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Physicochemical and Microbiological Characteristics of Thermal Healing Spring Waters in the Districts of Varna and Burgas, Black Sea Region, Bulgaria

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Abstract

Defined are the physicochemical properties of healing thermal spring waters in the area of Burgas District. It is shown that according to 18 controlled parameters included in the research, the thermal healing spring water village of Shivarovo, thermal healing spring water village of Polyanovo, 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 Burgas Mineral baths, thermal healing spring water village of Shivarovo, thermal healing spring water of village of Polyanovo, fulfill the standard requirements. The healing water of village Judge, District ofBurgas does not conform to the physicochemical indicators given for fluorides, and microbiological indicators with regards to coliform bacteria, *Escherichia coli* and enterococci.

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 №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 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.

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.

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In Bulgaria there are mineral and spring waters, which are not subjected to physicochemical and microbiological control by the Regional Health Inspectorate and microbiological control byt they are the most use springs for drinking from the population. Similar springs are located in the territory of Haskovo District, Stara Zagora District, Varna District (Valcheva et al., 2013).

Many of these sources do not carry out physicochemical and microbiological studies bu are used for drinking and household needs.

Although water is an unfavorable environment for the development of microorganisms and for the development of microorganisms, studies by many authors including heir, own, that microorganisms with valuable properties (enzymes, antibiotics, thermopile can acidophilic strains) are in mineral and non – thermal spring waters. This was proved by the results obtained from the experimental work carried out to determinal the microflora of medicinal and spring waters in Haskovo , Stara Zagora, Plovdiv (Tumbarski et al., 2014) and Varna region (Valcheva, Ignatov, 2019).

Isolated bacteria from the healing and spring regions have been identified by *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Bacillus methylotrophicus, Aeromonashydrophila.*

The isolated bacteria from the healing and spring waters in the Plovdiv region have been identified by molecular genetic methods suchas *Aeromonassobria, Klebsiellaoxytoca, Bacillus amyloliquefacienssubsp. plantarum, Bacillus thuringiensis, Bacillus cereus* (Valcheva et al., 2013, 2014).

Strains with high proteolytic, lipolytic and amylolytic activity were selected (Valcheva et al., 2013, 2014).

Antimicrobial activity of the strains of *Bacillus* sp., against the saprophytic and pathogenic microorganisms was detected: *Penicillium sp., Fusariummoliniforme, Rhizopus sp., Aspergillusniger, Aspergillusoryzae, Aspergillusawamori, Mucorsp. Enterococcus faecalis*, in the process of development and growt of the four *Bacillus – Bacillus cereus, Bacillus thuringiensis, Bacillus subtilis, Bacillus methylotrophicus*are the the most active strains – *Bacillus* methylotrophicus PY5, *Bacillus cereus LH1*, *Bacillus cereus WIF15* и *Bacillus thuringiensis B62* (Valcheva et al., 2013, 2014).

Pathogenic bacteria exhibit resistance and 4 retain their vitality in the process of develop pment and interaction between them and the strains of *Bacillus sp.* and at 37C°.

A relatively low bactericidal effect was demonstrated against the (Gr+) bacterium *Enterococcus faecalis*. The isolated strains are likely to have a higher inhibitory ability agains(Gr -) bacteria compared to (Gr+) bacteria (Valcheva et al., 2013, 2014).

The yeasts used in the genus *Candida* exhibit a simulating effect of two of *Bacillus sp. – Bacillus methylotrophicus PY5*, and *Bacillus cereus LH1*. This indicates that synergism has occurred between these microorganisms (Valcheva et al., 2013, 2014).

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 et al., 2012). 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 et al., 2012).

Mineral springs of Burgas

Burgas is the second largest seaside town in Bulgaria, located on the Southern Black Sea coast. In addition to the beautiful sea and spa – pious beaches, Burgas offers great opportunities for balneological treatment with mineral water, characteristic sea mud and lye.

This is one of the oldest balneological centers in Bulgaria. Mineral water is suitable for the treatment and prevention of diseases of the musculoskeletal system, peripheral nervous system, chronic gastritis and pyelonephritis infertility, gout.

Water is also beneficial for strengthening the general state of the body.

