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Original paper

The assessment of microbiological and physicochemical characteristics in drainage waters of the Siriu dam

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Abstract Based on the balneotherapeutic applications of mineral spring water and particularly sulphurous water, the aim of our research was to study the physicochemical and microbiological parameters of some drainage waters of Siriu dam that showed a strong hydrogen sulphide odour. In addition, due to the corrosive effect of some groups of microorganisms, such as iron-oxidizing bacteria and sulphate-reducing bacteria, the present paper also aimed to detect their presence in order to signal the need for some disinfection measures.

According to physicochemical analysis, there has been found an external drain that is suitable for use in balneotherapy, presenting a balanced content of mineral elements such as sulphur, calcium, silicon, chlorine and potassium. However, due to the presence of potentially toxic phytoplankton microorganisms such as *Microcystis* sp. and *Phormidium* sp. it is necessary to disinfect this water source before using it for any purpose. On the other hand, the identification of both sulphate-reducing bacteria and iron-oxidizing bacteria in the drainage waters of Siriu dam should be considered as an alarm signal as they may lead to bio-corrosion and deterioration of metallic or concrete structures, affecting the integrity of the dam and hydropower constructions.

Keywords Siriu dam, sulphurous mineral waters, drainage waters

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Introduction

The Siriu dam and its artificial lake are located on the Buzau River, 18 km upstream from Nehoiu village. The construction of the dam started in 1974 and was completed in 1994. It reaches a height of 122 m, the total length is 570 m and the volume of the reservoir is about 125 million m^3 water. The purpose of building the dam was to provide drinking water for the downstream localities, to irrigate an important agricultural area, and also to provide flood defence. On the other hand, the dam ensures the production of electricity through the construction of the Nehoiasu hydroelectric power plant that generates 120.6 GW h/year, having an installed power of 42 MW (DIACONU [1]). By comparison, the Iron Gate I Hydroelectric Power Station (one of the largest hydro power plants in Europe) has an installed power of 1068 MW and an average power production of 5250 GW h/year (NASTASE & al. [2]).

By investigating this area, it has been found that some drainage waters show a strong smell of hydrogen sulphide, suggesting both the danger to human and animal health when these springs are used as drinking water sources and the possibility of using these waters for therapeutic purposes. In this regard, scientific researches show that mineral springs and particularly sulphurous waters have been frequently used in balneotherapy, as a classical cure for a wide range of medical affections such as skin and respiratory disorders, arterial hypertension, inflammatory diseases, atherosclerosis and so on (CARBAJO & MARAVER [3], CARUBBI & al. [4], MATZ & al. [5], GÁLVEZ & al. [6]). Therefore, the location of mineral springs and the determination of their composition represent a first step in exploiting these water sources.

In addition, the presence of some groups of microorganisms, such as iron-oxidizing bacteria and sulphate-reducing bacteria, in the structure of hydroelectric constructions and installations may be associated with bio-corrosion processes that can severely affect the integrity of the dams and with the blockage of water pipes and drainage channels due to ferric iron precipitates (HEDRICH & al. [7]). Therefore, the identification of such groups of microorganisms in these environments must lead to the application of effective disinfection measures in order to avoid further problems related to the integrity of the hydropower plants structures.

In this context, the aim of our research was to study the physicochemical particularities and the microbiological parameters (bacteria, phytoplankton, and zooplankton) of some drainage waters of the Siriu dam in order to establish both the possibility of using these water sources in balneotherapy and the presence of microorganisms that may be involved in bio-corrosion of the dam's constructions and installations.

Materials and Methods

Sampling sites description

Water samples were collected in October 2018 from three sites (Figure 1): an external drain located in the vicinity of the Siriu dam (*Sample 1*), an underground drain located in the tenth gallery of the dam (*Sample 2*) and an accumulation of infiltrated water located in the eighth gallery (*Sample 3*). Samples were collected in sterile glass containers and were stored at 4˚C before analysis. *In situ* physicochemical analysis were performed exclusively for *samples 1* and *2*, the third sample being investigated only in laboratory, particularly from microbiological point of view due to the high content of a black precipitate that prevented *in vitro* chemical assays and the investigation of phyto- and zooplankton.

