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# Assessment of microbiological quality of groundwater in the Saïs plain (Morocco)

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## ABSTRACT

The groundwater of Saïs plain is the main drinking-water supply of Fez-Meknes region (Saïs basin) in Morocco. A large number of people living in this region depend on groundwater drawn from open dug wells for their daily water requirements. The main purpose of this study is to identify the major factors affecting groundwater quality by means an analysis of the microbial composition. The quality of 144 groundwater wells is evaluated in the Saïs plain according to microbiological measurements. The enumeration of total aerobic mesophilic flora, total coliforms, fecal coliforms, fecal streptococci, pseudomonas aeruginosa, salmonella and cholera vibrio were performed by the membrane filtration technique.

The results showed that the most of the water points studied are contaminated by Fecal coliforms, Total coliforms, Fecal streptococci and Pseudomonas aeruginosa, their number in the waters of the analyzed wells exceeds the WHO recommendation and the Moroccan standards (0 CFU/ 100 ml). The bacterial load varies from one well to another with a slight seasonal variation. The quality of the water Saïs plain may be improved by cleaning of the groundwater sources, removal of organic matter from the water, addition of a disinfectant or the boiling of drinking water before use. The study recommends regular monitoring of drinking water sources in this area for the presence of pathogenic bacteria.

## INTRODUCTION

Groundwater resources are among the most important sources of fresh water. Similar to surface water resources, water quality is an important environmental issue in groundwater worldwide, which largely threatened by a combination of microbiological and physicochemical contamination (**Pedley and Howard, 1997; Reid *et al.*, 2003; Ako *et al.*, 2012**).

With rapid increase in population and growth of industrialization, ground water quality is being increasingly threatened by agricultural chemicals and disposal of urban and industrial wastes. Several studies have demonstrated that once pollution enters the subsurface environment, it may take many years to be concealed, becoming dispersed over wide areas of groundwater aquifer and rendering groundwater supplies unsuitable for consumption and other uses (**Jain *et al.*, 2004; Jain *et al.*, 2016**).

Groundwater is used for domestic, agriculture and industrial purposes in most parts of the world. In addition to the increasing demands of rapidly growing population (**Awotwi *et al.*, 2017**), the human activities like agriculture and domestic release large numbers of pollutants into the water bodies and then influenced the quality of water (**Asare-Donkor, 2016**). Rivers and ground water are used for the domestic and agriculture purposes (**Tahri *et al.*, 2005**). Anthropogenic activities are pronounced in both urban and rural areas. In urban areas, contamination is mainly reliable to industrial activities and onsite sanitation whereas in sub-urban to rural areas it is mainly caused by agriculture activities (**Mjemah *et al.*, 2011; Elisante and Muzuka, 2016**). Such activities and processes may include but not limited to leachates from agricultural fields that utilize agro-chemicals and leachates from waste disposal sites.

The vulnerability of groundwater qualitatively reflects the natural ability of the aquifer to be reached and affected by pollution from surface (landfill, cemetery, industrial wastewater discharge, chemical fertilizers, pesticides, herbicides, application of domestic wastewater, etc.). Whatever the method of disposal used, the notion of vulnerability incorporates the physical parameters determining the degree of exposure to groundwater pollution from the soil surface. The deterioration of groundwater quality by microbiological contamination has been the subject of a number of research papers (**Lamrani Alaoui *et al.*,2008; Bahri and Saibi 2012; Douagui *et al.*, 2012; Belghiti, M.L. *et al.*, 2013; Saber *et al.*, 2013; Merghem *et al.*, 2016; Al-Barakah, F. *et al.*, 2017; Mallick, 2017; Unnisa, S. and Zainab B., 2017**).

The Saïs basin is a major agriculture area in Morocco and contains two major cities: Meknes and Fez. These urban areas play a decisive role in the socio-economic development of the region. They are among the large and important cities in central Morocco, because of their economic activities such as industry, craft, tourism and trade. Agriculture and urban activities in these areas influence the local environmental equilibrium and groundwater quality in their context. Problems in groundwater quality appear in several regions. These problems are increasing over time, following the economic activities concentration, and may constitute obstacles to sustainable development.

