STUDIA UBB CHEMIA, LXX, 4, 2025 (p. 157-180)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2025.4.09

EX VIVO EXPERIMENTAL AND SPECTROSCOPIC ANALYSIS OF URINARY STONE DISSOLUTION BY EL-MAATYA SPRING WATER: A MULTI-ANALYTICAL APPROACH

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ABSTRACT. El-Maatya spring water, sourced from Algeria, is locally esteemed for its therapeutic attributes and has undergone scientific scrutiny to evaluate its litholytic potential on urinary calculi. Thorough physicochemical and microbiological assessments were performed to analyze its composition and ensure its safety. The microbiological evaluation yielded excellent results, demonstrating an absence of coliforms, *E. coli*, and *streptococci*, thereby affirming the water's sanitary integrity. Spectroscopic techniques, including UV and FTIR, were employed to ascertain the composition of the urinary stones, primarily consisting of calcium oxalate and uric acid. *Ex vivo* dissolution experiments indicated a markedly elevated rate of mass loss in uric acid stones when compared to calcium oxalate stones, which exhibited negligible solubility—underscoring the selective efficacy of the water. Microscopic examinations revealed significant morphological changes in the structure of the calculi following exposure. Finally, a survey involving 241 participants corroborated the favorable perception of El-Maatya water regarding urinary health, thereby reinforcing its traditional utilization.

Keywords: *El-Maatya water, urolithiasis, dissolution, uric acid, calcium oxalate*

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INTRODUCTION

Nephrolithiasis is a common condition in men that leads to kidney stones mostly made of calcium oxalate. It is linked to metabolic problems and an imbalance in the pH of the urine [1, 2]**.** Urinary lithiasis creates crystalline concretions, blocking urine flow [3]. Urolithiasis, a kidney or bladder stone-producing condition, is gaining prevalence and requiring medication to alleviate pain and naturally expel stones [4, 5]. Pathogenic theories include precipitation and crystallization, nucleation, and suppression of inhibitory mechanisms [6]. Common drugs like allopurinol, citrate, cystone, and thiazide diuretics are used to prevent and treat the condition, but they don't always work because it can happen again and have bad side effects [5]. Among these, citrate lowers the formation of calcium stones by connecting to calcium and stopping the crystallization of calcium oxalate and calcium phosphate [7]. As a consequence, urolithiasis can lead to pyonephrosis and hydronephrosis, especially in individuals with type 2 diabetes and obesity [8, 9].

The consumption of spring water offers numerous health advantages [10]**.** Several water springs with specific mineral compositions can help prevent urolithiasis. For example, Fiuggi Water reduces urinary parameters such as calcium, phosphate, and uric acid [11], while Cerelia Water increases urinary pH and citrate excretion [12], and Serebryanyy Klyuch Water reduces calcium oxalate deposits [13].

Excess minerals in drinking water, including magnesium [14], calcium, sulfates, and fluorides, are essential in preventing urinary calculi, which are primarily calcium oxalate-based [15]. Prevention relies on hydration [16], calcium consumption [17], and a balanced diet [18]. Mineral waters are effective in treating illnesses like renal lithiasis, with minimal stone development risks if medical advice is followed [6]. Environmental factors, such as climate, socio-economic status, and dietary habits, influence the use of thermomineral waters in cryotherapy for various ailments [19, 16]. Long-term use of hydrogen-rich water lowers high uric acid levels and uric acid stones [20], while alkaline water raises the body's ability to get rid of uric acid and use purines [21, 22]. Alkalinization of urine to a pH between 6.5 and 7.0 effectively dissolves uric acid stones and helps prevent the formation of calcium oxalate stones [15, 23].

The study by Karagülle et al. found that using bicarbonate-rich mineral water can prevent recurrent calcium oxalate urolithiasis by enhancing urinary pH, citrate and magnesium excretion, and decreasing calcium oxalate supersaturation [24]. Uric acid stones, the second most prevalent type of renal stones [25], can be managed with oral pharmacological treatment through urine alkalinization [23]. Diluting urine stops crystal-forming substances like oxalate and calcium from forming in the urinary tract. This lowers the saturation of kidney stone-forming substances and raises the calcium oxalate nucleation threshold [26]. In addition, different minerals commonly present in natural waters, such as calcite, magnesian calcite, aragonite, halite, and sylvite, exhibit variable solubility in aqueous environments, which can influence alkalinization processes and, consequently, the solubility of urinary calculi [27, 28].

In Algeria, many studies on urolithiasis have been done, looking at how the chemical makeup of urinary calculi changes depending on where the patients live, their age, and their gender [29, 30]. Further research has investigated the impact of plant extracts from Algeria on stone dissolution [31-33], as well as the impacts of thermal spring water from various locations in the western region of the country [34, 15].

This study aims to assess the physicochemical and microbiological effects of spring water from El-Maatya in the Sétif province. We focus on evaluating the dissolution of urinary calculi retrieved from patients following surgical intervention using this water.

We are conducting a statistical analysis to assess public awareness and attitudes of this water, considering variables such as age and gender.

RESULTS AND DISCUSSION

Analysis Of El-Maatya water

Physicochemical tests

The findings derived from volumetric dosing and spectroscopic analysis are encapsulated in **Table 1**. It is observed that the conductivity measurement is approximately 1764 µS/cm, which remains within the acceptable limit (2800 µs/cm) for potable water according to Algerian standards, but is higher than the conductivity values of spring waters reported by Ennaghra [10]. This elevation may be attributable to an augmented concentration of dissolved ions, such as minerals and salts, present in this water. The mineral concentrations align with the established standards detailed in the table, with the exception of sodium, nitrates, bicarbonates, and the total alkaline titer, which are significantly heightened relative to the normative values. This elevation can be elucidated for each parameter as follows:

The water analyzed in our study has a sodium concentration greater than 200 mg/L, as well as a bicarbonate content exceeding 600 mg/L, thus classifying it among *'waters with sodium'* and *'waters with bicarbonates'* according to the classification of the European Directive 2009/54/EC [35].

Bicarbonate-rich mineral waters, such as those analyzed in our study, possess alkaline properties that provide several health benefits, including improved digestion, enhanced diuresis, and reduced bone resorption. Additionally, these waters could play a role in regulating cardiometabolic risks and in urinary alkalinization, which favors the dissolution of kidney stones [35]. Specifically, bicarbonate-rich waters like Cerelia water have been shown to increase urinary pH and citrate excretion, making the urinary environment less conducive to the formation of both uric acid and calcium stones [12, 36]. Moreover, experimental studies indicate that alkaline mineral water can reduce oxidative stress and inflammation, offering further protection against kidney stone formation [36].

