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¹Lazar A. D., ¹Gushcha S. G., ¹Koieva K. A., ²Muratov V. N.**FEATURES OF THE INFLUENCE ON ALLOCHTHONOUS MICROORGANISMS OF SPECIFIC BIOLOGICALLY ACTIVE COMPONENTS OF PACKAGED MINERAL WATERS**¹SI «Ukrainian Scientific-Research Institute of Medical Rehabilitation and Balneology, the Ministry of Public Health of Ukraine» (Odesa, Ukraine)²O.V. Bogatsky Physical-Chemical Institute National Academy of Sciences of Ukraine (Odesa, Ukraine)
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To date the issue of safety of bottled natural mineral water receives a lot of attention, as it's consumption increases every year. As any other product used by humans, it must be safe. Research from previous years confirms the importance of constant monitoring of the sanitary and microbiological safety of packaged mineral waters. The purpose of the work was to assess the influence of specific biologically active components (metasilicic and orthoboric acids) on the sanitary condition of packaged mineral waters (saturated and unsaturated), which were sold in Ukraine from 2017 to 2021. Sanitary-indicative microorganisms in the studied mineral water were detected in several stages. During the research methods with solid and liquid nutrient media were used. Liquid glucose-peptone medium, Endo's medium, oxidase test and microscopy were used to determine the number of coliform bacteria (CB). The total microbial count was determined on nutrient agar at 37°C for 24 hours. To determine *Pseudomonas aeruginosa*, a liquid glucose-peptone medium (GPM) was used as an accumulation medium, followed by cultivation on CPC agar, at 37°C for 24-48 hours, King-A medium, and nutrient agar at 42°C and 4°C, oxidase test and microscopy. Hugh-Leifson medium was used to determine oxidation and fermentation. A regularity was determined in the number and degree of contamination of MW samples depending on the type and concentration of the specific biologically active component. Metasilicic and orthoboric acids have a unidirectional antibacterial effect, but in turn, the effect on sanitary indicator microorganisms of orthoboric acid is more pronounced. Carbon dioxide and autochthonous microbiota of the most natural MW act as factors enhancing the antibacterial action. Was determined that the studied specific biologically active components of natural packaged mineral waters are able to show antibacterial properties by enhancing the effects of each other.

Key words: packaged natural mineral waters, carbon dioxide, metasilicic acid, orthoboric acid, allochthonous microbiota.

Connection of the publication with planned research works. The work is a fragment of the research work «Medical and biological assessment of the quality and value of underground waters of wells No. 10-K and No. 13-R of the State Enterprise Sanatorium «Sonyachne Zakarpattya» in Transcarpathian region» (state registration № 0107U003503), «Medical and biological assessment of the quality and value of groundwater well No. 33-p of the LLC «Ploskiv Mineral Water Plant» of Svalyava district, Transcarpathian region on the basis of preclinical studies and clinical trials» (state registration № 0110U00592), «Medical and biological assessment of the quality and value of groundwater well No. 3766 m. Zinkiv, Poltava region, to substantiate the possibility of their industrial spill» (state registration № 0108U011321).

Introduction. Natural mineral waters (MW) as a resource used by humanity have been known for many centuries. The healing properties of MW have been empirically determined since the times of ancient civilizations [1], but the active study of their biological effects depending on the chemical composition and peculiarities of the microbiological state in the laboratory began only in the first half of the twentieth century and continues to this day [2]. Despite such a long period of studying this issue of MW, therefore, since the beginning of the XXI century, interest in research on natural MW is only growing [3]. It is caused, on the

one hand, by the absence of side effects during the use of natural CF, on the other hand, by a high positive effect with a minimum amount of bioactive elements. To date, studies that allow us to form unambiguous conclusions about the effect of specific biologically active components of CF on the state of autochthonous microbiota, its physiological and bactericidal properties, are rare in the available literature [4-7]. Water from centralized sources of consumption has long established itself as a rather problematic product. It may contain a number of factors that adversely affect human health. These include: residual chlorine after chemical disinfection of water, particles of substances that are components of pipes, and in some cases – a high level of sanitary microorganisms [8, 9]. Considering that the recommended daily intake of drinking water for humans, depending on age and gender, is approximately 0.8-2.0 liters [10], and tap water is not always available and meets certain requirements, packaged natural MW remains a liquid that can effectively meet the physiological needs of the body [11].

