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# Bacteriological Quality Status of Spring Waters from the Taanzoult Plain (Aguelmam Sidi Ali Wetland, Morocco)

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**ABSTRACT**
The Taanzoult plain of Aguelmam Sidi Ali wetland contains several permanent springs of water. They are intended for watering livestock, supplying drinking water and hydrotherapy. Nevertheless, the water consumption from sources in the raw state, without any pre-treatment or hygienic measures, certainly threatens the health of humans and particularly that of traditional medicine patients. From this perspective, the present work is based on a monthly monitoring of eight bacterial germs in the waters of four sources (Aghbalou Aberchane, Aghbalou Dkhiss, Aghbalou Akjdate and forestry post) during a hydrobiological cycle of 12 months from January to December 2017. The results of analyses have shown that bacterial contamination is significant at the water sources, used mainly for watering livestock, compared to those dedicated to drinking and hydrotherapy. The mean content of total coliforms and fecal coliforms at all the stations are higher than the Moroccan standards for surface water intended for the drinking water production. Except for the wet period for Aghbalou Aberchane and Aghbalou Dkhiss waters, the value of the bacterial parameters studied are higher than the Moroccan standards. In general, the anarchic frequentation of the area negatively influences the water quality and compromises its potability. Consequently, it directly threatens the health of users and hydrotherapy patients, especially during the dry season. These findings could call on the health authorities and the competent authorities responsible for controlling the quality of surface water to make users aware of the health risks and provide for appropriate arrangements to improve the quality of the widely sought-after natural waters.

**Keywords:** bacteriology; spring waters; drinking water; livestock watering; water quality; Taanzoult plain; Aguelmam Sidi Ali wetland; Morocco.

## INTRODUCTION

The natural water resources of the Taanzoult plain constitute a biotope for the survival of various organisms having a key role in the trophic chain to maintain the balance of the natural ecosystems of the site. Thus, any thoughtless human intervention could cause imbalance and impact human and animal health [Sammoudi 2021].

Thus, the water consumption from sources in the raw state, without any pre-treatment or hygienic measures, coming or in situ from the Taanzoult plain springs, certainly threatens human health and especially that of hydrotherapy patients.

Aware of the essential role of microorganisms for the proper functioning of aquatic ecosystems and of the impact of the bacterial load resulting from human and animal activities, the knowledge of the bacteriological properties of water resources is a capital necessity to assess its quality and identify its state of pollution both for human consumption and for livestock watering. The bacteriological state of water resources would be an asset in predicting and regulating the proliferation of waterborne diseases that could have an impact on human and animal health.

Therefore, the quantitative and functional importance of microorganisms in aquatic trophic chains has only been seriously considered for about forty years [Pomeroy 1974]. By their abundance, in the digestive tract of humans and warm-blooded animals, fecal coliforms and intestinal enterococci are used as good and very useful indicators for assessing the level of fecal bacteriological contamination of waters [MDDEFP 2013].

This paper focused on the bacteriological characterization of spring waters used for drinking water supply and livestock watering according to current international and national standards and previous studies of similar environments. Thus, a spatiotemporal typology of water quality was highlighted in order to inform the competent authorities about possible health risks.

## MATERIAL AND METHOD

### Study area

The Taanzoult plain is an integral part of the Aguelmam Sidi Ali wetland and requires several conservation statuses (Figure 1). It has been recognized as a site of biological and ecological interest since 1996, classified among the RAMSAR sites since 2005, and has been an integral part of Khenifra National Park since 2012 and of the territory of the Atlas Cedar Biosphere Reserve since 2016. It is crisscrossed by several permanent and intermittent streams fed by meteoritic waters, melting snow fed and giving rise to several permanent and intermittent turgor [Sammoudi 2021].

