Physicochemical and Microbiological Characteristics of Thermal Healing Spring Waters in the District of Varna

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Abstract
Defined are the physicochemical properties of healing thermal and non-thermal spring waters in the area of Varna District. It is shown that according to 18 controlled parameters included in the research, the thermal healing spring water drilling №Р-83хKK “Saints Constantine and Helena”, thermal healing spring water Р-1x “Aquarium”, thermal healing spring water P-106 x “Dom Mladost”, thermal healing spring water P-161x Varna at “Primorski” swimming pool, fulfill the required conditions for drinking water. The spring waters from the given four water sources are characterized by microbiological indicators, as the pathogenic micro-organisms are defined by the membrane method. It is established that thermal healing spring water drilling №P-83xKK “Saints Constantine and Helena”, thermal healing spring water P-1x “Aquarium”, thermal healing spring water P-106 x “Dom Mladost”, thermal healing spring water P-161x Varna at “Primorski” swimming pool, fulfill the standard requirements. “The healing water” of village Goren Chiflik, District of Varna does not conform with the physicochemical indicators given for nitrates, and microbiological indicators with regards to coliform bacteria and enterococci.

Keywords: spring water, drinking water, physicochemical properties, microbiological indicators
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1. Introduction
Bulgaria is one of the richest in mineral springs countries in Europe. It takes second place after Iceland. Their total number is around 225. Based on their location are observed certain specifics. The ones to the north of Balkan are with lower temperatures, and are reached usually via drilling. Their total number is almost half the amount of the ones to the south of Old Mountain. There are around 148 known springs from Southern Bulgaria. Predominant in them are the ones with natural origin and higher temperature of the water. The causes for that lie in the combination between hydrological conditions of the continuing tectonic processes in the Earth’s crust [Ignatov, 2010]. 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, the second one ranges between 37°C and 60°C, and the third one with over 60°C. The hottest mineral spring in Bulgaria is the one at Sapareva Banya with temperature of 101,4°C. 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].

Mineral springs in Varna district
Health resort “Saints Constantine and Helena” is the first Bulgarian resort at Black Sea. One of the most important conditions for the resort development is the availability of 7 mineral springs with no analogue in Europe. They are calcium-magnesium, with low mineralization and come from depth of 1800 to 2050 meters under the ground with total flow rate of 175 l/sec. The temperature of the water varies between 40 and 60 degrees centigrade, it can heal successfully cardiovascular diseases, the endocrine system, illnesses of the musculoskeletal system and the functional nervous system, myocardial infarction.

Thermal healing spring, city of Varna 2 ( P-1x “Aquarium”)
- Healing prophylactic properties of the mineral water-the drinking thermal cure has positive influence over gastro-intestinal tract, biliary liver system and kidney excretory system. The presence of calcium proves to be suitable for application of mineral water for treatment of dental caries, as well as osteoporosis of any kind.

Thermal healing spring Varna (P-106 x “Dom Mladost”)
The water comes via drilling with depth 1980 m, and it is thermal with temperature of 47°C. It cures conditions of cardiovascular system, of peripheral nervous system, digestive system, gynaecological diseases and post-traumatic stress disorders.
Non-thermal healing spring “Healing water”, village of Goren Chiflik
Healing water that can be ignited and can burn, it springs up in the locality Botevo near Dolni Chiflik. That is due to the methane contained within it. The water comes up years ago after drilling for natural gas. The phenomenal liquid springs up like a geyser from 600 meters depth. Research shows that the water contains around 30% iodine and helps for gastro-intestinal conditions, arthritis, skin and eye diseases.

2. Materials and methods
In the work are used thermal healing waters from the district of Varna – thermal healing spring Varna 1 (Healing resort “Saints Constantine and Helena”) with water temperature of 48°C, thermal healing spring city of Varna 2 (P-1x “Aquarium) with water temperature of 47°C, thermal healing spring Varna 3 (P-106 x “Dom Mladost”) with water temperature of 40°C, thermal healing spring Varna 4 (P-161x Varna at swimming pool “Primorski”) with water temperature of 50°C, non-thermal healing spring “Healing water”, village of Goren Chiflik.

