MICROBIOLOGY AND CHEMISTRY QUALITY ASSESSMENT OF SEVERAL MINERAL WATERS FROM THE EASTERN CARPATHIAN AREAS – ROMANIA

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Abstract. The present study aimed at assessing the quality of mineral water samples taken from various sites in the Eastern Carpathian region through microbiological and chemical analyses. The results of the microbiological analysis revealed the presence in some of the water samples of the following groups of bacteria: - mesophilic heterotrophic aerobes, - ferrobacteria, - denitrifying bacteria. The obtained results showed the absence of pathogenic bacteria from the *Enterobacteriaceae* family of total coliforms, thermotolerant coliform bacteria (faecal coliforms, *Escherichia coli* strains, from the family *Enterobacteriaceae*, also), and intestinal enterococci (faecal streptococci) in all analysed water samples. These results are in accordance with the norms regarding the microbiological conditions for the natural mineral waters of SR 4450:1997 and the HG 1.020/2005. The results obtained from the chemical analysis of the samples revealed concentrations of nitrates and nitrites that fall within the admissible limits for mineral water samples (SR 4450:1997). Thus, based on the results of the microbiological analysis it can be appreciated that the analysed water samples had a good bacteriological quality. In some analysed mineral water samples, high concentrations of Ca, Mg, Na and K ions were determined. The rich content of these minerals, as well as their multiple effects and implications in the efficient functioning of the main metabolic pathways of the organism, can give to the analysed samples a beneficial action on the body, most likely by enhancing metabolic processes. In conclusion, the correlated values of the physicochemical and bacteriological parameters lead to the qualification of good quality natural mineral waters of the investigated samples.

Keywords: mineral waters, water quality assessment, microbiological and chemical analysis.

Rezumat. Evaluarea microbiologică şi chimică a calităţii unor ape minerale din unele zone ale Carpaţilor Estici - România. Prezentul studiu a avut ca scop evaluarea calităţii unor probe de apă minerală prelevate din diferite situsuri din zona Carpaţilor Estici prin analize microbiologice şi chimice. Rezultatele analizei microbiologice au evidenţiat prezenţa în unele dintre probele de apă a următoarelor grupe de bacterii: - aerobe heterotrofe mezofile, - ferobacterii, – bacterii denitrificatoare. Rezultatele obţinute au indicat absenţa bacteriilor patogene din grupurile coliformilor totali din familia *Enterobacteriaceae*, a bacteriilor coliforme termotolerante (coliformi fecali, din specia *Escherichia coli*, de asemenea din familia *Enterobacteriaceae*) şi a enterococcilor intestinali (streptococilor fecali) în toate probele de apă analizate. Aceste rezultate sunt în concordanţă cu normele privind condiţiile microbiologice pentru apele minerale naturale din SR 4450:1997 şi HG 1.020/2005. Rezultatele obţinute în urma analizei chimice a probelor au evidentiat concentraţii de nitraţi şi nitriţi care se încadrează în limitele admisibile pentru probele de apă minerală (SR 4450/1997). Astfel, pe baza rezultatelor analizei microbiologice se poate aprecia, că probele de apă analizate au prezentat o calitate bună din punct de vedere bacteriologic. În unele probe de apă minerală analizate au fost determinate concentraţii ridicate ale ionilor de Ca, Mg, Na şi K. Conţinutul bogat în mineralele menţionate, precum şi multiplele efecte şi implicaţii ale acestora în funcţionarea eficientă a principalelor căi metabolice ale organismului, pot conferi probelor analizate o acţiune benefică asupra organismului, cel mai probabil prin potenţarea proceselor metabolice. În concluzie, valorile corelate ale parametrilor fizico-chimici şi bacteriologici conduc la calificativul de ape minerale naturale de bună calitate a probelor analizate.

Cuvinte cheie: ape minerale, evaluare calitate apă, analiza microbiologică şi chimică.

INTRODUCTION

The Eastern Carpathians are famous for their mineral water resources, exploited since ancient times due to their special chemical composition and therapeutic effects. The chemical composition of mineral waters is mostly influenced by the type of rocks they cross and the geological structure of the area (KIS & BACIU, 2014).

The water samples analysed in this study belong to the category of groundwater, characterized, in general, by higher mineralization.

Depending on the mineralogical composition of the crossed areas, some groundwater sources contain significant amounts of iron, calcium, magnesium, manganese, hydrogen sulphide and sulphides, nitrogen compounds, etc (VASELLI et al., 2002; KIS & BACIU, 2014).