Physicochemical analysis

Physicochemical parameters were measured *in situ* by a portable multi-parameter instrument for water analysis (HI Hanna Instruments) and the chemical composition was determined by X-ray spectrometry (X-Ray Supermini, Rigaku Corporation) according to the manufacturer's protocol, using 10 mL of each water sample.

In order to carry out chemical analysis, samples were filtered through Whatman GF/F glass fibre filters. Nutrients were determined spectrophotometrically following a modified Berthelot method for NH_4-N (KROM [8]), a Griess-Ilosvay modified method for NO_2-N (KEENEY & NELSON [9]), and the TARTARI & MOSELLO [10] method for NO_3-N, PO_4-P, and total phosphorus (TP). The analysis of total organic carbon (TOC) was performed using a Multi NC 3100 Analyser from Analytik Jena.

The intensity of microbial enzymatic activities was determined by estimating the substrate consumption, according to the protocol described in our previous article (PĂCEȘILĂ & al. [11]). In this regard, the activities of α-glucosidase (EC 3.2.1.20), β-glucosidase, alanine aminopeptidase (EC 3.2.1), and alkaline phosphatase (EC 3.1.3.1) were evaluated using the following substrates: p-nitrofenyl-α-D-glucopyranoside, p-nitrophenyl-β-D-glucopyranoside, L-alanine-4-nitroanilide hydrochloride, and 4-nitrofenyl phosphate, respectively.

Biological investigations

The presence of phytoplankton and zooplankton species was investigated by inverted microscopy, using a Zeiss Axio Vert microscope. Microbiological investigations involved the cultivation of different groups of microorganisms (aerobic mesophilic bacteria, total coliforms, faecal coliforms, faecal streptococci, sulphate-reducing bacteria, ammonifying bacteria and iron-oxidizing bacteria) on different growth media, both solid and liquid. The inoculation of undiluted samples and their decimal dilutions on agar growth media was made in triplicate, by pour-plate technique. Quantification of microorganisms was performed by determining the number of colony-forming units per millilitre (CFU/mL) or by the most probable number (MPN) method. Table 1 shows the growth media used for each group of microorganisms and the incubation conditions (temperature and time).

Table 1. Growth media used in the microbiological analysis and incubation conditions.

Groups of microorganisms	Growth media		Incubation	
			Temperature (˚C)	Time
Mesophilic aerobic bacteria	Nutrient agar [12]	Solid	37	48 h
Total coliforms	Eosin Methylene Blue Agar [13]	Solid	37	24 h
Faecal coliforms	MacConkey [12]	Liquid	45	24 h
Faecal streptococci	Bromocresol-purple Azide Broth [12]	Liquid	45	48 h
Sulfate-reducing bacteria	Postgate medium [14]	Liquid	28	7 days
Ammonifying bacteria	Peptone broth [15]	Liquid	28	15 days
Iron-oxidizing bacteria	Winogradsky medium [16]	Liquid	28	14 days

Results and discussion

Physicochemical properties of water samples

Near the first two sampling sites (Figure 1A-B) there could be noticed a strong smell of sulphur and the collected water samples were characterized by a high degree of turbidity and an opaque-whitish colour. In the third sampling site (Figure 1C), the sulphur odour was absent and the water sample was black coloured, probably due to the precipitation of iron from the structure of the concrete walls of the dam.

Figure 1. Sampling sites (A – the external drain; B – the tenth gallery; C – the eighth gallery)

Comparing the water from the external drain (*Sample 1*) with the water from the underground drain (*Sample 2*) by physicochemical analysis performed *in situ* (Table 2), it was noted that the conductivity, the total dissolved solids content (TDS) and the salinity are approximately twice as high in *Sample 1* as in *Sample 2*. All of these parameters indicate both a higher amount of salts and a higher turbidity of *Sample 1* compared to *Sample 2*. The water has a neutral-slightly alkaline pH (7.4-7.89), and there are no risks to human health from this point of view. The negative Oxidation Reduction Potential (ORP) indicates that the analysed water has an antioxidant capacity which can be beneficial, helping the body to neutralize acidity.