In our research, the nitrate concentration slightly exceeded the prescribed threshold, indicating a potential environmental impact, especially from anthropogenic activities such as agriculture or urban development, as highlighted in previous studies [16, 37]. The nitrate contamination in the water of Dar Bentata, as reported by Amara-Rekkab (2023) [16], aligns with our observations and points to the risks posed by agricultural runoff. Nitrates in drinking water primarily result from sources like nitrogen-based fertilizers, improper waste disposal, and untreated wastewater discharge, which contribute to contamination of groundwater, a key drinking water source in many regions [38-40]. Research in various regions, such as the Poyang Lake Plain and the North East Alluvial Plains of Bihar, shows that agricultural practices, particularly the excessive use of fertilizers, are significant contributors to elevated nitrate levels [38, 39].

In relation to the pollution parameters, we distinctly noted the minimal concentrations of ammonium, nitrite, and phosphate compounds, recorded at 0.03 mg/L, 0.03 mg/L, and 0.02 mg/L, respectively, which substantiates the lack of contamination or pollution in this water, thereby favorably impacting our study. Iron is an essential micronutrient, closely involved in haemoglobin synthesis and oxygen transport. According to the World Health Organization [41], the recommended daily intake of iron for adults ranges from 10 to 50 mg. The total iron concentration measured in El-Maatya water (0.02 g/L, i.e., 20 mg/L) remains below the average daily requirement, indicating that this water may slightly but positively contribute to daily iron intake without posing any toxicological risk.

Table 1. Results of physicochemical parameters

Parameters		**Algerian standard**
Physicochemical parameters		
pH	7.62	6.5-9
Temperature (°C)	23.6	25
Electrical conductivity (µs/cm)	1764	2800
Turbidity (NTU)	1.17	5
TDS (mg/L)	902	1500
Global mineralization		
Calcium (mg/L)	44	200
Magnesium (mg/L)	17.01	150
Chloride (mg/L)	76.68	500
Nitrates (mg/L)	67	50
Total hardness (°F)	18	50
Sodium (mg/L)	390	200
Potassium (mg/L)	1.5	12
Bicarbonate (mg/L)	671	610
Sulfate (mg/L)	230	400
Complete alkaline title (°F)	55	50
Pollution parameters		
Ammonium (mg/L)	0.03	0.5
Nitrites (mg/L)	0.03	0.2
Phosphates (mg/L)	0.02	0.5
Heavy metals		
Total iron (mg/L)	0.02	0.3

Bacteriological analyses

These bacteriological findings entirely correspond to those published by Ennaghra *et al*. 2024 [10], which indicate 0 CFU/100 mL for *total coliforms*, *Escherichia coli*, and *fecal streptococc*i. Every tested parameter was negative, hence ensuring the great microbiological quality of El-Maatya water.

Analysis of urinary calculi

Dissolution test

After about three hours, we observed that stone **2** found in El-Maatya **(M)** water began to dissolve, and the others after one day, while those in the negative and positive control groups remained unchanged. After one day, the stones in the El-Maatya group were completely dissociated (**Figure 1a**).

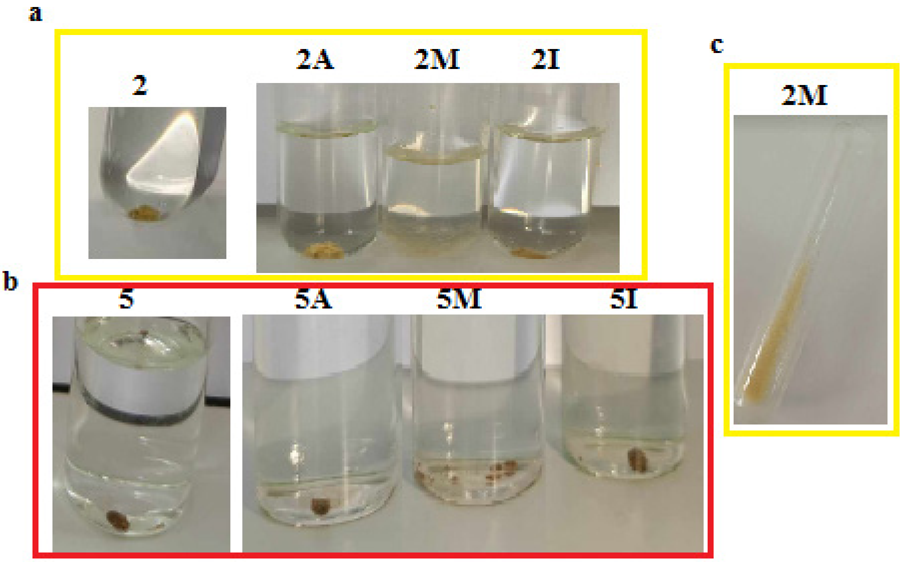


Figure 1. Photographs showing the dissolution of stones 2 **(a)** and 5 **(b)** in the three media (A, I, and M), and the precipitate of stone 2M **(c)** after one month of observation.

Some urinary stones found in the citrate group showed a slight dissolution, while the mineral water remained a negative control (unchanged) **(Figure 1b)**. After one month, the previous observations remained the same. Note: no dissolution but very fine powders obtained as a precipitate (**Figure 1c**).

Monitoring the dissolution of urinary calculi

In order to gain a better understanding of the inhibitory effect of El-Maatya **(M)** water, this method is based on monitoring the loss of mass of urinary calculi under the effect of different media over an incubation period of 3 weeks. The dissolution rates, calculated as a percentage, are shown in **Figure 2**. These results show that :

- The dissolution kinetics appear to be different for each incubation medium.
- A slight variation in mass loss was observed during the first 7 hours, especially in the case of calculus number **5**.
- Calculus number 2 dissolved easily from the start of immersion and reached 90% dissolution on day 23.
- No variation was observed when mineral water (I) was used as the medium.
- After 15 days, dissolution began in the sodium citrate medium (A) with a dissolution rate that was very low.

- Calculus number 4 is made up of several layers (in the form of a dragée). It looks completely different from the others. After one day, the first outer layer of the 4M stone dissociated and its color began to lighten. However, for calculus 4A and 4I, no change was observed (neither loss of mass nor dissolution of the outer layer).
- According to the results obtained, the size and weight of the stone had no impact on the dissolution test. Calculi 2 and 5, which are large, were solubilized, whereas calculus **4A** was not. So it's the environment that plays a decisive role.
- El-Maatya **(M)** water proved to be the best medium for stone dissolution. The dissolution rates for 2M, 3M, 4M, and 5M stones are 90.28%, 76%, 73.15%, and 21.18%, respectively. Calcium oxalate is known to be slightly more difficult to dissolve than uric acid, which is why the dissolution rate of stone No. 5 is the lowest compared with the others.
- When comparing this *ex vivo* dissolution test using plant extracts, they found that dissolution continued until the eighth week [31].

