

Seasonal Bacterial Contamination of Groundwater in the Zagora Area, Morocco

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ABSTRACT

The assessment of the bacterial quality of groundwater is a critical step in ensuring safe drinking water. A total of nine (9) water samples were obtained from wells located within the study area, during the wet and dry seasons between December 2020 and December 2021. The parameters measured in this study include total germs at 22 °C (TG-22 °C) and 37 °C (TG-37 °C), total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), and the sulfite-reductor clostridium (SRC). The results show that the contamination levels vary widely between the different wells and between seasons. The highest levels of contamination were found in W6, with the highest values of all parameters measured except for TC. W1 and W7 were the only wells that were protected, and they had lower levels of contamination compared to unprotected wells. The sources of pollution were mainly related to agricultural activities, such as animal waste and metal waste. The findings of this study suggest the need for improved management practices to reduce contamination and protect the groundwater resources in the study area.

Keywords: groundwater, bacterial quality, contamination, Zagora, Morocco.

INTRODUCTION

Groundwater plays a vital role as a primary water source in the major region, especially arid and semi arid areas and currently, is the most resource used in the different activities such as domestic, irrigation and industrial (Prasad and Narayana., 2004; Danielopol et al., 2003). Indeed, water is a renewable resource that faces constant threats of pollution from various sources, including population growth, agricultural activities, industrial operations, and more. Ensuring access to safe drinking water is essential for maintaining good health, safeguarding basic human rights, and implementing effective health protection

policies. Safe drinking water plays a crucial role in preventing water-related diseases within communities. It has a significant impact on reducing the risk of waterborne illnesses and improving overall public health (WHO, 2011). A significant portion of the population relying on groundwater tends to be impoverished and resides in polluted environments, thereby facing heightened health risks (Andreasen, 1996). The utilization of shallow groundwater for drinking and other household purposes is prevalent among many low-income urban communities in developing regions. (Pedley et al., 1997).

Morocco is among the developing countries that have succeeded relatively well in the

management of water resources. However, this finding should not hide the problems that remain and are likely to worsen and compromise the sustainability of development. Bacteriological groundwater quality plays crucial roles in the intended use of groundwater. The issue of bacterial contamination causing the degradation of groundwater quality has been the subject of numerous studies (Lamrani et al., 2008; Belghiti et al., 2013; Bengoumi et al., 2015; Hameed et al., 2017; Benyoussef et al., 2021; Sammoudi et al., 2022; Hicham et al., 2022; El hammioui et al., 2022).

Water is a fundamental element for human life and activities. It is indispensable for various aspects of our existence, including hydration, sanitation, agriculture, industry, and overall well-being. Most of the people of the Zagora region use groundwater from wells for their daily use and for drinking. The problem of water is a problem of quantity and quality, which are resources and pollution. Natural water sources face the potential danger of contamination from a wide range of contaminant sources (Lukubye et al., 2017). Yet, several natural and anthropogenic factors are known to negatively affect water quality. However, this water is subject to overexploitation and various forms of human-induced water pollution (Boudellah et al., 2022). However, these resources, influenced by both natural and human factors, face daily challenges that contribute to a decline in their sanitary quality. Specifically, indicators of fecal contamination such as total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) have been detected. Total coliforms have traditionally served as indicators of microbial water quality due to their association with fecal pollution. In the Zagora area, the understanding of groundwater quality, specifically from a bacteriological perspective, is limited or non-existent. Few studies have been conducted to assess the quality of groundwater as a drinking water source. This study focuses on investigating the seasonal variation in microbiological contamination of groundwater in the Zagora area.

MATERIALS AND METHODS

Study area

The study site of Bleïda is located in the extreme west of the province of Zagora at the

border with the province of Tata; it is bordered to the west by the province of Tata on the side Souss Massa. This site is part of the dry desert and semi-desert climate. The copper mining site of Bleïda is located in the southeast of the Bou Azzer-El Grâara buttonhole (GPS: 30°21'39.57"N; 6°27'39.37"W), about fifteen kilometers as the crow flies from the village of Bleïda. It can be reached by the road, 50 km long, which branches off to the south-east from the Tazenakht-Bou Azzer-Agdz road. Figure 1 shows the map of sampling station locations at the Bleida Study Site.

