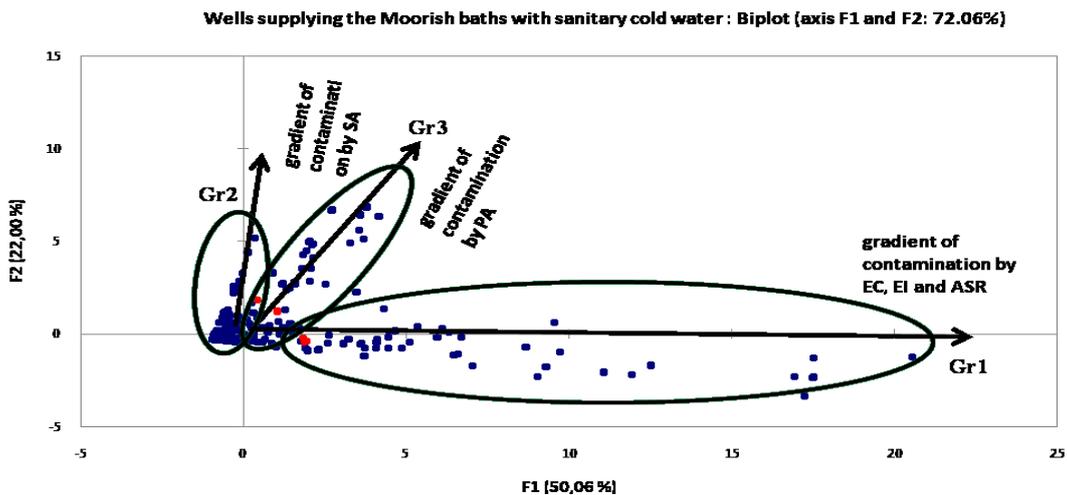
Parameter	% non conformity	% non conformity / season			
		winter	spring	Summer	autumn
<i>E. coli</i>	61	65	58	63	56
Intestinal enterococci	44	46	61	56	30
Anaerobic sulphite-reducing	32	33	28	33	34
<i>S. aureus</i>	30	30	35	34	22
<i>P. aeruginosa</i>	48	54	41	55	44
% total of non conformity	74	77	70	77	70
Total of samples	568	142	142	142	142

**Table 2:** intercorrelation matrix (Pearson (n) between the five pathogens searched EC, EI, ASR, PA, and SA

Variables	EC	EI	ASR	PA	SA
EC	1				
EI	0,729	1			
ASR	0,681	0,606	1		
PA	0,040	0,053	0,129	1	
SA	0,303	0,240	0,231	0,214	1



**Figure 1:** Mapping of the variables correlation (PCA)



**Figure 2:** Principal Components 1 and 2 of all bacteriological Wells supplying the Moorish baths with sanitary cold water

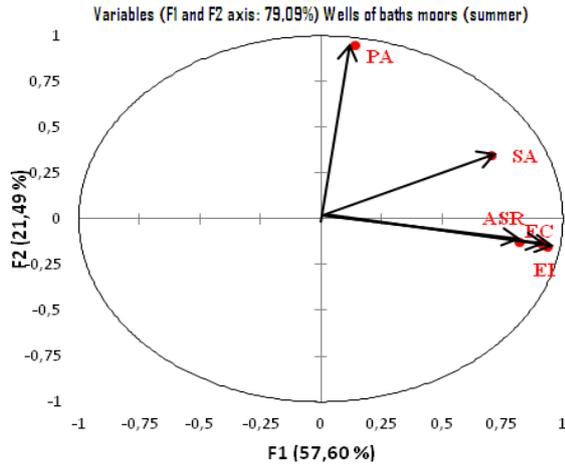


Figure 3: Circle of the correlation of variables in the summer

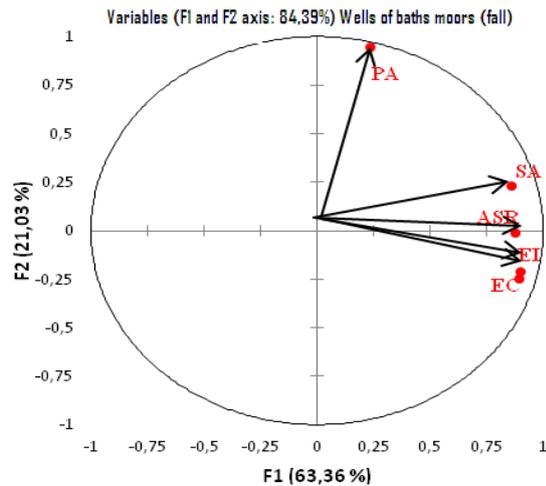


Figure 4: Circle of the correlation of variables in the fall

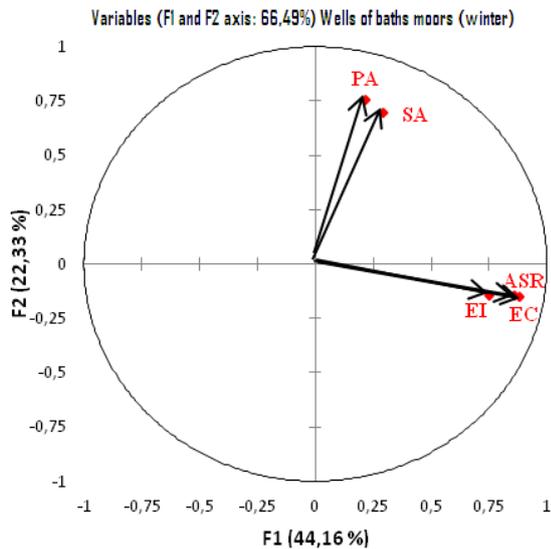


Figure 5: Circle of the correlation of variables in the winter

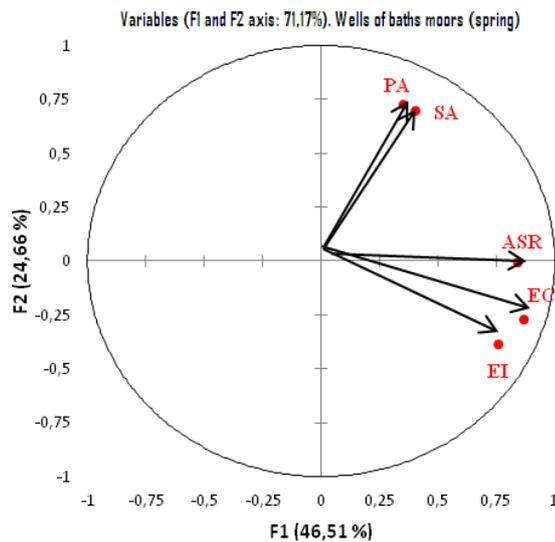


Figure 5: Circle of the correlation of variables in the spring

## V. Conclusion

Could be determined three groups of bacteriological contamination (i) Gr1 wells of collective baths which is moving towards a contamination by fecal indicators (*E. coli*, intestinal *enterococci*, anaerobic sulphite-reducing); (ii) Gr 2 wells Moorish baths which is moving towards a contamination with *P. aeruginosa*; (iii) Gr 3 wells baths which is moving towards a contamination by *S. aureus*. Also, a seasonal gradient of correlation between these pathogens increases slightly in the winter, spring and summer to autumn.

The repetition of such non-conformities should lead to questions about the health and safety applicable to the collective's baths and the exposure of users to microbiological health risk. In view of these findings and in light of the results and to identify some of the corrective actions to be carried for ensure better safety to users, regulation and organization of these institutions are required.

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