Therefore, every year the demand of the population for packaged MW is growing, so naturally the variety of both packaged therapeutic and table MW, which can be used in the medical field, and table MW, suitable for daily consumption, is increasing [12, 13, 14]. Due to the aseptic conditions of packaging after filling into containers, natural MW becomes a closed system that

has no connection with the environment. This favorably affects its shelf life [15].

Like every food product consumed by the population, packaged natural MW should be systematically checked for compliance with sanitary and hygienic conditions, and in particular, sanitary and microbiological control. MW used in industrial packaging must meet the requirements of DSTU 878-93: «Packaged mineral waters. Technical specifications» and Directive 2009/54/EU of the European Parliament and of the Council on the exploitation and marketing of natural mineral waters [16, 17].

The importance of safety of packaged natural mineral waters cannot be overestimated. The number of publications on this topic remains at a high level. Even with the improvement of the quality of life in the world, the problem of safety of water consumed by humans is still extremely acute and does not allow to abandon its constant monitoring [18, 19, 20].

The methods used in the packaging of water are important factors for maintaining its safety, but in addition to artificially improving the sanitary condition and saturation with carbon dioxide (CO₂), natural MWs have components that contribute to natural purification. Such factors can be some biologically active compounds in a certain concentration – orthoboric (H₃BO₃) and metasilicic (H₂SiO₃) acids, and the presence of autochthonous microbiota of mineral water also plays an important role [21, 22].

The purpose of the work was to analyze the effect of specific biologically active components (methosilicic and orthoboric acids) on the sanitary condition of packaged mineral waters sold in Ukraine from 2017 to 2021.

Object and methods of research. In this study, the analysis of the sanitary and microbiological condition of packaged carbonated and non-carbonated mineral natural drinking and therapeutic drinking waters of Ukraine, which contained methasilicic or orthoboric acid as a specific biologically active component, was carried out.

To determine the number of coliform bacteria (CB), first of all, liquid glucose-peptone medium was used, and at the next stage – Endo medium. Colonies obtained on Endo medium were inoculated into semi-liquid glucose-peptone medium, the presence of oxidase was determined and microscopy was performed. The number of saprophytic microorganisms as a total microbial count was determined on nutrient agar at 37°C

for 24 hours. For the determination of *Pseudomonas aeruginosa*, liquid glucose-peptone medium (LPM) was used as an accumulation medium, then cultivation was carried out on *Pseudomonas* isolation medium with the addition of N-cetylpyridinium chloride (CPC agar) at 37°C for 24-48 hours. The resulting colonies continued to be cultured on King-A medium, as well as on nutrient agar at 42°C and 4°C, and at the final stage, oxidase test and microscopy were performed. Hugh-Leifson medium was used to determine oxidation and fermentation. Methodological techniques and methods used in the research are given in the manual [23].

The following natural packaged MWs were used in the study:

1. Natural table non-carbonated MW, H₂SiO₃ content is 35-48 (mg/l);
2. Natural table highly carbonated MW, H₂SiO₃ content is 35-48 mg/l;
3. Natural medicinal strongly carbonated table water MW, H₂SiO₃ content is 50-150 mg/l;
4. Natural medicinal strongly carbonated table water MW, the content of H₃BO₃ is 35-120 mg/l;
5. Natural medicinal strongly carbonated table water MW, the content of H₃BO₃ is 100-250 mg/l;
6. Natural medicinal table non-carbonated MW, H₃BO₃ content is 50-160 mg/l;
7. Natural medicinal table carbonated water, the content of H₃BO₃ is 50-160 (mg/l).

Boric mineral waters are those in which content of boron in the form of H₃BO₃ is from 0.035 g/l [24]. Silicon MWs are considered to be those with silicon content in the form of H₂SiO₃ from 0.050 g/l [24].

Research results and their discussion. Table and medical-table waters containing orthoboric and methasilicic acids as a specific component were selected from the array of data obtained in the process of sanitary-microbiological study of packaged mineral waters of Ukraine during 2017-2021. Among the studied waters there were both those containing CO₂ and non-carbonated (**table**).

During the study, samples of mineral table water containing low concentrations of H₂SiO₃ (35-48 mg/l) were found to contain CB and *P. aeruginosa*, even in samples containing CO₂. In the mineral therapeutic and table water containing H₂SiO₃ in concentrations of 50-150 mg/l, the presence of sanitary microorganisms was also detected, although in lower concentration and with lower frequency.

