

Assessment of the Microbiological, Physicochemical, Toxic and Radioactive Quality of Thermal Waters for Therapeutic Purposes

Tirinsi Mohamed^{1,2*}, Doha Berraaouan³, Bouchra El Guerrouj^{1,2}, Yousra Hammouti¹ and Mohammed Melhaoui¹

1. Laboratoire d'Amélioration des Productions Agricoles, Biotechnologie et Environnement (LAPABE), Faculté des Sciences, Université Mohammed Premier, Oujda 60000, MOROCCO

2. Centre de l'Oriental des Sciences et Technologies de l'Eau et de l'Environnement (COSTEE), Université Mohammed Premier, Oujda 60000, MOROCCO

3. Physical Chemistry of Natural Substances and Process Research Team, Laboratory of Applied Chemistry and Environment (LCAE-CPSUNAP), Faculty of Sciences, Université Mohamed Premier, Oujda, MOROCCO

*tirinsim@yahoo.fr

Abstract

Thermal springs in Morocco represent an invaluable asset frequently used by the population for various therapeutic purposes. However, due to its parameters and therapeutic effects, the Fezouane Spring, which has been one of the best-known spas in Morocco's eastern region for several decades, may be exposed to various pollution risks. In this context, we conducted this study to improve physicochemical, bacteriological and radioactive quality from January 2021 to December 2021, following standardized protocols for spa waters in Morocco. The results show that the water from this spring is mesothermal, with an average annual temperature of 37.54°C, neutral pH, high levels of bicarbonate (365.41mg/l), calcium (65.37mg/l) and magnesium (38.39mg/l). In addition, nitrate, lead and selenium were present at mean concentrations of 3.53 mg/l, 0.001 and 0.0006 ± 0.00 respectively. Conversely, nitrite, arsenic, manganese, copper and mercury were absent. Bacteriological analyses showed the absence of pathogenic microorganisms.

In addition, the results of radioactive analyses of the plant's water revealed compliance with Moroccan standards. These results underline the compliance of Fezouane spring water with Moroccan potability standards NM 03.7.001. According to the Piper diagram, the water type is bicarbonate-calcium-magnesium. Based on these results, we conclude that thermalism specifically in Fezouane in Berkane, will contribute to sustainable development and will serve as an effective complementary approach in the treatment of various kidney pathologies.

Keywords: Assessment, Quality, Microbiological, Physicochemical, Toxic, Radioactive, Thermal, Waters, Therapeutic.

Introduction

Water is a non-substitutable resource essential to the survival of all living beings. However, it can contain contaminants responsible for the transmission of water-borne

diseases^{10,12,16}. It is a vital nutrient involved in numerous crucial physiological functions such as digestion, absorption, thermoregulation and waste elimination^{2,6}. Therefore, a special attention must be paid to the potabilization of water intended for human consumption. This water must be free from hazardous chemical substances and harmful pathogens that could, in the short or long term, harm human health.

Moreover, groundwater reservoirs, often geologically sheltered, are exposed to agricultural, industrial, and/or urban pollutants, precipitating alteration of their physico-chemical composition¹³. As a result, they can act as conduits for potentially harmful agents, including heavy metals and pathogenic microorganisms, precipitating cases of waterborne disease^{8,11,17}. While the benefits of mineral-rich thermal waters are widely documented^{5,21}, few studies have examined the potential risks associated with contamination of these waters by microbiological, chemical and radioactive pollutants.

Previous studies suggest that the composition of these waters is related to complex underground processes. Still, their vulnerability to pathogens and toxic substances remains largely unexplored³. In Morocco, the oriental region boasts important thermal sites, prompting government entities to define objectives and strategic plans to foster thermal tourism development²⁰. However, the Fezouane thermal spring is widely used by the local population and visitors for therapeutic purposes without any knowledge of the health risks associated with the contamination of these waters by various microbiological, chemical and radioactive contaminants.

In this context, the present study aims to assess the quality of water from the Fezouane Spring in terms of health safety by verifying the contamination of these waters by pathogenic microorganisms and also to examine the compliance of these waters with current Moroccan standards (standard 03.7.001) concerning physicochemical, toxicological and radiological parameters.

By filling this gap, this study aims to shed light on potential health risks and provide recommendations for the sustainable management of thermal springs in the region.