The purpose of this study was to assess the quality of 36 groundwater bodies in twelve stations localized in Saïs basin according to microbiological measurements. This study also focuses on the relationship between risk factors and contamination of wells. literature survey or a summary of the results.

## MATERIALS AND METHODS

### Study area

The study areais located in the middle of Morocco (Fig. 1). It is characterized by intensive agricultural and human activities. The climate of this area is semi-arid, with a mean annual temperature and precipitation of 15.2°C and 296 mm/year, respectively (**RBAS, 2018**).

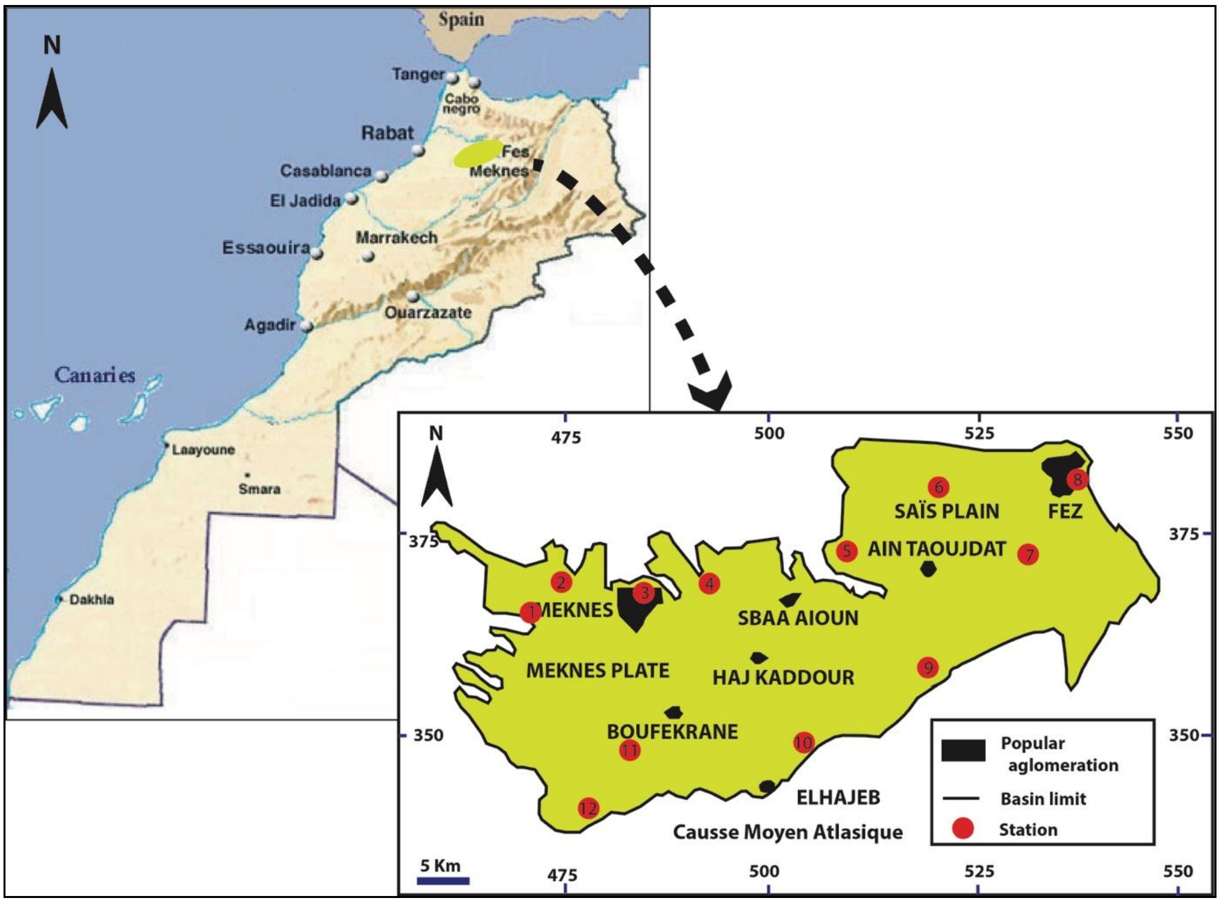


**Fig. 1.** Location of the study area and Spatial distribution of 12 stations

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The climate of Saïs region is semi-arid. It is characterized by a variant rainfall at the spatial and temporal level (**Essahlaoui *et al.*, 2001**). According to the data of the Sebou watershed recorded during the period 1970-2003(**RBAS 2018**), the annual contributions vary between 643 mm per year in the west of Meknes and 363 mm per year in the east of Fez. Generally, the water slide fell in the Saïs basin is approximately 1,247 billion m3 per year.

