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Physicochemical and Microbiological Analysis of Still And Carbonated Mineral Waters from Romania

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ABSTRACT

Even if vast surfaces of the Earth are covered with water, a huge number of people are confronted with serious lack of fresh and safe drinking water. Unsafe water is a worldwide public health threat endangering people. Water pollution, particularly the presence of pathogenic organisms, may be one of the main sources of infectious diseases. In this paper we present the results from the physicochemical and microbiological analysis of a number of still and carbonated mineral waters found in Romania. We report that in terms of dissolved minerals, all analyzed waters were found within the limits imposed by the present laws. The microbiological analysis revealed that a number of still water samples had some No.of colonies at 22 °C is 37 °C present.

KEYWORDS mineral water, quality, norms

Introduction

Unsafe water is a worldwide public health threat endangering people, placing them at risk for diarrhea and other diseases as well as chemical intoxication [1]. Although access to clean water has generally increased, this progress masks significant disparities within and between countries, between urban and rural areas as well as between high- and low-income groups [2]. For this reason the control of water quality has to be of a great concern.

Natural mineral waters usually differ in the degree of mineralization [3, 4]. Mineral waters are generally perceived as pure, clean and potable [5] and are usually defined as: microbiological pure waters which originate from a phreatic layer or from a subterranean aquifer and which are extracted from one or more natural emergences or bores. [6].

According to World Health Organization (WHO) the physical parameters that are likely to give rise to complaints from consumers are color, taste, odor and turbidity. Low pH causes corrosion and high pH results in taste complaints. In fact, the drinking water should be examined on microbiological and physicochemical quality [7]. Both bottled and tap water can be oligotrophic environments with sufficient nutrients to maintain bacterial growth, releasing organic matter and providing additional substrates for the microbial growth [8].

Water pollution, particularly the presence of pathogenic organisms, may be one of the main sources of infectious and intestinal diseases [9]. Heavy metals are stable and persistent environmental contaminants since they cannot be degraded nor destroyed. They produce adverse effects on health of human and other life forms in terrestrial and aquatic environment and also affect the food chain [10]. Lately more and more people believe that bottled water is safer than tap water [11].

Investigators have demonstrated the potential for bacterial growth in spring water for up to three weeks after bottling if the microbes are not adequately removed, resulting in microbial loads of up to 10^4 colony forming units (cfu) mL^{-1} at 37°C [12]. Also there is an increasing concern about the microbial quality of bottled water marketed worldwide. Several studies have documented the detection of coliforms and heterotrophic bacteria in bottled water in counts which far exceeded national and international standards set for potable water for human consumption [13].

Many heavy metals are detrimental to microorganisms even at the low concentrations present in natural waters. There are also reports on the adaptation of microorganisms to heavy metals [14].

There are commercially available two main types of bottled water: drinking water that can have its source in surface waters (rivers, lakes), rainwater or groundwater and must comply with the drinking water standards [11] and natural mineral water that can be still or carbonated [15].

Natural mineral water has no concentration limits for the majority of chemical constituents, but potentially toxic compounds are strictly regulated. Contamination indicators (original purity indicator) have the most restrictive values of all bottled waters. All natural mineral waters must be bottled at source. In Romania there is a strong tradition linked to the consumption of natural carbonated mineral water, although in recent years more and more Romanians got a taste for still water [6].

The purpose of this paper is the analysis of physical parameters, heavy metals and microbiological analysis for 20 natural mineral carbonated and still waters of Romania, from different springs presented in Figure 1.

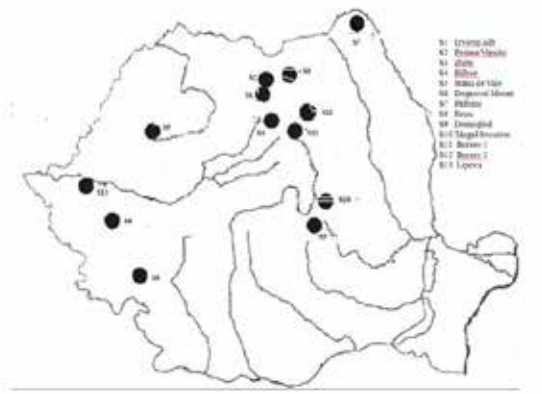

Figure 1. The map of still and carbonated mineral water springs.

