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RESEARCH ON SULPHUR-BACTERIA AND PHYSICAL-CHEMICAL PARAMETERS FROM SULPHUROUS WATERS OF JIBOU AREA

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Abstract

Sulphur is the fourth major essential nutrient element, after nitrogen, phosphorus and potassium, involved in biogeochemical cycles in nature. Sulphur is essential in all organisms because it is an integral part of major metabolic compounds, such as amino acids (methionine and cysteine), and it is an active component of numerous co-factors and prosthetic groups (Fe–S clusters, sulpholipids, glucosinolates, vitamins, etc), which are involved in metabolic processes of organisms. Medical research appears to confirm the validity of the use of sulphurous spring waters as therapeutical and preventive remedies for a large variety of diseases. In order to evaluate the quality of sulphurous water, samples have been taken of the Sulphurous Springs in Jibou. The objectives of this study were to determine physical and chemical properties (pH, electrical conductivity, salinity, redox potential) of some sulphurous water samples of Jibou area, and also to determine the microbiota involved in S biogeochemical cycle. The physical analysis showed that the sulphurous water of Jibou falls within the very weakly acidic reaction class, and the salinity was 0.2% in all samples. The aerobic heterotrophic bacteria show a maximum value in the sample 4 (2630x10^3 bacteria/ml). The microbial sulphurous community was detected in all water samples. This information should improve our understanding on adaptation mechanisms of bacteria involved in the sulphur cycle in correlation with physical-chemical parameters.

***Key words**: sulphurous water, Jibou, physical-chemical parameters, sulphate-reducing bacteria, sulphur-oxidation bacteria.*

INTRODUCTION

Jibou is an important region for occurrence of therapeutic sulphurous waters in North-Western Romania. Natural sulphurous mineral waters from Jibou have a treatment potential mostly used in balneotherapy.
Sulphur is present in all organisms and occurs in organic compounds with catalytic, structural and regulatory functions, such as amino acids, proteins, enzymes, etc. On the other hand, the biological functions of inorganic sulphur compounds are limited. These compounds are needed in assimilation (prokaryotes, plants and fungi) and synthesis of organic compounds because they provide sulphur. Sulphate, sulphide and elemental sulphur are the most abundant sulphur substances, and provide evidence for the simultaneous occurrence of dissimilatory sulphate reduction and sulphide oxidation (at *Eubacteria* and *Archaebacteria*). Hydrogen sulphide is rarely present in natural waters, with the exception of special sulphurous mineral springs. Hydrogen sulphide (H_2S) is the active component of sulphurous waters (Pokorna and Zabranska, 2015).
Balneotherapy treats many diseases, including immune-mediated skin diseases. Current medical research confirms the effect of sulphurous spring waters use, as preventive remedies and therapeutics for a large variety of diseases affecting the respiratory tract, skin, liver, intestine, gynaecological apparatus and osteoarticular system (Frosch, 2007). The different studies followed the effect of sulphurous medicinal waters in a murine dermatitis and on psoriatic patients. The role of hydrogen sulphide in releasing somatostatin during bathing treatment was also studied. Some results point out the anti-inflammatory role of sulphurous medicinal waters in psoriasis (Tsoureli-Nikita et al., 2002).
According to the World Health Organization (WHO, 2013) and US Environmental Protection Agency (EPA), water for human consumption should contain less than 250 ppm

of sulphates (Balintova et al., 2015; US EPA, 2017). When the sulphate concentrations for human consumption are compromised, the human health concerns would naturally arise (Backer, 2000; Fernando et al., 2018).

Other studies on sulphurous mineral waters revealed anti-oxidant properties (Albertini et al., 2007) and, under the same oral treatment, no pathological changes on biochemical oxidative enzymatic markers and hematochrome were noticed in human healthy volunteers (Albertini et al., 2008). H_2S is known as an endogenous inter- and intracellular signaling molecule with multiple effects. Its most investigated roles are related to cardiovascular, nervous system and inflammatory diseases (Matz et al., 2003). The effect of H_2S on various clinical pathology parameters has been so far restricted to acute hematologic and enzyme alteration studies.