Bacteriological analysis

The sampling campaigns were conducted over a period of one year. Glass bottles were sterilized in an oven at 120 °C for one hour and used for sample collection. At each well, a one-liter water sample was collected once a month. The collected water was stored in 500 mL sterile bottles and transported in a cooler to the laboratory. The analyses were performed within four hours of sampling following the bacteriological analysis protocols specified in the Moroccan standards (NM 03.7.001, 2006).

The study focused on the investigation and enumeration of bacteriological pollution indicator microorganisms, including total bacteria (at 22 °C and 37 °C), total coliforms (TC), fecal coliforms (FC), fecal streptococci (SF), and sulfite-reducing clostridia (SRC). The microbiological analysis was carried out using the membrane filter method. A volume of 100 mL of the water sample was filtered, and the membrane was then incubated on specific agar media as described by Rodier et al. (2009).

TTC-Tergitol agar medium was utilized for enumerating total coliforms and fecal coliforms, while Slanetz-Bartley agar was used for fecal streptococci enumeration (Hmid et al., 1997). Incubation of these media at 37 °C for 24 to 48 hours allowed for the enumeration of total coliforms and fecal streptococci, while fecal coliforms were incubated at 44 ± 0.5 °C. The colonies were then counted to determine the microbial load. The outcome is quantified as the number of colony-forming units (CFU) per specified volume. Then, a Principal Component Analysis (PCA) of the mean values of the results was performed. The software used was version 10 of the STATISTICA software.

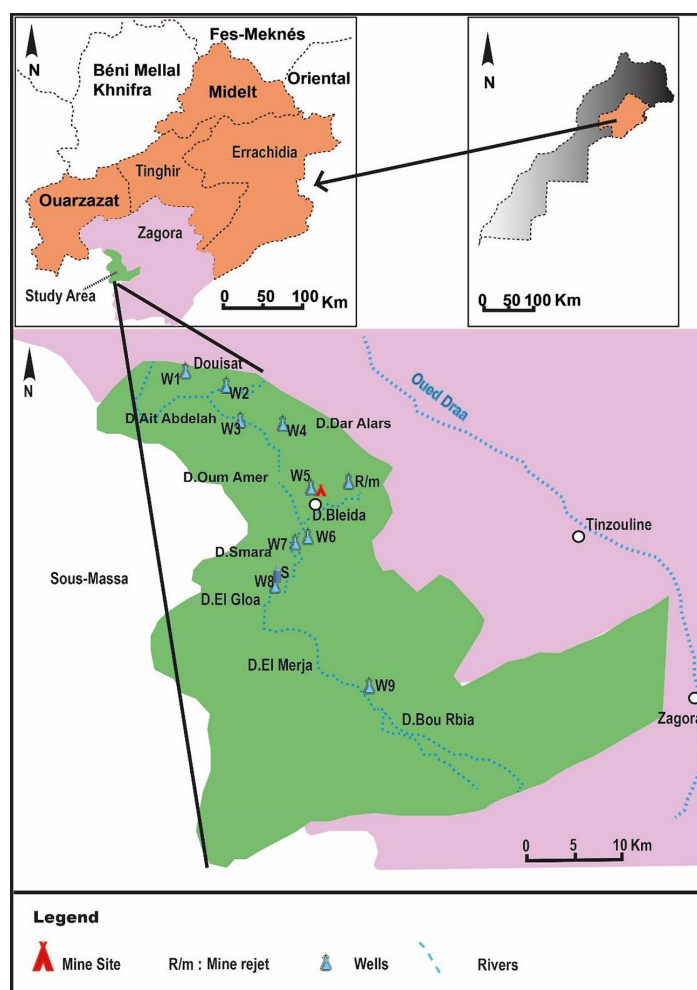


Figure 1. Study area (W = wells)

RESULTS AND DISCUSSION

The results of the bacteriological parameters of the studied water stations are summarized in Table 1.

Total germs

The results presented show the total germs (TG) content of groundwater at 22 °C and 37 °C

in different seasons (summer, winter, spring, and autumn) for nine different wells (W1–W9). Total germs (at 22 °C and at 37 °C) are a measure of the total bacterial population in water and are an important parameter for assessing the microbiological quality of water.

