Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2023, **24** (2), pp. 097 – 110

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

ASSESSMENT OF THE MICROBIOTA OF SOME MINERAL WATER FORM SLANIC MOLDOVA (ROMANIA): SEASONAL EVALUATION AND AFTER STORAGE IN DIFFERENT CONDITIONS

Lavinia Misăilă[1,2], Dumitra Răducanu[3*], Narcis Bârsan[4], Irina-Claudia Alexa[4], Oana-Irina Patriciu[4], Luminiţa Grosu[4*]

[1] "Vasile Alecsandri" University of Bacău, Doctoral School, 157, Calea Mărăşeşti, 600115, Bacău, Romania
[2] "Dimitrie Ghika" Technical College Comanesti, 1, Liceului Street, 605200, Comanesti, Romania
[3] "Vasile Alecsandri" University of Bacău, Faculty of Sciences, 157, Calea Mărăşeşti, 600115, Bacău, Romania
[4] "Vasile Alecsandri" University of Bacău, Faculty of Engineering, 157, Calea Mărăşeşti, 600115, Bacău, Romania

*Corresponding authors: dumitra_manea@yahoo.com, lumig@ub.ro

Received: March, 22, 2023
Accepted: June, 07, 2023

Abstract: The mineral waters that come from the springs in the area of Romanian mountain resort Slanic Moldova, heterogeneous from a physical-chemical point of view, are used for external or internal therapeutic purposes. A survey of the microbiological quality of mineral waters collected from 7 springs in Slanic Moldova was conducted concerning the seasonal quantitative variation of the total number of heterotrophic bacteria (HPC) and the evolution of some representative microbiological parameters (HPC, *Clostridium perfringen*s and *Pseudomonas aeruginosa*) in samples bottled and stored at (20 - 22 °C) or (4 °C). for a period of 1 - 360 days. All values for HPC determined at 22 °C were within the reference limits of 100 CFU·mL^{-1} for the investigated sources, in accordance with the requirements of EU and national regulations. At 37 °C, the HPC was within normal values (20 CFU/mL), for five (1 bis, 5, 10, 14 and 15) of the seven sources studied, both in the seasonal analysis and after bottling and storage in different conditions. *Clostridium perfringens* and *Pseudomonas aeruginosa* were not detected in all of the mineral water's samples. Microbiological assessment and proper management of mineral water resources can lead to a good prediction of water quality and avoidance of health risks.

Keywords: *bottling, Clostridium perfringens, HPC, microbiological quality, mineral water, Pseudomonas aeruginosa*

INTRODUCTION

The use of water for human consumption supposes that it is safe from a bacteriological point of view [1 – 3]. The EU's zero pollution plan contains measures to stop and / or prevent water pollution, discussed in the European Green Pact - EU Green Week 2021 [4] at Digital World Water Congress organized by International Water Association [5].
Directive 2009/54/EC of the European Parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters applied in Romanian regulation as Romanian Government Decision No. 1020 of 1 September 2005, clearly distinguishes between natural mineral water and regular drinking water [6, 7]. Interventions and treatment of natural mineral waters are not permitted, except the removal and/or adding of carbon dioxide and the decantation and/or filtration of unstable constituents such as iron, manganese, sulfur, or arsenic compounds [6, 7].
Natural mineral waters are distinguished by the rich and varied composition of major components and trace elements, specific to the geographical area to which they belong, by a local microbiota specific to each source that does not alter its therapeutic qualities and microbiological safety [8 – 11].
The physicochemical, organoleptic, and biological characterization gives a certain natural fingerprint to the mineral waters. The description of the microbiota presents in these waters, from a quantitative and qualitative point of view, made with classical techniques of determination (cultural methods), but also by using modern methods of molecular biology are of great interest nowadays [8, 12].
The increase of mineral water consumption at a global level has stimulated the emergence of many studies dedicated to microbiological evaluation of mineral water or bottled spring water, with geographical indication, marketed under different names and brands and in various categories of containers [8, 13 – 22].
The physicochemical and microbiological changes of the springs can have various reasons, among which are the non-compliance with good practices in the bottling and storage process that can alter the natural qualities of the mineral water and its beneficial properties [3, 8, 10, 12, 13, 18, 19, 23 – 30].
The presence of bacteria in glass or plastic containers has become an important subject of study for all categories of water used for consumption spring water, mineral water or tap water [1, 3, 14, 21, 24, 28, 31].
Contrary to many people's beliefs, bottled mineral water is not free of microorganisms [17, 18], so monitoring the microbiological quality of bottled water is a priority because the presence of excess bacteria can affect its quality and thus its safety.
The standards that regulate the assessment of the microbiological quality of mineral waters provides for the determination of heterotrophic bacteria and their load with fecal-domestic pollution bacteria (total coliforms, fecal coliforms, fecal streptococci), sulfur-reducing anaerobic bacteria (*Clostridium perfringens*) and *Pseudomonas aeruginosa*.
The total number of coliforms, often referred as heterotrophic plate count (HPC), comes from the source water itself. Although there are insufficient clinical data on consumer risk due to the high presence of HPC in mineral waters, it is believed that certain species of microorganisms may affect vulnerable people (e.g. children, pregnant women, the elderly or those with low immunity). Also, their increase may alter the taste and the odor of drinking waters, indicating the presence of nutrients and biofilms. In this context, HPC

monitoring is recommended as a possible indicator of bottled water production, as it is in public water supply [32 – 34].