## Samples and Sampling

144 well water samples were collected from twelve randomly selected stations at Saïs plain from January, 2018 to January, 2019 (Fig. 2) in clean and sterile polypropylene plastic bottles, which were previously soaked in 10% nitric acid solution and thoroughly rinsed several times with distilled water and finally with a portion of the water sample. These bottles were covered by aluminum foil and sent at a temperature of about 4 ° C in the laboratory for analysis. The time between sampling and analysis was not more than 6 hours (**Kazi *et al.*, 2009**). The **table 1** presents some characteristics of the twelve stations.

**Table 1 : Charactersitics of stations.**

| Stations | | Well diameter (m) | Well depth (m) | Piezometric level (m) | well status | Use of wells | Sources of pollution |
|---|---|---|---|---|---|---|---|
| **1** | Ain Arma | 1.45 | 28 | 20-26 | Protected | Drinking water, domestic use and irrigation | Domestic wastewater, agricultural activities, animal faeces. |
| **2** | Toulal | 1.2 | 26 | 18-25 | Not protected | Domestic use, and irrigation | Tannery, domestic wastewater |
| **3** | Wislane | 1.5 | 25 | 16-24 | Not protected | Drinking water, domestic use, and irrigation | Domestic and industrial wastewater, agricultural activities, animal faeces. |
| **4** | Sbaa Ayoun | 1.62 | 30 | 26-29 | Protected | Drinking water, domestic use, and irrigation | Domestic wastewater, agricultural activities, animal faeces. |
| **5** | Taoujdat | 1.23 | 35 | 32-34 | Not protected | Drinking water, domestic use, and irrigation | Domestic wastewater and agricultural activities |
| **6** | Ain Lahnach | 1.53 | 27 | 19-25 | Not protected | Drinking water, domestic use, and irrigation | Domestic wastewater, agricultural activities, animal faeces. |
| **7** | Ain Chqaf | 1.60 | 29 | 23-26 | Not protected | Drinking water, domestic, and irrigation | Domestic wastewater, agricultural activities, animal faeces. |
| **8** | Sidi Hrazem | 1.58 | 23 | 15-21 | Not protected | Domestic use | Tannery, domestic wastewater and agricultural activities, animal faeces. |
| **9** | Bitit Ait Oualal | 1.30 | 31 | 26-29 | Not protected | Drinking water, Usage domestique, and irrigation | Domestic wastewater, agricultural activities, animal faeces. |
| **10** | EL Hajeb | 1.25 | 26 | 19-24 | Not protected | Drinking water, domestic use, and irrigation | Domestic wastewater, agricultural activities, animal faeces. |
| **11** | Mejjat | 1.45 | 24 | 13-22 | Not protected | Domestic use and irrigation | Industrial and domestic waste water, animal faeces. |
| **12** | Agourai | 1.20 | 32 | 26-29.5 | Not protected | Drinking water, domestic use, and irrigation | Domestic wastewater, agricultural activities, animal faeces. |

## Analytical procedures

The bacteriological analysis has been done by using the filter membrane method. This method consists in collecting, identifying and enumerating on the surface of a 0.45 μm porosity sterile filter membrane (**Rodier *et al.*, 2009**) (in cellulosic ester for example) the microbial germs present in a water sample, the filter is then placed on suitable media and incubated at suitable temperatures for each microbial seed for a sufficient total incubation time. The advantage of membrane filtration is the use of a larger volume to define the concentration and diversification (quantitative and qualitative) of the bacteria present in the water sample analyzed. to do this it is necessary to filter through the membrane a determined volume of the sample to be analyzed. In our work, research and enumeration of total aerobic mesophilic flora, total coliforms, fecal coliforms, fecal streptococci, *Pseudomonas aeruginosa*, salmonella and cholera vibrio were performed by the membrane filtration technique (**Balogun, 2000**).