Based on the results, it is evident that the total germ content (at 22 °C and 37 °C) exhibits significant variations across different seasons and

Table 1. Results of bacteriological analysis of water from the surveyed stations in the study region (average values)

Sample	Total germs (at TG-22°C)	Total germs (at TG-37°C)	Total coliforms (TC)	Fecal coliforms (FC)	Fecal streptococci (SF)	Sulfito-reductor clostridium (SRC)
W1	440.5	303.5	1356.75	700.25	1409.75	35
W2	504.5	323.75	849.75	559.33333	913.25	64.5
W3	602.75	359.75	1692.5	885	1541	89
W4	836.75	536	1319.5	897.41667	1355	109.5
W5	304.75	191.25	1891.5	795.833333	1486.5	79
W6	1051.25	760.75	3574	1795.3333	7118.75	137
W7	877	651.5	2609.25	1379.25	2635	106.5
W8	659.5	358.75	437	485.08333	303.5	36
W9	421.25	308.25	1967	898.833333	2477.5	11.75

wells. For example, in summer, the TG content of W7 is much higher than that of W1, W2, and W3. Similarly, in winter, the TG content of W6 is much higher than that of W2, W3, and W8 (Table 1). This suggests that there may be seasonal and spatial variations in the microbial content of groundwater. It is also important to note that some wells show high TG content across all seasons, such as W6 and W7, while others show consistently lower levels, such as W1, W2, W3, and W8. This may be due to differences in the sources of contamination or the quality of well construction and maintenance. In fact, the total germs content at 37 °C is generally lower than that obtained at 22 °C, which may be due to the fact that some microorganisms prefer a lower temperature range for growth and reproduction. The high values of the total amount of bacteria obtained in the sampling wells of the study region are comparable to the values recorded in the aquifer of the Plateau of Saïs by Said Lotfi et al., (2020), and similar findings have been reported by El Moustaine et al. (2014) in the Meknes aquifer.

The results indicate the presence of potential seasonal and spatial variations in the microbial content of groundwater. Therefore, it is crucial to conduct regular monitoring and assessment of the bacterial quality of groundwater to ensure the safety of drinking water. In cases where high total germ content is detected, appropriate corrective measures should be implemented to prevent future contamination and ensure that the water is safe for consumption.

Total coliforms

The results presented in Figure 2c show the total coliform (TC) content of groundwater in different seasons (summer, winter, spring, and autumn) for five different wells (W1-W5) measured in CFU/100ml. Total coliforms are a collection of bacteria that are frequently encountered in the natural surroundings, and their detection in water signifies the possible existence of additional detrimental bacteria, viruses, and parasites. Moreover, coliforms are of interest because a very large number of them live in abundance in the feces of hot-blooded animal feces and therefore constitute fecal indicators of primary importance (Rodier et al., 2009). It is crucial to emphasize that the presence of coliform bacteria does not automatically imply that the water is unfit for consumption. However, it

does indicate that the water may be at a higher risk of contamination by harmful microorganisms. Further testing and assessment, including testing for specific pathogenic microorganisms, should be conducted to determine the safety of the water for consumption.

The TC content varies widely across different seasons and wells. For example, in spring, W4 shows the highest TC content, while in winter, W2 shows the highest TC content. Wells W6, W7 and W9 contained most of the total coliforms positive results. The seasonal variation of these germs showed a significant microbial load during the winter season. This suggests that there may be seasonal and spatial variations in the presence of coliform bacteria in groundwater. The results suggest that regular monitoring and assessment of the bacterial quality of groundwater is necessary to ensure the safety of drinking water. If high TC content is detected, appropriate corrective measures should be taken to prevent future contamination.

Fecal coliforms

The results depicted in Figure 2d display the levels of fecal coliform (FC) in groundwater during different seasons (summer, winter, spring, and autumn) across nine distinct wells (W1-W9), measured in CFU/100ml. fecal coliforms are a subset of coliform bacteria that originate in the intestinal tracts of warm-blooded animals. They are commonly used as an indicator of fecal contamination in water (Elmund et al., 1999). Fecal coliforms are the most frequently employed bacterial group in water bacteriological analysis. The presence of fecal coliforms in water suggests the potential presence of microorganisms that can cause gastrointestinal illnesses. Consequently, the detection of fecal coliforms in water intended for consumption indicates a serious fecal contamination issue (Goodman et al., 1982).

Referring to our result, we can see that the FC content varies widely across different seasons and wells. For example, in summer, W6 and W7 show the highest FC content, while in winter, W9 shows the highest FC content. This suggests that there may be seasonal and spatial variations in the presence of fecal coliform bacteria in groundwater.