In contrast, the other quality microbiological parameters mentioned indicate contamination at source or during bottling: for treated drinking water, they demonstrate the effectiveness of treatment and for untreated groundwater (mineral water) indicate a possible deficiency of natural hydrogeological protection mechanisms (indicator vulnerability) [17, 18].

The presence of coliform bacteria and fecal streptococci in water is a good indicator of water contamination with fecal residues resulting from their infiltration into the groundwater [15, 22, 30, 33 – 37].

Clostridium perfringens (formerly known as C. welchii) is a Gram-positive which can be found in nature as a normal component of the soil, decaying vegetation, marine sediments, human and other vertebrates' intestinal tract [12, 38 – 40]. Among the approximately 60 known species of clostridia, half have the ability to cause disease to both human and animal hosts [1 – 3, 38 – 40].

Reducing sulfite clostridia and fecal streptococci (FS), although they have the value of indicators in water quality standards in some studies, are debatable, especially in terms of bottled water and storage conditions and use [21, 41 – 43].

Pseudomonas aeruginosa is a common bacterium that can cause disease in animals, including humans. It is found in soil, water, skin flora, and in most environments around the world. It thrives not only in normal environments, but also in hypoxic atmospheres [1, 34, 37, 44, 45].

Although the risk of infection with *Pseudomonas aeruginosa* occurs only in the consumption of water that has a very high content of this pathogen, the literature mentions that *P. aeruginosa* represent a common indicator of contamination during the bottling process that can compromise the consumer's immune system, in time [17, 46 – 48].

Romania is one of the EU countries, beneficiary of numerous springs of mineral waters and natural spa resorts, among which is the resort Slanic Moldova, also called the Pearl of Moldavia [49]. The mineral water springs from Slanic Moldova (located in eastern Romania) are a valuable resource due to the internal or external therapeutic properties that can be exploited for the benefit and increase of human life's quality [10, 11, 16, 50].

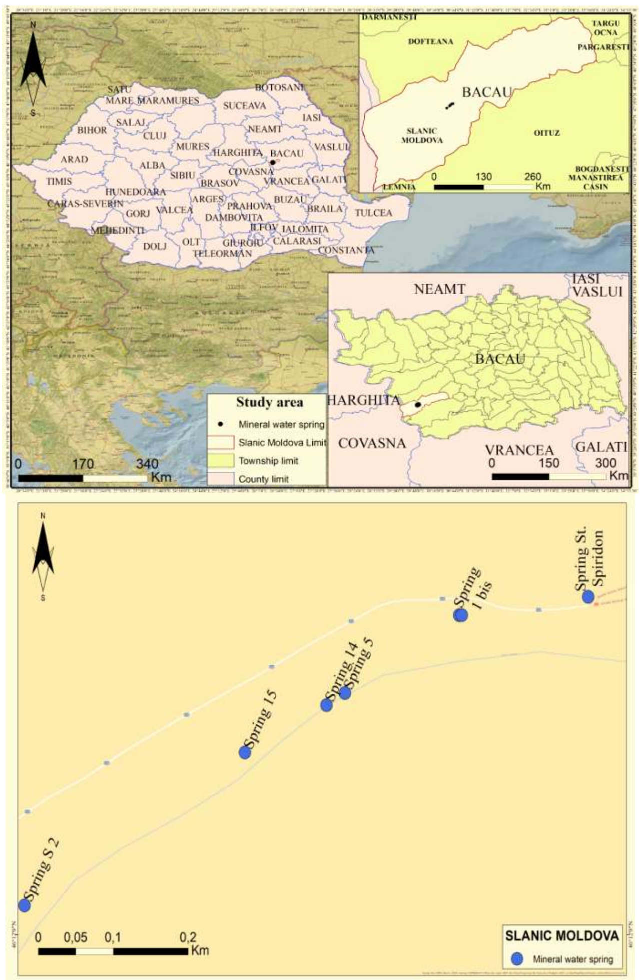
In the context of the increased interest in the use of mineral waters with potential therapeutic effect, in conjunction with a dynamic and globalized market for products such as bottled water, this study aimed to assess the quantitative evolution of the microbiota of the mineral waters from Slanic Moldova, after bottling and storage under different conditions. Experimental planning consisted of detecting quantitative variations in HPC, as well as detecting the presence of *Clostridium perfringens* and *Pseudomonas aeruginosa* as microbiological indicators during the shelf life of bottled mineral waters.

MATERIALS AND METHODS

Sampling of selected mineral waters

Mineral water samples were collected from 7 of the 20 springs in the Slanic Valley [10, 11, 42].

Mineral water was taken at the source of the springs, where access to the aquifer was allowed. The springs from Slanic Moldova are protected from contact with surface waters. Well drilling brings water to the surface for springs 14, 15, Sonda 2 (S2). The waters of springs 1 bis, 10 come to the surface from an accumulation basin. Sfantul Spiridon (SS) and spring 5, come from an upper underground aquifer system, influenced by external factors: precipitation, anthropogenic activities. Figure 1 presents the general location of the study area and the evaluated springs.



a

b

Figure 1. *Geographical framing of the study area and marking of the studied mineral water springs: a) map of the region; b) analyzed springs localization*

The selected mineral waters are distinguished by the originality of the chemical composition and the therapeutic potential as can be seen from Table 1.