This indicates that CO₂ does not guarantee the microbiological safety of water. Although it affects some enzymatic and biochemical reactions, it only slows down the growth rate of bacteria, but not completely stops it [25].

Silicic acids have a bacteriostatic effect based on the ability to change the surface charge and communication between cells and, therefore, to prevent bacterial attachment and the formation of bacterial groups. [26, 27].

A certain number of saprophytic microorganisms was determined in the MB when determining the total microbial count (TMC), in the case of waters containing H₂SiO₃ in concentrations of 35-48 mg/l,

Table – Sanitary and microbiological condition of natural packaged MW, which contain methosilicic and orthoboric acids

№ Sample of MW	2017 year			2018 year			2019 year			2020 year			2021 year		
	T M C	C B	P. a.	T M C	C B	P. a.	T M C	C B	P. a.	T M C	C B	P. a.	T M C	C B	P. a.
MW containing H ₂ SiO ₃															
1	2	0	2-3	0	75	0	0	0	0	1-25	20	0	1-16	7	0
2	0	0	0	0	24	0	0	0	0	2-9	75	0	1-2	3	0
3	0	0	0	0-25	20	0	1-45	0	0	0	0	0	0	0	0
MW containing H ₃ BO ₃															
4	0-1	0	0	0	0	0	0-10	0	0	0-1	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0-1	0	0	0	0	0
6	0	0	0	0	9	0	0	0	0	0	0	0	0-1	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	1-5	0	0

Notes: TMC – total microbial count (CFU/ml); CB – coliform bacteria as coli-index; P.a. – *Pseudomonas aeruginosa* (CFU/ml).

the number of colonies varied from 1 to 25 CFU/ml, indicating the presence of autochthonous microbiota.

H₃BO₃ in amounts less than 10 g/l has a bactericidal effect. The mechanism of its action on microorganisms has not been finally determined. In turn, boric acid in vitro inhibits the growth of yeast, gram-positive and gram-negative bacteria, as well as the formation of biofilms, modulating the metabolites released by microorganisms [28].

MW containing H₃BO₃ and CO₂ were determined to be safe according to the studied indicators, with the exception of MW containing H₃BO₃ at concentrations of 50 mg/l – 160 mg/l without the addition of CO₂. In 2018, the presence of MW in the amount of 9 bacterial cells per 1 liter of MW was determined. The results obtained indicate that H₃BO₃ or H₂SiO₃ with CO₂ in the complex mutually enhance the antibacterial properties of each other.

In other types of MW containing H₃BO₃, the presence of microorganisms was observed while determining the total microbial count within the normal range, on average 1-10 CFU/ml. Determination of the presence of a certain number of non-pathogenic microorganisms indicates that even MW with a bactericidal component has its own microbiota, which has adapted to exist in this environment.

The influence of such factor as autochthonous microbiota is still under active study. As for the exact determination of its ability to antibacterial action is not enough to study all possible ways of influence of each of the species of microorganisms of the autochthonous microbiota, because it is necessary to take into account their interspecific interactions. In the studied types of water, the autochthonous microbiota can be considered not as a direct antibacterial factor, but as a concomitant

one. Since representatives of autochthonous microbiota are natural inhabitants of these waters, they have mechanisms that help to survive in the environment. While representatives of CB and *P. aeruginosa*, introduced from outside, do not have such mechanisms [15, 22, 29].

Thus, the mechanisms of interaction of microorganisms within communities, as well as the impact of these communities on opportunistic pathogens require further detailed study.

Conclusions.

1. It was determined that at a concentration of 35 mg/l to 48 mg/l of methosilicic acid in the composition of the MW, it has no antibacterial properties, even in combination with carbon dioxide, and at a concentration of 50 mg/l to 100 mg/l it has a bacteriostatic effect, affecting the formation of bacterial communities.

2. It was found that the presence of orthoboric acid in the composition of CF at a concentration of 35 mg/l-250 mg/l with the addition of carbon dioxide causes bactericidal effect of CF in all cases. However, without CO₂, the antibacterial effect of mineral natural packaged water containing H₃BO₃ is reduced.

3. It was determined that orthoboric acid and metasilicic acid in combination with CO₂ enhance the antibacterial effect of each other.