### Sampling plan

The spatiotemporal sampling plan is based on the abundance of water resources, hydrological

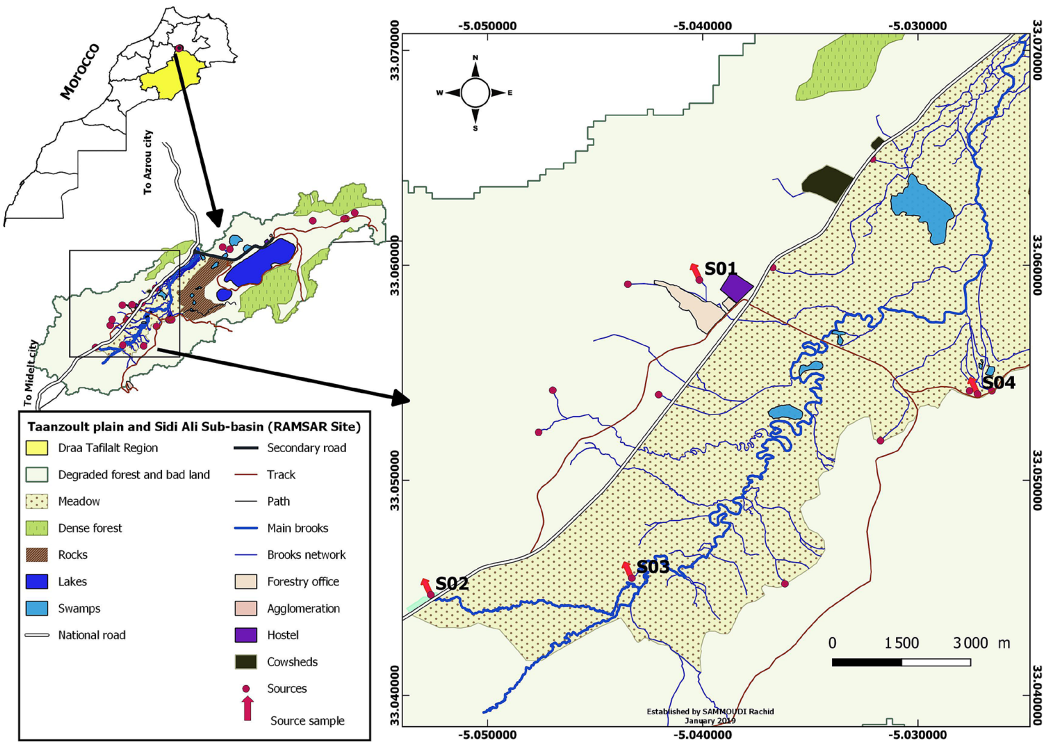


**Figure 1.** Study site and sampling stations

**Table 1.** Description of sampling stations

| Station | Designation | Coordinates | |
|---|---|---|---|
| S01 | Forest post office of Aguelmam Sidi Ali | 33.059324N | -5.040141W |
| S02 | Aghbalou Dkhiss | 33.04468N | -5.052645W |
| S03 | Aghbalou Akjdate | 33.045459N | -5.0433W |
| S04 | Aghbalou Aberchane | 33.054038N | -5.027228W |

properties, topographic and geographic accessibility. For this purpose, the selection focused on four sources gushing out at the level of the Taanzoult plain (Table 1). Sampling was carried out during the hydrobiological cycle from January to December 2017 with a monthly frequency. The sample analysis included 48 samples.

### Monitored parameters

The bacteriological analysis of the sample stations relates to the search for and the enumeration of eight bacteriological indicators of pollution. Therefore, these are total aerobic mesophilic flora (FMAT) at 22 °C and 37 °C, total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), sulfite-reducing anaerobes (ASR), *Staphylococcus aureus* (STA) and *Pseudomonas aeruginosa* (PSE) in accordance with AFNOR standards and protocols by Rodier et al. [Rodier et al. 2009].

### Statistical analysis and typology

All statistics were performed using SPSS V.20 software and results are reported as mean ± standard deviation. The distribution was tested for normality by the Shapiro-Wilk test ($p \leq 0.05$), and the homogeneity of variances was tested by the Levene test. Spatiotemporal variability of bacteriological contamination was tested by means of an analysis of variance (one-way ANOVA) supplemented by Tukey's post-hoc test if the variability was found to be significant. When homogeneity of variance was not achieved, a multiple comparison using Dunnett's T3 post-hoc test was used to explain the bacterial contamination of the spring waters.