Material and Methods

Study area: The Fezouane thermal spring is in the center of the Fezouane commune, part of the Berkane province in the Oriental region. Geographically, Fezouane is at coordinates: Latitude: 34.9164, Longitude: -2.20417 (34° 54' 59" North, 2° 12' 15" West), at an altitude of 258 meters, with a population of 5,089 inhabitants as depicted in figure 1.

Sampling: Sampling was conducted monthly throughout the year 2021 at the Fezouane Spring. Samples were collected aseptically in sterile bottles of 1000, 500, 250 and 50 ml, following the standards described in Rodier et al¹⁸. Then, they were transported to the Hydrology and Laboratory of Thermalism at the Department of Biology and Toxicology at the National Institute of Hygiene (INH) in Rabat, Morocco, in isothermal containers at 4°C and analyzed within eight hours of collection.

Physicochemical, Microbiological and Radiological Analyses: Physicochemical parameters for Fezouane water were assessed on-site for qualitative measurements and in the laboratory. Temperature, pH and conductivity were measured on-site and complemented in the laboratory. The

pH and electrical conductivity measurement of Fezouane water were carried out according to Moroccan standards 03-7-009 (2012) and NM 03-7-011 (2001) respectively. Nitrates and nitrites were measured in the laboratory by molecular absorption spectrometry and automated continuous flow analysis based on electromagnetic radiation ranging from ultraviolet to radio waves with a wavelength of $\lambda=415$ nm.

Elements such as Na^+ and K^+ were determined by flame atomic emission spectrophotometry using a Perkin Elmer 430 spectrophotometer, while Ca^{2+} and Mg^{2+} were quantified using volumetric complexation with Ethylene-Diamine-Tetraacetic-Acid (EDTA) described by Rodier et al¹⁸. Chloride (Cl^-) and bicarbonate (HCO_3^-) were measured by volumetric titration using the mercuric nitrate method and titrimetry method, respectively, according to Moroccan standards for water NM03-7-024 (1990).

The electro thermal atomic absorption technique was employed to analyze trace metallic element pollutants (TMEP), including Cu, Mn, As, Se, Ba, Cd, CN⁻, Cr, Mn, fluoride, Pb, Se, B, Ni and Hg. Pesticide residues were determined using gas chromatography (GC).