The* in vitro *and physicochemical effects of El-Maatya water

Our *in vitro* analyses showed that uric acid and calcium oxalate stones dissolved on contact with the spring water tested. These results should be interpreted in the light of the physico-chemical properties of this water, in particular its pH of 7.6, its high concentration of bicarbonates, its high total alkaline titre (55°F) and its sodium content, which exceeds Algerian standards.

High concentrations of sodium and bicarbonates can have an impact on the dissolution of kidney stones, in particular by influencing the alkalinisation of urine and thus promoting the dissolution of certain types of stone, as observed in our in vitro tests. This observation is in line with the study by Alsinnawi et al, who reported complete dissolution of stones in 39% of patients treated with sodium bicarbonate alone, with urinary pH maintained above 7 for around 9 weeks [42].

Despite epidemiological evidence indicating that elevated sodium intake correlates with an increased likelihood of urinary stone formation [43], our *ex vivo* investigations demonstrate that the sodium-rich spring water examined exhibits a noteworthy capacity for stone dissolution. This seemingly paradoxical finding may be elucidated through intricate physico-chemical processes associated with the diverse array of minerals contained within the water, particularly emphasizing the buffering effect rendered by bicarbonate and the pronounced alkalinity. These findings imply that the litholytic properties of water are not exclusively contingent upon its sodium concentration, but rather also depend on its comprehensive ionic equilibrium, which warrants further investigation.

The dissolution rate of urinary calculi in different media over 23 days is shown in **Figure 2**.

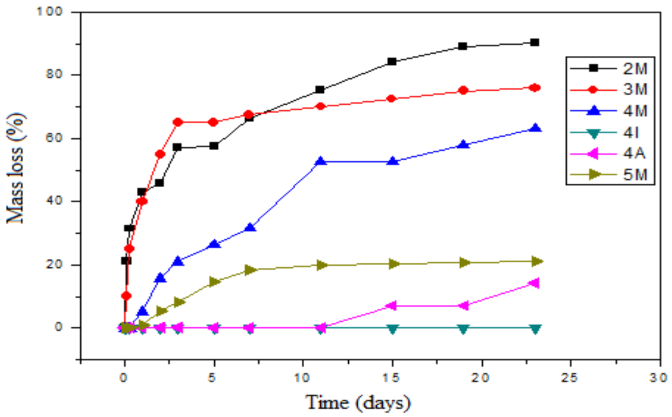


Figure 2. Evolution of mass loss over time for the different media.

a. Variation in pH

The pH of El-Maatya water is more basic than that of sodium citrate and mineral water, with a value of 7.95 ± 0.01. During the incubation of urinary calculi, the pH of the media varied with time, gradually increasing to reach values of 8.65, then stabilizing at the end, whether in M, I, or A (**Figure 3**).

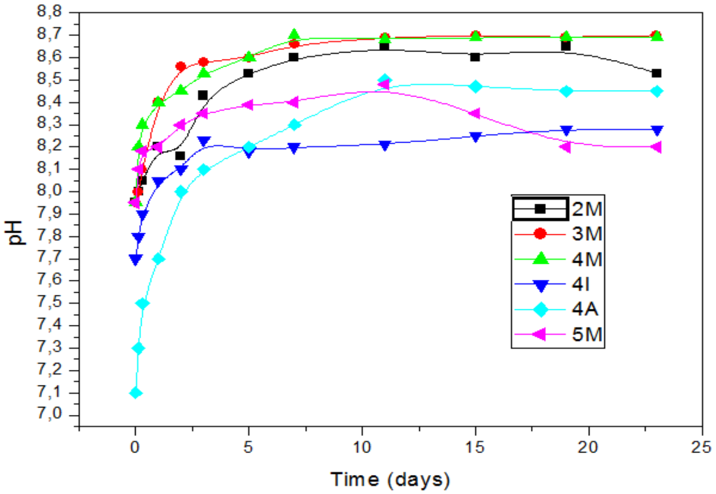


Figure 3. Variation in pH as a function of time.

b. Variation in stone size

Figure 4 and **5** illustrate the evolution of urinary stone size over time, showing a remarkable reduction for the **2M** stone, which decreased from 20 mm to 9 mm. For the **3M** stone, the size was reduced by approximately half. The size of the **4M** stone changed slightly, from 13 to 5 mm.

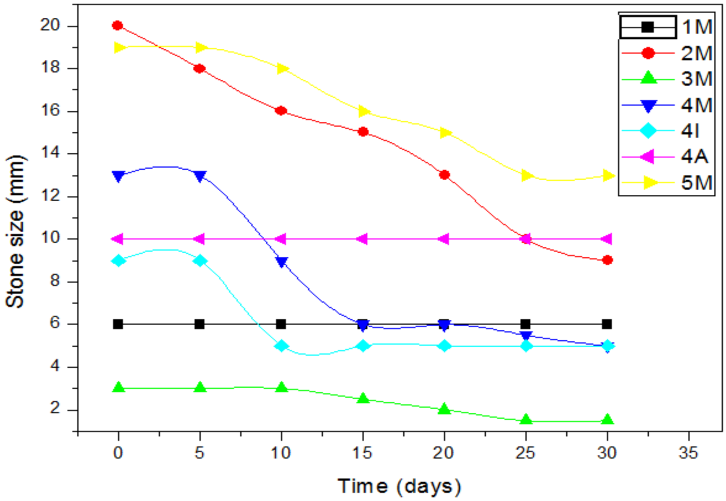
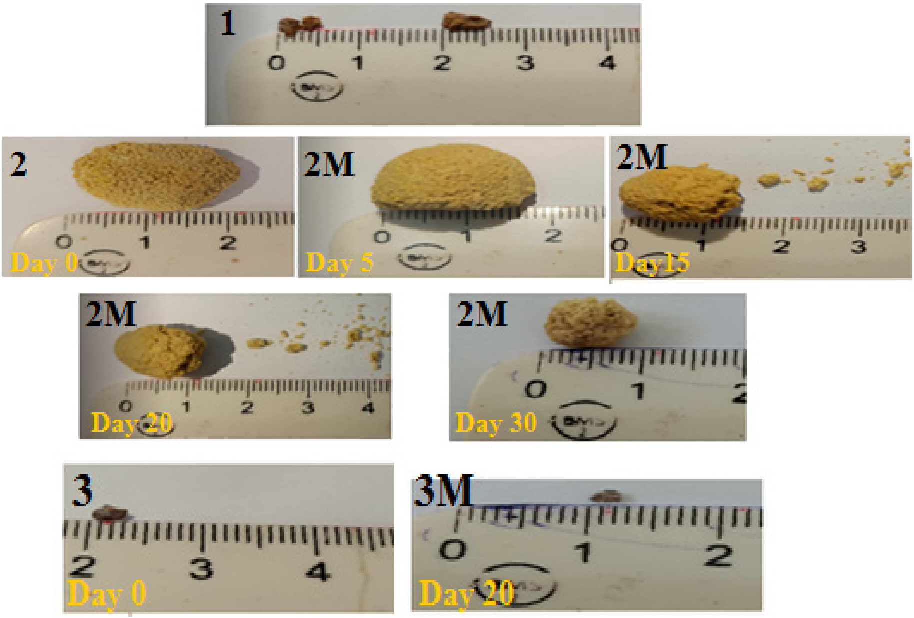


Figure 4. Variation in the size of urinary stones as a function of time.