2.1. Nutrient media
Nutrient agar (MPA) 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$_2$SO$_3$ – 1,4; K$_2$HPO$_4$ – 3,0; fuchsine – 0,14; agar – agar – 12,0 pH 7,5 – 7,7.
Nutrient gelatine (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% Nutrient agar; 100 cm$^3$ 20% solution Na$_2$SO$_3$; 50 cm$^3$ 20% glucose solution; 10 cm$^3$ 8% solution of Fe$_2$SO$_4$.

2.2. Methods for analysis
2.2.1. Methods for physicochemical analysis
1. Method for determination of colour 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 No UB10128148;
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) – NO$_3$ – No 2, technical device photometer "NOVA 60 A" ID No 08450505;
8. Method for determination of nitrites – VLM NO$_3$ – No 3, technical device photometer "NOVA 60 A" ID No 08450505;
9. Method for determination of ammonium ions – VLM - NH$_4$ – No 1, technical device photometer "NOVA 60 A" ID No 08450505;
10. Method for determination of general hardness – BDS ISO 6058;
11. Method for determination of sulphates – VLM - SO$_4$ – No 4, technical device photometer "NOVA 60 A" ID No 08450505;
12. Method for determination of calcium – BDS ISO 6058;
13. Method for determination of magnesium – BDS 7211 : 1982;
14. Method for determination of phosphates – VLM - PO$_4$ – 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;
18. Method for determination of electrical conductivity – BDS EN 27888, technical device – conductivity meter inoLab cond 720 ID No 11081137.
2.2.2. Methods for determination of microbiological indicators

1. Methods for evaluation of microbiological indicators 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.
2. Method for determination of *Escherichia coli* and coliform bacteria – BDS EN ISO 9308 – 1: 2004;
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 – titre by fermentation method – Ginchev’s method
7. Determination of coli – bacteria over Endo’s medium – membrane method.

Determination of sulphite reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

3. Results and discussion

It is done a comparative physicochemical analysis of mineral spring waters at the territory of Varna District by the main indicators (colour 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). The results from these examinations are given in Table 1.

### Table 1. Comparison of the examined spring waters in Varna District by physicochemical properties

| Controlled parameter | Measuring unit | Maximitm Limit Value | Result Varna 1 (drilling №Р-83хКК „Saints Constantine and Helena”) | Result Varna 2 (P-1x "Aquarium") | Result Varna 3 (P-106 x "Dom Mladost") | Result Varna 4 (P-161x Varna "Primorski" swimming pool) |
|----------------------|----------------|----------------------|-------------------------------------------------|---------------------------------|---------------------------------|-------------------------------------------------|
| 1. Color according to Rublyovska Scale | Chromaticity Values | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers |
| 2. Smell at 20°C | Rating | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers |
| 3. Turbidity | NTU | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers | Acceptable to consumers |
| 4. pH | pH единици | ≥ 6,5 и ≤ 9,5 | 7,79 | 7,6 | 9,48 | 9,46 |
| 5. Oxidisability | mgO2/dm³ | 5,0 | 2,1 | 1,6 | 1,9 | 1,2 |
| 6. Chlorides | mg/ dm³ | 250 | 64,41 | 104 | 130 | 96 |
| 7. Nitrates | mg/ dm³ | 50 | 0,9 | 2,9 | 2,1 | 4,9 |
| 8. Nitrites | mg/ dm³ | 0,50 | 0,04 | 0,04 | 0,00 | 0,004 |
| 9. Ammonium ions | mg/ dm³ | 0,50 | 0,04 | 0,04 | 0,21 | 0,85 |
| 10. Hardness | mgekv/ dm³ | 12 | 4 | 3,9 | 11,5 | 11,5 |
| 11. Sulphates | mg/ dm³ | 250 | 36,21 | 77 | 62 | 76 |
| 12. Calcium | mg/ dm³ | 150 | 53,11 | 43 | 118 | 120 |
| 13. Magnesium | mg/ dm³ | 80 | 32,83 | 27 | 68 | 67 |
| 14. Phosphates | mg/ dm³ | 0,5 | 0,02 | 0,03 | 0,02 | 0,02 |
| 15. Manganese | mg/ dm³ | 50 | 0,01 | 0,001 | 0,009 | 0,05 |
| 16. Iron | µg/ dm³ | 200 | 0,05 | 0,59 | 481 | <5 |
| 17. Fluorides | mg/ dm³ | 1,5 | 0,43 | 0,71 | 0,55 | 0,62 |
| 18. Electrical conductivity | µS/ dm³ | 2000 | 694 | 768 | 350 | 350 |