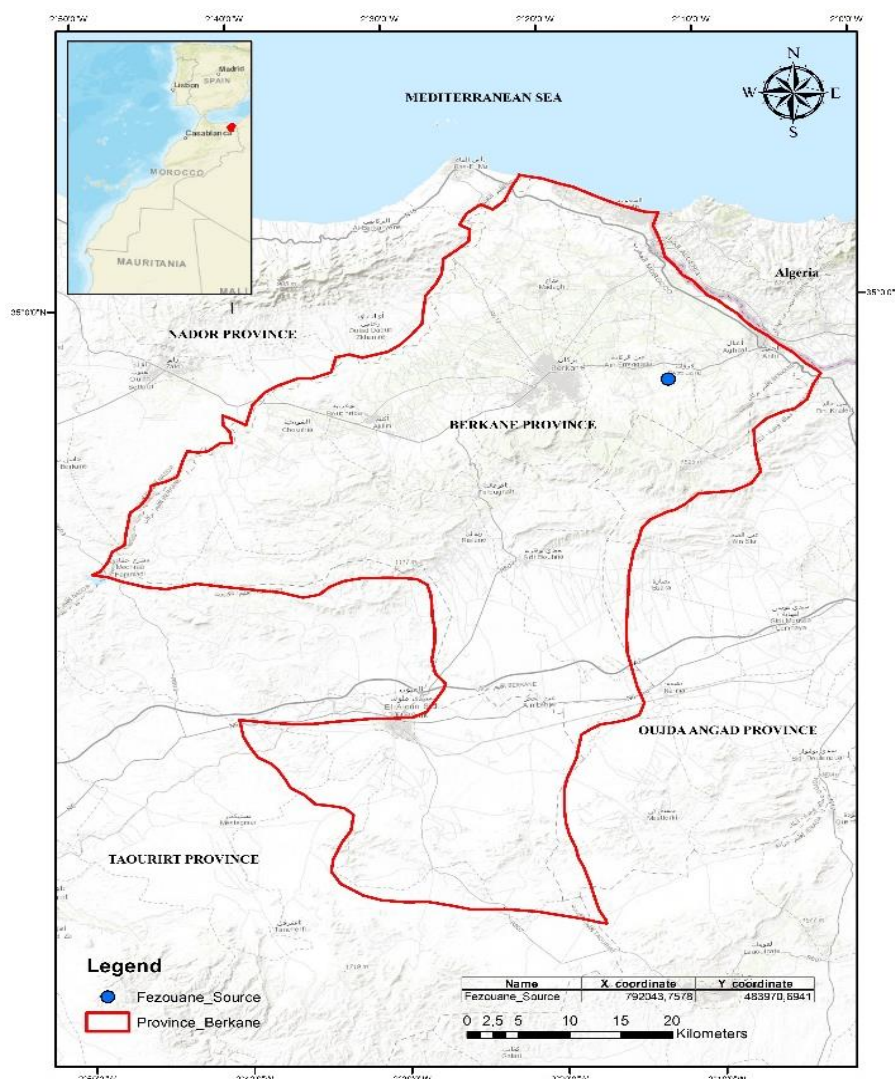


Figure 1: Fezouane study area (Berkane province)

Microbiological analysis included enumeration of viable microorganisms at 22°C and 37°C, fecal coliforms, *Escherichia coli*, Enterococcus intestinal, anaerobic sulfite-reducing spores at +37°C and +55°C, *Pseudomonas aeruginosa*, fecal coliforms, fecal Streptococcus, Salmonella, Legionella and Legionella pneumophila using filtration methods according to ISO standards 9308-1, ISO 7899-2, ISO 6461-2 and ISO 6222.

Radiological control of total activity values of α and β elements was conducted at the National Radioprotection Center in Salé, Morocco, using the evaporation reduction method of large volumes of filtered water with a gas detector according to NM 03.7.001.

Statistical analysis: In order to simplify the results, a descriptive statistic was conducted using Microsoft Office Excel. Also we used principal component analysis (PCA) to identify the various relationships between the analyzed parameters⁹.

Results and Discussion

Statistical analysis of physicochemical parameters:

According to table 1, all parameters fall within the acceptable limits defined by the Moroccan Standard NM 03.7.001 (MAV).

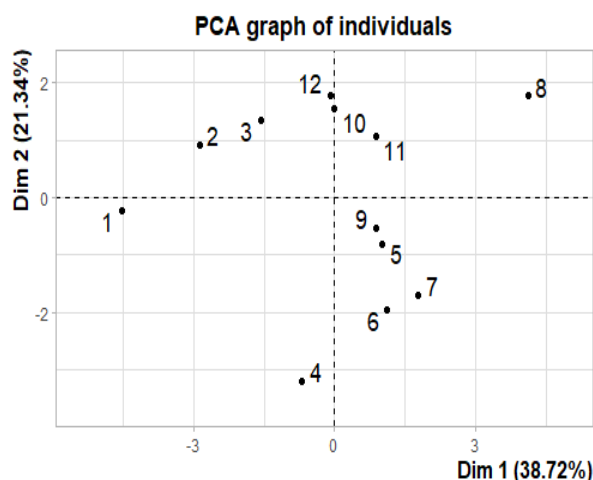
Additionally, the recorded temperature values range from 37.4°C to 37.8°C, with an average of 38.37°C. Based on the spring's emergence temperature, water can be classified into four categories: cold water, which is below 20°C; hypothermal water, ranging between 20°C and 35°C; thermal water, between 35°C and 50°C; and hyperthermal water, exceeding 50°C¹. These values confirm the thermal nature of Fezouane waters, a characteristic previously identified in the studies of Maamri et al⁷. These results are in line with the findings of Houti et al⁵ who report that water from the Ain Allah station is mesothermal (39°C), has average conductivity (870 $\mu\text{S}/\text{cm}$) and lower sodium and calcium levels⁵.

Table 1
Descriptive Statistics for Physicochemical Parameters of Fezouane Water in 2021.

	Unit	n		mean	sd	Min	max	se	MAV
Temperature	°C	12		38.37	0.56	37.40	39.53	0.16	Acceptable
pH	-	12		7.63	0.08	7.48	7.72	0.02	6.5-8.5
Conductivity (EC)	$\mu\text{S}/\text{cm}$	12		673.75	3.25	670.00	679.00	0.94	2700
DR525°C	mg/l	12		333.50	6.47	327.00	343.00	1.87	--
TH	°f	12		32.30	1.44	30.80	35.28	0.42	--
Ca ²⁺	mg/l	12		65.37	0.71	64.33	66.78	0.21	--
Mg ²⁺	mg/l	12		38.39	1.22	36.55	41.13	0.35	--
HCO ₃ ⁻	mg/l	12		365.41	3.92	360.18	370.78	1.13	--
Cl ⁻	mg/l	12		33.06	1.37	30.08	35.01	0.40	750
Na ⁺	mg/l	12		24.07	0.27	23.82	24.71	0.08	--
K ⁺	mg/l	12		0.88	0.04	0.80	0.93	0.01	--
SO ₄ ²⁻	mg/l	12		32.89	0.22	32.60	33.25	0.06	400
NO ₃ ⁻	mg/l	12		3.60	0.14	3.38	3.87	0.04	50