The spatiotemporal typology of spring water quality from a bacteriological point of view is based on principal component analysis (PCA) using SPSS 20.0. This analysis made it possible to visualize the spatiotemporal covariation, to know the global links between the different parameters and to establish a spatiotemporal typological structure. In order to do this, the authors first checked whether the considered data are factorizable so that this analysis can be applied to the conducted study [Benzécri 1973; Kaiser 1960]. This involves (i) the existence of correlation between several variables, (ii) the KMO index, which is of the order of 0.8, and (iii) the significance of Bartlett's test of sphericity. According to Kaiser's criterion, the PCA can only be significant if the number of factorial axes retained is less than or equal to 4, with an initial eigenvalue greater than 1 [Kaiser 1960].

## RESULTS AND DISCUSSION

The results of the eight bacteriological parameters monitored are reported in Table 2. Their statistical analysis shows that the homogeneity of variance is significant ($P > 0.05$) spatially for TC, FS, ASR and PSE and only for FMAT-22

**Table 2.** Descriptive statistics (mean ± SD and range (min-max) of bacteriological parameters of spring waters in the Taanzoult plain

| Bacteriological parameters | | S01 | S02 | S03 | S04 |
|---|---|---|---|---|---|
| FMAT_37 (CFU/1mL) | Mean±SD | 406±264 | 109±121 | 255±169 | 289±224 |
| | Min–Max | [18–917] | [16–464] | [52–536] | [12–620] |
| FMAT_22 (CFU/1mL) | Mean±SD | 470±189 | 168±109 | 622±302 | 412±254 |
| | Min–Max | [77–772] | [35–349] | [116–1155] | [84–840] |
| TC (CFU/100mL) | Mean±SD | 2385±4715 | 1954±3397 | 5226±3585 | 2185±3787 |
| | Min–Max | [55–14760] | [85–10380] | [197–11375] | [148–11590] |
| FC (CFU/100mL) | Mean±SD | 66±50 | 35±31 | 110±100 | 48±29 |
| | Min–Max | [3–170] | [3–102] | [17–324] | [2–88] |
| FS (CFU/100mL) | Mean±SD | 17±10 | 9±7 | 14±11 | 8±7 |
| | Min–Max | [1–39] | [1–25] | [1–42] | [1–27] |
| PSE (CFU/100mL) | Mean±SD | 302±444 | 349±303 | 953±660 | 351±237 |
| | Min–Max | [28–1595] | [5–920] | [10–2230] | [22–750] |
| ASR (CFU/20mL) | Mean±SD | 2±3 | 6±15 | 6±11 | 8±27 |
| | Min–Max | [0–12] | [0–51] | [0–38] | [0–97] |
| STA (CFU/100mL) | Mean±SD | 4±5 | 8±8 | 120±114 | 27±27 |
| | Min–Max | [0–15] | [0–25] | [0–280] | [0–80] |

temporally. Thus, the ANOVA is significant ($P<0.05$) only for PSE and FMAT22 in the spatial and temporal planes, respectively.

### Total aerobic mesophilic flora (FMAT)

The total aerobic mesophilic flora (TAMF) is used as an indicator of the overall load. It represents all indigenous and non-indigenous microorganisms capable of multiplying at a temperature varying between 22 and 40 °C [Rodier et al. 2009].

The FMAT count at 37 °C indicates that the average annual content varies between 109±121 CFU/100mL and 406±264 CFU/1mL with a minimum of 12 CFU/1mL recorded in December at S04 and a maximum of 917 CFU/1mL recorded in October at S01. In turn, that at 22 °C indicates that the annual average content varies between 168±109 CFU/1mL and 622±302 CFU/100mL with a minimum of 35 CFU/1mL recorded in December in S02 and a maximum of 1155 CFU/1mL recorded in October in S03. The values recorded in the study area are lower than those recorded in the different wells of the Middle Atlas [Benghuouni et al. 2015] as well as those of Oued Moulouya and Ansegmir and higher in the waters of Hassan II dam in Midelt [Chahboune et al. 2014].