Microbiological Analysis
For the culture media we used Tergitol 7 Agar, and TTC solution 1% produced by Oxoid for the identification of Coliforms and *Escherichia coli,* Slanetz - Bartley medium from Oxoid and Esculin BALL azide agar produced by Laboratorios CONDA for the identification of intestinal Enterococci, Pseudomonas agar base and Pseudomonas CN supplement and yeast extract agar produced by Oxoid for the identification of *Pseudomonas aeruginosa* at 22°C and 37°C.

Two methods were used for microbiological analysis of water samples: membrane filtration for the detection and enumeration of microorganisms and plate incorporation for colony growth at 22°C and 37°C.

After incubation, the plates have been immediately examined. For colony count (from the surface and inside the agar), we used for this paper the WE BZG30 colony counter from WTW GmbH.

Physicochemical Analysis:
A Perkin Elmer Sciex ELAN DRC-e ICP-mass spectrometer, with the operating conditions summarized in the Table 1, was used for all measurements of the heavy metals: Pb, Cd, Ni, Cu, Cr, Mn, Ba and B. Metal analysis was performed according to a previous work by ICP-MS Determination of heavy metals and other toxic elements in drinking water from rural Several Areas of Romania (18). A multi-element standard reference solution of 10 µg mL-1 was used as internal standard for all the metals. This method of analysis has been used in.

The pH and conductivity were determined from an electronic pH/conductivity meter Cond 720 from WTW GmbH.

Table 1. ICP-MS operating conditions

Parameter	Value
Rf power	1500 W
Plasma gas flow	15 L min^{-1}
Aerosol gas flow	1.2 L min^{-1}
Resolution	Normal
Sweeps/reading and	5
Points/spectral peack	1
Dwell time	500 ms
Readings per replicate	400

Results and discussions
The physical parameters: pH, conductivity, heavy metals contents and microbiological parameters of 10 samples of still mineral water (represented in this paper with W)and 10 samples of carbonated mineral water were (represented in this paper with C) determined and compared with national and international limits presented in Table 2.

Table 2. Drinking water limits for heavy metals and other toxic elements (µg L^{-1}), pH, conductivity (µS cm^{-1}) and number of colonies at 22 and 37°C (cfu mL^{-1})

Standard	RGD 1020/2005	WHO
pH	according to the natural state of water	6.5-9,9
Conductivity	according to the natural state of water	2500
B	The maximum limit for boron will be determined by the competent national authority, where harmonized European standards will be adopted in this respect, in accordance with them	2000
Cr	50	50
Mn	500	400
Ni	20	70
Cu	1000	2000
Cd	3	3
Ba	1000	700
Pb	10	10
No. of colonies at 22°C	20 (100*)	-
No. of colonies at 37°C	5 (20*)	-
*12h after bottling		

Our results, presented in Table 3 and Table 4 show that the majority of the samples, with the exception presented below, don't exceed the limits.

Table 3. Metal concentrations (µg L^{-1}), pH, conductivity (µS cm^{-1}), and the number of colonies at 22 and 37°C (cfu mL^{-1}) in still water

Sample	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10
pH	7.68	7.2	7.05	7.26	7.5	7.78	6.94	7.72	7.65	7.51
Conductivity	320	476	106.5	128	515	324	90.2	297	662	798
B	4.17	163.92	14.13	53.54	13.27	5.40	19.68	10.96	199.76	84.43
Cr	10.06	15.58	2.96	5.67	16.00	8.39	4.56	6.02	19.72	16.89
Mn	6,54	4.26	21	0.10	0.22	0.02	0.18	0.02	0.54	0.11
Ni	0.14	0.48	0.64	0.05	0.39	0.32	0.13	0.12	0.30	0.09
Cu	0.03	0.44	0.05	0.15	0.24	0.53	0.25	0.31	0.57	0.07
Cd	0.02	0.53	0.05	0.02	0.03	0.02	0.01	0.09	0.06	0.03
Ba	60.37	11.86	11.50	20.06	16.53	10.35	5.23	5.11	117.56	16.74
Pb	0.01	0.20	0.03	0.08	0.05	0.11	0.18	0.52	0.24	0.87
No. of colonies at 22°C	0	45	0	567	724	170	0	4	18	38
No. of colonies at 37°C	0	0	0	342	431	0	0	0	0	0