Table 2
Eigen values and percentage contribution of variables

Dimensions	Initial Eigen values		
	Eigen value	% of variance	% Cumulative
1	4.61	38.72	38.72
2	2.54	21.34	60.06
3	1.62	13.63	73.69
4	1.18	9.93	83.61
5	0.80	6.75	90.36
6	0.46	3.85	94.21
7	0.29	2.43	96.63
8	0.20	1.67	98.30
9	0.11	0.91	99.21
10	0.08	0.67	99.89
11	0.01	0.11	100.00

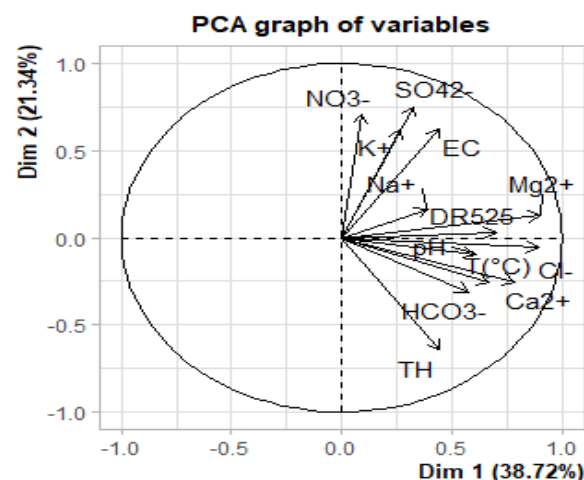
**Figure 2: Individuals (months) factor map (PCA)**

The confirmation of these thermal properties underscores the unique characteristics of Fezouane spring water, contributing to its appeal and potential therapeutic benefits. Additionally, the absence of harmful chemical substances within permissible limits further underscores the suitability of Fezouane water for human consumption and highlights its potential as a valuable resource for various purposes, including therapeutic and recreational uses. The analysis using Principal Component Analysis represented in the figures 2 and 3 estimated that the first two dimensions of analysis account for 60.06% (table 2) of the total dataset inertia.

According to figures 2 and 3, the dimension 1 contrasts different months, with August (positioned to the right of the graph with a strongly positive coordinate) opposed to January and February (situated to the left with strongly negative coordinates). The group associated with August, characterized by a positive coordinate, shares high values for Na^+ , Mg^{2+} and Ca^{2+} , listed in descending order of strength. In contrast, January's group, which has a negative coordinate, is associated with low values for Cl^- and pH. February's group, also positioned negatively on the axis, includes variables whose values do not significantly deviate from the mean.

Dimension 2 differentiates December (at the top of the graph with a strongly positive coordinate) from April, July and June (at the bottom with strongly negative coordinates). The group corresponding to December shares high values for NO_3^- and EC, ranked from strongest to weakest. April, on the other hand, is associated with low values for K^+ . Meanwhile, July and June, which also have negative coordinates, are linked to high values of TH and $T(^{\circ}\text{C})$, with the most influential variables listed first.

Quality control of Fezouane thermal waters: Controlling the quality of thermal waters involves a wide range of analytical techniques designed to determine the properties of the water and hence its quality. This is why it is so important to control the analytical quality of this water and its

**Figure 3: Variables factor map (PCA)**

compliance with the Moroccan standard (NM 03.7.001) which determines the quality of water used for human consumption.

This standard has identified two quality control parameters: (i) parameters with a health effect and (ii) undesirable substances that may give rise to complaints. These parameters may be the cause of water-borne diseases if they exceed the maximum admissible values laid down in NM 03.7.001. The aim of this study is to investigate the quality of Fezouane water through analysis of the parameters laid down in the Moroccan standard.