For salmonella, a volume of five liters is filtered through sterile 0.45 μm diameter membranes to reduce the volume, followed by pre-enrichment in buffered water followed by enrichment. in the selenite broth and then transplanted to the selective salmonella shigella agar, the suspect colonies are then subjected to biochemical and serological studies, the biochemical tests use a triple iron-sugar agar, to the production of indole, for decarboxylase and β-galactosidase activity, serological tests include agglutination assays with anti-H and anti-O sera after removal of autoagglutination strains (**Rodier *et al.*, 2009**).

For cholera vibrio, enrichment is performed with alkaline peptone water. it is followed by subculture on the selective medium: thiosulphate agar, citrate, bile salts and sucrose (TCBS). suspicious colonies are transplanted on iron kliger agar. after an incubation of 18h, vibrio cholerae produces a distinctive yellow without release of gas. the urease and oxidase activities are subsequently sought. the urease-negative and oxidase-positive strains are retained to complete the biochemical and serological tests (**Rodier *et al.*, 2009**).

## Data Analysis

Analysis of variance (ANOVA) at 5 % level of significance to compare means of the microbiological water quality among all the sampled ground water sources between seasons, using statistical analysis software (SPSS).

Factor analysis (FA) was employed to interpret the structure of the underlying data set through a reduced new set of variables (principal components, PCs), arranged in decreasing order of importance. The distribution of individual association of microbiological parameters in groundwater was determined by principal component method.

## RESULTS AND DISCUSSION

### Fecal coliforms (FC)

Fecal coliforms (CF) are searched in water for faecal contamination according to **Gaujous** (**1995**). The results of the analysis of different water points studied at the level of the study area showed that most of the wells are highly contaminated, especially at station 2, station 5, station 7, station 8, station 10 and station 12, while station 2, station 6, station 8, station 9 and station 11 record low concentrations relative to the other points studied.

The fecal coliform values are between 34 CFU / 100ml and 440 CFU / ml with an average value of 177CFU/100ml and a standard deviation of 10 CFU/100ml (**Fig 2a**).

The values found in fecal coliforms exceed the recommended standards (**WHO, 2000**) and **Moroccan standards**, **2006)** (0 CFU / 100ml)). The very high concentrations could be explained by the presence of a source of microbial pollution due to the accumulation of faeces (manure used in agriculture, excretions of animals that fall into the water due to poor protection of the dots). The same results have been found by **Belghiti, M. L. *et al.*** (**2013**) in Meknes.

### Total coliforms TC

Total coliforms (TC) are bacteriological parameters that indicate a fecal pollution. total coliform count results showed that most of the samples show high contamination, especially at stations 6, 7, 8 and 9, the total coliform values are between 21 CFU / ml and 372 CFU / ml with an average value of 138 CFU/ml and a standard deviation of 8,6

CFU/ml the results obtained showed that the TCs undergo large seasonal fluctuations during the study period ($p<0.05$) (Fig 2b).

The majority of the waters analyzed during this study do not meet the standards, (**WHO, 2000** and **Moroccan standards, 2006** (0 CFU / 100 ml)) the presence of the very high total coliforms shows a deterioration of the quality of the groundwater the high levels of total coliforms could be explained by the poor protection of these water points, the spreading of manure near the water points and infiltration of surface water. These results are similar to their found by **Belghiti, M. L. *et al.* (2013)** in Meknes.

### Total germs

According to our results, most of the waters analyzed in this study show total germs in very high concentration, with reference to **Moroccan standards** (**2006**) and **WHO standards** (**2004**), (for total amounts at 22 ° C the Moroccan standard (NM0061) (100 CFU / ml) and for the total standards at 37 ° C the Moroccan standard (20 CFU / ml) the majority of the analyzed waters far exceed the recommended standards. we also noticed that the bacterial load varies from one point to another with a slight seasonal variation (Fig 2c,d). contamination of groundwater by total sprouts could be the source of water contamination by germs from several sources of pollution near these water points (waste accumulation, septic tank farmland, animal barns, manure storage on agricultural land without precautionary precaution ...), the situation of water points, and the poor protection of wells. the very high presence of total germs in the groundwater could also be explained by the presence of a major source of pollution and which is at the origin of the construction of environment favorable to the bacterial development.