The spatiotemporal variability of both indicators is statistically significant ($p<0.05$). In a second round, the Tukey post-hoc test of FMAT at 22 °C to 37 °C states that there is a significant difference between sources frequented for drinking (S02 and S04) and those for livestock watering (S01 and S03). Similarly, it is also significant between the dry and wet seasons. This could be explained by the increase in temperature, the decrease in water flow and the intensity of human and animal activity in the dry season.

### Total coliforms (TC)

The monitoring results showed that the annual mean TC content varies between 1954±3397 and 5226±3585 CFU/100mL with a minimum of 55 CFU/100mL recorded in January and a maximum of 14760 CFU/100mL in October at station S01 [Aziz et al. 2017; Derfoufi et al. 2020]. The temporal variability is statistically significant ($p<0.05$), while it is spatially insignificant ($p>0.05$). Therefore, Dunnett's T3 post-hoc test states that the TC contamination of spring waters in autumn is different from other seasons. This is justified by the high use of springs for fresh water supply (S02 and S04) and livestock watering (S01 and S03) as well as the lack of dilution caused by the low water flow during the low water period (in autumn).

With regard to water quality, all the values recorded are higher than the guide value of Moroccan standards (<50 CFU/100mL) for surface water likely to be used for drinking water production [MCATUHE 2002]. Overall, a good quality is attributed to the S01, S02 and S04 spring waters and a medium quality to the S03 spring waters.

### Fecal coliforms (FC)

The enumeration of FC in water does not only indicate the degree of contamination of the water by human or animal excreta but also indicates the possible presence of other potentially pathogenic micro-organisms (bacteria, viruses and protozoa...) [Painchaud 2007; WHO 2017]. The presence of these germs in drinking water puts human health at risk [APHA et al. 2017].

The annual average FC content varies between 35±31 CFU/100mL and 100±110 CFU/100mL with a minimum value of 2 CFU/100mL recorded at S04 in December and a maximum content of 324 CFU/100mL recorded at S03 in July. Indeed, the FC contamination of the Taanzoult plain waters is much lower than that of the Hassan II dam waters in Midelt [Chahboune 2015] and that of the Ain Hamma spring (Ben Moussa 2014). The spatiotemporal variability is statistically significant ($p<0.05$). Nevertheless, in a second round, the Dunnett's T3 post-hoc test demonstrates the similarity of springs contamination. Furthermore, this contamination is low in winter compared to the other seasons of the year. This could only be explained by the inaccessibility of the water sources in the winter period, except for source S02 (located in the resting air). Moreover, even if it is accessible, by passengers on the N13 national road, the extreme cold (2 °C to -15 °C) and snow prevent open defecation upstream of source S02.

According to Moroccan quality standards for surface water that could be used for drinking water production [MCATUHE 2002], the quality of the water in springs S01, S02 and S04 is excellent in winter, with a FC content below the Moroccan guide value (<20UFC/100mL), and good quality (20 < <2000UFC/100mL) during the other seasons for the same springs, and during all seasons for spring S03.

### Fecal streptococci (FS)

The annual average FS content oscillates between 8±7 CFU/100mL and 17±10 CFU/100mL with a minimum value of 1 CFU/100mL at all springs in December and a maximum content of 42 CFU/100mL recorded in October at S03. It is therefore lower compared to spring and groundwater in the Meknes area [Belghiti 2015], Oued Khoumane and the Ain Hamma spring [Ben Moussa 2014] and the waters of Lake Batllava [Kashtanjeva et al. 2022].

The spatial variability is statistically insignificant ($p > 0.05$), hence the quasi-similarity of the contamination of the spring waters. In contrast, the temporal variability is statistically significant ($p < 0.05$). Thus, the Dunnett's T3 post-hoc test shaded three statistically different seasonal subgroups of FS contamination, namely G1 (summer and autumn seasons), G2 (spring season) and G3 (winter season). This gradient evolves proportionally with the rate of water source use over the seasons (winter to autumn), which is conditioned by the climate of the area and the accessibility of the water sources (freezing of the meadow and inaccessibility in winter).

Compared to the Moroccan quality standards for surface water that could be used for drinking water production [MCATUHE 2002], the water quality of all the springs is excellent, with FS content below the Moroccan guide value (<20 UFC/100mL), except in September at the level of springs S02 and S04, in autumn at the level of S03 and in summer and autumn at the level of S01 where the quality is good (20 < <2000 UFC/100mL).