XRF analysis revealed the presence of silicon, sulphur, potassium, calcium, chlorine and germanium oxides in the external drain, this result explaining the strong smell of sulphur near the first sampling site. Generally, the presence of these chemical elements in appropriate concentrations has beneficial effects on human or animal health, being a dietary requirement for almost any organism (QUATTRINI & al. [17]). Silica and calcium, for example, are particularly beneficial for bones. However, too high concentrations of these elements often have negative effects (SENGUPTA [18]). High sulphur concentrations, for example, cause alteration of organoleptic properties of water and often provoke stomach and gastrointestinal disorders. Sample 2, taken from the underground drain, is distinguished by a relatively high content of barium, this chemical element being abundant in the Earth's crust. However, high concentrations of barium compounds that dissolve in water may have harmful effects on human health (NIELSEN & LADEFOGED [19]). Sample 3, on the other hand, shows a high content of ferric oxide and small amounts of calcium and sulphur oxides. In this case, the presence of iron is also supported by the black colour of the sample.

By comparing the chemical composition of the external drain (Sample 1) with that of other sulphurous springs in Romania (that are already used in balneotherapy), some differences can be observed. For example, the mineral springs of Pucioasa and Vulcana Bai (Dambovita county) have a high content of sulphur, bicarbonate, calcium, magnesium and chlorine, but also varying concentrations of metals such as Cr, Mn, Fe, Co, Ni, Cu and Pb (MURARESCU & al. [20]). In the external drain of the Siriu dam there were not identified such metals but the presence of germanium and silicon in this water source, besides the other chemical elements, could have positive effects in balneotherapy (DOBRZYNSKI & al. [21]). Also, many other Romanian balneal resorts are well-known for sulphurous waters (Calimanesti, Caciulata, Cozia, Govora, Harghita Bai, Olanesti, Santimbru Bai) and although their therapeutic effects are exhaustively discussed in the literature, the chemical composition of these springs has not been accurately reported yet (TATARU [22]; KIS & BACIU [23]).

Table 2. Physicochemical parameters of water samples.

	pH	**Temperature (ºC)**	**Conductivity (µS/cm)**	**TDS (mg/L)**	**PSU**	**ORP (mV)**
Sample 1	7.4	13.19	847	419	0.41	-242.9
Sample 2	7.89	15.11	476	238	0.23	-231.4

TDS: total dissolved solids; PSU: practical salinity unit; ORP: oxidation reduction potential;

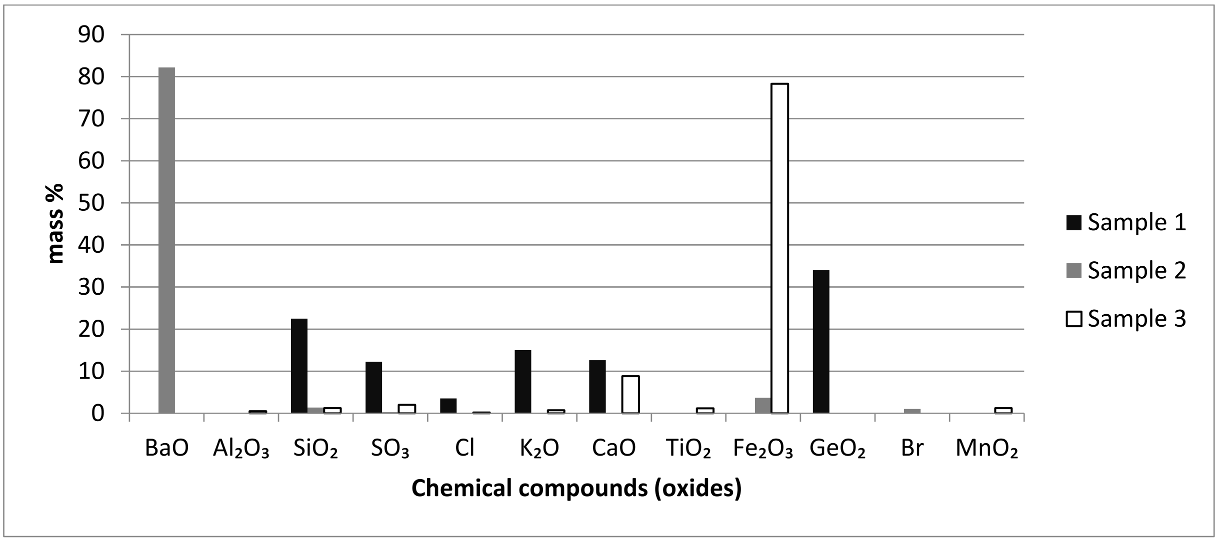

Figure 2. Chemical compounds identified by XRF analysis; samples are described in the *Materials and Methods* section.