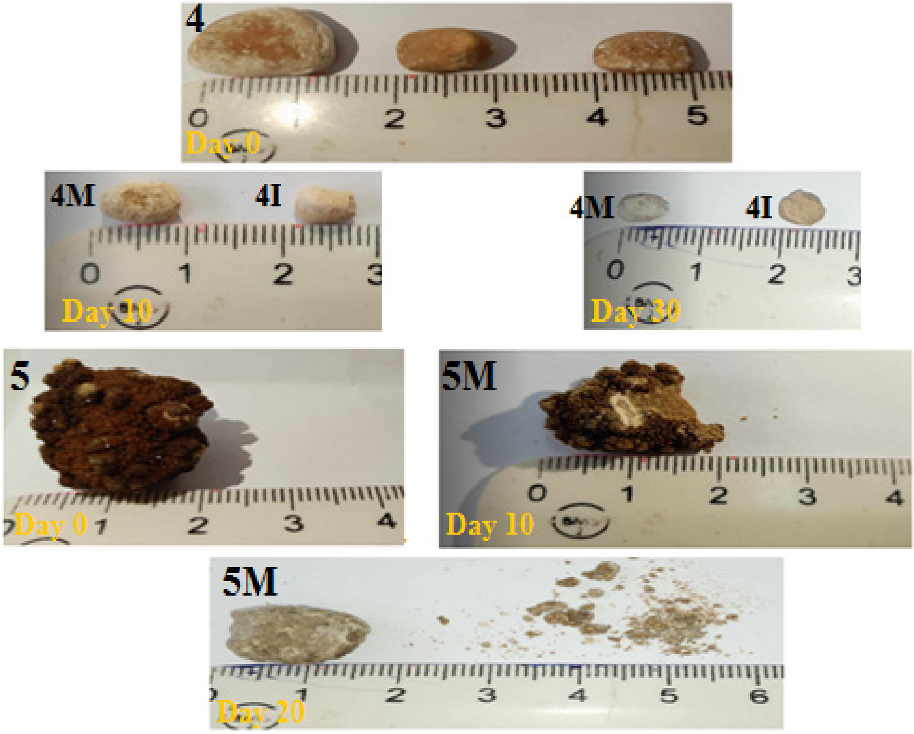


Figure 5. Changes in the size of urinary calculi over time.

Study by UV-VIS spectroscopy

a. Study conducted on El-Maatya water (M)

The UV spectrum (**Figure 6**) shows two characteristic bands at 215 nm and 280 nm, typical of uric acid [44], observed for urinary calculi numbers **1**, **2**, **3** and **4**. Furthermore, two additional bands at 350 nm and 400 nm are observed in the UV spectrum corresponding to urinary stone number **2**. The first is probably related to impurities, and the second band could be correlated to the yellow colour obtained (**Figure 7**) during the immersion of urinary calculus number **2**. This additional band at 400 nm suggests the presence of other compounds in the stone, such as dyes or other chemical substances in addition to uric acid. It is also possible that the presence of bilirubin or bilirubin derivatives contributes to the yellow colouration, particularly in the case of liver problems.

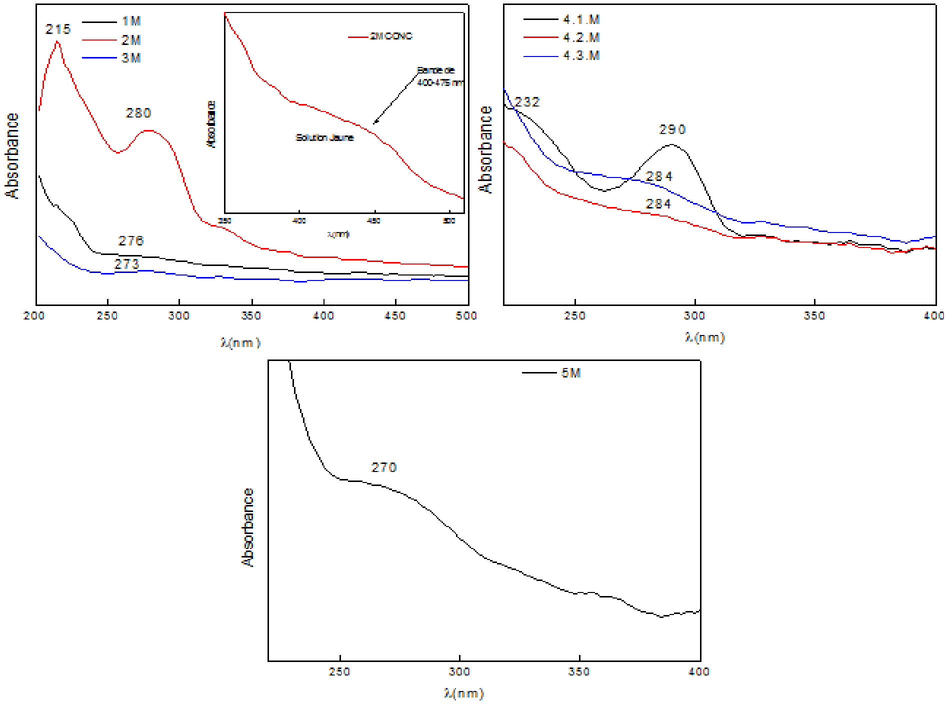


Figure 6. UV spectrum of M water after 24 hours of dissolution of urinary calculi numbers **1**, **2**, **3**, and **4.1**, **4.2**, and **4.3** corresponding to uric acid and urinary stone number **5** corresponding to calcium oxalate.