Mineral spring of District Sudievo

Sudievo is a village in southeastern Bulgaria. It is located in Aytos municipality. Water helps diseases of urinary system, disorders of locomotory system, endocrine diseases. It is suitable for daily use as drinking water. This water in Sudievo is hydrocarbonate, sodium, but contains fluoride. According to the requirements for drinking water, not mineral water, water should contains not more than 1.5 milligrams per liter of fluoride. The water in Sudievo contains much more in quantity than this chemical element.What those who consume this water need to know is that excessive ingestion of this fluoride per day can accordingly damage tooth enamel in young children.In the northern part of Aytosko Polje there are several mineral springs along the fault line: the "Smelly Fountain" near the village of Shivarovo and those near the villages of Cherry, Yabulchevo and Saedinenie. Geothermal water with a flow rate of 30 L/s and a temperature of 51 °C emerges from deep drilling in the village of Polyanovo, which flows freely without being used. Analyses show that the sources have extremely good healing properties.

Medicinal properties of water: in diseases of the locomotory system, gastrointestinal, liver-bile and renal diseases.

Mineral springs of 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 Burgas – thermal healing spring Burgas Mineral baths with water temperature of 41°C, thermal healing spring village of Shivarovo with water temperature of 47°C, thermal healing spring village of Polyanovo with water temperature of 51°C, thermal healing spring village of Judge with water temperature of 51°C.

Nutrient media

Nutrientagar (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_2SO_3 – 1,4 ; K_2HPO_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_2SO_3 ; 50 cm^3 20 % glucose solution; 10 cm^3 8% solution ofFe_2SO_4.

Methods for analysis

Methods for physicochemical analysis

Method for determination of color according to Rublyovska Scale – method by Bulgarian State Standard (BDS) 8451: 1977;

Method for determination of smell at 20°C — method BDS 8451 : 1977 technical device – glass mercury thermometer, conditions № 21;

Method for determination of turbidity - EN ISO 7027, technical device turbidimeter type TURB 355 IR ID № 200807088;

Method for determination of pH – BDS 3424 : 1981, technical device pH meter type UB10 ID № UB10128148;

Method for determination of oxidisability – BDS 3413 : 1981;

Method for determination of chlorides – BDS 3414 : 1980;

Method for determination of nitrates – Validated Laboratory Method (VLM) – NO_3 – № 2, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of nitrites – VLM NO_3 –№ 3, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of ammonium ions – VLM - NH_4 – № 1, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of general hardness – BDS ISO 6058;

Method for determination of sulphates – VLM - SO_4 – № 4, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of calcium – BDS ISO 6058;

Method for determination of magnesium – BDS 7211: 1982;

Method for determination of phosphates – VLM - PO_4 – № 5, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of manganese – VLM – Mn – № 7, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of iron – VLM – Fe – № 6, technical device photometer ,, NOVA 60 A " ID № 08450505;

Method for determination of fluorides – VLM – F – № 8, technical device photometer, NOVA 60 A " ID № 08450505;

Method for determination of electrical conductivity – BDS EN 27888, technical device – conductivity meter inoLabcond 720 ID № 11081137.

Methods for determination of microbiological indicators

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.

Method for determination of *Escherichia coli* and coliform bacteria –BDSEN ISO 9308 – 1: 2004;

Method for determination of enterococci – BDS EN ISO 7899 – 2;

Method for determination of sulphite reducing spore anaerobes – BDS EN 26461 – 2: 2004;

Method for determination of total number of aerobic and facultative anaerobic bacteria – BDS EN ISO 6222: 2002;

Method for determination of *Pseudomonas aeruginosa* – BDS EN ISO 16266: 2008.

Determination of coli – titre by fermentation method – Ginchev's method

Determination of coli – bacteria over Endo's medium – membrane method.

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

3. Results and discussion

3.1. Results of mineral springs in Burgas region

It is done a comparative physicochemical analysis of mineral spring waters at the territory of Burgas 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.

The trial data reveal that thermal healing spring water village of Shivarovo, thermal healing spring water village of Polyanovo 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(RZI (Regional Health Inspection) – Burgas).