Table 1. *Chemical characteristics and properties of selected mineral waters from Slanic Moldova*

Spring	Mineralization degree [mg·L^{-1}]	CO_2 content [mg·L^{-1}]	Type of water	Way of usage
1 bis	6060.00	1450.00	Hypertonic water, with high content of essential minerals for the human body and trace elements with known biological uses. Source 1 bis, contains small amounts of U and Hg, far below the maximum allowed limit (0.001 mg·L^{-1}) and amounts of Ag	Recommended for internal use, having antiseptic and tissue repair effects
5	238.00	1114.00	Siliceous and ferruginous water, containing K, Al, Ca, Fe, Mg, Si, Mn, very rich in trace elements (B, Li, Cr, Cu, Zn, Se, Sr, Ba, Be, V, Co, Ga, As, Cs)	Recommended for external use, having astringent and anti-inflammatory properties
10	6061.00	1034.00	Hypertonic, carbonated, bicarbonated, chlorinated water, that contains significant amounts of Li and Mn	Recommended for internal use in the sphere of gastric and biliary disorders
14	9200.00	1045.00	Hypertonic, carbonated, bicarbonated, ferruginous water, that contains the highest amounts of Li, Mn, Cu, and Ag of all mineral waters in Slanic Moldova	Recommended for internal use, indicated for iron deficiency anemia and digestive tract disorders
15	7230.00	1047.00	Composition similar to spring 14 of carbonated, bicarbonated, sodium-chlorinated, ferruginous water, with a more content in Ag	Recommended for internal use, such as gastrointestinal diseases
S2	1270.00	132.00	Hypotonic, alkaline water (pH = 8), with an average concentration of Na, K, Ca, B, Mg, Al, and rich in trace elements (Li, Cr, Cu, Se, Sr, Ba, Be, V, Ga, Rb)	Recommended for internal use, in kidney and liver disease
SS	62.72	26.23	Oligomineral water, low in Na, aerated, with a pleasant taste	Recommended as still water

Microbiological analysis was carried out seasonal (winter, spring, summer and autumn) and after at 1, 7, 30, 90, 180 and 360 days of storage, according to SR EN ISO 6222, 2004.
A total of 196 samples were collected from the seven springs in containers made of sterile glass (84) and polyethylene terephthalate - PET (84), in January 2020 (for storage) and 28 in sterile glass during 2020 year for seasonal analysis (January, April, July and November). All samples were transported to the laboratory, in the same day.
The glass containers (mark LB) used have the following characteristics: made of borosilicate glass LBG 3.3, with blue threaded lid (GL 45), graduated, transparent, autoclavable, with a capacity of 500 mL, according to ISO 4796.
PET containers (brand LABBOX) with a capacity of 500 mL were sterile, without thiosulphate, with polyethylene lid with safety ring, according to ISO 19458.
Bottling of the water took place directly, without any chemical treatment and the samples were stored for a maximum period of 360 days, in absence of light, in various conditions of temperature: room temperature (20 - 22 °C) and refrigeration conditions (4 °C).

Microbiological analyses of selected mineral waters

The monitoring of the microbiological parameters for the mineral water samples, performed in triplicate, using the decimal dilution (10-3), aimed determining the total number of bacteria, sporulated sulphite-reducing anaerobes bacteria (*Clostridium perfringens*) and *Pseudomonas aeruginosa*.
The mineral water samples were examined by filtration of 100 mL sample volumes through mixed cellulose esters membranes of 0.45 µm pore size (except Clostridia - 0.22 µm) and 47 or 50 mm diameter (Millipore, Bedford, MA, USA) followed by plating on selective media prepared in accordance with manufacturer's instructions.

Determination of Heterotrophic Plate Count (HPC)

For HPC the pour-plate technique was employed as described in the standard methods for the water examination [51 – 55]. One milliliter of mineral water sample was mixed with melted Plate Count Agar (PCA, MERCK, Germany) at 44 °C and 2 sets of plates were prepared for all samples. One set was incubated 22 °C for 48 – 72 hours and the other set was maintained for 24 hours at 37 °C. All colonies formed on each plate were counted and the results were expressed in colony-forming units (CFU)·mL^{-1} of water sample [14, 20, 25, 53].

Anaerobic reducing sulfite bacteria

The identification of the vegetative forms of *Clostridium perfringens* was performed according to national standard SR EN 26461-2/2002 [56] by placing the membranes filters inversely (face down) in sterile conditions on Tryptose Sulfite Cycloserine Agar - TSC (Merck, Germany) medium followed by anaerobic incubation. Anaerobic conditions were generated by pouring another layer of liquefied and cooled TSC medium over the membrane at 45 - 50 °C (sandwich type). The evaluation of the results was done by counting the colonies and reporting them to the volume of filtered water [38 – 40].

***Pseudomonas aeruginosa* evaluation**

Pseudomonas CN Agar Base medium (Merck, Germany) was used for the identification of *Pseudomonas aeruginosa* by membrane filtration technique, based on the detection of pyocyanin (PYO) production, according to the standard ISO 16266/2006 [57]. The membranes were plated onto specific medium and were incubated at 37 °C for 24 - 48 hours *Pseudomonas aeruginosa* develops blue-green colonies due to its selective enzymatic activity (PYO). Other heterotrophic bacteria present in the sample may develop transparent or yellow-colored colonies [38, 40, 44, 45].
The results were compared to the reference criteria established by international and Romanian regulations on the exploitation and marketing of mineral waters [51, 52, 56].