The trial data reveal that thermal healing spring water drilling №P-83xKK “Saints Constantine and Helena”, thermal healing spring water P-1x “Aquarium”, thermal healing spring water P-106 x “Dom Mladost”, thermal healing spring water P-161x at “Primorski” swimming pool are in compliance with the controlled parameters set out in Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes. The “Healing water”, village of Goren Chiflik, District of Varna is not in compliance with regards to nitrates – higher than 130 milligrams per liter (RZI (Regional Health Inspection) –
For the same spring waters are determined their microbiological indicators by the membrane method. In Table 2 are shown the experimental studies from the determination of total number of mesophilic aerobic and facultative anaerobic bacteria.

**Table 2**

| Examined water source                                                                 | Indicator, cfu/cm³ |
|--------------------------------------------------------------------------------------|--------------------|
| 1. Thermal healing spring Varna 1 (drilling №Р-83х Health Resort „Saints Constantine and Helena”) with water temperature of 48 °C | 4± 1               |
| 2. Thermal Healing Spring Varna 2 P-1x "Aquarium”) with water temperature of 47 °C | 5 - 7              |
| 3. Thermal Healing Spring P-106 x „Dom Mladost“ with water temperature of 40 °C      | 5± 1               |
| 4. Thermal Healing Spring P-161x Varna at "Primorski“ swimming pool with water temperature of 50 °C | 5-8                |
| 5. Healing spring „Healing water“ village of Goren Chiflik                          | 170-180            |

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

The presence of coliforms and *Escherichia coli* is determined by the membrane method, and according to Ginchev’s method. The trial results (Table 3 and Table 4) reveal that thermal healing spring drilling №Р-83х Health Resort “Saints Constantine and Helena” with water temperature of 48°C, thermal healing spring P-1x “Aquarium” with water temperature of 47°C, thermal healing spring P-106 x “Dom Mladost” with water temperature of 40°C, thermal healing spring P-161x Varna at “Primorski” swimming pool, are in compliance with the requirements for presence of coli bacteria. Non-thermal healing spring “Healing water” village of Goren Chiflik does not comply with the requirements for presence of coliform bacteria and enterococci. The given results are also confirmed by the analyses via the membrane method (Table 4). All the remaining indicators are determined by the membrane method.