Health-related Parameters

Bacteriological Parameters: The bacteriological analyses carried out on samples collected from the Fezouane spring yielded noteworthy results. Specifically, the absence of several key pathogenic microorganisms was observed. *Escherichia coli*, a common indicator of fecal contamination and a causative agent of gastrointestinal infections, was notably absent. Similarly, the absence of intestinal *Enterococci* and fecal coliforms, which are indicators of fecal pollution and potential pathogens themselves, suggests a favorable microbial quality of the water. Additionally, the absence of anaerobic sulfite-reducing spores at both $+37^{\circ}\text{C}$ and $+55^{\circ}\text{C}$ in 50 ml samples further underscores the absence of specific fecal contaminants.

Moreover, the absence of *Salmonella*, a genus of bacteria notorious for causing food borne illnesses such as salmonellosis, is significant from a public health standpoint. Likewise, the absence of *Legionella pneumophila*, the causative agent of Legionnaires' disease, a severe form of pneumonia often associated with water sources, is reassuring in terms of preventing respiratory infections.

These findings indicate that the Fezouane spring water meets stringent microbiological standards, mitigating the risk of various waterborne diseases. Such absence of pathogenic bacteria contributes to the overall safety and suitability of the water for consumption and recreational purposes, thus

ensuring the well-being of the population reliant on this valuable natural resource¹⁵.

Chemical and toxic substances: Water pollution by chemical substances represents a prevalent and multifaceted challenge, characterized by a diverse array of inorganic compounds, each with potential health ramifications. Notably, contamination by sodium, in elevated concentrations, is linked to arterial hypertension. Nitrates, originating predominantly from agricultural activities, can induce varying degrees of health complications due to impaired tissue oxygenation. Heavy metals such as lead, mercury and cadmium, primarily sourced from industrial or artisanal activities, tend to accumulate in sediments at a specific river or coastal locations. These metals bioaccumulate within aquatic flora and fauna, posing significant risks to human health through historical or recent intoxication events via the food chain.

In the context of the Fezouane spring, comprehensive analyses of chemical substances and heavy metals (Table 4)

have been conducted to assess water quality against Moroccan potability standards (NM 03.7.001). Results indicate that nitrates, barium, fluorides, lead and selenium were present within average concentrations of 2.79 ± 0.00 mg/l, 0.001 ± 0.00 , 0.15 ± 0.00 , 0.001 ± 0.00 and 0.0006 ± 0.00 respectively. Conversely, nitrites, arsenic, cadmium, cyanides, chromium, manganese, copper, mercury, boron and nickel were notably absent in the analysis. These findings underscore the compliance of Fezouane spring water with established regulatory thresholds for chemical substances and heavy metals, as outlined in Moroccan potability standards.

Such adherence highlights the criticality of ongoing monitoring efforts and regulatory enforcement to uphold the integrity of water resources and safeguard public health. This underscores the significance of continued vigilance and adherence to regulatory standards to ensure the sustained health and well-being of the populace reliant on this vital water source⁴.

Table 3
Results of bacteriological parameter analyses for Fezouane water in 2020

Parameters	Average (12 months) \pm SD	VMA*
<i>Escherichiacoli</i> 100 ml ⁻¹	0.00 \pm 0.00	00
Intestinal enterococci 100 ml ⁻¹	0.00 \pm 0.00	00
Faecal coliforms 100 ml ⁻¹	0.00 \pm 0.00	00
Sulfite-reducing anaerobes + 37 °C 50 ml ⁻¹	0.00 \pm 0.00	00
Spores of sulfite-reducing anaerobes + 55 °C 50 ml ⁻¹	0.00 \pm 0.00	00
Salmonella	0.00 \pm 0.00	00
<i>Legionella pneumophilie</i>	0.00 \pm 0.00	00

Note: * The maximum acceptable value according to Moroccan Standard NM 03.7.001 (VMA).

Table 4
Results of chemical analyses of Fezouane water in 2020

Parameters	Unit	Minimum	Maximum	Mean \pm Standard	VMA *
Nitrites	mg/l	00	00	0.00 \pm 0.00	0.5
Nitrates	mg/l	2.79	2.79	2.79 \pm 0.00	50
Arsenic	mg/l	00	00	0.00 \pm 0.00	0.01
Barium	mg/l	0.01	0.01	0.01 \pm 0.00	0.7
Cadmium	mg/l	00	00	0.00 \pm 0.00	0.003
cyanides	mg/l	00	00	0.00 \pm 0.00	0.07
chromium	mg/l	00	00	0.00 \pm 0.00	0.05
Manganese	mg/l	00	00	0.00 \pm 0.00	0.5
Copper	mg/l	00	00	0.00 \pm 0.00	2
Fluorides	mg/l	0.15	0.15	0.15 \pm 0.00	1.5
Mercury	mg/l	00	00	0.00 \pm 0.00	0.001
Lead	mg/l	0.001	0.001	0.001 \pm 0.00	0.01
Selenium	mg/l	0.0006	0.0006	0.0006 \pm 0.00	0.01
Boron	mg/l	00	00	0.00 \pm 0.00	0.3
Nickel	mg/l	00	00	0.00 \pm 0.00	0.02

* The maximum acceptable value according to Moroccan Standard NM 03.7.001 (VMA).