Statistical analysis

The results of the analysis of the 196 samples were statistically analyzed using IBM SPSS Statistics software, version 20.0 (SPSS Inc., Chicago, IL, USA) and OriginPro 2019b software (OriginLab, OriginPro 2019b, Northampton, MA, USA). The maps were drawn using ArcGIS Maps for SharePoint 4.2 software (Esri Inc.) and our own application for Android systems specially created for the identification, classification and location of mineral water springs - WaterShed [9].

RESULTS AND DISCUSSION

Seasonal evaluation of the mineral water microbiota

In order, to provide a first impression of the richness and relative abundance of microorganisms, the seasonal monitoring of the microbiota of the 7 mineral water springs was performed, which allows a holistic understanding and an ecological assessment of the microbiological status of these mineral waters.
Seasonal monitoring of the microbiological quality of the springs from Slanic Moldova began in the cold season of 2020 (January), continued in the spring (April), summer (July), and autumn (November).
The total number of heterotrophic bacteria determined at 22 °C was within the limits allowed by the European regulations (Table 2) for all studied sources.

Table 2. *Microbiological limits for natural mineral waters according to national and UE regulations*

Parameter	HPC		*C. perfringens*	*P. aeruginosa*
	22 °C	**37 °C**		
Level regulation	≤ 100 CFU·mL^{-1}	≤ 20 CFU·mL^{-1}	ND/50 mL	ND/250 mL

The average variations of seasonal analyzed mineral water microbiota are presented in Table 3. For HPC determined at 37 °C, there was a slight exceedance for S2 in the summer season and in all seasons in the case of SS spring.

Table 3. *Seasonal heterotrophic plate count (HPC) dynamics of the studied mineral waters from Slanic Moldova*

Spring	Type of water	HPC [CFU·mL^{-1}]							
		winter		spring		summer		autumn	
		22 °C	37 °C	22 °C	37 °C	22 °C	37 °C	22 °C	37 °C
1 bis	Carbonated	< 1	2	0	2	1	3	< 1	< 4
5		0	3	0	1	< 1	2	1	1
10		< 1	2	0	0	3	0	0	< 1
14		0	0	0	0	2	0	0	0
15		1	0	0	0	< 2	< 2	1	1
S2	Non-carbonated	5	5	< 10	11	< 8	21	< 10	17
SS		13	< 24	< 41	25	67	< 72	35	34

The increase in the total number of germs, in the summer season, may be a first warning regarding accidental pollution or other situations favorable to the development of microorganisms, anthropogenic activities that lead to environmental damage (grazing, excessive tourism, etc.) [15, 30, 34, 58].
In the case of the SS spring, the unsatisfactory results recorded in all seasons could be also due to the geographical position (proximity to the intensely circulated road and accommodation area).

Bacterial evolution of bottled mineral waters in different storage conditions

Knowing the behavior of the microbiota in conditions of mineral water storage prevents the elimination of health risks for consumers. Additional to HPC determination, two important pollutant indicators (spore-forming sulfite-reducing anaerobes (*clostridia*) and *Pseudomonas aeruginosa*) were quantitatively analyzed, completing thus the monitoring sheet of microbiological parameters.

Heterotrophic Plate

The results of HPC determination at 22 °C for mineral water samples stored and kept in different conditions (Table 4) show that all studied waters comply with the standards set by EU and national legislations.
The determination of HPC at 37 °C indicates a very small exceedance of the standard values allowed for only two (S2 and SS) of the seven springs.
As can be seen in Figure 2, for S2 spring only two small exceedances of HPC at 37 °C were recorded for samples stored at room temperature: after 30 days (in glass packaging) and at 90 days of storage (in plastic).
In the case of SS spring (Figure 3), a maximum value was recorded in the plastic containers kept at room temperature (40 CFU·mL^{-1}) after 1 day and a minimum of 8 CFU·mL^{-1} for samples stored in the refrigerator for 90 days.
The data collected show no notable influence in HPC values depending on the nature of the bottle in which the samples were kept, plastic or glass. The lowest HPC values were recorded for all refrigerated samples stored at 4 °C.

Table 4. *HPC variation range of the studied mineral waters from Slanic Moldova, stored in glass (G) and plastic (P) containers for 1, 7, 30, 90, 180 and 360 days at room temperature (20 - 22 °C/RT) and refrigerated (4 °C/Ref)*

Spring	Type of water	HPC [CFU·mL^{-1}]							
		GRT		GRef		PRT		PRef	
		22 °C	37 °C	22 °C	37 °C	22 °C	37 °C	22 °C	37 °C
1 bis	Carbonated	0-1	0-3	0	0-2	0	0-3	0	0-2
5		0	0-1	0	0	0	0	0	0
10		0-1	0-2	0-1	0-1	0-2	0-1	0	0-2
14		0-1	0	0-1	0-1	0	0-2	0	0-1
15		0	0-2	0-2	0-2	0-2	0-3	0-1	0-1
S2	Non-carbonated	7-14	6-21	5-13	7-16	6-10	10-21	6-12	4-11
SS		8-20	24-35	15-27	10-32	8-13	17-40	12-28	8-26