### Sulfite-reducing anaerobes (ASR)

The mean ASR content varies between 2±3 CFU/20 mL, recorded at station S01, and 8±27 CFU/20 mL, recorded at station S04, with a maximum content of 97 CFU/20mL recorded in April. This proliferation is remarkable in spring at all stations and in summer at S01 and S03. Moreover, the variability is statistically significant temporally ($p < 0.05$) and spatially non-significant ($p > 0.05$). This observation is inversely proportional to the orthophosphate ($PO_4^{3-}$) content measured at the same stations and same periods [Sammoudi et al. 2020]. Similarly, this contamination is lower compared to that of spring and groundwater in the Meknes area [Belghiti 2015], Oued Khoumane and Ain Hamma spring [Ben Moussa 2014] and surface water in eastern Morocco [Derfoufi et al. 2020] where climatic conditions are less favorable than in the Taanzoult plain.

### Pseudomonas aeruginosa (PSE)

The mean PSE content ranged from 302±444 CFU/100 mL recorded at station S01 to 953±660 CFU/100 mL recorded at station S03. The minimum value of 5 CFU/100 mL was recorded at S02 in January and the maximum of 2230 CFU/100 mL was recorded at S03 in June. The spatiotemporal variability is statistically significant ($p < 0.05$). In fact, the contamination is significantly higher at station S03 than at stations S01, S02 and S04 with a very low contamination in winter compared to other seasons. Compared to similar studies, the contamination found in our sites is lower compared to those of spring and groundwater in the Meknes area [Belghiti et al. 2013] and those of Oued Khoumane and the Ain Hamma spring [Ben Moussa 2014].

### Staphylococcus aureus (STA)

The average STA content ranged from 4±5 CFU/100mL, recorded at station S01, to 120±114 CFU/100mL, recorded at station S03, with a maximum content of 280 CFU/100mL recorded in June. Thus, the spatiotemporal variability is statistically significant ($p < 0.05$). Moreover, the Dunnett's T3 post-hoc test states that there is a significant difference between S03 and the other stations as well as no significant difference between seasons except the winter season which is marked by the absence of this germ. This could be explained by the absence of anthropogenic activity due to inaccessibility during the glaciation period (in winter). Despite the detection of STA in running water, there is no proven health risk from water use to date [LeChevallier and Seidler 1980; WHO 2017].

### Typology

In order to elucidate the confusion of certain spring water samples and to better explain the causality of the impact of human use, particularly during the non-winter period, a typology of bacterial contamination is highlighted. Thus, the principal component analysis highlighted two factorial axes with an initial eigenvalue >1 that

**Table 3.** Total explained variance of bacteriological results of spring waters of the Taanzoult plain

| Component | Initials eigenvalues | | | Extraction sum of squares of the retained factors | | |
|---|---|---|---|---|---|---|
| | Total | % of variance | % cumulatif | Total | % of variance | % cumulatif |
| 1 | 4,460 | 55,749 | 55,749 | 4,460 | 55,749 | 55,749 |
| 2 | 1,106 | 13,827 | 69,576 | 1,106 | 13,827 | 69,576 |
| 3 | ,876 | 10,951 | 80,527 | | | |
| 4 | ,654 | 8,172 | 88,699 | | | |
| 5 | ,335 | 4,182 | 92,881 | | | |
| 6 | ,277 | 3,462 | 96,342 | | | |
| 7 | ,197 | 2,468 | 98,810 | | | |
| 8 | ,095 | 1,190 | 100,000 | | | |

could explain 69.58% of the total variance of the parameters studied (Table 3). Then, the graphical representation will be without rotation on the first two axes, while the other factorial axes with initial eigenvalues less than 1 are neglected due to their non-significance [Kim and Mueller, 1978].