Organic substances: pesticides, polycyclic aromatic hydrocarbons (PAHs) and trihalomethanes (THMs):

The water from the Fezouane spring exhibited significantly low levels of pesticide residues (Table 5), which do not exceed the maximum concentrations prescribed by specific Moroccan standards outlined in the norm (NM03.7.001) for potable water. This observation underscores the absence of any pollution attributable to agricultural activities. This finding is indicative of the efficacy of measures implemented to mitigate pesticide contamination in the surrounding environment.

It suggests that agricultural practices within the catchment area of the Fezouane spring adhere to regulatory guidelines concerning pesticide usage. Additionally, it reflects positively on the overall environmental stewardship efforts aimed at preserving water quality and minimizing potential health risks associated with pesticide exposure. The absence of pesticide residues exceeding regulatory thresholds highlights the importance of continuous monitoring and adherence to established standards in safeguarding the integrity of water resources.

Such findings are crucial in informing policy decisions and management strategies aimed at ensuring the sustainability and safety of water sources, thereby protecting public health and environmental well-being.

Radiological parameters: The analyses of alpha and beta global radioactivity in the waters of Fezouane are represented in table 6, these results are compliant with Moroccan standard 03.07.001. This finding is significant as it reflects the absence of elevated levels of alpha and beta radiation in the water, ensuring that it meets regulatory standards for safe consumption. Compliance with established norms underscores the absence of significant radioactive contamination in the Fezouane water source, thus mitigating potential health risks associated with exposure to harmful radiation.

The adherence of Fezouane water to Moroccan regulatory standards for radioactivity underscores the effectiveness of measures implemented to monitor and manage radiation levels in water sources.

Such findings provide reassurance regarding the safety and suitability of Fezouane water for various purposes, including drinking and recreational activities. Continuous monitoring of radioactivity levels in water sources remains imperative to ensure ongoing compliance with regulatory standards and to safeguard public health and environmental well-being. These findings contribute to the broader efforts aimed at maintaining the integrity of water resources and ensuring the provision of safe and potable water for communities reliant on the Fezouane spring.

Table 5
Values of pesticide molecules in Fezouane waters

Pesticidemolecules	Concentration(µg/l)	VMA *
Alachlor	< 0.01	0.1
Aldehydic endrin	< 0.01	0.1
Aldrin	< 0.01	0.03
Alfa-HCH	< 0.01	0.1
Beta-HCH	< 0.01	0.1
Bifenox	< 0.01	0.1
Captane	< 0.01	0.1
Carbaryl	< 0.01	0.1
Chlorfenviphos-Ethyl	< 0.01	0.1
Chlortal-dimethyl	< 0.01	0.1
Cypermethrin	< 0.01	0.1
Dieldrin	< 0.01	0.03
Heptachlor	< 0.01	0.03
Lindane	< 0.01	0.1
DDT	< 0.01	0.1
Ethyl parathion	< 0.01	0.1
Propazine	< 0.01	0.1
Permethrin	< 0.01	0.1
Carbendazim	< 0.01	0.1
Malathion	< 0.01	0.1

Table 6
Radiological parameters of Fezouane waters

Parameters	Unit	FEZOUANE water	VMA *
Global alpha activity	Bq/l	0.06	0.1
Global beta activity	Bq/l	0.78	1

Hydro chemical classification of water

Water classification according to the Piper diagram: The Piper diagram (Figure 3) revealed the predominance of bicarbonate, calcium and magnesium ions over sodium in the Fezouane water, imparting it with bicarbonate-calcium-magnesium facies¹⁴.

This geochemical characterization is indicative of the water's chemical composition, highlighting the prevalence of bicarbonate, calcium and magnesium ions compared to sodium ions. Such facies are commonly associated with waters originating from carbonate-rich geological formations. The dominance of bicarbonate ions suggests a significant contribution from carbonate dissolution processes in the aquifer, while the presence of calcium and magnesium ions underscores the leaching of these minerals from surrounding geological strata.