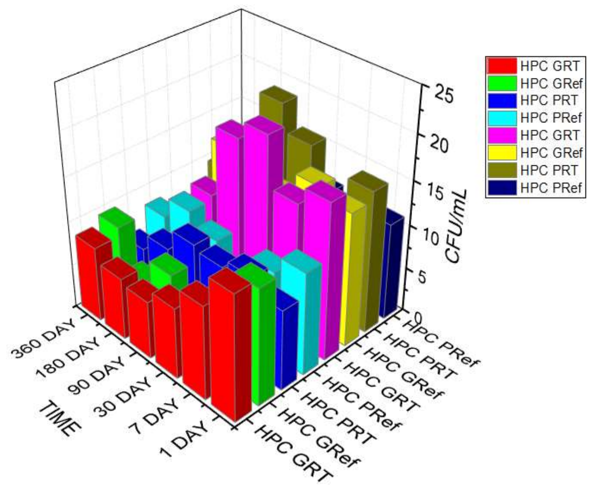


Figure 2. *HPC variation spring Sonda 2*

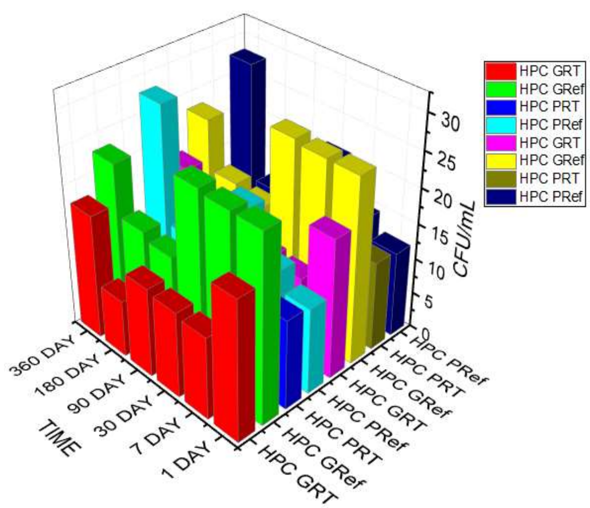


Figure 3. *HPC variation spring Sfantul Spiridon*

Anaerobic sulfite-reducing bacteria/clostridia

In the bottled samples of waters from springs 1 bis, 5, 10, 14, 15, S2 and SS, regardless of storage conditions, bacteria of the genus *Clostridium perfringens* are absent.

Pseudomonas aeruginosa

The presence of *Pseudomonas aeruginosa* was not detected in mineral waters samples of springs 1, 5, 10, 14, 15, S2 and SS, bottled in different types of containers and stored at the room temperatures or in refrigeration conditions.

Our results on the behavior of S2 and SS sources are consistent with the literature studies which show that high levels of HPC in non-carbonated mineral waters are usually indicators of the natural proliferation of native bacteria present in small numbers in the source water [59 – 61] able to multiply well even with a very limited supply of nutrients [17, 62].

Also, the low number of bacteria recorded for sources 1 bis, 5, 10, 14 and 15 can be correlated with the level of carbonation, knowing that carbonation lowers the *p*H of the water, being an obstacle to the multiplication of HPC bacteria [17, 63 – 66].

It should be noted that the special behavior of the SS source can be attributed to its oligomineral character, the literature indicating that the degree of mineralization has an important impact on the development of microorganisms [67].

Knowing that sulfite-reducing clostridia, common components of the intestinal microbiota of humans and other mammals which is widely used to evaluate the sanitary quality of water and P. aeruginosa, sensitive indicators of surface contamination [18] their absence in the studied mineral waters both in the seasonal evaluation and during storage, is a supplementary indicator of the safety of these products from the perspective of bottling these waters.

This monitoring offers the possibility of making decisions in order to protect the microbiological quality of the mineral water of the investigated springs [12, 13, 18, 33, 34, 36, 68].

CONCLUSIONS

A complex microbiological study concerning the seasonal determinations and monitoring of the changes occurred during the bottling and storage in different conditions of 7 springs mineral waters from Slanic Moldova has been performed for the first time.

The microbiological indicators studied in accordance with national and European regulations, concerned the determinations of heterotrophic plate count, sulfur-reducing anaerobic bacteria (*Clostridium perfringens*) and *Pseudomonas aeruginosa*.

The results obtained by determination of total number of heterotrophic bacteria (HPC) at 22 °C and 37 °C for seasonal analyses and bottled mineral waters in different storage conditions (glass or plastic containers at room temperature or refrigerated at 4 °C) reflect the fact that there were no large increases in bacteria number for five (1 bis, 5, 10, 14, and 15) out of the seven springs, respectively these waters comply with the standards set by EU and national legislations.

HPC exceedances for the two non-carbonated sources S2 and SS were recorded in the summer season, when higher water temperatures favor the development and activity of microorganisms. For the investigated area, in the case of S2 spring, the pollution is seasonal, evidenced in the summer season, meanwhile for SS spring it is maintained in summer, autumn and winter, but within more narrow limits.

For most samples the HPC values are slightly higher in the case of samples kept at room temperature but without a significant differentiation between plastic and glass containers. Regarding the indicators *Clostridium perfringens* and *Pseudomonas aeruginosa*, their absence in the mineral water samples kept for a maximum period of 360 days and analyzed under the conditions mentioned in this study underlines the microbiological stability of these waters.

These analyzes complete the fingerprint and peculiarities of the studied springs and mineral waters. Also, in addition to the necessity to determine the physicochemical parameters, the results of this study highlight the importance of systematic monitoring of the microbiological quality of valuable aquifer resources in the case of current exploitation, as well as the evolution of the bottled water microbiota in different moments of time during their subsequent capitalization.