Sulphur bacteria are prokaryotes that use reduced or oxidized sulphur compounds in their metabolism. Reduced sulphur compounds (sulphide and elemental sulphur) are involved as electron donors in photosynthesis of anoxygenic phototrophic sulphur bacteria, or as energy and electron sources by colourless sulphur-oxidizing bacteria. In opposition, oxidized sulphur compounds (sulphate), are involved as electron acceptors for the oxidation of organic matter by sulphate-reducing bacteria. When the sulphur level in water is above the needs of plants and microbes the excess is settled down. All of it or only a part will further circulate through oxidation to sulphate or reduction to sulphide. Thus, it is important to know the bacteria involved in these reactions. Each group of sulphur bacteria needs available sulphur compounds in a specific redox status. Sulphur bacteria are a heterogeneous group with taxa belonging to different phylogenetic branches within *Bacteria* and *Archaea*.

Biological oxidation of sulphur is important in the natural sulphur cycle. The oxidation reactions are performed by sulphur-oxidizing bacteria from *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, Chloroflexia* and *Chlorobia* classes. The electrons resulted from sulphide oxidation are then used by aerobic chemotrophic colorless sulphur-oxidizing bacteria (*Beggiatoa, Thiobacillus,* etc) in respiration and for autotrophic CO_2 reduction. The anaerobic phototrophic sulphur-oxidizing bacteria (green sulphur bacteria - *Chlorobium,* etc; purple sulphur bacteria - *Halochromatium, Thiocapsa*, etc) transfer electrons from sulphur or other donors for autotrophic CO_2 reduction, using light energy (Zang et al., 2018).

Sulphur reducers are able to reduce elemental sulphur, and are widespread among Bacteria and Archaea (*Desulfurococcus, Clostridium, Methanococcus, Geobacter* etc). They use elemental sulphur as an electron acceptor in oxidizing organic matter (formate, acetate, lactate, pyruvate, sugars etc). The sulphide produced can further pass to autotrophic sulphide-oxidizing bacteria (*Thiobacillus, Halothiobacillus*) which oxidize it back to elemental sulphur (Ghosh and Dam, 2009; Zang et al., 2018).

This paper aims to investigate the bacteria involved in sulphur cycle (aerobic and anaerobic bacteria) in relation to physical-chemical parameters in sulphurous waters from Jibou zone (Salaj County).

MATERIALS AND METHODS

Water samples. Different sulphurous mineral water samples were collected from several local springs from Jibou town area. For the sample collection ground-glass apparatus was used. All samples were kept in the lab at 4°C, waiting processing. The waiting time before processing didn't exceed 24 hours. The water samples for physical and chemical analysis were analysed according to SR EN ISO 5667-1:2007/AC, 2007 and SR EN ISO 5667-3, 2013, and they were also microbiologically studied according to SR EN ISO 19458, 2007.

Physical and chemical analyses. A portable multiparameter was used for the physical and chemical analyses. The physical and chemical analysis consists in detection of some parameters as: pH, electrical conductivity (EC), salinity (%), redox potential (Eh) and dissolved oxygen (SR EN 27888, 1997; SR EN ISO 10523:2012).

Microbiological analyses. Bacteriological parameters for water samples were established, namely: the total number of the mesophilic heterotrophic bacteria (cfu = colony-forming units), aerobic and anaerobic sulphur-reducing, sulphur-oxidising, sulphate-reducing bacteria. In order to determine these bacterial indicators from the water samples we used the methods according to Carpa et al., 2014 and different standards for water samples. The culture media used were: nutrient agar (for heterotophic bacteria) (SR EN ISO 6222, 2004); peptone water medium (for aerobic sulphur reducing bacteria) (Carpa et al., 2014); Oppenheimer and Gunkel culture media (for anaerobic sulphur-reducing bacteria (Atlas, 2010); Starkey culture media (for sulphate-reducing bacteria *Desulfovibrio*) (Cusa, 1996); Postgate culture media (for sulphur-oxidizing bacteria (Atlas, 2010)).

Water samples were subjected to serial dilutions and the first three dilutions were used to inoculate all culture media. After inoculation, the samples were incubated for 7 days at a temperature of 25ºC.