In regard to nutrients, the results of chemical analysis revealed medium concentrations of nitrogen and phosphorus compounds (Table 3). In this respect, according to STAS 161/2006, the water samples are mesotrophic and are included in the first water quality class. On the other hand, the total organic carbon (TOC) is absent (*sample 1*) or present in very low concentrations (*sample 2*), the inorganic carbon (IC) being the dominant form in both samples. The absence of TOC is an indicator of the fact that the analysed water sources are not contaminated especially with domestic or industrial waste (FLORESCU & al. [24]). Relatively high concentrations of IC, such as carbonate, bicarbonate and dissolved carbon dioxide, may result from the solubilisation of minerals, the action of microorganisms or from the atmosphere, these compounds being able to affect the pH and mineral content of the water (KUBALA & al. [25]).

Table 3. Concentrations of nutrients in water samples.

	NH_4 mg N/L	**NO_2** mg N/L	**NO_3** mg N/L	**PO_4** mg P/L	**TP** mg/L	**IC** mg/L	**TOC** mg/L
Sample 1	0.02	0.00	0.75	0.001	0.005	98.71	0.00
Sample 2	0.00	0.002	0.60	0.003	0.009	36.25	1.40

TP: Total Phosphorus; IC: Inorganic Carbon; TOC: Total Organic Carbon;

Relating to the extracellular enzymatic activity, the results presented in Figure 3 show that alanine aminopeptidase activity is more intense compared to alkaline phosphatase or glucosidases activities. The reduced activity of glycoside hydrolases as compared to enzymes involved in the degradation of proteins can be explained as a consequence of low levels of carbohydrates based organic matter in the analysed water sources, which is why the native microorganisms do not synthesize large amounts of this type of enzymes.

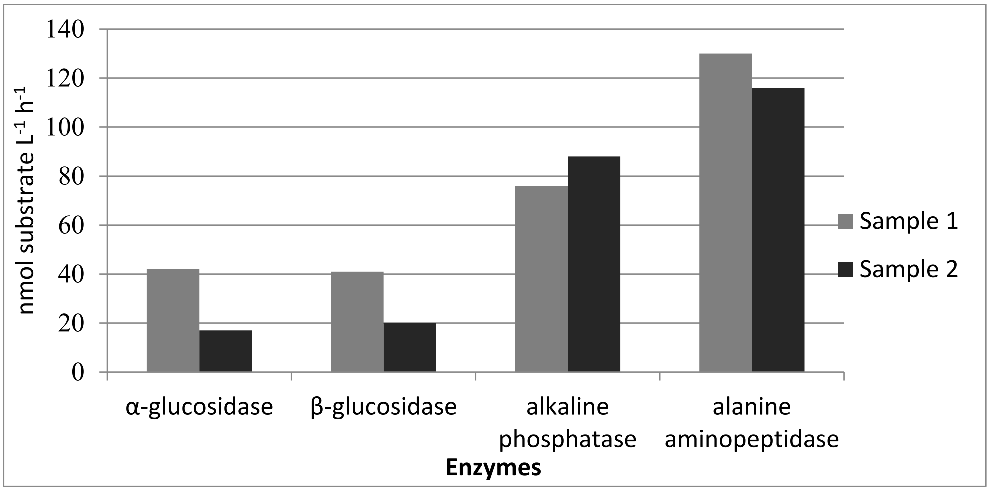

Figure 3. Enzymatic activity in water samples; samples are described in the *Materials and Methods* section.

Microbiological parameters of water samples

The phytoplankton species identified in *Sample 1* are represented by two species of cyanobacteria *(Microcystis* sp. and *Phormidium* sp.) and a species of diatom (*Cocconeis placentula*) (Figure 4). Their existence is possible only on the outer end of the drainage pipe (Figure 1A), where the light and temperature conditions are favourable for the installation of these types of aquatic organisms. They are not found in the underground springs because there are no favourable conditions for their growth. However, due to the toxic potential of these cyanobacterial species, it is advisable to disinfect the water catchment system before water consumption or bathing.

