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 Vestilovska 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; Vestilovska 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 Vestilovska. 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 (Ignotov, Mosin, 2012).

Whole mountain and mineral springs in Zlatishko-Tetevenska and Vestilovska mountain are colds with temperature less than 37°C. The waters from mountain springs have the biggest local extremum at (λ=8.95 μm) (ν=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 long-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]. Ignotov and Mosin [26] have shown that the optimal values of Calcium if between 20 and 40 mg/ dm² are connected with human life span. Example is Nova Scotia in Canada, where are living hypercentenarians’ (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, Vestilovska 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).

\[\text{Wilson - Bleer medium} \quad \text{(for defining of sulphite reducing spore anaerobes \textit{Clostridium perfringens}) with contents \((g/dm^3) - 3\%} \]

\[\text{Endo's Medium} \quad \text{(for defining of \textit{Escherichia coli} and coliform bacteria) with contents \((g/dm^3) - \text{peptone} - 5,0; \text{triptone} - 5,0; \text{lactose} - 10,0; \text{Na}_2\text{SO}_3 - 1,4; \text{K}_2\text{HPO}_4 - 3,0; \text{fuchsin} - 0,14; \text{agar} - \text{agar} - 12,0 \text{pH} 7,5 - 7,7} \]

\[\text{Nutrient gelatin (MPD) \quad \text{(for defining of \textit{Pseudomonas aeruginosa}) with contents \((in \%) - \text{Peptic digest of animal tissue} - 25\% \text{gelatin; pH} = 7, 0 - 7, 2}} \]

\[\text{Medium for defining of enterococci (esculin - bile agar)} \]

\[\text{Medium for defining of sulphite reducing bacteria (Iron Sulfite Modified Agar)} \]

\[\text{2.2 Methods for Analysis} \]

\[\text{2.2.1 Methods for physicochemical analysis} \]

1. Method for determination of color according to Rublyovska Scale – method by Bulgarian State Standard (BDS) 8451: 1977;
3. Method for determination of turbidity - EN ISO 7027, technical device turbidimeter type TURB 355 IR ID No 200807088;
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 NO2 – No 3, technical device photometer "NOVA 60 A" ID No 08450505;
9. Method for determination of ammonium ions – VLM – NO4 – No 1, technical device photometer "NOVA 60 A" ID No 08450505;
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;
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 No 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;

\[\text{2.2.2 Methods for determination of microbiological indicators} \]

6. Determination of coli –liter by fermentation method – Ginchev’s method (Bulgarian standard)
8. Determination of sulphite reducing anaerobic bacteria (Clostridium perfringens) – membrane method.

3. RESULTS AND DISCUSSION

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 / dm³), alkaline reaction (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 / dm³), alkaline reaction (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 / dm³), alkaline reaction (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