The quality of natural waters is generally determined by the totality of mineral or organic substances contained, dissolved gases, suspended particles, and living organisms present. This can be defined as a conventional set of physical, chemical and biological characteristics, expressed as values, which allow the water sample to be included in a certain category, thus acquiring the property to serve a specific purpose (JOSEPH et al., 2018; ALEXANDER et al., 2019; SARKER et al., 2019).

In order to establish the quality of a water sample, from the multitude of physical, chemical and biological characteristics that can be determined by means of laboratory analyses, practically a limited number of parameters are used, considered significant: - organoleptic indicators (taste, smell); - physical indicators (pH, electrical conductivity, colour, turbidity); - chemical indicators; - radioactive indicators; - biological indicators (a category which also includes bacteriological indicators) (ĆIRIĆ et al., 2018).

Determining the physical, chemical and biological parameters characteristic of drinking water from different sources is very important to establish the type and quality of water (PANTELIĆ et al., 2017).

From a bacteriological point of view, the most important condition for a water sample to be considered drinking water is the total absence of pathogenic bacteria. Given the relatively laborious methods of highlighting and the lack of their constant presence in water, the highlighting of indicator bacteria is used. These are represented by: - thermotolerant coliform bacteria (faecal coliforms, *Escherichia coli* strains), - intestinal enterococci (faecal streptococci), - coliform bacteria (total coliforms of the *Enterobacteriaceae* family) and - total number of aerobic mesophilic heterotrophic bacteria (QUATTRINI et al., 2016; ĆIRIĆ et al., 2018).

Total coliforms and thermotolerant coliform bacteria are used as indicators of water quality, and the absence of these bacteria as cultivable colonies on selective culture media are an indicator of good quality water (MOREIRA et al., 1994).

According to World Health Organization (WHO) regulations, drinking water should be clear, colourless, odourless, tasteless, and free from pathogenic bacteria or other toxic chemicals (***. OMS, 1994; PANTELIĆ et al., 2017).

Thus, the present study aimed at assessing the quality of mineral water samples taken from various sites in the Eastern Carpathian region through microbiological and chemical analyses.

MATERIAL AND METHODS

In order to determine the physical, chemical, and bacteriological indicators of water quality, 12 samples of bottled mineral water randomly selected from commercial sources were analysed.

The mineral water samples came from sources located in six distinct areas:

1) Vatra Dornei - Suceava county: BUp and BUc samples,
2) Bilbor - Harghita county: BIp and BIc samples,
3) Borsec - Harghita county: BOp and BOc samples,
4) Suceava - Suceava county: DOp and DOc samples,
5) Stâna de Vale - Bihor county: IMp and IMc samples,
6) Sâncrăieni, Harghita county: PHp and PHc samples

From each source, there was a sample of still mineral water and one of carbonated mineral water (Table 1).

Table 1. Mineral water samples investigated.

Sample	Indicative water sample	pH
1	BUp (still mineral water)	7.43
2	BUc (carbonated mineral water)	6.07
3	BOp (still mineral water)	8.00
4	BOc (carbonated mineral water)	6.70
5	IMp (still mineral water)	7.56
6	IMc (carbonated mineral water)	5.25
7	PHp (still mineral water)	6.91
8	PHc (carbonated mineral water)	6.22
9	DOp (still mineral water)	8.05
10	DOc (carbonated mineral water)	6.04
11	BIp (still mineral water)	7.40
12	BIc (carbonated mineral water)	6.09

Determination of physical and chemical indicators: The temperature, conductivity, and salinity of the samples were determined using a WTW 340i multiparameter kit (Germany). Nitrites, nitrates, and phosphates were determined spectrophotometrically, as follows:

- nitrites: in the presence of sulfanilic acid and α-naphthyl amine. NO_2^- ions react with sulfanilic acid in a strongly acidic environment, and in the presence of α-naphthyl amine a compound with a red coloration is formed (***. Romanian Law no. 458 / 2002);

- nitrates: in the presence of sodium salicylate and Seignette salt, NO_3^- ions react with sodium salicylate in acidic medium and then form sodium hydroxide salts and Seignette salt, yellow nitro salicylic acid salts are formed (TARTARI & MOSELLO, 1997);

- phosphates: in the presence of antimony and potassium tartrate and ascorbic acid, the determination of the reaction leads to the formation of an Sb-phospho-molybdenum complex, reduced in turn by L-ascorbic acid, to a blue complex (TARTARI & MOSELLO, 1997).

The concentration of alkali metals and heavy metals was determined by the atomic absorption spectrometry method, using a ContrAA 700 atomic absorption spectrophotometer (Analytik Jena, DE).

The pH value of the water samples was determined with a laboratory pH meter (Table 1).