REFERENCES

1. Curutiu, C., Iordache, F., Gurban, P., Lazar, V., Chifiriuc, M.C.: Main microbiological pollutants of bottled waters and beverages, *Bottled and Packaged Water*, **2019**, **4**, 403-422;
2. Gerba, C.P., Pepper, I.L.: Chapter 13: Microbial Contaminants, in *Environmental and Pollution Science* (Editors: Brusseau, M.L., Pepper, I.L., Gerba, C.P.), Third Edition, Elsevier Academic Press, San Diego (CA), **2019**, 191-217, https://doi.org/10.1016/B978-0-12-814719-1.00013-6;
3. World Health Organization, Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum, Geneva: World Health Organization, 24 April 2017, Guideline, **2017**, https://www.who.int/publications/i/item/9789241549950 (Accessed date: 11 January 2022);
4. EU Green Week Zero Pollution for healthier people and planet, **2021**, https://www.eugreenweek.eu/ro (Accessed date: 20 January 2022);
5. Digital World Water Congress, Virtual conference for water professionals globally covering the full water cycle, from water resources to wastewater recovery - connecting the dots for a water wise world, **2021**, https://digital.worldwatercongress.org (Accessed date: 23 January 2022);
6. European Parliament, Directive 2009/54/EC of the European Parliament and of the Council of 18 June 2009 on the exploitation and marketing of natural mineral waters, **2009**, https://op.europa.eu/en/publication-detail/-/publication/41c107dd-8061-4bb5-bf05-d12ecf8c4697 (Accessed date: 21 January 2022);
7. Romanian Government, Decision no. 1020 for the approval of the Technical Norms for the exploitation and marketing of natural mineral waters. Romanian Official Monitor no. 854 from 22th September (GD 1020: 2005), **2005**, http://www.namr.ro/legislation/romanian-legislation/water-domain (Accessed date: 18 January 2022);
8. Carstea, E.M., Levei, E.A., Hoaghia, M.A., Savastru, R.: Quality assessment of Romanian bottled mineral water and tap water, *Environmental Monitoring and Assessment*, **2016**, **188**, 521, https://doi.org/10.1007/s10661-016-5531-9;
9. Misăilă, L., Nedeff, F.M., Barsan, N., Grosu, L., Patriciu, O.I., Gavrilă, L., Fînaru, A.L.: WATERSHED - Android application for the mineral waters classification, *Revista de Chimie*, **2019**, **70** (6), 2212-2217;
10. Munteanu, C.: *Therapeutic mineral waters* (in Romanian), Balneara Publishing House, Bucharest, **2013**, 1-64;
11. Saraiman, A.: *Slanic Moldova the Pearl of Moldova* (in Romanian), Alexandru Ioan Cuza University, Iasi, **1999**, 1-75;

12. Carraturo, F., Del Giudice, C., Compagnone, M., Libralato, G., Toscanesi, M., Trifuoggi, M., Galdiero, E., Guida, M.: Evaluation of microbial communities of bottled mineral waters and preliminary traceability analysis using NGS microbial fingerprints, *Water*, **2021**, **13** (20), 2824;
13. Biadglegne, F., Tessema, B., Kibret, M., Abera, B., Huruy, K., Anagaw, B., Mulu, A.: Physicochemical and bacteriological quality of bottled drinking water in three sites of Amhara Regional State, Ethiopia, *Ethiopian Medical Journal*, **2009**, **47** (4), 277-284;
14. Ferreira, A.C., Morais, P.V., Da Costa, M.S.: Alterations in total bacteria, iodonitrophenyltetrazolium (INT)-positive bacteria, and heterotrophic plate counts of bottled mineral water, *Canadian Journal of Microbiology*, **1994**, **40** (1), 72-77;
15. Frank, C., Werber, D., Cramer, J.P., Askar, M., Faber, M., An der Heiden, M., Bernard, H., Fruth, A., Prager, R., Spode, A., Wadl, M., Zoufaly, A., Jordan, S., Kemper, M.J., Follin, P., Müller, L., King, A.A., Rosner, B., Buchholz, U., Stark, K., Krause, G.: Epidemic profile of Shiga-toxin producing *Escherichia coli* O104:H4 outbreak in Germany, *The New England Journal of Medicine*, **2011**, **365** (19), 1771-1780;
16. Guillot, E., Leclerc, H.: Bacterial flora in natural mineral waters: characterization by ribosomal ribonucleic acid gene restriction patterns, *Systematic and Applied Microbiology*, **1993**, **16** (3), 483-493;
17. Varga, L.: Bacteriological quality of bottled natural mineral waters commercialized in Hungary, *Food Control*, **2011**, **22** (6), 591-595;
18. Leclerc, H., Moreau, A.: Microbiological safety of natural mineral water, FEMS, *Microbiology Reviews*, **2002**, **26** (2), 207-222;
19. Macerata, U.M., Levré, E., Armani, G., Agostini, G., Molinari, G., Caroli, G.: Bacterial species detected in some bottled mineral waters sold in Italy, *International Journal of Clinical Pharmacology Research*, **1988**, **8** (1), 31-37;
20. Schwaller, P., Schmidt-Lorenz, W.: Bacterial flora of four different french non-carbonated mineral waters in bottles. I. Colony counts, rough differentiation of the bacterial flora, and characterization of the group of gram-negative, yellow-pigmented bacteria, *Zentralblatt für Bakteriologie:* I. Abt. Originale C: Allgemeine, angewandte und ökologische Mikrobiologie, **1980**, **1** (4), 330-347;
21. September, S.M., Els, F.A., Venter, S.N., Brozel, V.S.: Prevalence of bacterial pathogens in biofilms of drinking water distribution systems, *Journal of Water and Health*, **2007**, **5** (2), 219-227;
22. Zeenat, A., Hatha, A.A., Viola, L., Vipra, K.: Bacteriological quality and risk assessment of the imported and domestic bottled mineral water sold in Fiji, *Journal of Water and Health*, **2009**, **7** (4), 642-649;
23. Akhbarizadeh, R., Dobaradaran, S., Schmidt, T.C., Nabipour, I., Spitz, J.: Worldwide bottled water occurrence of emerging contaminants: A review of the recent scientific literature, *Journal of Hazardous Materials*, **2020**, **392**, 122271, https://doi.org/10.1016/j.jhazmat.2020.122271;
24. Al Moosa, M.E., Ali Khan, M., Alalami, U., Hussain, A.: Microbiological quality of drinking water from water dispenser machines, *International Journal of Environmental Science and Development*, **2015**, **6** (9), 710-713;
25. Da Silva, M.E.Z., Santana, R.G., Guilhermetti, M., Filho, I.C., Endo, E.H., Ueda Nakamura, T., Nakamura, C.V., Dias Filho, B.P.: Comparison of the bacteriological quality of tap water and bottled mineral water, *International Journal of Hygiene and Environmental Health*, **2008**, **211** (5-6), 504-509;
26. Haas, C., Rose, J., Gerba, C.: *Quantitative Microbial Risk Assessment*, Wiley Publishing House, New York, **1999**, 150-464;
27. Loy, A., Beisker, W., Meier, H.: Diversity of bacteria growing in natural mineral water after bottling, *Applied and Environmental Microbiology*, **2005**, **71** (7), 3624-3632;
28. Schindler, P.R., Vogel, H., Back, W.: Recommendations for changing microbiological examination parameters in filling bottled water to comply with the mineral and drinking water regulation, *Gesundheitswesen*, **1995**, **57** (12), 806-811;
29. Sasikaran, S., Sritharan, K., Sandrasegarampillai, B., Arasaratnam, V.: Physical, chemical and microbial analysis of bottled drinking water, *The Ceylon Medical Journal*, **2012**, **57** (3), 111-116;
30. Some, S., Mondal, R., Mitra, D., Jain, D., Verma, D., Das, S.: Microbial pollution of water with special reference to coliform bacteria and their nexus with environment, *Energy Nexus*, **2021**, **1**, 1-9;