The detection of fecal coliform bacteria in water suggests that it has been compromised by fecal contamination, which may harbor detrimental

microorganisms like *E. coli*, *Salmonella*, and *Giardia*. These microorganisms have the potential to induce gastrointestinal illnesses, diarrhea, and other health complications, especially among susceptible groups such as young children, the elderly, and individuals with weakened immune systems. It is crucial to acknowledge that the presence of fecal coliform bacteria in groundwater signifies a potential health hazard, and it is necessary to implement suitable measures to prevent future contamination. The results suggest that regular monitoring and assessment of the bacterial quality of groundwater is necessary to ensure the safety of drinking water. If high fecal coliform content is detected, appropriate corrective measures should be taken to prevent future contamination and ensure that the water is safe for consumption.

Fecal streptococci

The presence of fecal streptococci is generally indicates of fecal pollution. Our results show the Fecal Streptococci (FS) content of groundwater in different seasons (summer, winter, spring, and autumn) for nine different wells (W1–W9) measured in CFU/100 ml. Fecal streptococci are a collection of bacteria typically present in the intestines of humans and other warm-blooded animals, and they are utilized as an indicator of fecal contamination in water. Additionally, coliforms and fecal streptococci are the predominant bacterial groups employed in water bacteriological analysis, as stated by Poole and Hobson (1979). The fecal streptococci were detected in several wells, especially in W6, W7 and W9. Moreover, the FS content varies widely across different seasons and wells. For example, in summer, W6 shows the highest FS content, while in winter, W4 shows the highest FS content. This suggests that there may be seasonal and spatial variations in the presence of fecal streptococci bacteria in groundwater. In fact, the seasonal variation of these germs showed a significant microbial load during all seasons. This finding underscores the importance of regular monitoring and evaluation of the bacterial quality of groundwater to guarantee the safety of drinking water. In cases where elevated levels of fecal streptococci are detected, it is crucial to implement appropriate corrective measures to prevent future contamination and ensure the water's suitability for consumption.

Based on our surveys of the studied wells, we have noticed the presence of manure, some septic tanks, latrines, and various types of waste in the surrounding areas of the wells. These elements could be the main factors responsible for the contamination of these water resources, resulting in fecal contamination.

Sulfito-reductor clostridium

Sulfito-reductor clostridium (SRC) bacteria are classified as strict anaerobes. They are Gram-positive, spore-forming microorganisms capable of reducing sulfites to hydrogen sulfide. SRC bacteria have a longer survival duration in water compared to other fecal pollution indicator microorganisms such as coliforms and streptococci (Regnault, 1990). Due to their exceptional resistance, the detection of SRC spores can indicate past or sporadic fecal pollution events (Rodier et al., 2009). SRC bacteria are a group of anaerobic bacteria that are commonly found in soil and water. SRC bacteria play a significant role in the biogeochemical cycle of sulfur, an essential element for various biological processes. However, certain strains of SRC can pose health risks, such as causing gastroenteritis, especially when found in high concentrations in drinking water. It is crucial to monitor and control the levels of SRC bacteria to ensure the safety of drinking water and minimize potential health issues.

The results of the Sulfito-reductor clostridium (SRC) analysis show that the microbial population varies significantly between seasons and wells. The highest SRA counts were observed in spring and autumn, with wells W1 and W3 showing the highest levels. In contrast, the lowest counts were observed in winter, with wells W1 and W8 showing the lowest levels. The presence of SRA bacteria in the groundwater can be attributed to several factors, such as agricultural activities, sewage contamination, and geological characteristics of the area. The high counts observed in spring and autumn may be due to increased precipitation and runoff, which can carry contaminants from the surface into the groundwater. The low counts observed in winter may be due to decreased biological activity in the cold temperatures. These results corroborate those found in another prospected region by El moustaine et al. (2014) in the Meknes region.

In summary, the findings of this study indicate that the microbial characteristics of