The analysis of the dispersion of the parameters and individuals (observations) represented by the two-dimensional PCA made it possible to conclude that the impact of the season and anthropic activity are preponderant on the contamination of the Taanzoult plain spring waters (Figures 2 and 3). Bacterial pollution of the spring water is therefore remarkable during the summer and autumn seasons, particularly at S03. This could be explained by its open location in a vast meadow amply coveted by herds for grazing during the dry period, thus exceeding the equilibrium load. This overload induces the fecal matter concentration, resulting from animal dejection and micturition, in the absence of dilution due to the low flow rate of springs. This observation is amply confirmed by the results of physicochemical analyses of spring waters during the same

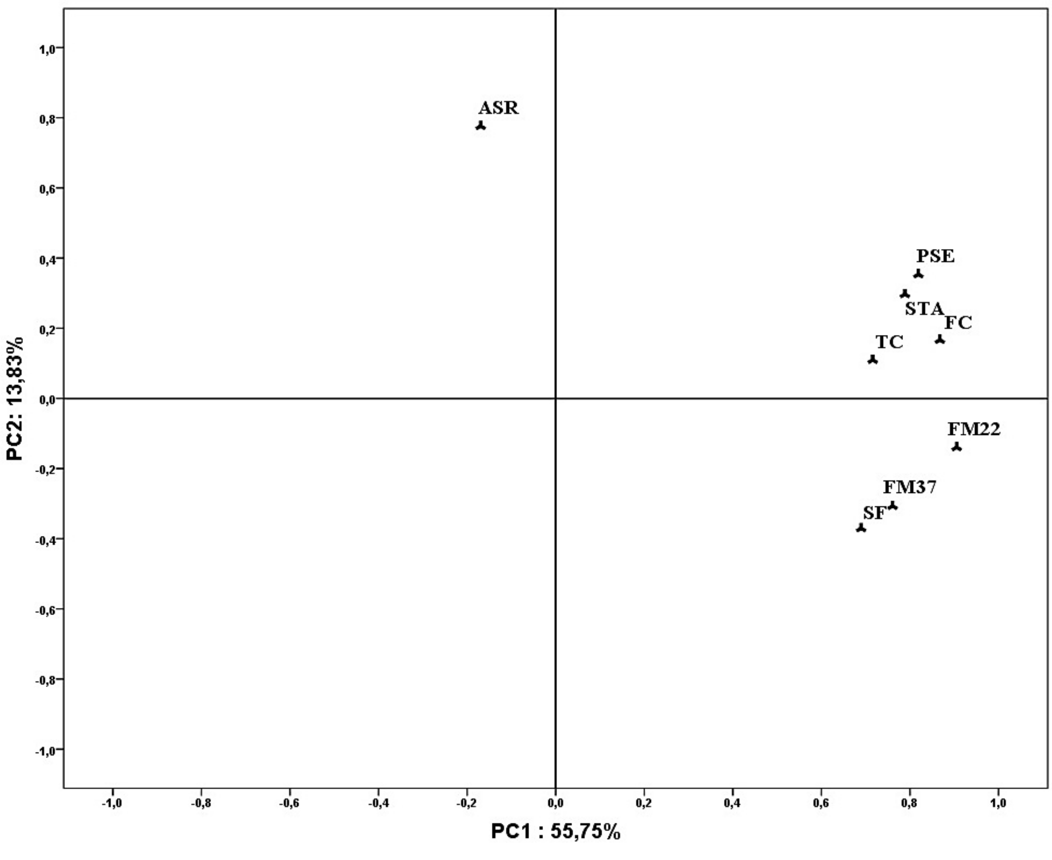


**Figure 2**. Graphical representation of the bacteriological parameters of the Taanzoult plain spring waters in relation to the factorial axes