The results confirm the presence of a major source of pollution and which has led to the establishment of favorable environments for bacterial growth. The same results have been found by **Belghiti, M. L. *et al.* (2013)** in Meknes.

### Sulphite-reducing clostridia SRC

The results of the bacteriological analysis of the water sampled in this study reveal the presence of sulphite-reducing clostridia in most water points studied. The contents of clostridia sulfito-reducers vary between 0CFU / 20 ml and 26CFU / 20ml with a mean value of 8 CFU/20ml and a standard deviation of 0,6 CFU/20ml.

The concentrations obtained are very variable from one water point to another with a slight seasonal fluctuation (Fig. 2e). The values found in most of the water points studied exceed the standards recommended by the World Health Organization (**2004**), 0 CFU / 20ml. the presence of sulphite-reducing clostridia spores in natural water is reminiscent of past fecal contamination (**WHO, 2004**). It should be pointed out that Clostridia sulphite-reducing bacteria are often considered as witnesses to fecal pollution. the spore form, which is much more resistant than the vegetative forms of fecal coliforms and fecal streptococci, would thus make it possible to detect an old to intermittent fecal pollution according to **El haissoufi *et al.* (2011)**. The same results have been found by **Belghiti *et al.* (2013)** in Meknes.

***Pseudomonas aeruginosa* (Pa)**

*Pseudomonas* aeruginosa are bacteriological indicators indicative of water pollution (**Gellatly. *et al.*, 2013**). the values found showed that pseudomonas aeruginosa concentrations are between 3CFU / 100 ml and 54 CFU / 100 ml with a mean value of 22CFU/100ml and a standard deviation of 1 CFU/100ml.

The number of Pseudomonas aeruginosa in the waters of the analyzed wells exceeds the norm (**WHO, 2004**) (0 CFU / 100ml) in the analyzed waters the maximum concentrations are recorded at the wells in the station 2, station 10, and station 4. and the minimum concentrations are recorded at wells in the station 6, station 8, station 9 and station 12 (Fig. 2f).

The contamination of water points by PA could be explained by fecal pollution of animal or human origin (sewage spreading, cattle breeding, use of animal waste as fertilizer for agricultural land adjacent to water points) and poor protection of these water points. The presence of PAs could cause the transmission of many infectious and parasitic diseases to water transport including: cholera typhoid, hepatitis, schistosomiasis, malaria (WHO 2004). the results obtained are in agreement with those found by Nola et al. (2001). The same results have been found by **Belghiti *et al.* (2013)** in Meknes.

**Fecal streptococci (SF)**

Fecal streptococci (SF) are the most common group of bacteria used for bacteriological examination of water (**Poole and Hoberson, 1979**). the enumeration of fecal streptococci in the study zone subterranean revealed that well water supports station 1, station 2, station 4, station 5, station 9, station 10 are the most loaded fecal streptococci. while the sinks station 1, station 2, station 3, station 7, station 8 and station 11 have the lowest degree of contamination. the values found showed that fecal streptococci concentrations are between 21 CFU / 100 ml and 454 CFU / 100 ml with an average value of 158 CFU/100ml and a standard deviation of 8,6 CFU/100ml (Fig. 2g).

The number of fecal streptococci in the majority of the water points analyzed far exceeds the WHO recommendation and the Moroccan standards (0 CFU / 100ml). the very large number of fecal streptococci in the analyzed waters could be explained by fecal animal or human pollution (sewage spreading, use of wastewater disposal wells, livestock breeding, use of animal waste as fertilizer for agricultural land) and the poor protection of these water wells.

The detection of enterococci in groundwater or in wells should give rise to a serious suspicion of fecal contamination and the presence of enteropathogenic microorganisms (**Simmons *et al.*, 2001**). The same results have been found by **Belghiti *et al.* (2013)** in Meknes.

**Pathogenic germs (Salmonella and Vibrio cholerae)**

Salmonella and Vibrio cholerae are the germs responsible for more or less serious pathologies in humans (**Giannella *et al.*, 1973, 1975; Rubin and Weinstein, 1977; Stephen, 1985; Mishu *et al.* 1994; Harris *et al.*, 2012**). their research does not automatically take place in routine bacteriological examinations of water. However, it is necessary during particular situations, especially during epidemics or during the evaluation of a new resource. Research is done on large quantities of water.