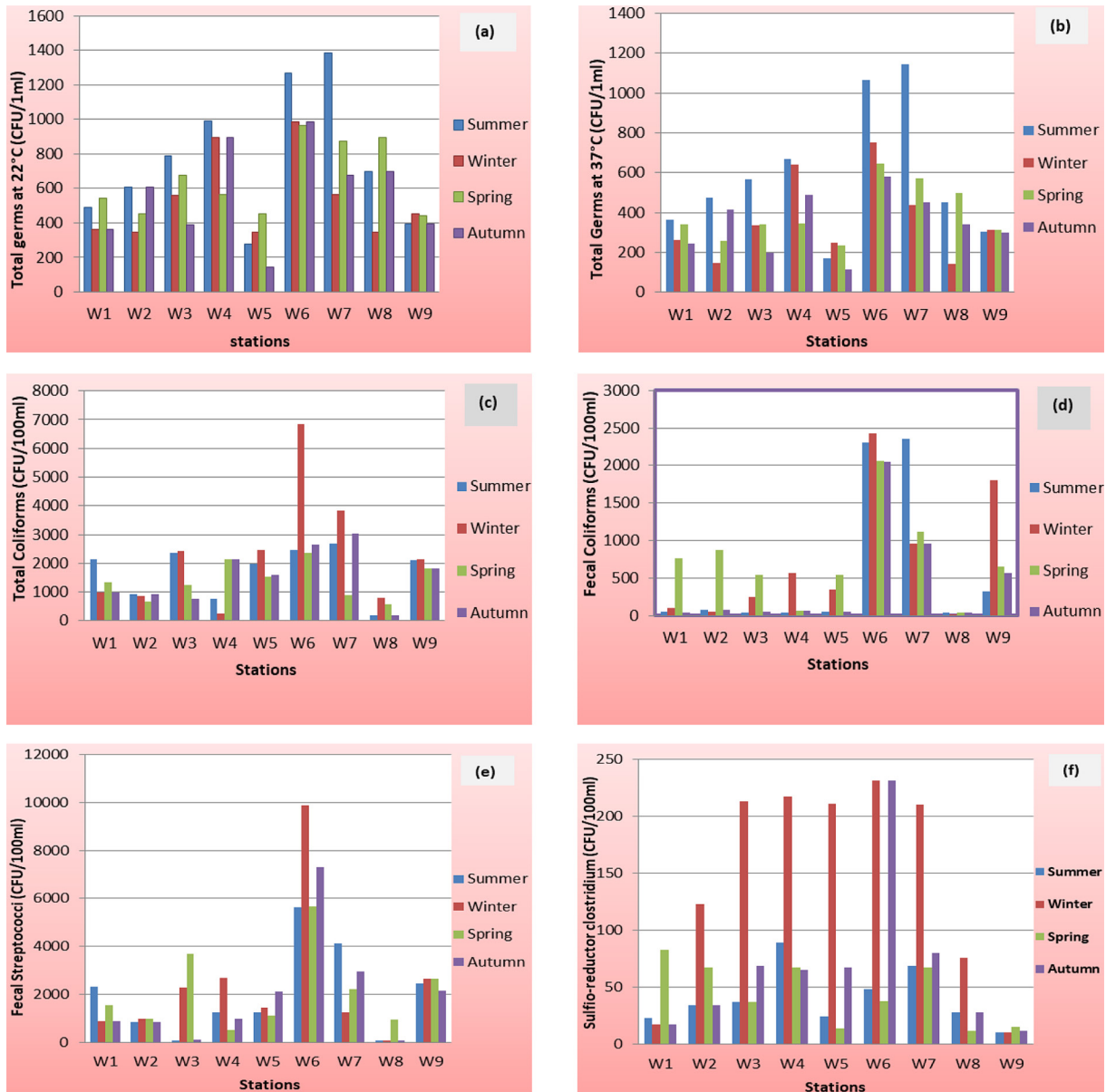


Figure 2. Seasonal bacterial variation in the 9 sampled station

groundwater in the Zagora region are subject to seasonal variations and the geological attributes of the area. To ensure the safety of drinking water sources in the region, it is crucial to consistently monitor the levels of SRA and other microbial populations in the groundwater. Regular monitoring efforts are essential in maintaining the quality and safety standards of the area’s drinking water sources.

Typology of the investigated wells

The projection of the variables on the F1–F2 factorial plane, as depicted in Figure 3, reveals a negative correlation between (TG at 22 °C), (TG at 37 °C), (CT), (CF), (SF), and (SRA) with

respect to F1. F1 represents the primary axis that indicates the major parameters influencing the level of bacteriological contamination in the well waters. It distinguishes highly polluted waters from those with lower or minimal contamination. The combination of F1 and F2 in the Principal Component Analysis (PCA) accounts for a satisfactory variance, explaining 92.82% of the total variance, as illustrated in Figure 4.

Furthermore, the hierarchical classification analysis (HCA) was conducted on the stations based on the PCA results, resulting in the identification of three spatially distinct sample groups (Figure 5).

