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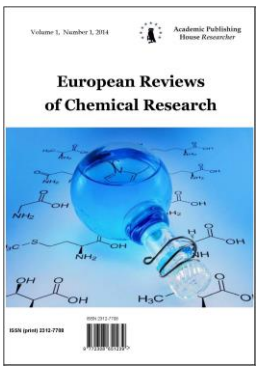

Physicochemical and Microbiological Characteristics of Thermal Healing Spring Waters in the District of Burgas

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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.

Keywords: spring water, drinking water, physicochemical properties, microbiological indicators.

1. Introduction

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).

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*

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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* and *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 37 C°.

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).

Mineral springs Burgas city

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 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.

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.

2.1. 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.

2.2. 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.

2.3. 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

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 Shivarovowith water temperature of 41 °C	11 – 17
3. Thermal Healing Springvillage of Polyanovowith 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 50cm³	Culture volumes 10cm³	Culture volumes 10cm³	Culture volumes 10cm³	Culture volumes 10cm³	Culture volumes 10cm³
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 water temperature 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 BurgasDistrictby 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.

4. Conclusion

The research shows the effects of hot mineral water from Burgas region, Bulgaria.

There are the results with

- Comparison of the examined spring waters in Burgas District 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 District.

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Standards

Ordinance №9/2001, Official State Gazette, issue 30.

Decree № 178/23.07.2004 about the quality of water, intended for drinking purposes.

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.

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BDSEN ISO 7899 – 2 – determination of nitrates.

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