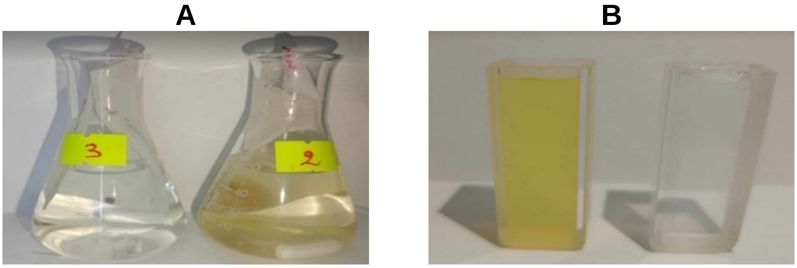


Figure 7. Representation of Erlenmeyer flasks **(A)** and UV-Vis cuvettes **(B)** containing M water after 24 hours of dissolution of urinary calculi (samples **3** and **2**), showing the color change.

The UV spectrum corresponding to urinary calculus number **5** shows a single characteristic band at 270 nm, typical of calcium oxalate [45]. This absorption band at 270 nm is consistent with the presence of calcium oxalate, a common compound in kidney stones. The absence of other significant bands in the spectrum suggests a relative purity of the sample analyzed, highlighting mainly the presence of CaC_2O_4. *It should be noted that a yellow color similar to that observed for calculi number **2** is obtained, but no absorbance in the visible range is observed.*

b. Study using sodium citrate (A)

The study carried out with sodium citrate **(A)** reveals that the UV spectra of urinary calculi placed in this medium, compared with those in El-Maatya water **(M)**, show a shift of the bands towards higher wavelengths (*bathochrome effect*) with a difference in λ_{max} between 2 nm and 20 nm (**Figure 8**). For example, the band at 215 nm in the case of **2M** is shifted to 236 nm, and a further schifting from 280 nm to 290 nm is observed. These observations could be attributed to the difference in pH and ionic strength of the salts present in El-Maatya water, which differ significantly from sodium citrate. Another observation concerns the presence of two characteristic dual-chromophore bands linked to uric acid in the spectrum of sodium citrate, whereas in the spectrum of El-Maatya water a single chromophore is generally observed, particularly in the cases of **3M**, **4.2M**, and **4.3M**.

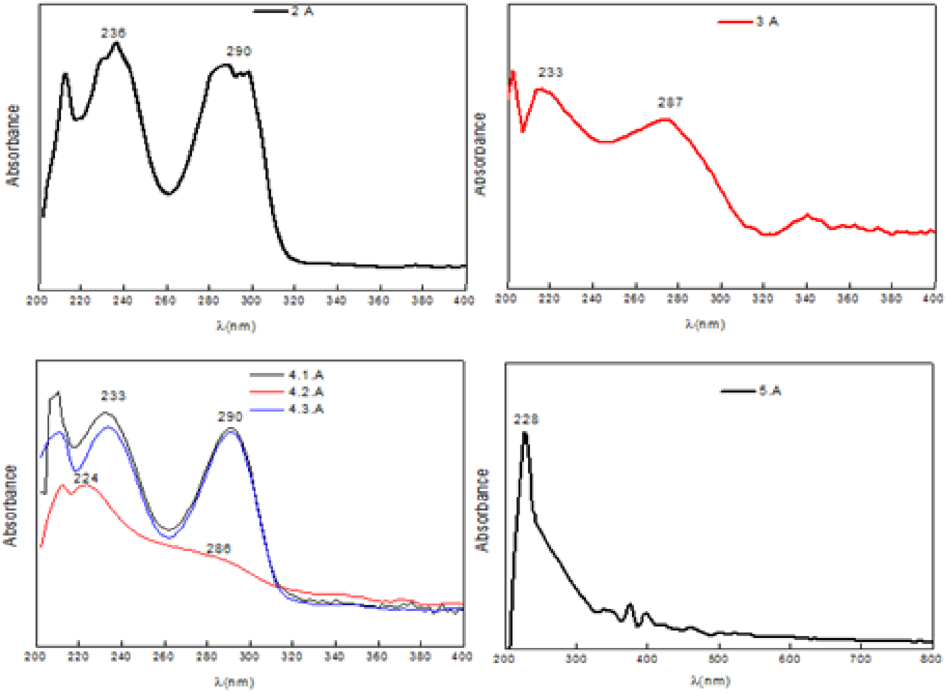


Figure 8. UV spectrum in sodium citrate after 24 hours of dissolution of urinary calculi number **2**, **3**, and **4.1**, **4.2**, **4.3**, and **5** corresponding to uric acid.

Contrary to the previous interpretation, we observe a decrease in λ_{max} of the CaC_2O_4 characteristic band of urinary calculus N°**5** (**Figure 6**). It is of the order of 270 nm in **M** water and 228 nm in **A** water, corresponding to a *hypsochromic effect*

c. Study using mineral water (I)

The UV spectra corresponding to mineral water **(I)** are not clear when compared with those of citrate **(A)** and El-Maatya **(M)**. In addition, this water was unable to dissolve the stones, so no changes were apparent after treatment.

Infrared spectroscopy

Identification of the components of urinary calculi is essential to provide information on the aetiological factors responsible for their formation, making therapy and prevention possible. Morphological examination combined with infrared spectroscopy can provide useful information about their chemical composition.

a. Urinary calculi N° 1, 2, 3, and 4 (4-1; 4-2 and 4-3)

After visual analysis of the peaks obtained in the infrared vibration spectra of urinary calculi **1, 2**, **3**, and **4**, we were able to determine that they were all of the same type, i.e., uric acid.

The salt form of uric acid most commonly found in urinary calculi is the anhydrous form; it has a characteristic infrared spectrum that is easily recognized by the presence of an N-H distortion vibration in the 1637 - 1624 cm^{-1} frequency region. However, Mujahid et al. have suggested that this elongation vibration is linked to the C=O group [46].

Numerous N-H elongation bands are observed in the 3600 to 2600 cm^{-1} range, as well as other bands attributed to hydrogen bonds (OH). A band at 1588 cm^{-1}, attributed to carbonyl deformation of the conjugated amide, is absent. In agreement with Sekkom et al., C-C elongation appears at 1449 cm^{-1} **(Figure 9a)** and 1410 cm^{-1} **(Figure 9b)** due to the hypsochromic effect of the amide and carbonyl groups [47].

The peaks observed at [1449-1410 cm^{-1}] and 1109 cm^{-1} are due to O-H deformation and C-O elongation, respectively. The C-N elongation and deformation vibrations appear in the frequency region 1021 - 1000 cm^{-1} and at 858 cm^{-1}, respectively [48].

b. Urinary stone No. 05

After analysis and close examination of the peaks in the spectrum shown in **Figure 10**, we concluded that this urinary calculus is a calcium oxalate.

Pure calcium oxalate monohydrate was characterized by five bands: The absorption band observed at 3500 - 3021 cm^{-1} is due to the elongation vibration of the O-H function, which is in agreement with Sekkoum et al. [47]. Two strong absorbances at 1651 and 1322 cm^{-1} correspond to the C=O and C-O elongations, respectively [49].