Microbiological investigations: The water samples were subjected to microbiological analysis regarding the highlighting and quantitative estimation of:

1) aerobic mesophilic heterotrophic bacteria (expressed as a total number);

2) pathogenic bacteria represented by: a) coliform bacteria (total coliforms from the *Enterobacteriaceae* family), b) thermotolerant coliform bacteria (faecal coliforms, from the species *Escherichia coli*) and c) intestinal enterococci (faecal streptococci);

3) physiological groups of microorganisms involved in the biogeochemical cycle of iron (iron bacteria and neutrophilic iron-oxidizing bacteria);

4) bacteria involved in the biogeochemical cycle of nitrogen (denitrifying bacteria);

5) bacteria involved in the biogeochemical cycle of sulphur (sulphate-reducing bacteria).

The quantification of the types of bacteria studied was performed according to microbiological norms in stages that involve: inoculation of the sample or its dilutions, incubation, and determination of the total number of bacteria. Thus, serial decimal dilutions were made from the water samples. In order to determine the aerobic heterotrophic bacteria and total coliforms, the samples or dilutions were then inoculated in Petri dishes on agar growth media in triplicate, by the pour-plate technique. Also, in the case of other groups of bacteria, the water sample or the corresponding dilutions were inoculated, in triplicate, into test tubes containing liquid or semisolid culture media, specific to the respective groups of microorganisms.

The culture media used to determine each group of microorganisms were the following: - nutrient agar for aerobic mesophilic heterotrophic bacteria; - Eosin Methylene Blue Agar medium (EMB Agar or Levine) for total coliforms; - MacConkey medium for faecal coliforms; - Bromocresol purple Azide Broth medium for intestinal enterococci (faecal streptococci); - Postgate medium for sulphate-reducing bacteria; - Pochon medium for denitrifying bacteria; - Vinogradski medium for iron bacteria and - a selective culture medium containing thiosulfate and ferrous sulphate as a source of Fe^{2+} (pH value of 7.0 - 7.2) for neutrophilic iron-oxidizing bacteria (***. Romanian standard STAS 3001-91; ***. Romanian standard SR 4450:1997; GUYARD et al., 1999; LAZĂR et al., 2004; ***. Romanian Government Decision 1.020/2005).

The Petri dishes and test tubes were incubated at different temperature values (28° C, 37° C, and 45° C, respectively) for a variable period (24 - 48 hours or 7 - 14 days), depending on the type of microorganisms investigated.

The quantification of microorganisms was performed by determining the number of colony-forming units per millilitre (CFU / mL) or the number of microorganisms / mL of a water sample by the McCrady method (or MPN - the Most Probable Number method) (LAZĂR et al., 2004).

RESULTS AND DISCUSSIONS

The conductivity of the samples (an indicator of the total amount of salts dissolved in water, a parameter that largely depends on the geological composition of the crossed layers) varies in the limits of 80-500 µS / cm for still mineral waters and 130 - 2500 µS / cm for carbonated mineral waters (Table 2), the maximum values being registered for BOp and BOc samples. In the case of the carbonated mineral water sample IMc, the value is very low (137 µS / cm), close to that of the still mineral water sample IMp (127 µS / cm), while for the still mineral water sample PHp, the value is very high (1190 µS / cm).

Nitrate and nitrite concentrations are within the permissible limits (SR 4450:1997). The phosphate concentration fluctuates between 1-30 µgP / l, the minimum values being registered for the water samples DOp (still mineral water) and DOc (carbonated mineral water), and the maximum ones in the samples BIp and BIc (Table 2).

Table 2. Physico-chemical analysis of still and carbonated mineral water samples.

Parameter Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Conductivity [uS/cm]	83	1075	497	2520	127	137	1190	987	325	1349	97	1555
Temperature [C]	21.7	21.6	21.8	21.7	21.8	21.7	21.8	21.8	21.9	21.8	21.8	21.8
Salinity [PSU]	0	0.3	0	1.2	0	0	0.4	0.3	0	0.5	0	0.6
Nitrates [mgNO3/l]	6.5	3.5	2	2	5	4.2	8.5	11	3	1	1.3	7.2
Nitrites [mgNO2/l]	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01
Phosphates [mgP/l]	0.018	0.011	0.019	0.020	0.003	0.005	0.006	0.010	0.001	0.003	0.030	0.024
Na [mg/l]	2.79	19.21	2.73	85.3	1.24	1.15	69.7	61	0.86	19.02	2.74	91.6
K [mg/l]	1.25	3.34	0.81	18	0.72	0.71	9.86	10.27	0.38	2.85	2.01	31.8
Ca [mg/l]	12.8	207	49.1	577.5	20.53	14.87	159.3	110.7	65	370	12.6	156.9
Mg [mg/l]	1.08	19.5	17	46	1.9	1.9	13.3	19.3	0.98	5.3	1.2	22
Mn [mg/l]	-	0.004	-	0.05	-	-	0.07	0.01	-	-	-	0.015
Cu [ug/l]	-	-	-	-	-	-	-	-	-	-	-	-
Zn [ug/l]	-	-	-	-	-	-	-	-	-	-	-	-
Pb [ug/l]	-	-	-	-	-	-	-	-	-	-	-	-
Ni [ug/l]	-	-	-	1.5	-	-	-	-	-	1	-	-
Cd [ug/l]	-	-	-	-	-	-	-	-	-	-	-	-