- Group 1 (C1) – this cluster brings the water samples (W6, W7, W9), which present a bacteriological quality very poor, relatively more

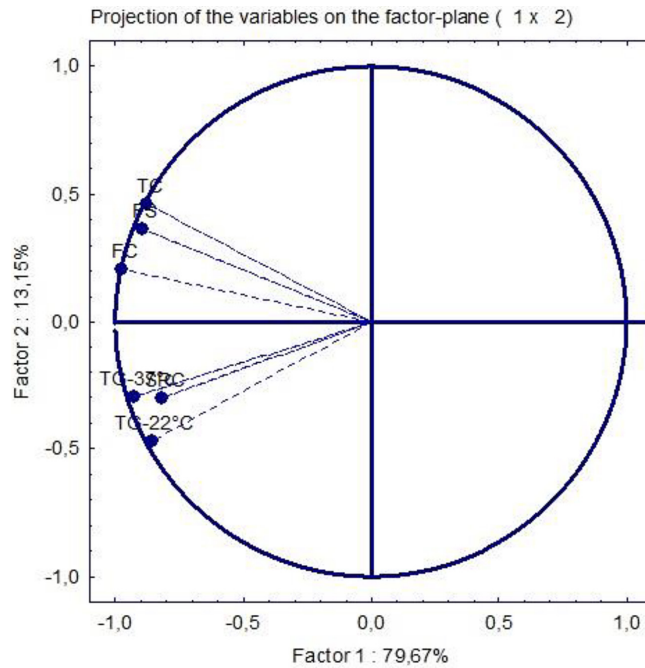


Figure 3. Projection of the bacteriological parameters of water from the 9 stations listed in Table 1 onto the plane defined by the first two factorial axes of the PCA

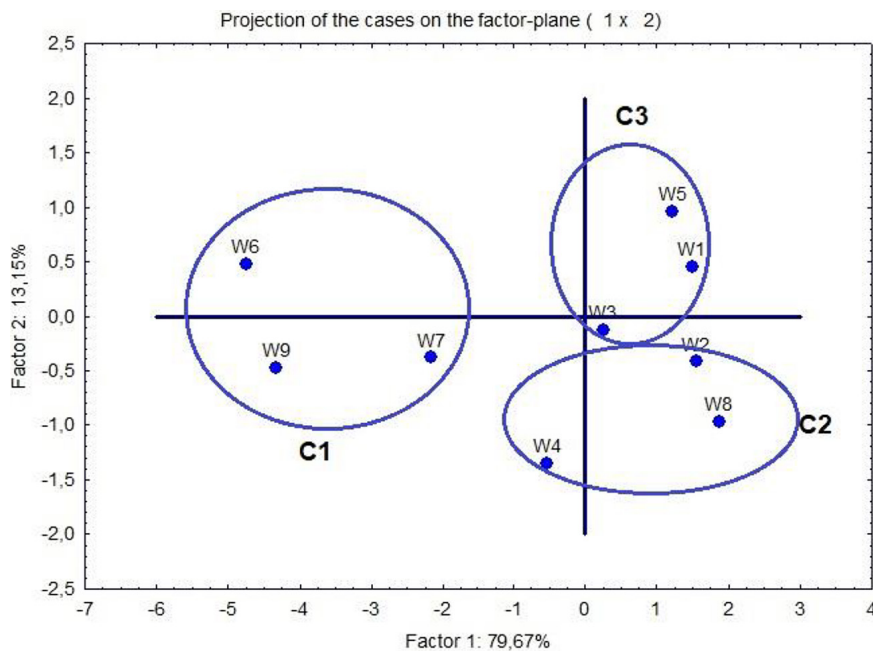


Figure 4. Projection of the 10 stations in Zagora onto the plane defined by the first two factorial axes of the PCA obtained from the matrix of stations/ bacteriological parameters of water from the 9 wells

contaminated with all germs of fecal contamination: GT at 22 °C, GT at 37 °C, total coliforms, fecal coliforms, fecal streptococci and Sulfitorreductor anaerobes; which greatly exceeds Moroccan standards (NM 03.07.001, 2006).

- Group 2 (C2) – the second cluster contains the wells (W2, W4, and W8), with water characterized by a very high rate in the concentration

of the germs indicators of fecal pollution in particular the fecal coliforms (FC).

