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Microbiological and Physicochemical Research of Mountain Spring Waters in Vasiliovska Mountain, Municipality of Teteven, Bulgaria

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Authors' contributions

This work was carried out in collaboration between both authors. Author II designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors II and NV managed the analyses of the study. Author NV managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Defined are the physicochemical properties of three mountain spring waters in the area of Vasiliovska mountain, Municipality of Teteven, Bulgaria. Vasiliovska mountain is in the central part of Stara Planina (Balkan) mountain.

The methodology is including research of physicochemical parameters and microbiological indicators. It is needed the whole parameters to corresponds to Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes in Bulgaria, European Union [1,2,3].

The spring waters from the given three water sources are characterized by microbiological indicators, and the pathogenic micro-organisms in the samples from the springs water sources mentioned above are determined by the membrane method [4,5].

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It is shown that according to 18 controlled parameters included in the study, the following springs in Vasiliovska mountain - “Babintsi” with water temperature 12.3°C, “Gechovoto” with water temperature 13.5°C, “Ignatov izvor” with water temperature 14.1°C, correspond to all controlled parameters according to Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes.

Keywords: Spring mountain water; Vasiliovska mountain; physicochemical properties; microbiological indicators.

1. INTRODUCTION

The causes for that lie in the combination between hydrological conditions of the continuing tectonic processes in the Earth’s crust [6,7,8]. By their nature the springs can be separated in cold, warm and hot springs. The first group includes the ones with temperature up to 37°C and this is cold mineral water. The second one ranges between 37°C and 60°C and this is warm mineral water. The third one with over 60°C and this is hot mineral water. The hottest mineral spring in Bulgaria is the one at Sapareva Banya with temperature of 101.4°C. In Bulgaria there are a lot of mountain spring sources. Municipality of Teteven is in the skirts of two mountains – Zlatishko-Tetevenska and Vasiliovska. There are more than 30 mountain sources and 2 with mineral water in village of Golyam izvor. The springing waters have different mineralogical characteristics. Their content is defined by the ones of the rocks, where the water has been flowing through, and the solubility of the minerals within them (Ignatov, Mosin, 2012).

Whole mountain and mineral springs in Zlatihsko-Tetevenska and Vasiliovska mountain are colds with temperature less than 37°C. The waters from mountain springs have the biggest local extremum at (λ=8.95 μm) ($\tilde{\nu}$=1117 cm^{-1}). This local extremum corresponds with spectrum of water as factor for health and longevity [9,10,11].

In Bulgaria, there are mineral and spring waters, which are not subjected to physicochemical and microbiological control by the Regional Health Inspectorates, yet they are the most widely used springs by the population as sources of drinking water. There are springs located in the territory of Haskovo District [12,13], Stara Zagora District [14,15], Burgas District [16], Varna District [17].

For many of these sources physicochemical and microbiological studies have not been conducted, yet they are used for drinking and household needs [5].

Water is an environment for the development of microorganisms. Studies by many authors, including our research team, demonstrate that microorganisms with valuable properties (enzymes, antibiotics, thermophilic and acidophilic stains) are present in mineral and mountain spring waters.

This was proved by the results obtained from the experimental work carried out to determine the micro flora of medicinal and spring waters in Haskovo, Stara Zagora, Burgas [12-17] and Varna region [3].

There are different studies of physicochemical and microbiological properties of spring mountain water as factor of longevity. The municipality of Teteven [18] and Smolyan [19] are popular in Bulgaria as area of l*ong*-living people. The researches have shown that water is main factory for health and longevity with physicochemical composition and structure [20,21,22,23]. The levels of mountain spring water of Calcium (Ca), Magnesium (Mg), Zinc (Zn) and Manganese (Mn) are essentials for health and longevity [24,25]. Ignatov and Mosin [26] have shown that the optimal values of Calcium if between and 20 and 40 mg/ dm^3 are connected with human life span. Example is Nova Scotia in Canada, where are living 'hyper-centenarians' (people aged over 110 years). There were performed studied of glacier water from Swiss Alps and Chilean Andes [27].

The sources of spring mountain water are from Municipality of Teteven, Vasiliovska mountain, Bulgaria for research are: “Babintsi”, “Gechovoto” and “Ignatov izvor”.