No zooplankton organisms were observed in any of the investigated samples. This is a proof of the fact that there are no planktonic communities in the spring water, but only a few species favoured by light in the areas where the springs reach the surface.

In regard to bacterial contamination of water sources, no total coliforms, faecal coliforms or faecal streptococci (0 CFU/mL) were found in any of the three samples. This result indicates that there is no recent faecal contamination. Mesophilic bacteria are present in low densities in *Sample 1* and *2*, but a larger number was identified in *Sample 3* (Table 4).

Ammonifying bacteria and sulphate-reducing bacteria were identified in all three samples, the highest level being in *Sample 3*. The presence of ammonifying microorganisms is correlated with the degradation of organic matter and the formation of ammonia (NH_4^+-N) through ammonification, this group of microorganisms having an important role in the nitrogen cycle in ecosystems (ZHAO & al. [26]). Sulphate-reducing microorganisms have also a great importance in the degradation of organic materials, being involved in the sulphur cycle. Their presence can cause serious corrosion problems of metallic and concrete structures due to hydrogen sulphide resulting from the metabolic activities of these microorganisms. This compound, through its "rotten egg" odour, is often a marker for the presence of sulphate-reducing bacteria in natural habitats (BARTON & FAUQUE [27]).

The presence of iron-oxidant bacteria in the eighth gallery (*Sample 3*) also indicates a risk for the integrity of the dam's metal structures, this group of microorganisms being involved in bio-corrosion processes, as reported by the literature (CHAMRITSKI & al. [28]; LIU & al. [29]).

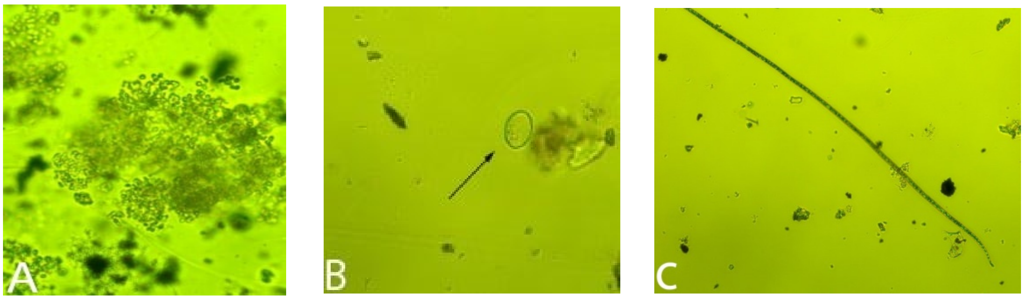

Figure 4. Phytoplankton species: *Microcystis* sp. (A), *Cocconeis placentula* (B), *Phormidium* sp. (C)

Table 4. Bacterial density of water samples.

	Ammonifying bacteria (cells/mL)	**Sulfate-reducing bacteria** (cells/mL)	**Iron-oxidizing bacteria** (cells/mL)	**Mesophilic bacteria** (CFU/mL)
Sample 1	4.5 x 10	11 x 10^2	NS	4.6
Sample 2	15 x 10^2	9.5 x 10	NS	13
Sample 3	14 x 10^3	20 x 10^3	4.5 x 10^3	1330

NS: not studied; CFU: colony forming unit; samples are described in the *Materials and Methods* section.

Conclusions

In conclusion, according to physicochemical analysis, water from the external drain is suitable for use in balneotherapy, presenting a balanced content of mineral elements such as sulphur, calcium, silicon, chlorine and potassium. However, it is not recommended to consume it because of the relatively high content of sulphur which is responsible for altering its organoleptic properties and which may have adverse effects on human or animal health. The absence of coliforms in this source of water indicates that no recent faecal contamination has occurred, but the presence of potentially toxic phytoplankton microorganisms such as *Microcystis* sp. and *Phormidium* sp. suggests the need to disinfect this water source before using it for any purpose.

The water from the underground drain shows a high level of barium compounds and an unbalanced content of beneficial chemical elements. Therefore, this water source is not recommended for therapeutic purposes or as source of drinking water.

The identification of both sulphate-reducing bacteria and iron-oxidizing bacteria in the drainage waters of Siriu dam should be considered as an alarm signal as they may lead to bio-corrosion and deterioration of metallic or concrete structures, affecting the integrity of the dam and hydropower constructions.

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