According to Asyana et al. [48], two bands at 771 and 667 cm^{-1} correspond, respectively, to the deformation of C-H bonds and the out-of-plane deformation of O-H bonds. In-plane deformation of the O-C bond appears at 514 cm^{-1} (**Figure 10**).

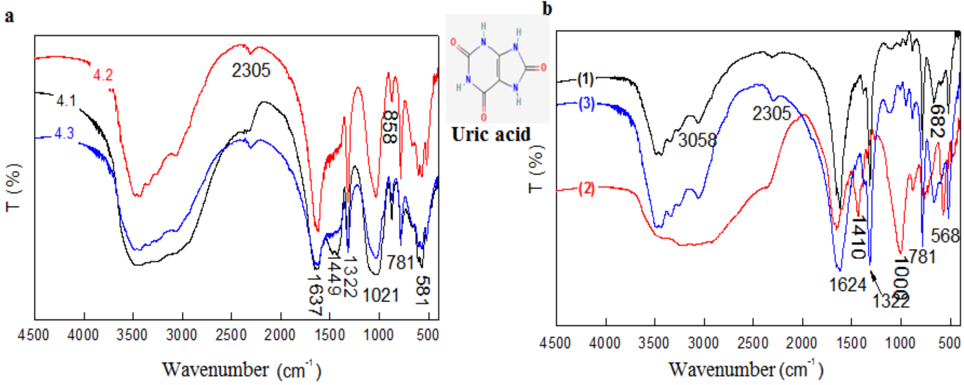


Figure 9. Infrared spectra of urinary stones number **4 (a)** and **1**, **2**, and **3 (b)** corresponding to uric acid.

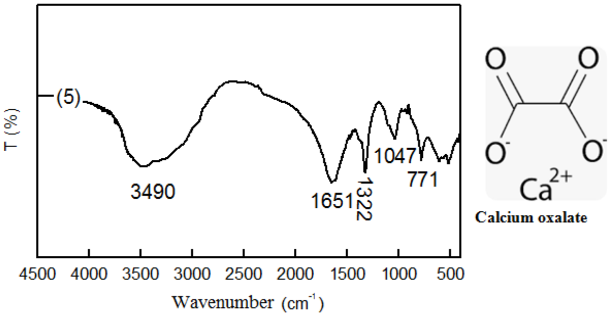
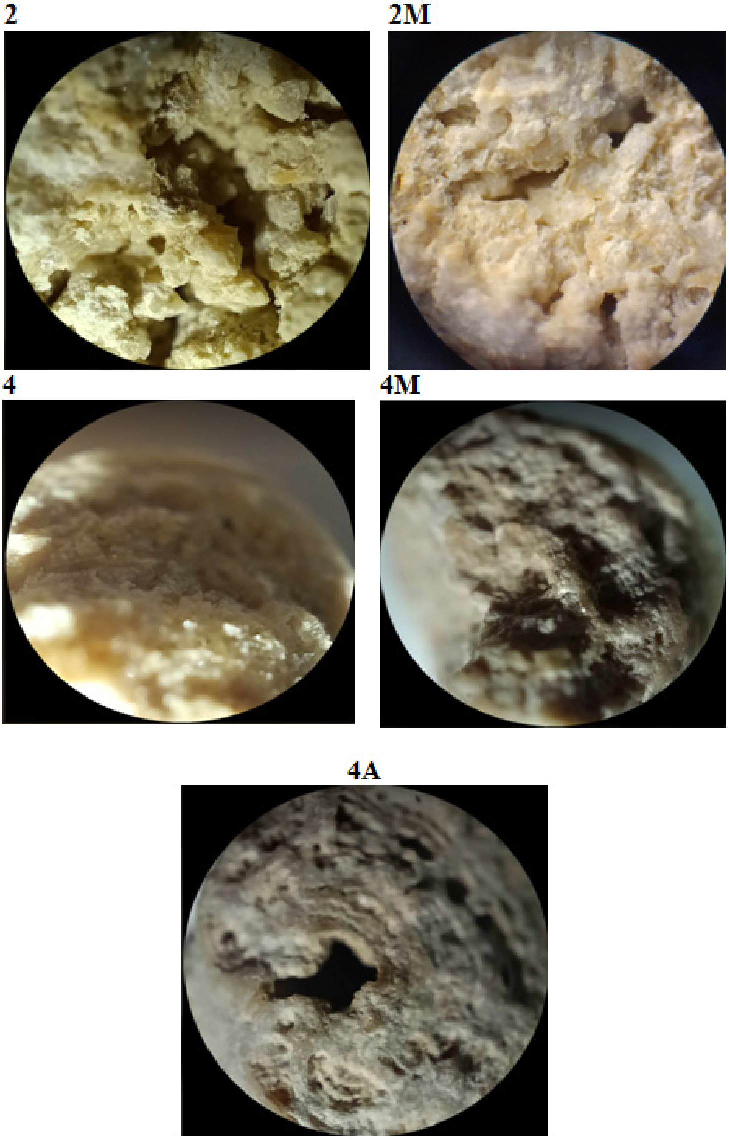


Figure 10. Infrared spectrum of urinary stone number **5** corresponding to calcium oxalate.

Light microscopic examination

Microscopic examination of the peripheral layers of the stone can be essential in identifying its morphological type and the metabolic disorder responsible for its nucleation or growth process. In addition, comparing the urinary calculus before and after a dissolution treatment allows morphological changes to be highlighted (**Figure 11**).

Figure 11 depicts the microscopic morphology of kidney stones before and after treatment, revealing a significant difference. Stone **5** had a brilliance, both microscopically and macroscopically, that has since disappeared. On a macroscopic scale, the color of calculus **4** shifted to light gray when the outer layer of lithiasis disappeared. On a microscopic scale, we see the same change, confirming the transformation. The similar phenomenon is seen with urinary stone **2**.



2

2M

4

4M

4A

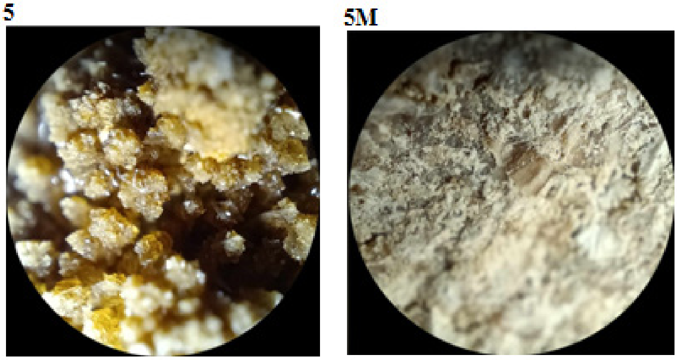


Figure 11. Optical microscope image of stones **2**, **4**, and **5** before and after treatment at 20X scale.