Growth examination. In order to assess the number of heterotrophic aerobic bacteria, the number of clusters on each Petri dish was counted and then the cfu/ml was computed. Bacteria involved in sulphur cycle were determined by multiple tubes method and data were interpreted according to the Alexander statistic table – 1965 (Carpa et al., 2014).

RESULTS AND DISCUSSIONS

The physical and chemical parameters were measured in all water samples with the portable multiparameter. The number of bacteria belonging to the following ecophysiological groups was assessed: aerobic mesophilic heterotrophs, aerobic sulphur-reducing bacteria, aerobic sulphur-oxidizing bacteria, anaerobic sulphur-reducing bacteria and anaerobic sulphate-reducing bacteria. The results indicated that the number of bacteria from each group varied depending on the sampling sites.

Physical and chemical analyses. The actual acidity (pH) is very important because it influences physiological groups of bacteria. In the water samples the pH ranged between 6.73 and 6.88 (Table 1). These values belong the very weakly acidic reaction classes. The redox potential (Eh) is used in water assessments because measures the capacity of chemical elements to acquire electrons and by that be reduced. A high redox potential indicates a greater affinity of chemical species for electrons and a greater tendency to be reduced, therefore, an aerobic environment. Sulphur bacteria are critical mediators of redox transformations occurring within the biogeochemical sulphur cycle of the biosphere. The values of redox potential in our water samples were very low. Values of electrical conductivity varied between 845 and 867 µS/cm. In all water samples was measured a salinity of 0.2%, which means that the sulphurous waters from Jibou area contain salts.

Table 1. Physical and chemical parameters in water samples

Samples	pH	Eh (redox potential) (mV)	Electrical conductivity (µS/cm)	Salinity (%)	Dissolved O_2 (mg/l)
1	6.73	-3	845	0.2	0.11
2	6.81	-5	858	0.2	0.250
3	6.84	-7	862	0.2	0.30
4	6.87	-9	860	0.2	0.10
5	6.88	-10	867	0.2	0.23

Microbiological analyses. The total number of mesophilic heterotroph bacteria was measured by the growth layers method. Based on the results, colony forming units (cfu/ml) were calculated.

Viable counts of aerobic and anaerobic sulphur-reducing, sulphur-oxidising, sulphate-reducing bacteria were assessed by most probable number counts (MPN). The results are presented in Table 2, where one can observe that the predominant bacteria involved in the sulphur cycle are aerobic the sulphur-reducing bacteria, followed by sulphur-oxidizing ones. The number of anaerobic bacteria is quite low in all analysed water samples.

Table 2. Microbial communities in water samples

Samples	Bacteria					BIWQ
	Heterotrophic bacteria	Sulphur oxidizing aerobic	Sulphur reducing aerobic	Sulphur reducing anaerobic	Sulphate reducing anaerobes	
	(cfu/ml)	*(bacteria/ml)*				
1	$1146x10^3$	130	170	40	120	2.167
2	$1446x10^3$	380	440	68	91	2.835
3	$2093x10^3$	170	210	78	100	2.753
4	$2630x10^3$	170	310	82	94	2.806
5	$965x10^3$	140	260	61	82	2.649

The aerobic heterotrophs constituted the largest eco-physiological group of bacteria and it is used as a global indicator of microbiological water quality (Figure 1).
The number of aerobic heterotrophs varied in those five water samples, exhibiting values between $965x10^3$ cfu/ml and $2630x10^3$cfu/ml. One can observe that the greatest abundance of aerobic heterotrophs was in water sample 4 and the smallest value was found in water sample 5.