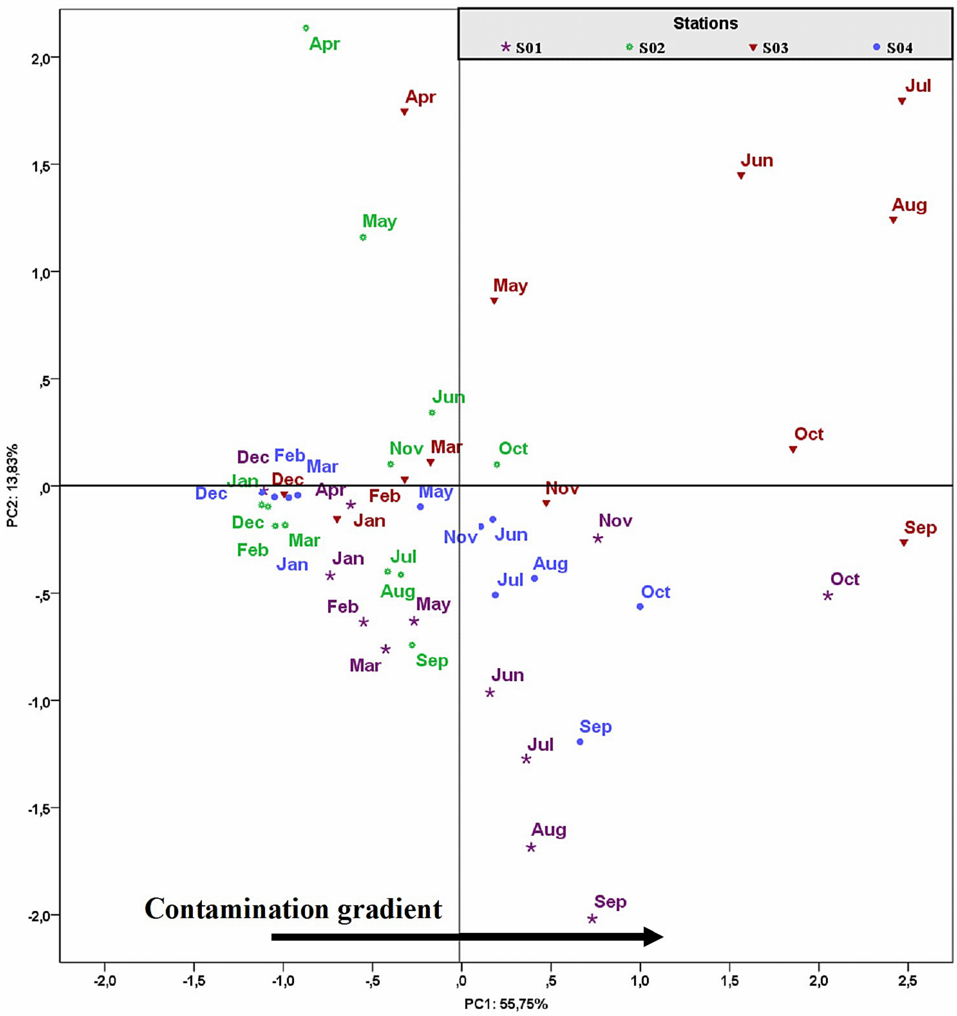


**Figure 3.** Spatiotemporal plot of individuals (observations) of spring waters in relation to the factorial axes

period [Sammoudi et al. 2019]. Despite the low bacterial contamination of spring waters S04, S02 and S01, the impact of the anarchic frequentation of the site by users to obtain fresh drinking water was noted, especially in the hot season when the springs near the settlements dry up. Similarly, because of its alleged therapeutic virtues, S04 is coveted by visitors from all walks of life to obtain water for consumption in situ and/or at home, which increases the degree of pollution of the site (open defecation and accumulation of household waste from their camps in situ) in the absence of any appropriate development that could preserve the ecological role of the Ramsar site.

## CONCLUSIONS

The bacteriological study of the Taanzoult plain waters was able to highlight the site frequentation impact to the evolution of bacterial contamination over the seasons. Indeed, the dry seasons are marked by a strong waters bacterial contamination compared to the humid ones.

The bacterial contamination of spring water is accentuated at sources S01 and S03 (dedicated to watering livestock) compared to source S02 (frequented for drinking water supply) and S04 (frequented mainly for drinking and hydrotherapy). As for the Moroccan standards, only the sources intended for drinking had contents conforming to the Moroccan standards, except for the TC in October and in November, the CF in dry seasons and the SF during the month of September.

Since the site is an essential source of drinking and watering water, it would be wise for the competent authorities to consider appropriate arrangements of the permanent water sources in order to prevent health risks, to improve the quality of the widely sought-after natural and to perpetuate the conservation status attributed by the Ramsar Convention to the Aguelmam Sidi Ali wetland.

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