The bicarbonate-calcium-magnesium facies is often associated with alkaline earth metals and is characteristic of waters with moderate to high mineralization levels. This geochemical signature is valuable in understanding the hydro geochemical processes governing water-rock interactions within the Fezouane aquifer system. Overall, the Piper diagram provides valuable insights into the chemical composition and hydro geochemical facies of Fezouane water, aiding its characterization and understanding its suitability for various purposes, including drinking, agricultural and therapeutic uses.

Water classification by Schöller¹⁹: The Schöeller-Berkaloff diagram (Figure 4) indicates that the water exhibits values for bicarbonate, calcium and magnesium. These findings partially align with those of the Piper

diagram (Figure 5), which also suggests bicarbonate-calcium-magnesium facies¹⁹. The Schöeller-Berkaloff diagram provides further insight into the chemical composition of the water, specifically highlighting the presence of bicarbonate, calcium and magnesium ions. These ions are significant contributors to the overall hydro geochemical signature of the water.

The partial alignment between the results of the Schöeller-Berkaloff and Piper diagrams underscores the consistency in characterizing the Fezouane water as having bicarbonate-calcium-magnesium facies. These facies are indicative of the dominant influence of carbonate dissolution processes and the leaching of calcium and magnesium ions from geological formations within the aquifer.

Overall, the Schöeller-Berkaloff diagram complements the findings of the Piper diagram, providing additional confirmation of the bicarbonate-calcium-magnesium facies observed in the Fezouane water. This hydro geochemical characterization is essential for understanding the water's origin, geochemical evolution and potential applications in various domains. Examination of the characteristics of this water leads to the conclusion that it circulated solely through the carbonate formations.

Conclusion

The study sought to control the quality of thermal waters and assessing the health risks associated with their use has revealed potential risk factors for water contamination. However, a qualitative assessment of health risks was conducted, affirming the absence of health risks associated with the consumption and therapeutic use of thermal waters.