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

Controlled parameter	Measuring unit	Maximum Limit Value	Result Burgas Mineral baths	Result Shivarovo	Result Polyanovo	Result Judge
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 indicator	pH values	≥ 6,5 и ≤ 9,5	9,95	9	9,11	9,1
5. Oxidisability	mgO_2/dm^3	5,0	0,50	0,4	0,5	0,5
6. Chlorides	mg/ dm^3	250	30,7	26,3	26,3	26,0
7. Nitrates	mg/ dm^3	50	0,2	2,10	0,1	0,15
8. Nitrites	mg/ dm^3	0,50	0,007	0,00	0,006	0,005
9. Ammonium ions	mg/ dm^3	0,50	0,111	0,150	0,154	0,158
10. General hardness	$mgekv/ dm^3$	12	0,4	0,4	0,4	0,4
11. Sulphates	mg/ dm^3	250	37	34	35	36
12. Calcium	mg/ dm^3	150	120	118	116	117
13. Magnesium	mg/ dm^3	80	68	66	67	66
14. Phosphates	mg/ dm^3	0,5	0,015	0,016	0,016	1,018
15. Manganese	mg/ dm^3	50	0,0005	0,0008	0,0007	0,0009
16. Iron	$\mu g/ dm^3$	200	0,0016	0,0020	0,0022	0,0037
17. Fluorides	mg/ dm^3	1,5	7,73	0,4	1,48	5,5
18. Electrical conductivity	$\mu S/ dm^3$	2000	633	620	612	615

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

Examined water source	Indicator, cfu/cm^3
1. Thermal healing spring Burgas Mineral baths with water temperature of 41°C	6 ± 1
2. Thermal Healing Spring village of Shivarovo with water temperature of 41 °C	11 - 17
3. Thermal Healing Springvillage of Polyanovo with water temperature of 51°C	5 - 8
4. Thermal Healing Springvillage of Judge with water temperature of 51 °C	120-150

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 Burgas Mineral baths with water temperature of 41°C, thermal healing spring village of Shivarovo with water temperature of 41°C, thermal healing spring village of Polyanovo with water temperature of 51°C swimming pool, are in compliance with the requirements for presence of coli bacteria. Thermal healing spring village of Polyanovo 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 50 cm³	Culture volumes 10 cm³	Culture volumes 10 cm³	Culture volumes 10 cm³	Culture volumes 10 cm³	Culture volumes 10 cm³
1. Thermal healing spring Burgas Mineral baths with water temperature of 41°C	> 100	–	–	–	–	–	–
2. Thermal Healing Spring village of Shivarovo with water temperature of 41 °C	> 100	–	–	–	–	–	–
3. Thermal Healing Springvillage of Polyanovo with water temperature of 51°C	> 100	–	–	–	–	–	–
4. Thermal Healing Spring village of Judge with water temperature of 51 °C	80	+ Acid	+ Acid	+ Acid and gas	+ Acid and gas	+ Acid and gas	–

Table 4. Microbiological indicators of spring waters in Burgas District

Indicators	Measuring unit	Thermal healing spring Burgas Mineral baths with water temperature of 41°C	Thermal healing springvillage of Shivarovo with water temperature of 41 °C	Thermal healing spring village of Polyanovo with watertemperature of 51 °C	Thermal healing springvillage of Judge with water temperature of 51 °C
Coliforms	cfu/cm³	0/100	0/100	0/100	10/100
Escherichiacoli	cfu/cm³	0/100	0/100	0/100	10/100
Enterococci	cfu/cm³	0/100	8/100	0/100	8/100

Sulphite reducing anaerobic bacteria*(Clostridium perfringens)*	cfu/cm³	0/100	0/100	0/100	0/100
Pseudomonas aeruginosa	cfu/cm³	0/250	0/250	0/250	0/250

Based on the conducted physicochemical and microbiological evaluations it is established that from the four examined springs at the territory of Burgas District by physicochemical parameters only thermal healing spring water village of Shivarovo, thermal healing spring water village of Polyanovo 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. With regards to microbiological parameters thermal healing water Burgas Mineral baths, thermal healing spring village of Shivarovo, thermal healing spring water village of Polyanovo swimming pool are in compliance with the requirements for drinking water.