1 = BUp; 2 = BUc; 3 = BOp; 4 = BOc; 5 = IMp; 6 = IMc; 7 = PHp; 8 = PHc; 9 = DOp; 10 = DOc; 11 = BIp; 12 = Bic.

Low values of alkaline and alkaline-earth metals content were recorded in water samples: BUp, IMp and IMc, and BIp, in accordance with the low conductivity values.

The carbonated mineral water sample BOc has a high content of calcium and magnesium (over 500 mg Ca / L and 46 mg Mg / L). High concentrations of Ca and Mg are also found in carbonated mineral water samples: - BUc (207 mg Ca / L, respectively 19.5 mg Mg / L), - DOc (370 mg Ca / L) and - BIc (157 mg Ca / L, 22 mg Mg / L respectively), the latter also having a high content of Na and K (91.6 mg Na / L and 32 mg K / L) (Table 2).

Except for the BOc and DOc carbonated mineral water samples, which contain traces of Ni ion, whose values are well below the acceptable limit values for drinking water (Law no. 458 / 2002), none of the analysed water samples contains Pb, Ni, or Cd, priority hazardous substances according to the EU Water Framework Directive (WFD) (***. EC WFD 60/2000).

The results of the microbiological analysis showed the presence, in some of the investigated water samples, of the following groups of microorganisms: - aerobic mesophilic heterotrophic bacteria, - iron bacteria, and - denitrifying bacteria (Table 3).

The aerobic mesophilic heterotrophic bacteria were determined only in the still mineral water samples BOp and PHp, at the level of of 1 CFU / ml and 4 CFU / ml, respectively (Table 3). These values are within the limits allowed for the total content of microorganisms forming colonies at a temperature of 37^{o} C, according to the norm of microbiological indicators in the HG 1020/2005.

The obtained results indicated the absence of pathogenic bacteria: - total coliforms from the *Enterobacteriaceae* family, - thermotolerant coliform bacteria (faecal coliforms, of the *Escherichia coli* species) and - intestinal enterococci (faecal streptococci) from all 12 mineral water samples investigated (Table 3). These results are in accordance with the norms regarding the microbiological conditions for natural mineral waters from SR 4450/1997 and HG 1020/2005.

Table 3. Quantitative estimation of aerobic mesophilic heterotrophic bacteria, coliforms (total and faecal), and intestinal enterococci present in the water samples investigated.

Water samples	**Microorganisms (CFU / mL or the number of microorganisms / mL of sample)**			
	Mesophilic heterotrophic bacteria	Total coliforms	Faecal coliforms	Intestinal enterococci
BUp	0	0	0	0
BUc	0	0	0	0
BOp	1	0	0	0
BOc	0	0	0	0
IMp	0	0	0	0
IMc	0	0	0	0
PHp	4	0	0	0
PHc	0	0	0	0
DOp	0	0	0	0
DOc	0	0	0	0
BIp	0	0	0	0
BIc	0	0	0	0

CFU / mL = the number of colony-forming units per millilitre of water sample

From the group of microorganisms involved in the biogeochemical cycle of iron, the iron bacteria (which reduce Fe^{3+} to Fe^{2+}) were present in the still mineral water samples: BUp (2.5 microorganisms / mL), BOp (2.5 X 10^2 microorganisms / mL), IMp (1.1 x 10^3 microorganisms / mL) and PHp (4.5 microorganisms / mL). Comparatively, in the carbonated mineral water samples from the same sources, the iron bacteria were not present.

The iron bacteria were present in the still mineral water sample DOp at the level of 1.1 x 10^3 microorganisms / mL and respectively, in the carbonated mineral water sample DOc (1.5 microorganisms / mL). The iron bacteria were not present in the mineral water samples BIp and BIc (Tables 4, 5).

The neutrophilic iron-oxidizing bacteria (which oxidize Fe^{2+} to Fe^{3+}) were not present in any of the analysed mineral water samples (Tables 4, 5).