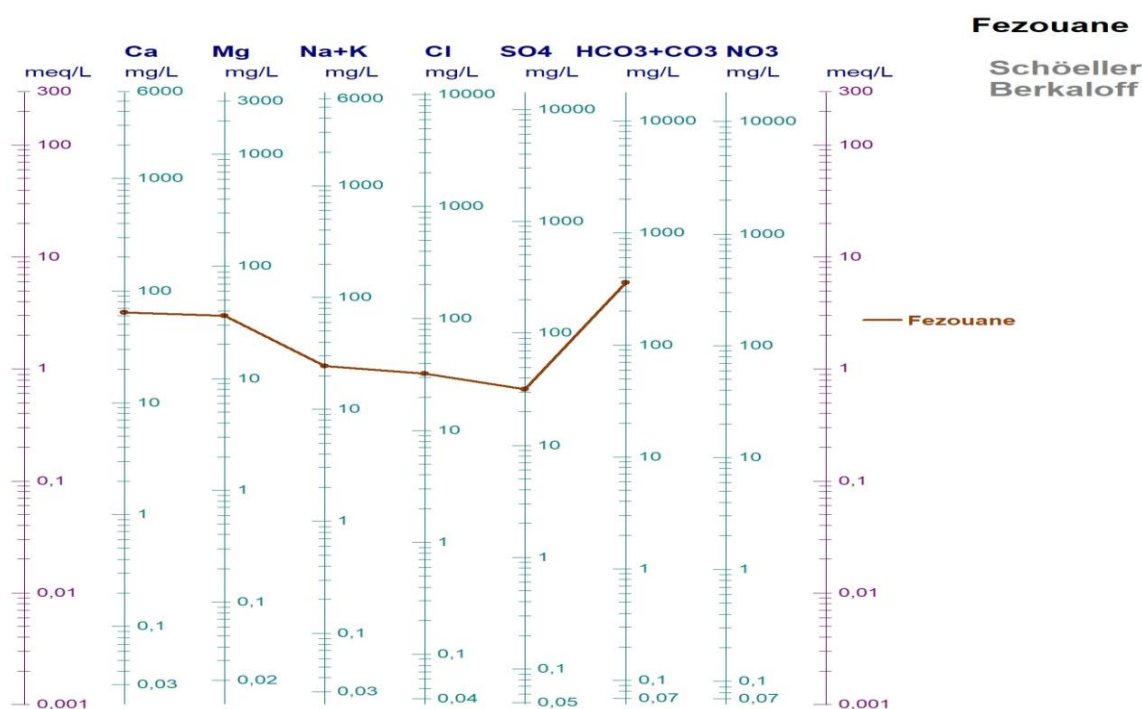


Figure 4: Diagramme de Schöller-Berkaloff de l'eau Fezouane

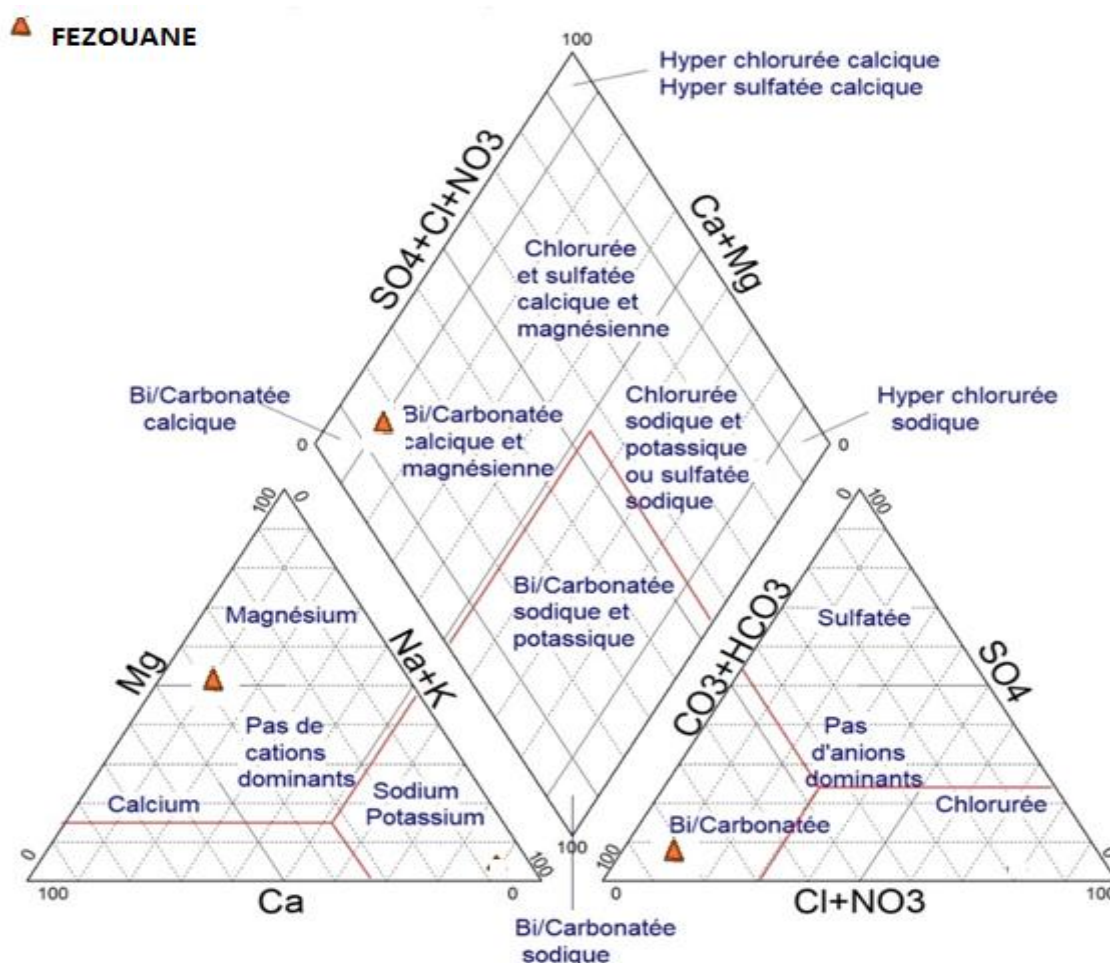


Figure 5: Piper diagram of Fezouane water

Furthermore, the study suggests that the current risks and practices observed at the thermal spring site can, for the time being, adequately manage the health risks at the Fezouane Springs. Looking ahead, future risk management studies should aim to precisely describe and analyze these risks. Moreover, they should provide the opportunity to adapt existing regulations to better protect the thermal spring source in the future.

This proactive approach underscores the importance of ongoing research and regulatory adaptation to ensure the continued safety and sustainability of thermal water resources like those at the Fezouane Springs. By continually evaluating and updating risk management strategies, authorities can safeguard public health and preserve these valuable natural assets for future generations.

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