The disappearance of the brightness of stone **5** after treatment probably indicates a change in its chemical composition, suggesting dissolution or a change to a less bright and more brittle crystalline phase. In addition, the change in color of stone **4** to light gray, associated with the disappearance of its outer layer, suggests a significant alteration in its structure, perhaps due to the action of the treatment.

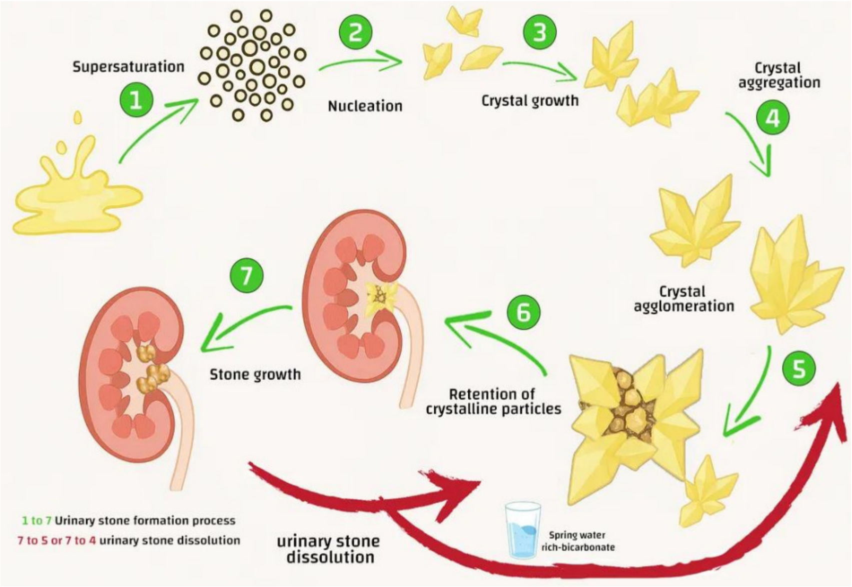


Figure 12. Proposed mechanism of urinary stone dissolution by spring water.

These changes suggest possible mechanisms of action (**Figure 12**) of the treatment on kidney stones but would require further study to be confirmed.

Survey on the use of El-Maatya water

241 people responded to the survey on the use of El-Maatya water. Of these, 76% were women and 23.7% men.

The majority of respondents were young people aged between 20 and 40, and 82.6% were university graduates. Participants came from various wilayas, in particular 70.12% from Sétif, 6.64% from Borj Bouaririj, 4.56% from Algeria, 3.73% from Mila, 1.66% Skikda, 1.24% Tlemcen, 0.83% Boumerdes and Oum Bouaki, and 0.41% from Batna, Djelfa, Bejaia, Jijel, Blida, Tiaret, Khenchela, Guelma, and Mascara

Only 40.7% of respondents were familiar with El-Maatya water. Of these, 26.1% said that the water is used mainly for cooking, making it easier to cook chickpeas and white beans. A further 5.4% use it for cosmetic purposes and 21.7% for medical treatments. Among the respondents, 38.6% suffer personally from lithiasis or have relatives who do and use El-Maatya water as a treatment. Of these, 9.5% consult a urologist during their water treatment. A further 13% combine the water with other medicines under the guidance of their general practitioner.

17.4% of participants reported negative effects of this water, such as brittle bones, frequent urination, and the risk of abortion in pregnant women.

In addition, 43.2% knew of other waters used to treat urinary lithiasis, including:

29 people knew of Maa Zdim, 10 in Ain Bouglez, 9 in Borj Bouaririj, 7 in Ain Ben Tata (Tlemcen), 3 in Nguaouas (Batna), 5 in Ouled Yalass, 2 in Algeria, and 1 in Annaba.

Opinions differ on the taste of the water. 22 people say it is tasteless, 4.98% find it bitter, 36.1% describe it as concentrated water, and 36.93% don't know what it tastes like.

The majority strongly recommend the use of this water.

Future investigations should include clinical trials on voluntary subjects to assess the dissolution dynamics of urinary stones through periodic CT scans. Such studies would provide direct evidence of the curative efficacy of El-Maatya water and support its potential medical application in urolithiasis prevention and treatment.

CONCLUSIONS

El Maatya's water has favorable physicochemical and microbiological characteristics for healthy consumption, although certain mineral values, such as sodium, nitrates, bicarbonates, and total alkalinity, exceed standard norms.

The high concentrations of some minerals, such as sodium, can be attributed to the geology of the area of this spring and to other environmental factors.

Microbiological tests show a total absence of *coliforms*, *E. coli*, and *streptococci,* guaranteeing good sanitary quality.

Dissolution tests indicate that this water can be effective in dissolving certain types of urinary stones, mainly those composed of uric acid (bands at 215 nm and 280 nm), but is less effective for calcium oxalate stones (band at 270 nm).

The type of urinary stone (uric acid and calcium oxalate) was identified by UV and infrared spectroscopy based on the presence of chromophores and associated functional groups.

Light microscopic analysis confirmed a complete change in the morphology of the stones, both microscopically and macroscopically.

The survey of the local population, based on a sample of 241 people, shows that the water is well accepted and used for its perceived benefits, despite some potential drawbacks linked to the high concentrations of certain minerals.

Future studies should focus on a detailed mineralogical analysis of El-Maatya water using XRD and optical microscopy, as well as on correlating its mineral composition with the nutritional and metabolic needs of the local population.

EXPERIMENTAL SECTION

Material

The following reagents were used in the practical session:

Sodium citrate tribasic dihydrate (Biochem, purity ≥ 99%); distilled water; Ifri mineral water; El-Maatya spring water; sodium hydroxide (NaOH), pellets (Sigma-Aldrich, purity ≥ 98%); sodium salicylate (Sigma-Aldrich, purity ≥ 99%); sulphuric acid (H_2SO_4, Sigma-Aldrich, 95–98%); silver nitrate ($AgNO_3$, Sigma-Aldrich, purity ≥ 99.8%); potassium chromate (K_2CrO_4, Sigma-Aldrich, purity ≥ 99%); hydroxylamine hydrochloride ($NH_2OH\cdot HCl$, Sigma-Aldrich, purity ≥ 98%); hydrochloric acid (HCl, Sigma-Aldrich, 37%). Other reagents required for the titrimetric determinations were used according to the ISO standards cited in Table 2.

Analysis of El-Maatya water

The water of El-Maatya, also known as Ain Al-Shifa, is located in the village of El-Maatya, in the commune of Draa Kbila, to the north of the province of Sétif, Algeria (Google Maps – Plus Code: C234+7X). This well was dug in 2001, and a spring structure was built for it in 2002.