3.2. Results of mineral springs in Varna region

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 5.

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) – Varna).

Table 5. Comparison of the examined spring waters in Varna District by physicochemical properties

Controlled parameter	Measuring unit	Maximum Limit Value	Result Varna 1 (drilling №P-83xKK „Saints Constantine and Helena")	Result Varna 2 (P-1x "Aquarium")	Rsult Varna 3 (P-106 x „Dom Mladost")	Result Varna 4 (P-161x Varna "Primorski" swimming pool)
1. Colour 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	mgO_2/dm^3	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	0,29	0,21	0,85
10. General 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	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

For the same spring waters are determined their microbiological indicators by the membrane method. In Table 6 are shown the experimental studies from the determination of total number of mesophilic aerobic and facultative anaerobic bacteria.

Table 6. Determination of total number of mesophilic aerobic and facultative anaerobic bacteria

Examined water source	Indicator, cfu/cm³
1. Thermal healing spring Varna 1 (drilling №P-83x 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 (Murgov, Denkova, 2010).

The presence of coliforms and *Escherichia coli* is determined by the membrane method, and according to Ginchev's method. The trial results (Table 7 and Table 8) reveal that thermal healing spring drilling №P-83x 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 ate "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 7. 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 №P-83x Health Resort„Saints Constantine nd 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 8. Microbiological indicators of spring waters in Varna District

Indicators	Measuring unit	Thermal healing spring Varna 1 (drilling №P-83x Health Resort „Saints Constantine and Helena”) with water temperature of 48 °C	Thermal healing spring Varna 2 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 with water temperature of 50 °C	Non-thermal healing spring „Heling watera“ village of Goren Chiflik
Coliforms	cfu/cm^3	0/100	0/100	0/100	0/100	15/100
Escherichia coli	cfu/cm^3	0/100	0/100	0/100	0/100	15/100
Enterococci	cfu/cm^3	0/100	0/100	0/100	0/100	10/100
Sulphite reducing anaerobic bacteria *(Clostridium perfringens)*	cfu/cm^3	0/100	0/100	0/100	0/100	0/100
Pseudomonas aeruginosa	cfu/cm^3	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 №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 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 №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 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.

4. Conclusion

The research shows the effects of hot mineral water from Burgas and Varna Districts, Bulgaria. There are the results with:

- Comparison of the examined spring waters in Burgas and Varna Districts by physicochemical properties;
- Determination of total number of mesophilic aerobic and facultative anaerobic bacteria;
- Coli – titre of thermal healing spring waters;
- Microbiological indicators of spring waters in Burgas and Varna Districts.

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BDS8451: 1977 – defining of colour according to Rublyovska Scale, determination of smell at 20 °C.

EN ISO 7027 –determination of turbidity.

BDS3424: 1981 – determination ofpH.

BDS3413: 1981 –determination of oxidisability.

BDS3414: 1980 –determination of chlorides.

BDS ISO 6058 – determination of calcium, determination of general hardness.

BDS EN 27888 – determination of electrical conductivity.

VLM – NH_4 – № 1 –determination of ammonium ions.

VLM –NO_3 – № 2 –determination of nitrates.

VLM – NO_2 – № 3 – determination of nitrites.

VLM– SO_4 – № 4 –determination of sulphates.

VLM– PO_4 – № 5 – determination of phosphates.

VLM– Fe – № 6 – determination of iron.

VLM–Mn– № 7 – determination of manganese.

VLM– F – № 8 – determination of fluorides.

BDS 7211: 1982 – determination of magnesium.

BDSEN ISO 7899 – 2 –determination of nitrates.

BDSEN ISO 9308 – 1: 2004 – determination of*Escherichia coli*andcoliformbacteria.

BDSEN26461 – 2: 2004 – determination of sulphite reducing anaerobic bacteria*(Clostridiumperfringens)*.

BDSEN ISO 16266 – determination of*Pseudomonas aeruginosa*.

BDSEN ISO 7899 – 2 – determination of eneterococci.

BDS EN ISO 6222: 2002 – determination of total number of aerobic and facultative anaerobic bacteria.