<table>
<thead>
<tr>
<th>Controlled parameter</th>
<th>Measuring unit</th>
<th>Maximum limit value</th>
<th>Result Babintsi</th>
<th>Result Gechovoto</th>
<th>Result Ignatov izvor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Color Rublyovska Scale</td>
<td>Chromacity values</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Acceptable</td>
</tr>
<tr>
<td>2. Turbidity</td>
<td>Rating</td>
<td>Acceptable</td>
<td>Acceptable to</td>
<td>Acceptable</td>
<td>Acceptable</td>
</tr>
<tr>
<td>3. pH</td>
<td>pH values</td>
<td>≥ 6.5 or ≤ 9.5</td>
<td>8.00±0.10</td>
<td>7.94±0.10</td>
<td>6.82±0.10</td>
</tr>
<tr>
<td>4. Oxidisability</td>
<td>mgO₂/dm³</td>
<td>≤5.0</td>
<td>0.96±0.10</td>
<td>0.62±0.06</td>
<td>0.78±0.08</td>
</tr>
<tr>
<td>5. General hardness</td>
<td>mg/ dm³</td>
<td>≤12</td>
<td>2.86±0.10</td>
<td>3.45±0.35</td>
<td>2.76±0.28</td>
</tr>
<tr>
<td>6. Chlorides</td>
<td>mg/ dm³</td>
<td>≤250</td>
<td>2.9</td>
<td>4.1±0.4</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>7. Nitrites</td>
<td>mg/ dm³</td>
<td>≤50</td>
<td>2.9±0.3</td>
<td>9.8±1.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>8. Nitrites</td>
<td>mg/ dm³</td>
<td>≤0.5</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>9. Ammonium ions</td>
<td>mg/ dm³</td>
<td>≤0.5</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
</tr>
<tr>
<td>10. Sulphates</td>
<td>mg/ dm³</td>
<td>≤250</td>
<td>19.1±1.9</td>
<td>15.9±1.6</td>
<td>17.9±1.8</td>
</tr>
<tr>
<td>11. Calcium</td>
<td>mg/ dm³</td>
<td>≤150</td>
<td>54.0±5.0</td>
<td>66±6.6</td>
<td>40.4±4.04</td>
</tr>
<tr>
<td>12. Magnesium</td>
<td>mg/ dm³</td>
<td>≤80</td>
<td>2.48±0.25</td>
<td>2.1±0.2</td>
<td>2.46±0.25</td>
</tr>
<tr>
<td>13. Phosphates</td>
<td>mg/ dm³</td>
<td>≤0.5</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>14. Manganese</td>
<td>mg/ dm³</td>
<td>≤50</td>
<td>&lt;0.1</td>
<td>1.0±0.1</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>15. Iron</td>
<td>µg/ dm³</td>
<td>≤200</td>
<td>5.0±5.0</td>
<td>11.4±1.1</td>
<td>13.9±1.4</td>
</tr>
<tr>
<td>16. Fluorides</td>
<td>mg/ dm³</td>
<td>≤1.5</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>17. Electrical conductivity</td>
<td>µS/ dm³</td>
<td>≤2000</td>
<td>274±8</td>
<td>326±10</td>
<td>244±8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Examined water source</th>
<th>Indicator, cfu/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mountain spring “Babintsi” with temperature 12.3°C</td>
<td>4±1</td>
</tr>
<tr>
<td>2. Mountain spring “Babintsi” with temperature 13.5°C</td>
<td>3±1</td>
</tr>
<tr>
<td>3. Mountain spring “Babintsi” with temperature 14.1°C</td>
<td>5±1</td>
</tr>
</tbody>
</table>
### Table 3. Coli – titre of thermal healing spring waters in Vasiliovska mountain, Municipality of Teteven, Bulgaria

<table>
<thead>
<tr>
<th>Name of water source</th>
<th>Coli- titre</th>
<th>Culture volumes 50 cm³</th>
<th>Culture volumes 10 cm³</th>
<th>Culture volumes 10 cm³</th>
<th>Culture volumes 10 cm³</th>
<th>Culture volumes 10 cm³</th>
<th>Culture volumes 10 cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mountain spring &quot;Babibtsi&quot; with temperature 12.3°C</td>
<td>&gt; 100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2. Mountain spring &quot;Gechovoto&quot; with temperature 13.5°C</td>
<td>&gt; 100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3. Mountain spring &quot;Ignatov izvor&quot; with temperature 14.1°C</td>
<td>&gt; 100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Norm</th>
<th>Measuring unit</th>
<th>Mountain spring &quot;Babibtsi&quot; with temperature 12.3°C</th>
<th>Mountain spring &quot;Gechovoto&quot; with temperature 13.5°C</th>
<th>Mountain spring &quot;Ignatov izvor&quot; 14.1°C with temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms</td>
<td>0/100</td>
<td>cfu/cm³</td>
<td>0/100</td>
<td>0/100</td>
<td>0/100</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0/100</td>
<td>cfu/cm³</td>
<td>0/100</td>
<td>0/100</td>
<td>0/100</td>
</tr>
<tr>
<td>Enterococci</td>
<td>0/100</td>
<td>cfu/cm³</td>
<td>0/100</td>
<td>0/100</td>
<td>0/100</td>
</tr>
<tr>
<td>Sulphite reducing anaerobic bacteria (Clostridium perfringens)</td>
<td>0/100</td>
<td>cfu/cm³</td>
<td>0/100</td>
<td>0/100</td>
<td>0/100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0/250</td>
<td>cfu/cm³</td>
<td>0/250</td>
<td>0/250</td>
<td>0/250</td>
</tr>
</tbody>
</table>
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

The products used for this research are for scientific research and they are not products of companies. There is absolutely no conflict of interests. The research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Standards

3. BDS 8451: 1977 – defining of color according to Rublyovska Scale, determination of smell at 20°C.
5. BDS 3424 : 1981 – determination of pH.
8. BDS ISO 6058 – determination of calcium, determination of general hardness.
10. VLM – NH4 – № 1 – determination of ammonium ions.
11. VLM – NO3 – № 2 – determination of nitrates.
15. VLM – Fe – № 6 – determination of iron.
17. VLM – F – № 8 – determination of fluorides.
22. BDS EN ISO 16266 – determination of Pseudomonas aeruginosa.

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