**Table 3. Coli – titre of thermal healing spring waters**

| Name of water source                                                                 | Coli titre | Culture volumes 50cm³ | Culture volumes 10cm³ | Culture volumes 10cm³ | Culture volumes 10cm³ | Culture volumes 10cm³ | Culture volumes 10cm³ |
|--------------------------------------------------------------------------------------|------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| 1. Thermal healing spring Varna 1 (drilling №Р-83х Health Resort „Saints Constantine and Helena”) with water temperature of 48 °C | > 100      | –                      | –                      | –                      | –                      | –                      | –                      |
| 2. Thermal Healing Spring Varna 2 P-1x "Aquarium") with water temperature of 47 °C | > 100      | –                      | –                      | –                      | –                      | –                      | –                      |
| 3. Thermal healing spring P-106 x „Dom Mladost“ with water temperature of 40 °C      | > 100      | –                      | –                      | –                      | –                      | –                      | –                      |
| 4. Thermal healing spring P-161x Varna at "Primorski“ swimming pool with water temperature of 50 °C | > 100      | –                      | –                      | –                      | –                      | –                      | –                      |
| 5. Non-thermal healing spring „Healing water“ village of Goren Chiflik              | 70         | Acid                   | Acid                   | Acid and gas           | Acid and gas           | Acid and gas           | Acid and gas           |
## Table 4 Microbiological indicators of spring waters in Varna District

| Indicators                                                                 | Thermal healing spring Varna 1 (drilling №Р-83x КК “Saints Constantine and Helena”) with water temperature of 48 °С | Thermal healing spring Varna 2 P-1x "Aquarium" with water temperature of 47 °С | Thermal healing spring P-106 x „Dom Mladost“ with water temperature of 40 °C | Thermal healing spring P-161x Varna at "Primorski" swimming pool with water temperature of 50 °C | Non-thermal healing spring „Heling wateria“ village of Goren Chiflik |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------|
| Coliforms                                                                 | 0/100                                                                                                           | 0/100                                                                            | 0/100                                                                            | 0/100                                                                            | 15/100                                                                 |
| *Escherichia coli* Enteroceccii                                           | 0/100                                                                                                           | 0/100                                                                            | 0/100                                                                            | 0/100                                                                            | 15/100                                                                 |
| Sulphite reducing anaerobic bacteria (*Clostridium perfringens*)          | 0/100                                                                                                           | 0/100                                                                            | 0/100                                                                            | 0/100                                                                            | 10/100                                                                 |
| *Pseudomonas aeruginosa*                                                  | 0/250                                                                                                           | 0/250                                                                            | 0/250                                                                            | 0/250                                                                            | 0/250                                                                 |

Based on the conducted physicochemical and microbiological evaluations it is established that from the five examined springs at the territory of Varna District only thermal spring water, drilling №Р-83x КК “Saints Constantine and Helena”, thermal healing spring water P-1x "Aquarium", thermal healing spring water P-106 x “Dom Mladost”, thermal healing spring water P-161x Varna at “Primorski” swimming pool 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, and with regards to microbiological parameters thermal healing water, drilling №Р-83x КК “Saints Constantine and Helena”, thermal healing spring water P-1x "Aquarium", thermal healing spring water P-106 x “Dom Mladost”, thermal healing spring water P-161x at “Primorski” swimming pool are in compliance with the requirements for drinking water.

“Healing water” village of Goren Chiflik, Varna District does not comply with physicochemical indicators given for nitrates – them being higher than 130 milligrams per litre (Regional Health Inspection (RZI) – Varna), and with regards to microbiological indicators it is not in compliance with the requirements for presence of coliform bacteria and enterococci. 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, it is not suitable for drinking.

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BDS 3414 : 1980 – determination of chlorides.

BDS ISO 6058 – determination of electrical conductivity.
BDS 7211 : 1982 – determination of magnesium.
BDS EN ISO 7899 – 2 – determination of nitrates.
BDS EN ISO 9308 – 1: 2004 – determination of Escherichia coli and coliform bacteria.
BDS EN 26461 – 2 : 2004 – determination of sulphite reducing anaerobic bacteria (Clostridium perfringens).
BDS EN ISO 16266 – determination of Pseudomonas aeruginosa.
BDS EN ISO 7899 – 2 – determination of enterococci.
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VLM – SO₄ – № 4 – determination of sulphates.
VLM – PO₄ – № 5 – determination of phosphates.
VLM – Fe – № 6 – determination of iron.
VLM – Mn – № 7 – determination of manganese.
VLM – F– № 8 – determination of fluorides.

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