2. MATERIALS AND METHODS

This study used the water samples from the following springs in the district of Sliven. There are “Babintsi”, “Gechovoto”, “Ignatov izvor”.

A comparative physicochemical analysis of mineral spring waters at the territory of Sliven District was performed using the main indicators

(color according to Rublyovska Scale, smell at 20°C, turbidity, pH, oxidisability, chlorides, nitrates, nitrites, ammonium ions, general hardness, sulphates, calcium, magnesium, phosphates, manganese, iron, fluorides, electrical conductivity).

2.1 Nutrient Media

Nutrient agar *Nutrient agar* - agar *MPA* (total bacteria number and diagnostics) [28] with contents (in %) – meat water, peptone – 1%, agar – agar – 2%.

Endo's Medium (for defining of *Escherichia coli* and coliform bacteria) with contents (g/dm^3) – peptone – 5,0; triptone – 5,0; lactose – 10,0; Na_2SO_3 – 1,4; K_2HPO_4 – 3,0; fuchsine – 0,14; agar – agar – 12,0 pH 7,5 – 7,7.

Nutrient gelatin (MPD) (for defining of *Pseudomonas aeruginosa*) with contents (in%) – Peptic digest of animal tissue; 25% gelatin; pH = 7, 0 – 7, 2.

Medium for defining of enterococci (esculin – bile agar).

Medium for defining of sulphite reducing bacteria (Iron Sulfite Modified Agar).

Wilson-Bleer medium (for defining of sulphite reducing spore anaerobes (*Clostridium perfringens*) with contents (g/dm^3) – 3%.

2.2 Methods for Analysis

2.2.1 Methods for physicochemical analysis

1. Method for determination of color according to Rublyovska Scale – method by Bulgarian State Standard (BDS) 8451: 1977;
2. Method for determination of smell at 20°C — method BDS 8451: 1977 technical device – glass mercury thermometer, conditions No 21;
3. Method for determination of turbidity - EN ISO 7027, technical device turbidimeter type TURB 355 IR ID No 200807088;
4. Method for determination of pH – BDS 3424: 1981, technical device pH meter type UB10 ID NoUB10128148;
5. Method for determination of oxidisability – BDS 3413: 1981;
6. Method for determination of chlorides – BDS 3414: 1980;
7. Method for determination of nitrates – Validated Laboratory Method (VLM) – NO3 – No 2, technical device photometer "NOVA 60 A" ID No 08450505;
8. Method for determination of nitrites – VLM NO_2 – No 3, technical device photometer "NOVA 60 A" ID No 08450505;
9. Method for determination of ammonium ions – VLM – NO_4 – No 1, technical device photometer "NOVA 60 A" ID No 08450505;
10. Method for determination of general Method for hardness – BDS ISO 6058;
11. Method for determination of sulphates – VLM - SO4 – No 4, technical device photometer "NOVA 60 A" ID No 08450505;
12. Method for determination of calcium – BDS ISO 6058;
13. Method determination of magnesium – BDS 7211: 1982;
14. Method for determination of phosphates – VLM - PO4 – No 5, technical device photometer "NOVA 60 A" ID No 08450505;
15. Method for determination of manganese – VLM – Mn – No 7, technical device photometer "NOVA 60 A" ID № 08450505;
16. Method for determination of iron – VLM – Fe – No 6, technical device photometer "NOVA 60 A" ID No 08450505;
17. Method for determination of fluorides – VLM – F – No 8, technical device photometer "NOVA 60 A" ID No 08450505;
18. Method for determination of electrical conductivity – BDS EN 27888, technical device – conductivity meter inoLabcond 720 ID No 11081137.

2.2.2 Methods for determination of microbiological indicators

1. Methods for evaluation of microbiological indicators according to Ordinance No 9 / 2001, Official State Gazette, issue 30, and decree No 178 / 23.07.2004 about the quality of water, intended for drinking purposes.
2. Method for determination of *Escherichia coli* and coliform bacteria – BDS EN ISO 9308 – 1: 2004; Method for determination of enterococci – BDS EN ISO 7899 – 2;
3. Method for determination of sulphite reducing spore anaerobes – BDS EN 26461 – 2: 2004;
4. Method for determination of total number of aerobic and facultative anaerobic bacteria – BDS EN ISO 6222: 2002;
5. Method for determination of *Pseudomonas aeruginosa* – BDS EN ISO 16266: 2008.