The water sampling was conducted in May 2024 at the designated source, utilizing sterile containers that were subsequently stored in a cooler maintained at a temperature of 4°C. Following this, the samples were transported to the laboratory for comprehensive analysis. We performed both physicochemical and microbiological assessments of El-Maatya water at the ADE (Algérienne des Eaux) facility located in Sétif.

Physicochemical analyses

For the physicochemical parameters, an electrochemical approach was employed to assess the physical parameters, including pH, temperature, electrical conductivity, and turbidity, using benchtop instruments from HACH (USA): a pH meter (HACH, benchtop type), a conductivity meter (HACH, benchtop type), and a turbidity meter (Model 2100N, HACH, USA).

For the chemical parameters, volumetric methods were applied to determine chloride, calcium, total hardness, and alkalimetric titre, using standard glassware (burette, pipette, conical flask, and beaker).

Spectrophotometric methods were employed for the determination of nitrate, nitrite, ammonium, phosphate, sulfate, and total iron using a molecular absorption spectrophotometer (HACH DR 6000, USA). Sodium and potassium concentrations were measured using a flame photometer (Jenway, UK).

Table 2. Physicochemical water analysis methods

Parameters	**References to the analytical method**
Analysis by spectroscopic methods	
Nitrates (NO_3^-)	T 90-012, NF-August1975
Nitrite (NO_2^-)	ISO 6777, 1st edition, August 1984
Ammonium (NH_4^+)	ISO 7150, 1st edition, 1984
Phosphates (PO_4^{3}-)	ISO 6878, 1st edition, 1998
Sulfates (SO_4^{2}-)	Rodier [50]
Total iron	ISO 6332, 1st edition, August 1994
Sodium (Na^+) and Potassium (K^+)	ISO 9964, 3rd edition, 1993
electrochemical method	
Conductivity	ISO 7888, May 1985
pH	ISO 1052-3-2008
turbidity measurements	ISO 7027-1-1999
Analysis by volumetric assay	
Chlorides (Cl^-)	ISO 9297 T90014, 1989
Calcium (Ca^{2+})	ISO 6058, 1st edition, june 1984
Total Hardness (T.H.)	ISO 6059, 1st edition, 1984
Complete Alkaline Titration (C.A.T.)	ISO 9963, 1st February 1996

All spectrometric and volumetric measurements were performed according to the standard methods summarized in Table 2, with sulfate content determined spectrophotometrically using a calibration curve (solutions 0–7) and absorbance measured at 420 nm after addition of $BaCl_2$, following Rodier [50].

Bacteriological analysis

Bacteriological analyses were carried out in accordance with ISO 9308-1 and ISO 7899-2, by filtering 100 mL of El-Maatya water through a 0.45 µm membrane, followed by inoculation on Tergitol TTC agar for coliforms and *Escherichia coli*, and Slanetz and Bartley agar for *fecal streptococci*. Incubation was performed at 36 ± 2 °C for 21 ± 3 hours for *coliforms* and *E. coli*, and at 44 ± 4 hours for *intestinal enterococci*. Characteristic colonies were confirmed by subculturing on *Brilliant Green Bile Lactose (BGBL)* and tryptophan broths and BEA agar, in accordance with ISO 8199.

Urinary stone analysis

The monitoring of stones dissolution

In this method, we monitored the effect of the different media *ex-vivo* by following the variation in mass of natural stones from immersion to three weeks [33]. Calculi were collected from various patients suffering from urinary lithiasis, with weights ranging from 2 to 350 mg. Three media were chosen: El-Maatya water (M), sodium citrate (A) as a positive control and mineral water (I) as a negative control.

The urinary stones were placed in porous bags made of woven fiber, then in eight Erlenmeyer flasks containing 25 mL of different media.

Table 3 displays the urinary calculus contents and the corresponding media for each Erlenmeyer flask. The effectiveness of the various media is indicated by the percentage of dissolution [51], determined using the following formula :

$$a\% = \frac{w_{initial} - w_{final}}{w_{initial}} 100$$

Where a% is the dissolution rate of the urinary stone and $w_{initial}$ and w_{final} are the stone weights before and after incubation in the different solutions.

Table 3. Characteristics of each Erlenmeyer flask used with its corresponding contents: flask number, number of the urinary calculus, and volume of the medium.

Erlenmeyer N°	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Medium	M	A	M	M	M	A	I	M
Stone N°	01	01	02	03	04-1	04-2	04-3	05
Volume (mL)	25	25	50	25	25	25	25	50

At each time interval, the stones were sampled, dried, and weighed to study mass loss. At the same time, pH values were measured by a pH meter in the liquid media.

Note: We analyzed calculus N°01 by infrared and UV spectroscopy only because the solubility test did not work (in our opinion, calculus N°1 was fixed by formalin (formaldehyde) for an anapathological study).

Solubility test

Given that the urinary calculi available to us are unique, we planned to take three small pieces of each calculus to test their dissolution in a 5 ml volume of three different media (El-Maatya water: M, mineral water as a negative control: I, and sodium citrate as a positive control: A).

Ultraviolet analysis

After one day of immersion, 0.2 mL was withdrawn from each medium containing the stones. The samples were diluted and transferred into a UV cell for chromophore analysis. The measurements were performed using a UV–Vis spectrophotometer (Unicam 300, England).

Infrared analysis

Seven pellets were prepared using a mixture of 98% KBr and 2% of the powdered stones (samples 1–5). The infrared spectra were recorded using a Fourier-transform infrared spectrophotometer (Shimadzu IR Affinity-NF, Japan) within the mid-infrared range of 4000–400 cm^{-1}.

Qualitative analysis of urinary calculi using an optical microscope

The study was carried out on small pieces of urinary calculi N° 2, 4, and 5 before and after treatment with M water, using an optical microscope (20X magnification). The microscope used is from the brand Optika.

Survey on the water from the el-maatya region (draa kebila commune) for the treatment of urinary and kidney stones

Responses were collected via Google Forms on this site: https://docs.google.com/forms/d/12QRw4E_QDOGQt-H88zs9LrVALaHzDKdGuCnIIHWIM1U/edit?usp=sharing, as well as by paper questionnaires asking people questions and ticking their answers.

The survey was carried out taking into account various criteria, such as *the gender of the participants*, *the taste of El-Maatya water*, *the region of origin*, *age*, *level of education*, and *the perceived effects of water*. These aspects made it possible to analyse the perceptions and uses of water within the population.

ACKNOWLEDGEMENTS

The authors would like to thank the Laboratoire Public de l'Algérienne des Eaux, Unité Sétif, for performing the physicochemical and microbiological analyses of the El-Maatya spring water. Their technical support and expertise contributed significantly to the quality of this research.

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