31. Böhmer, H., Resch, K.L.: Mineral water or tap water? A systematic analysis of the literature concerning the question of microbial safety, *Forsch Komplementarmed Klass Naturheilkd*, **2000**, **7** (1), 5-11;
32. Falcone-Dias, M.F., Filho, A.F.: Quantitative variations in heterotrophic plate count and in the presence of indicator microorganisms in bottled mineral water, *Food Control*, **2013**, **31** (1), 90-96;
33. Altmann, M., Spode, A., Altmann, D., Wadl, M., Benzler, J., Eckmanns, T., Krause, G., An Der Heiden, M.: Timeliness of surveillance during outbreak of Shiga Toxin-producing *Escherichia coli* infection, Germany, *Emerging Infectious Diseases*, **2011**, **17** (10), 1906-1909;
34. Amanidaz, N., Zafarzadeh, A., Mahvi, A.H.: The interaction between heterotrophic bacteria and coliform, fecal coliform, fecal streptococci bacteria in the water supply networks, *Iranian Journal of Public Health*, **2015**, **44** (12), 1685-1692;
35. Carrillo-Gómez, J., Durán-Acevedo, C., García-Rico, R.: Concentration detection of the *E. coli* bacteria in drinking water treatment plants through an E-Nose and a Volatiles Extraction System (VES), *Water*, **2019**, **11** (4), 774, https://doi.org/10.3390/w11040774;
36. Desalegn, A., Temesgen, T.: Assessing the contamination level of water and determination of the major sources of contaminants among rural community of Dire Dawa administrative council, *International Journal of Advanced Research in Biological Sciences*, **2020**, **7** (9), 74-83;
37. Sefcová, H.: Survey of the microbiological quality of bottled water, *European Journal of Public Health,* **1998**, **6** (1), 42-44;
38. Foster, A., Rankin, M.: Evaluation and adaptation of a membrane filter technique for detection of *Clostridium perfringens*, *Journal of the American Society of Brewing Chemists*, **1989**, **47** (3), 70-72;
39. Stelma, G.N. Jr.: Use of bacterial spores in monitoring water quality and treatment, *Journal of Water and Health*, **2018**, **16** (4), 491-500;
40. Vierheilig, J., Frick, C., Mayer, R.E., Kirschner, A.K., Reischer, G.H., Derx, J., Mach, R.L., Sommer, R., Farnleitner, A.H.: *Clostridium perfringens* is not suitable for the indication of fecal pollution from ruminant wildlife but is associated with excreta from nonherbivorous animals and human sewage, *Applied and Environmental Microbiology*, **2013**, **79** (16), 5089-5092;
41. Coyne, M., Howell, J.M.: The fecal coliform/fecal streptococci ratio (FC/FS) and water quality in the Bluegrass region of Kentucky, *Soil Science News and Views*, **1994**, **15** (9), 34, https://uknowledge.uky.edu/pss_views/34;
42. Feru, A.: *Natural mineral water guide* (in Romanian), Novis Publishing House, Cluj Napoca, **2012**, 1-105;
43. Lamy, M.C., Sanseverino, I., Niegowska, M., Lettieri, T.: Microbiological parameters under the drinking water directive current state of art on somatic coliphages and *Clostridium perfringens* and spores, *Joint Research Centre*, **2020**, 1-111;
44. Pereira, S.G., Cardoso, O.: Antibiotic susceptibility of natural mineral water *Pseudomonas aeruginosa*: another evidence of a great plasticity, *Water Research Conference*, Lisbon, Portugal, **2020**, 1-10;
45. Rongchun, W., Shuizi, D., Cheng, L., Danhui, Y., Hong, L.: The contribution of *Pseudomonas aeruginosa* infection to clinical outcomes in bronchiectasis: a prospective cohort study, *Annals of Medicine*, **2021**, **53** (1), 459-469;
46. Hunter, P.R.: The microbiology of bottled natural mineral waters, *Journal of Applied Bacteriology*, **1993**, **74**, 345-352;
47. Rosenberg, F.A.: The microbiology of bottled water, *Clinical Microbiology Newsletter*, **2003**, **25** (6), 41-44;
48. Rusin, P.A., Rose, J.B., Haas, C.N., Gerba, C.P.: Risk assessment of opportunistic bacterial pathogens in drinking water, *Reviews of Environmental Contamination and Toxicology*, **1997**, **152**, 57-83;
49. Stoica, C.: Slanic Moldova "Pearl" of the Eastern Carpathians (in Romanian), *Magic Print Publishing House*, Onesti, **2021**, 308-347;
50. Misăilă, L., Bârsan, N., Nedeff, F.M., Răducanu, D., Grosu, L., Patriciu, O.I., Gavrilă, L., Fînaru, A.L.: Quality assessment of Slanic-Moldova mineral waters stored in reusable bottles, *Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2020**, **21** (2), 253-262;