- Cluster 3 (C3) – this group is composed of wells: W1, W3, and W5, which also present water with bacteriological quality poor, but relatively less contaminated with germs of fecal contamination, in comparison with the wells of the first cluster.

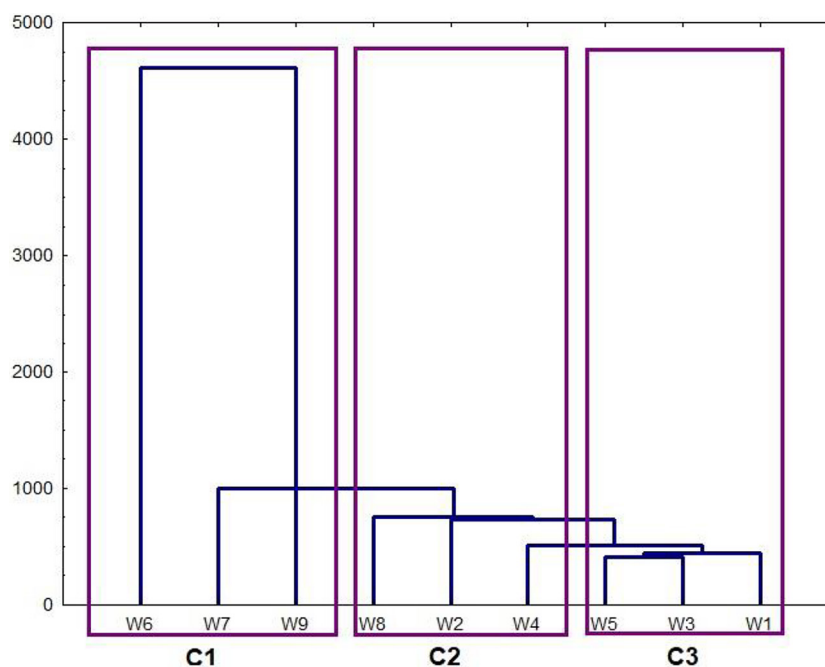


Figure 5. Dendrogram representing the hierarchical classification of the 9 stations in Zagora

CONCLUSIONS

The microbiological parameters examined, including total germs at 22 °C (TG-22 °C) and 37 °C (TG-37 °C), total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), and sulfite-reductor clostridium (SRC), are commonly used to evaluate the quality of water sources, including groundwater. Based on the analysis of these parameters, it can be inferred that the studied wells are most likely groundwater wells used for drinking water supply or irrigation purposes. The inclusion of samples from different seasons suggests that the study was conducted over a year to assess the seasonal variations in microbial contamination of the groundwater. The presence and levels of these microorganisms can indicate contamination from various sources, such as sewage, animal waste, or agricultural runoff. These contaminated waters pose a significant health risk to humans. Additionally, the high bacterial contamination of the wells may be attributed to poor well protection, such as open wells.

REFERENCES

- Andreasen, J. 1996. Urban tenants and community involvement. *Habitat Int*, 3, 359–65.
- Bengoumi, D., Chahlaoui, A., Belghiti, L., Taha, I., Samih, M., El Moustaine, R. 2015. Etude de la qualite bacteriologique de l'eau de certains puits dans les elevages avicole (Meknes et Gharb -Maroc). *Larhyss Journal*, 24, 209–226.
- Benyoussef, S., Arabi, M., El Ouarghi, H., Ghalit, M., Azirar, M., El Midaoui, A., Ait Boughrou, A. 2021. Impact of Anthropogenic Activities on the Quality of Groundwater in the Central Rif (North Morocco). *Ecological Engineering & Environmental Technology*, 22(6), 69–78. <https://doi.org/10.12912/27197050/141525>.
- Belghiti, M.L., Chahlaoui, A., Bengoumi, D., El Moustaine, R. 2013. Etude de la qualite physico-chimique et bacteriologique des eaux souterraines de la nappe plioquaternaire dans la region de Meknes (Maroc). *Larhyss Journal*, 14, 21–36.
- Boudellah, A., El Moustaine, R., Maliki, A., Moutaouakil, S., El Gharmali, A., Boulanouar, M., El Hachimi, Y and Ghamizi M. 2022. Impacts of Anthropogenic Factors on the Groundwater Ecosystem of Fezouata in South-East of Morocco". *Journal of Ecological Engineering*, 23(5), 30–43. <https://doi.org/10.12911/22998993/146678>
- Danielopol, D.L., Griebler, C., Gunatilaka, A., Nottenboom J. 2003. Present state and future prospects for groundwater ecosystems, *Environmental Conservation*, 30, 104-130.
- El Hammioui, Y., Anarghou, H., Belghiti, M., Hachi, T., Elhamdouni D., Laiboud, C., Essabiri, H., Boumalkha O., Khaffou M., and Abba, E.H. 2022. Evaluation of the metallic and bacteriological quality of well water in Khenifra province (Morocco). *IOP Conf. Series: Earth and Environmental Science*, 1090, 012031. DOI: 10.1088/1755-1315/1090/1/012031