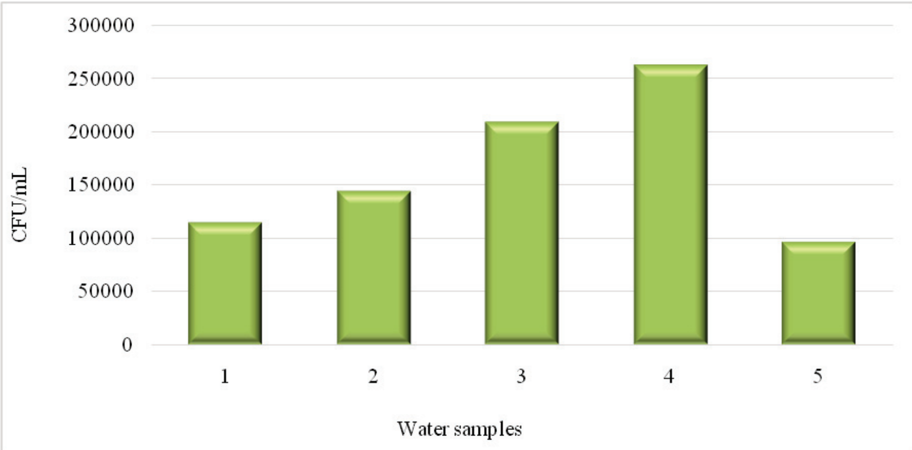

Figure 1. Aerobic heterotrophic bacteria in water samples

Bacteria in the Sulphur cycle removes the toxic effect of hydrogen sulphide and restore the sulphur to the biological use. They can be found on the bottom of standing waters and they are oxidizing the sulphur mineral compounds. Their energy source is hydrogen sulphide (H_2S), which they transform into sulphur (S), sulphurous acid (H_2SO_3) and sulphuric acid (H_2SO_4).
Sulphur-oxidizing bacteria are the main microorganisms that participate in the natural sulphur cycle. Sulphur-oxidizing bacteria are used for the continuous monitoring of toxicity and their use circumvents many of the obstacles associated with conventional assays (Luo et al., 2013). Sulphur-oxidizing bacteria, are chemoautotrophic bacteria that oxidize reduced sulphur compounds to sulphuric acid, in the presence of oxygen (O_2). Some of them, such as *Acidithiobacillus* genus, use elemental sulphur (S) particles as the electron donor (Sedky et al., 2013). Among aerobic bacteria involved in the sulphur cycle one can observe that aerobic sulphur-reducing bacteria are present in a slightly higher number compared to the number of aerobic sulphur-oxidizing bacteria (Figure 2).

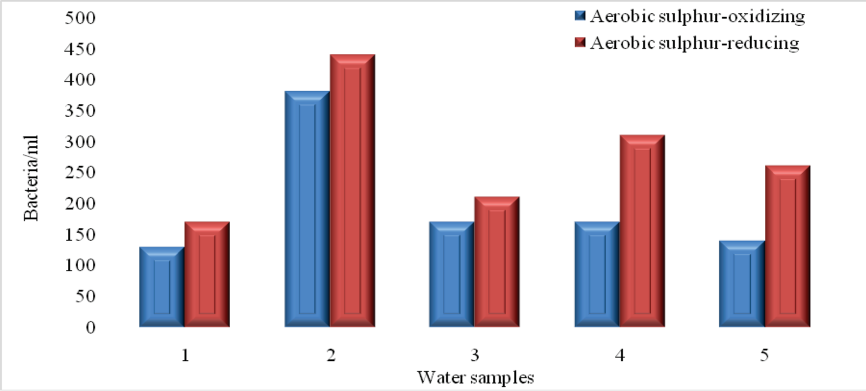

Figure 2. Aerobic sulphur bacteria (sulphur-oxidizing and sulphur-reducing) in water samples

Sulphur reducers can reduce elemental sulphur, and they can use elemental sulphur as the electron acceptor, to oxidize many organic matters (Ghosh and Dam, 2009).

Among the anaerobic bacteria, the anaerobic sulphur-reducing bacteria had the lowest count in the water samples, compared to anaerobic sulphate-reducing bacteria and aerobic bacteria (figure 3). The lowest count was discovered to be for anaerobic sulphur-reducing bacteria in sample 1 (40 bacteria/ml).