4. Autochthonous microbiota of natural mineral waters containing methosilicic or orthoboric acids may be an additional antibacterial factor.

Prospects for further research. Conducting research to determine the impact of each of the above components individually and in combination on the sanitary and microbiological condition of packaged mineral waters.

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ОСОБЛИВОСТІ ВПЛИВУ СПЕЦИФІЧНИХ БІОЛОГІЧНО АКТИВНИХ КОМПОНЕНТІВ НА АЛОХТОННУ МІКРОБІОТУ ФАСОВАНИХ МІНЕРАЛЬНИХ ВОД

Лазарь А. Д., Гуца С. Г., Коєва К. А., Муратов В. Н.

Резюме. На сьогоднішній день питанню безпеки фасованих мінеральних природних вод (МВ) приділяють значну увагу, оскільки щороку збільшується її споживання. Як і будь-який інший продукт, вживаний людиною, вона має бути безпечною. Дослідження попередніх років підтверджують важливість постійного моніторингу санітарно-мікробіологічного стану фасованих мінеральних вод (МВ). Метою роботи було оцінити вплив специфічних біологічно активних компонентів (метакремнієвої та ортоборної кислот) на санітарний стан фасованих природних МВ (насичених та ненасичених діоксидом вуглецю), що реалізовувалися в Україні з 2017 по 2021 роки. Санітарно-показові мікроорганізми в досліджуваних МВ виявляли в кілька етапів. Використовували методи посіву на щільні та рідкі поживні середовища. Для визначення кількості колиформних бактерій використовували рідке глюкозо-пептоне середовище, середовище Ендо, оксидазний тест та мікроскопія. Загальне мікробне число визначали на поживному агарі при 37°C протягом 24 годин. Для визначення *Pseudomonas aeruginosa*, як середовище накопичення, використовувалося рідке глюкозо-пептоне середовище, далі проводили культивування на середовищі з N-цетилпіридинієм хлоридом (при 37°C протягом 24-48 годин), і на середовищі Кінг-А, а також на поживному агарі (при 42° Ста 4°C). Далі проводили оксидазний тест та мікроскопію. Для визначення оксидації та ферментації використовували середовище Хью-Лейфсона. За результатами досліджень встановлено закономірність у кількості та ступені контамінації зразків МВ залежно від типу та концентрації специфічного біологічно активного компонента. Метаакремнієва та ортоборна кислоти мають односпрямовану антибактеріальну дію, але в свою чергу дія на санітарно-показові мікроорганізми ортоборної кислоти виражена сильніше. Діоксид вуглецю та аутохтонна мікробіота самих природних МВ виступають як посилюючі фактори антибактеріальної дії. Визначено, що досліджені специфічні біологічно активні компоненти природних фасованих МВ здатні проявляти антибактеріальні властивості посилюючи ефекти один одного.

Ключові слова: фасовані природні мінеральні води, діоксид вуглецю, метакремнієва кислота, ортоборна кислота, санітарно-мікробіологічний стан, антибактеріальна дія, алохтонна мікробіота.

FEATURES OF THE INFLUENCE ON ALLOCHTHONOUS MICROORGANISMS OF SPECIFIC BIOLOGICALLY ACTIVE COMPONENTS OF PACKAGED MINERAL WATERS

Lazar A. D., Gushcha S. G., Koieva K. A., Muratov V. N.

Abstract. Nowadays the issue of safety of bottled natural mineral water receives a lot of attention, as it's consumption increases every year. Like any other product used by humans, it must be safe. Research from previous years confirms the importance of constant monitoring of the sanitary and microbiological safety of packaged mineral waters. *The purpose of the work:* to assess the influence of specific biologically active components (metasilicic and orthoboric acids) on the sanitary condition of packaged mineral waters (saturated and unsaturated), which was sold in Ukraine from 2017 to 2021. *Materials and methods.* Sanitary-indicative microorganisms in the studied mineral water were detected in several stages. During the researching were used methods with solid and liquid nutrient media were used. Liquid glucose-peptone medium, Endo's medium, oxidase test and microscopy were used to determine the number of coliform bacteria (CB). The total microbial count was determined on nutrient agar at

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Key words: packaged natural mineral waters, carbon dioxide, metasilicic acid, orthoboric acid, allochthonous microorganisms.

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