Sample	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
pH	5.94	5.58	5.88	4.8	5.58	5.97	6.01	5.81	5.64	6.28
Conductivity	1588	418	1650	1312	532	1694	1805	1190	728	2.35
B	1185.31	108.38	1010.29	36.24	15.39	37.85	1173.97	617.38	1303.03	1469.16
Cr	18.79	27.19	13.98	11.59	26.45	12.21	25.32	18.15	16.11	19.84
Mn	203.82	2.23	151.17	0.11	0.29	920.9	230.79	240.12	6.36	176.26
Ni	4.64	0.44	0.82	0.06	0.56	13.08	2.72	1.1	0.55	1.75
Cu	0.55	0.53	0.74	0.07	2.27	0.32	0.31	1.12	0.57	0.65
Cd	0.03	0.61	0.08	0.03	0.02	0.03	0.02	0.1	0.07	0.03

Table 4. Metal concentrations (μg L^{-1}), pH, conductivity (μS cm^{-1}), and the number of colonies at 22 and 37°C (cfu mL^{-1}) carbonated water

The results were compared with the norms established by the Romanian Government Decision 1020/2005 [4], based on the European Council Directive 777/80/ECC [16] and WHO guidelines [2]. According to RGD1020/2005 heavy metals (Pb, Cd, Ni, Cu, Cr, Mn,Ba and B) are part of the constituents naturally present in natural mineral waters and the maximum limits, when exceeded, can be a risk factor for public health.

The heavy metal concentrations obtained from the water samples were much lower than the values of RGD 1020/2005 as well as the values recommended by WHO. In the case of carbonated waters, the concentrations are within the maximum specified limits but were higher than those obtained from still waters. The higher concentrations found in carbonated waters can be attributed to the higher acidity of these waters, which can naturally dissolve more material in their formation process. Also, the majority of analyzed carbonated water samples come from known volcanic areas of Romania. The only element that presented higher concentrations than the admitted by the Romanian law governing water quality (Law 458/2002), was Boron (1469.15 μg L^{-1} vs. max.1000 μg L^{-1}), but the value was still lower than the limit recommended by WHO. If we refer to the value of Law 458/2002 [17], it can be seen that we obtained higher values in half of carbonated water samples analyzed. The law 458/2002 governing the quality of drinking water other than mineral waters and spring waters (17).

Obtained values for pH are less for carbonated water than to still waters, as expected, due to acidic content of carbon dioxide. Conductivity values obtained are higher in carbonated than in still waters, as shown in Table 3 and Table 4.

Microbiological analysis of water samples analysis included the following parameters: Coliform bacteria, *E. coli*, Intestinal Enterococci, *P. aeruginosa*, and the determination of the number of colonies at 22°C and 37°C. For determination of: Coliform bacteria, E. *coli*, P. aeruginosa and Intestinal Enterococci, they were absent in all samples of mineral water analyzed.

The number of Colonies at 22 °C and 37°C were absent from all carbonated water samples. For the still water samples, we found the number of colonies at 22 °C present in 7 samples from 10 three of which are greater than the maximum limits established by RGD 1020/2005 and in 2 out of 10 samples, the number of colonies at 37 °C are also higher than the maximum limits of RGD 1020/2005. The absence of bacteria from the carbonated water samples is due to the bacterial inhibition effect of the carbon dioxide. Number of colonies at 37 ° C provides information on an entire bacterial population in the water, and the effectiveness of treatment processes, including disinfection and filtration (19).

Conclusions

Reviewing the results from the microbiological analysis of still and carbonated mineral water samples we identified a number of colonies that grow at 22°C and 37°C in a number of still water samples. This number of colonies at 22°C and 37°C were totally absent from carbonated mineral water samples, due to the inhibiting effect that carbon dioxide has on microorganisms. Analyzing the results of physicochemical determination we found that the values were within the maximum allowed concentrations of legislation in force.

Currently the legislation regulating bottled mineral water quality states that the tests must be performed before the bottling process. The legislation states that the water needs to have the potential sources for all microbiological parameters analyzed, noting the number of allowed colonies at 22°C and 37°C and the monitoring time - 12 hours after bottling. As we know from the literature microorganisms can grow in water up to 3 weeks after bottling. There is currently no legislation in force at national,European and international level to which they can relate quality mineral water we drink, when they buy in the store.

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