51. Romanian Standard SR EN ISO 6222, Water quality - Enumeration of culturable micro-organisms - Colony count by inoculation in a nutrient agar culture medium (in Romanian), **2004**, 1-10;
52. Romanian Standard SR EN ISO 8199, Water quality - General requirements and guidance for microbiological examinations by culture (in Romanian), **2008**, 1-70;
53. APHA - Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, **2005**, 1-8;
54. Guidelines for the safety assessment of natural mineral waters, *French Food Safety Agency*, **2008**, https://www.anses.fr/en/system/files/EAUX-Ra-EauxMineralesEN, (Accessed date: 28 January 2022);
55. Bartram, J., Cotruvo, J., Exner, M., Fricker, C., Glasmacher, A.: *The Significance of HPCs for water quality and human health*, IWA Publishing, London, UK, **2003**, 60-81;
56. Romanian Standard SR EN 26461-2. Water quality - Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 2: Method by membrane filtration (in Romanian), **2002**, 1-20;
57. International Standard ISO 16266. Water quality - Detection and enumeration of *Pseudomonas aeruginosa* - Method by Membrane filtration, **2006**, 1-12;
58. Andersen, H.: Children on the frontline against *E. coli*: typical hemolytic-uremic syndrome, *Clinical Laboratory Science*, **2005**, **18** (2), 90-95;
59. Kassenga, G.R.: The health-related microbiological quality of bottled drinking water sold in Dar Es Salaam, Tanzania, *Journal of Water and Health*, **2007**, **5** (1), 179-185;
60. Manaia, C.M., Nunes, O.C., Morais, P.V., Da Costa, M.S.: Heterotrophic plate counts and the isolation of bacteria from mineral waters on selective and enrichment media, *Journal of Applied Bacteriology*, **1990**, **69** (6), 871-876;
61. Stickler, D.J.: The microbiology of bottled natural mineral waters, *The Journal of the Royal Society of Health*, **1989**, **109**, 118-124;
62. Tsai, G.J., Yu, S.C.: Microbiological evaluation of bottled uncarbonated mineral water in Taiwan, *International Journal of Food Microbiology*, **1997**, **37** (2-3), 137-143;
63. King, A.D., Nagel, C.W.: Growth inhibition of a Pseudomonas by carbon dioxide, *Journal of Food Science*, **1967**, **32** (5), 575-579;
64. Venieri, D., Vantarakis, A., Komninou, G., Papapetropoulou, M.: Microbiological evaluation of bottled non-carbonated ("still") water from domestic brands in Greece, *International Journal of Food Microbiology*, **2006**, **107** (1), 68-72;
65. Warburton, D., Harrison, B., Crawford, C., Foster, R., Fox, C., Gour, L., Krol, P.: A further review of the microbiological quality of bottled water sold in Canada: 1992-1997 survey results, *International Journal of Food Microbiology*, **1998**, **39**, 221-226;
66. Warburton, D.W.: Methodology for screening bottled water for the presence of indicator and pathogenic bacteria, *Food Microbiology*, **2000**, **17** (1), 3-12;
67. Sevillano, D., Romero-Lastra, P.T., Casado, I., Alou, L., González, N., Collado, L., Domínguez, A.A., Arias, C.M., Corvillo, I., Armijo, F., Romero, M., Maraver, F.: Impact of the biotic and abiotic components of low mineralized natural mineral waters on the growth of pathogenic bacteria of human origin: A key to self-control of spa water quality, *Journal of Hydrology*, **2018**, **566**, 227-234;
68. Siddiqee, M.H., Hossain, N., Hasan, M.M., Siddiqi, U.R., Shah, M., Faruque, S.M.: Fecal coliform and *Salmonella* spp. in household drinking waters in Dhaka city: an overlooked health hazard, 20th Symposium on Health-Related Water Microbiology (HRWM), Vienna, Austria, **2019**, 63-64.