6. Determination of coli – titer by fermentation method – Ginchev's method (Bulgarian standard)
7. Determination of coli – bacteria over Endo's medium – membrane method.
8. Determination of sulphite reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

3. RESULTS AND DISCUSION

The results from the tests of physicochemical research with are given in Table 1.

The results are for the following mountain sources in Municipality of Teteven in Vasiliovska mountain.

3.1 Babintsi

The mountain spring "Babintsi" is near to village of Babintsi, 950 m altitude above sea level. The spring mountain water is cold (12,3°C), low-mineralized (236.6 mg / dm^3, alkalinereaction (pH 8.0). Water from this source has relaxing effect on the nervous system.

3.2 Gechovoto

The mountain spring "Gechovoto" is near to neighborhood Gechovoto, town of Teteven, 655 m altitude above sea level. The mountain spring water is cold (13,5°C), low-mineralized (217.2 mg / dm^3, alkalinereaction (pH 7.94). Water from this source has relaxing effect on the nervous system and stimulating effect of the smooth muscle.

3.3 Ignatov Izvor

The mountain spring "Gechovoto" is near to neighborhood Gechovoto, town of Teteven, 655 m altitude above sea level. The mineral water is cold (14,1°C), low-mineralized (187.4 mg / dm^3, alkalinereaction (pH 6.82). Water from this source has relaxing effect on the nervous system and stimulating effect of the smooth muscle.

Also, the microbiological indicators for the same spring waters were determined by the membrane method. The experimental studies from the determination of total number of mesophilic aerobic and facultative anaerobic bacteria are shown in Table 2.

Table 1. Comparison of the examined spring waters Vasiliovska mountain, Municipality of Teteven, Bulgaria

Controlled parameter	Measuring unit	Maximum limit value	Result Babintsi	Result Gechovoto	Result Ignatov izvor
1. Color Rublyovska Scale	Chromaticity values	Acceptable	Acceptable	Acceptable	Acceptable
2. Smell at 20°C	Rating	Acceptable	Acceptable to	Acceptable to	Acceptable
3.Turbidity	NTU	Acceptable	Acceptable	Acceptable	Acceptable
4. pH	pH values	≥ 6,5 и ≤ 9,5	8.00±0.10	7.94±0.10	6.82±0.10
5. Oxidisability	mgO_2/dm^3	≤5.0	0.96±0.10	0.62±0.06	0.78±0.08
6. General hardness	mgekv/ dm^3	≤12	2.86±0.10	3.45±0.35	2.76±0.28
7. Chlorides	mg/ dm^3	≤250	2.9	4.1±0.4	2.7±0.3
8. Nitrates	mg/ dm^3	≤50	2.9±0.3	9.8±1.0	<0.05
9. Nitrites	mg/ dm^3	≤0,50	<0.05	<0.05	5.9±0.6
10. Ammonium ions	mg/ dm^3	≤0,50	<0.013	<0.013	<0.013
11. Sulphates	mg/ dm^3	≤250	19.1±1.9	15.9±1.6	17.9±1.8
12. Calcium	mg/ dm^3	≤150	54.0±5.0	66±6.6	40.44±4.04
13. Magnesium	mg/ dm^3	≤80	2.48±0.25	2.1±0.2	2.46±0.25
14. Phosphates	mg/ dm^3	≤0,5	<0.10	<0.10	<0.10
15. Manganese	mg/ dm^3	≤50	<0.1	1.0±0.1	1.8±0.2
16. Iron	µg/ dm^3	≤200	5.0±0.5	11.4±1.1	13.9±1.4
17. Fluorides	mg/ dm^3	≤1,5	<0.10	<0.10	<0.10
18.Electrical conductivity	µS/ dm3	≤2000	274±8	326±10	244±8

Table 2. Determination of total number of mesophilic aerobic and facultative anaerobic bacteria

Examined water source	Indicator, cfu/cm^3
1. Mountain spring "Babibtsi" with temperature 12.3°C	4± 1
2. Mountain spring "Babibtsi" with temperature 13.5°C	3 ± 1
3. Mountain spring "Babibtsi" with temperature 14.1°C	5± 1