Pathogenic germs include Strains of Salmonella and Vibrio cholerae, which can cause diseases whose serious transmission of these germs is through the consumption of water or food polluted by stool sick or infected subject. According to the microbiological analyzes that we carried out, we are reassured of the absence of the pathogenic germs in all the analyzed waters in the basin of Saïs. The same results have been found by **Belghiti *et al.* (2013)** in Meknes.

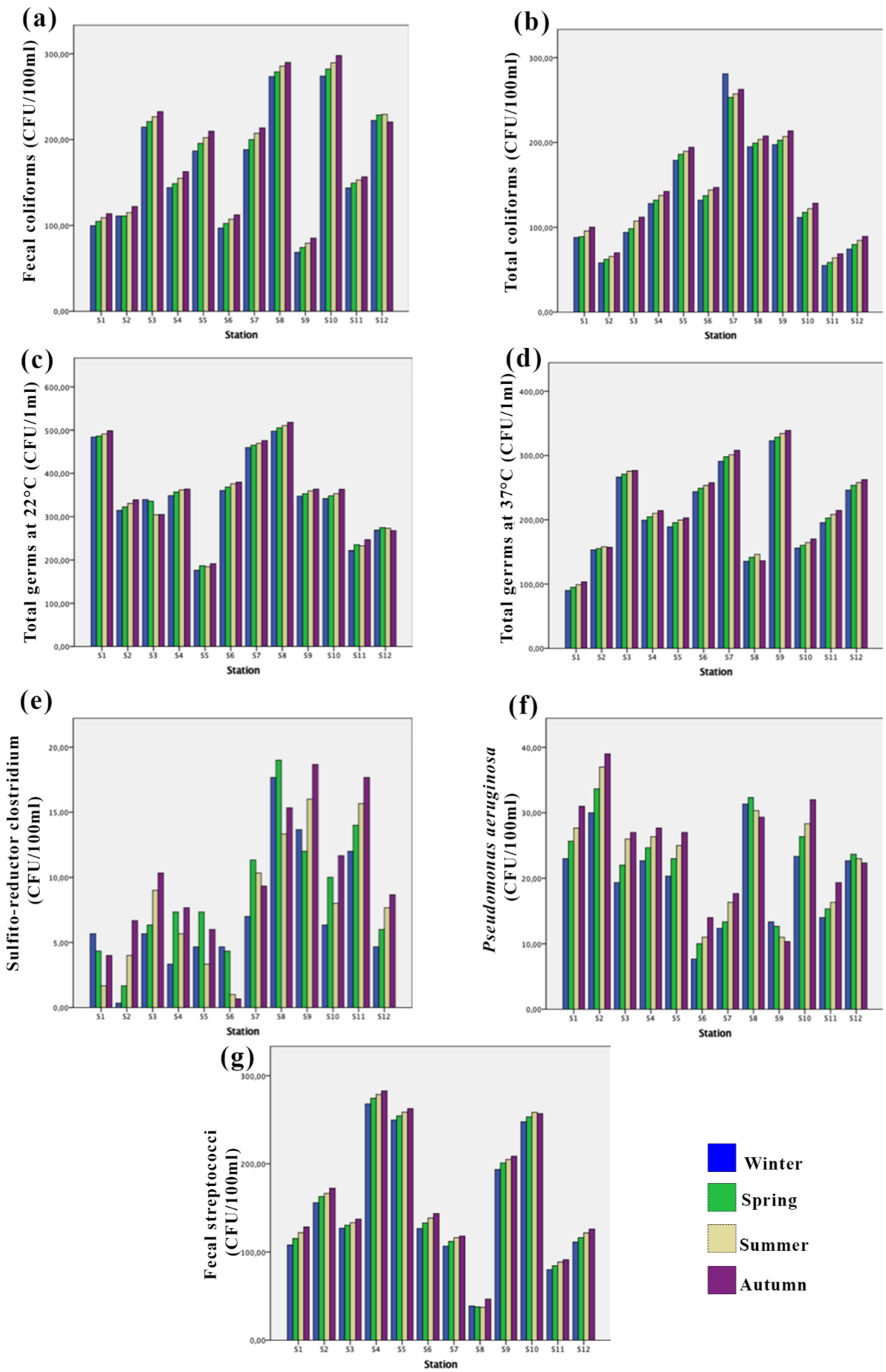


Fig. 2. Seasonal variation of bacterial parameters in the 12 stations.