8. El Moustaine, R., Chahlaoui A., Rour, E.H., Bengoumi D., Belghiti, L. 2014. Qualité bactériologique des eaux de quelques puits et sources dans la région de Meknès (Maroc). *Rev. Microbiol. Ind. San et Environn.*, 8(1), 39–53.
9. Elmund, G.K., Allen, M.J., Rice, E.W. 1999. Comparaison of *Escherichia coli*, total coliform and fecal coliform populations as indicators of wastewater treatment efficiency. *Water Environ. Res.*, 71, 332–339.
10. Goodman, R.A., Greenberg, H.B., Mckinley, T.E., Smith, J.D. 1982. Norwalk gastroenteritis with water system in rural Georgia community. *Archives of Environmental health* 37, 358–360.
11. Hameed, S., Abdelhakim, A.Y., Mohammed, J., Zouheir, C., Abdelhafid, C. 2017. Microbiological, physicochemical, and heavy metals assessment of groundwater quality in the Triffa plain (eastern Morocco). *Appl Water Sci.* <https://doi.org/10.1007/s13201-017-0598-1>.
12. Hicham, G., Mustapha, A., Mourad, B., Abdelmajid, M., Ali, S., Yassine, E. Y., Mohamed, C., Ghizlane, A., Zahid, M. 2022. Assessment of the physicochemical and bacteriological quality of groundwater in the Kert Plain, northeastern Morocco. *Int J Emerg Water Res*, 6, 133–147. <https://doi.org/10.1007/s42108-021-00157-x>.
13. Lamrani, A.H., Oufdou, K., Mezrioui, N. 2008. Environmental pollutions impacts on the bacteriological and physicochemical quality of suburban and rural groundwater supplies in Marrakesh area (Morocco). *Environ Monit Assess*, 145(3), 195–207.
14. Lukubye, B., Andama, M. 2017. Bacterial analysis of selected drinking water sources in Mbarara municipality, Uganda, *J. Water Resour. Prot.* 9, 999–1013. <https://doi.org/10.4236/jwarp.2017.98066>.
15. NM 03.7.001. 2006. Norme marocaine relative à la qualité des eaux d'alimentation humaine. *Bulletin Officiel N° 5404*.
16. Pedley, S., Howard, G. 1997. The public health implication of groundwater microbiology. *Q. J. Eng. Geol.*, 30(2), 179–88.
17. Poole, N.J., et Hobson, P.N. 1979. Water pollution and its prevention. *Microbial. Ecology.*, 226–245.
18. Prasad, B.G., Narayana, T.S. 2004. Subsurface Water Quality of Different Sampling Stations with Some Selected Parameters at Machilipatman Town, *Nature, Environment and Pollution Technology*, 3(1), 47–50.
19. Regnault, J.P. 1990. *Microbiologie générale. Microorganismes de l'eau*. Ed. Vigot. Paris, 580–609.
20. Rodier J., Legube B., Merlet N., et Coll. 2009. *L'analyse de l'eau. Eaux naturelles, eaux résiduaires, eau de mer*. Dunod, 9e éd., 1579.
21. Said, L., Miloud, C., Mohamed, N., Fatima Zahraa, T., Abdelftah, B., Khadija, E.K., Driss B. 2020. Assessment of microbiological quality of groundwater in the Saïs plain (Morocco). *Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt*, 24(1), 509–524.
22. Sammoudi, R., Chahlaoui, A., Ouarrak, K., Taha, I., Khaffou, M. 2023. Bacteriological Quality Status of Spring Waters from the Taanzoult Plain (Aguelmam Sidi Ali Wetland, Morocco). *Journal of Ecological Engineering*, 24(4), 184–191. <https://doi.org/10.12911/22998993/159634>
23. WHO. 2011. *Guidelines for drinking-water quality*, 4th Edn. World Health Organization, Geneva.