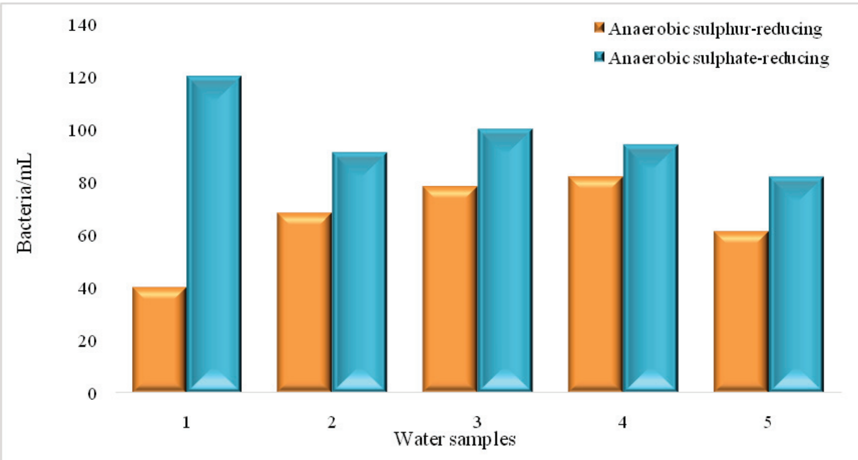

Figure 3. Anaerobic sulphur bacteria (sulphur-reducing and sulphate-reducing) in water samples

Results of the analyses indicated that the number of bacteria from each ecological group varied.

For a general characterisation of the sulphurous bacteria living in the water, we estimated the bacterial indicator of water quality (BIWQ), according to formula (Muntean, 1995-1996):

$$BIWQ = \frac{1}{n} \bullet \sum \log_{10} N$$

where:

BIWQ = the bacterial indicator of water quality;

n = the number of the physiological groups considered within the calculation;

N = the number of the bacteria belonging to each ecophysiological group.

For all samples, the BIWQ was very low and the calculated values ranged between 2.617 and 2.835. The highest BIWQ value was obtained in sample 2, collected from sulphurous waters from Jibou areas (Figure 4).

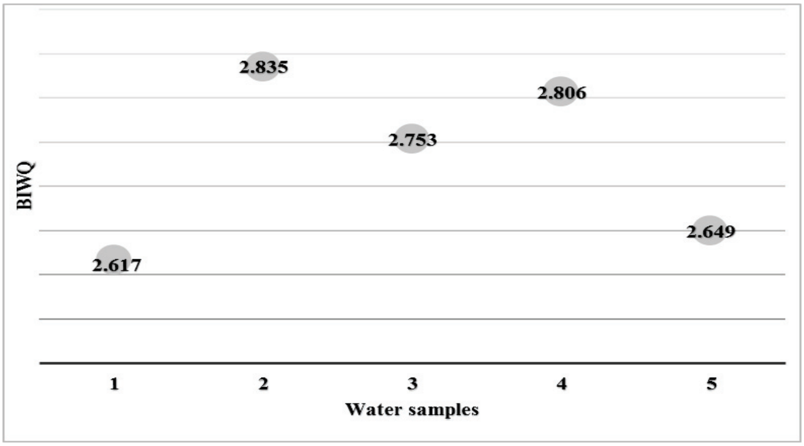

Figure 4. Bacterial indicators of water quality (BIWQ)

CONCLUSIONS

The physical analyses showed that the sulphurous water in Jibou area fits into the very weakly acidic reaction class. The values of redox potential in water samples were very low and these values affect the affinity of the nutrients and the presence of aerobic bacteria. Values of electrical conductivity varied between 845 and 867 µS/cm. In all water samples a salinity of 0.2% was detected.

Numerical distribution of aerobic heterotrophic bacteria shows higher values in the sample 4 ($2630x10^3$ cfu/ml), followed by sample 3 ($2093x10^3$cfu/ml) and the lowest value was found in sample 5 ($965x10^3$cfu/ml).

The microbial sulphur community is not very abundant but suggests that all analysed groups were present in all water samples. Even if aerobic and anaerobic sulphur bacteria were present in the water samples, the aerobic sulphur bacteria were predominant.

The presence of all the five studied bacterial ecophysiological groups studied was noticed in all the water samples. Their number decreases in the following order: aerobic mesophilic heterotrophs ($965x10^3$ - $2630x10^3$ cfu/ml) > aerobic sulphur-reducing bacteria (170 - 440 bacteria/ml) > aerobic sulphur-oxidizing bacteria (130 – 380 bacteria/ml) > anaerobic sulphate-reducing bacteria (82 - 120 bacteria/ml) > anaerobic sulphur-reducing bacteria (40 - 82 bacteria/ml). The bacterial indicator of water quality (BIWQ) had very low values, which varied between 2.617 and 2.835.

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