Table 4. Quantitative estimation of bacteria involved in the biogeochemical cycles of iron, nitrogen, and sulphur.

Water samples	**Microorganisms (number of microorganisms / mL of the sample)**			
	The iron cycle		The nitrogen cycle	The sulphur cycle
	Iron bacteria	Neutrophilic iron-oxidizing bacteria	Denitrifying bacteria	Sulphate-reducing bacteria
BUp	2.5	0	0	0
BUc	0	0	0	0
BOp	2.5 x 10^2	0	7.5	0
BOc	0	0	0.7	0
IMp	1.1 x 10^3	0	0	0
IMc	0	0	0	0
PHp	4.5	0	1.5 x 10	0
PHc	0	0	0	0
DOp	1.1 x 10^3	0	0	0
DOc	1.5	0	0.7	0
BIp	0	0	1.5 x 10	0
BIc	0	0	8.0	0

In the case of microorganisms involved in the biogeochemical cycle of nitrogen, the obtained results indicated the presence of denitrifying bacteria in low numbers (between 0.7 – 1.5 x 10 microorganisms / mL). The denitrifying bacteria were present in still mineral water samples: BOp (7.5 microorganisms / mL), PHp (1.5 X 10 microorganisms / mL) and in BIp (1.5 X 10 microorganisms / mL). Also, the presence in small numbers of this group of bacteria was determined in three samples of carbonated mineral water: BOc (0.7 microorganisms / mL), DOc (0.7 microorganisms / mL) and BIc (8.0 microorganisms / ml) (Table 4).

The denitrifying bacteria were absent in still mineral water samples: BUp, IMp, and DOp, as well as in the carbonated mineral water samples BUc, IMc, and PHc (Tables 4, 5).

The sulphate-reducing anaerobic bacteria were not present in any of the 12 mineral water samples investigated (Tables 4, 5).

Table 5. The groups of microorganisms present in the analysed still and carbonated mineral water samples.

No	Water samples	Physiological groups of microorganisms (CFU / mL or the number of microorganisms / mL of the sample)	
		Still mineral water	Carbonated mineral water
1	BU	iron bacteria (2.5)	-
2	BO	heterotrophic bacteria (1.0), iron bacteria (2.5 x 10^2), denitrifying bacteria (7.5)	denitrifying bacteria (0.7)
3	IM	iron bacteria (1.1 x 10^3)	-
4	PH	heterotrophic bacteria (4.0), iron bacteria (4.5), denitrifying bacteria (1.5 x 10)	-
5	DO	iron bacteria (1.1 x 10^3)	iron bacteria (1.5) denitrifying bacteria (0.7)
6	BI	denitrifying bacteria (1.5 x 10)	denitrifying bacteria (8.0)

- = absence of microorganisms investigated

CONCLUSIONS

The bacteriological quality of mineral water is based on the presence or absence of the bacteria indicating fecal pollution (*Escherichia coli*, intestinal enterococci) and the surface contaminants (total coliforms).

The determination of total coliforms, faecal coliforms, and intestinal enterococci is used as an indicator of drinking water quality, and the absence of these indicator bacteria certifies a good quality of the respective waters.

The results obtained after performing the microbiological analysis indicated the absence of total coliforms from the *Enterobacteriaceae* family, of thermotolerant coliform bacteria (faecal coliforms, of the *Escherichia coli* species) and intestinal enterococci (faecal streptococci) from all the 12 analysed mineral water samples.

Also, the results showed the presence of a very low number of aerobic mesophilic heterotrophic bacteria only in two investigated water samples: 1.0 CFU / mL in the still mineral water sample BOp and respectively, 4.0 CFU / mL in the still mineral water sample PHp. These values are below the reference values allowed for the total content of colony-forming microorganisms at a temperature of 37^o C (20 CFU / mL), according to the norms regarding the microbiological conditions for natural mineral waters from HG 1.020/2005.

Thus, based on these results, it can be appreciated that the still and carbonated mineral water samples investigated presented a good quality from a bacteriological point of view.

The results obtained from the chemical analysis of the samples revealed concentrations of nitrates and nitrites that fall within the admissible limits for mineral water samples (SR 4450/1997).

In some analysed mineral water samples, high concentrations of Ca, Mg, Na and K ions were determined. The rich content of these minerals, as well as their multiple effects and implications in the efficient functioning of the main metabolic pathways of the organism, can give to the analysed samples a beneficial action on the body, most likely by enhancing metabolic processes.

In conclusion, the correlated values of the physicochemical and bacteriological parameters lead to the qualification of good quality natural mineral waters of the investigated samples.

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