Table 3. Coli – titre of thermal healing spring waters in Vasiliovska mountain, Municipality of Teteven, Bulgaria

Name of water source	Coli- titre	Culture volumes 50 cm^3	Culture volumes 10 cm^3	Culture volumes 10 cm^3	Culture volumes 10 cm^3	Culture volumes 10 cm^3	Culture volumes 10 cm^3
1. Mountain spring "Babibtsi" with temperature 12.3°C	> 100	–	–	–	–	–	–
2. Mountain spring "Gechovoto" with temperature 13.5°C	> 100	–	–	–	–	–	–
3. Mountain spring "Ignatov izvor" with temperature 14.1°C	> 100	–	–	–	–	–	–

Table 4. Microbiological indicators of spring waters in Vasiliovska mountain, Municipality of Teteven, Bulgaria

Indicators	Norm	Measuring unit	Mountain spring "Babibtsi" with temperature 12.3°C	Mountain spring "Gechovoto" with temperature 13.5°C	Mountain spring "Ignatov izvor" 14.1°Cwith temperature
Coliforms	0/100	cfu/cm^3	0/100	0/100	0/100
Escherichia coli	0/100	cfu/cm^3	0/100	0/100	0/100
Enterococci	0/100	cfu/cm^3	0/100	0/100	0/100
Sulphite reducing anaerobic bacteria *(Clostridium perfringens)*	0/100	cfu/cm^3	0/100	0/100	0/100
Pseudomonas aeruginosa	0/250	cfu/cm^3	0/250	0/250	0/250

According to the standard requirements from the examined water samples from the four springs, the water is clean.

The presence of coli forms and Escherichia coli is determined by the membrane method, and according to Ginchev's method (Bulgarian standard). The experimental results (Table 3 and Table 4) reveal "Babintsi", "Gechovoto" and "Ignatov izvor", are in compliance with the requirements for presence of coli bacteria.

The present results for those springs are also confirmed by the analyses via the membrane method (Table 4). All the remaining indicators are determined by the membrane method.

4. CONCLUSION

Based on the conducted physicochemical and microbiological evaluations it is established that from the four examined springs at the territory of Sliven district, Bulgaria thermal healing mineral spring "Banya" and non - thermal healing spring "Babibtsi", "Gechovoto" and "Ignatov izvor" sources correspond to all controlled parameters according to Ordinance № 9/2001, Official State Gazette, issue 30, and decree № 178/23.07.2004 about the quality of water, intended for drinking purposes.

DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Standards

1. Ordinance № 9 / 2001, Official State Gazette, issue 30.
2. Decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes.
3. BDS 8451: 1977 – defining of color according to Rublyovska Scale, determination of smell at 20°C.
4. EN ISO 7027 – determination of turbidity.
5. BDS 3424 : 1981 – determination of pH.
6. BDS 3413 : 1981 – determination of oxidisability.
7. BDS 3414 : 1980 – determination of chlorides.
8. BDS ISO 6058 – determination of calcium, determination of general hardness.
9. BDS EN 27888 – determination of electrical conductivity.
10. VLM – NH4 – № 1 – determination of ammonium ions.
11. VLM – NO3 – № 2 – determination of nitrates.
12. VLM – NO2 – № 3 – determination of nitrites.
13. VLM – SO4 – № 4 – determination of sulphates.
14. VLM – PO4 – № 5 – determination of phosphates.
15. VLM – Fe – № 6 – determination of iron.
16. VLM – Mn – № 7 – determination of manganese.
17. VLM – F – № 8 – determination of fluorides.
18. BDS 7211: 1982 – determination of magnesium.
19. BDS EN ISO 7899 – 2 – determination of nitrates.
20. BDS EN ISO 9308 – 1: 2004 – determination of Escherichia coli and coliform bacteria.
21. BDS EN 26461 – 2 : 2004 – determination of sulphite reducing anaerobic bacteria (Clostridium perfringens) .
22. BDS EN ISO 16266 – determination of Pseudomonas aeruginosa.
23. BDS EN ISO 7899 – 2 – determination of eneterococci.
24. BDS EN ISO 6222: 2002 – determination of total number of aerobic and facultative anaerobic bacteria.

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