## Factor analysis

The principal component analysis (PCA) is a technique that attempts to explain the variance of a large dataset of intercorrelated variables with a smaller set of independent variables (**Simeonov *et al.*, 2003**). PCA permit the identification of the main factors that explain the variance of dataset. Two components of PCA analysis showed 58,5% of the variance on the resulted data of groudwater samples of Saïs basin stations (Fig. 3a). The first component (F1) accounted for 32% of the total variance in the dataset of groundwater; the germs, sulftite-reductor clostridium and total germs at 37°C were loaded. The second component (F2), explaining 26% of the total variance, has strong positive loadings with fecal coliforms and total germs at 22°C, indicates prese.

The scores plot (F1 and F2) for the groundwater samples (Fig. 3b) shows high distribution of sulfite redactor clostridium and total germs at 37°C in station 9, which are mostly appeared in the right quadrants while all the sampling sites are appeared in the left und upper quadrant. The fecal coliforms and total germs at 22°C are correlated with the station 10.

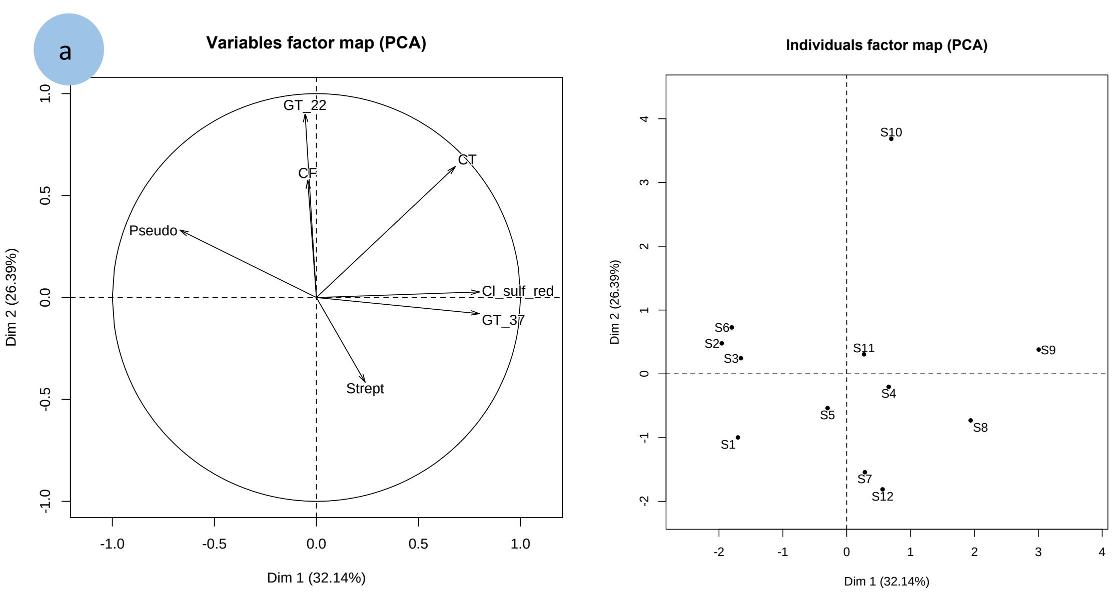


**Fig. 3.** Plots of PCA (a) scores for distribution of microbiological parameters in sub-districts of Saïs basin and (b) scores for combined dataset of groundwater samples.

## CONCLUSION

The results of microbiological analysis of groundwater in Meknes show a high concentration of contamination germs in all wells, constituting a danger for the population of this region where groundwater is the main source of drinking water. The microbiological contamination is due to risk factors such as human activities, lack of well protection structures and anthropogenic activities in the area. In public health terms, recognizing this vulnerability is clearly important in areas. The microbiological analyses do not fully relate with the specific vulnerability classes as a result of WHO recommendations and Moroccan standards.

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