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INVESTIGATION OF PHYSIOLOGICALLY ACTIVE IONS AND MICROBIOLOGICAL CHARACTERISTICS OF GASTRIC SPRING WATER IN THE SMALL TOWN OF TSKALTUBO

***Abstract**: for the first time, the examination of physiologically active ions and microbiological conditions in the gastric spring water of a small town of Tskaltubo was carried out. The content of magnesium, calcium, total iron, bicarbonate, chloride, sulfate ions, total arsenic, permanganate oxidizability, dissolved oxygen, dry residue, BOD_5 (biochemical oxygen demand), and carbon dioxide has been determined. For the determination, relatively simple and rapid chemical and physicochemical methods, which are well-proven in practice and have good reproducibility, were selected. Biogenic substances were determined using the photometric method. Based on the experimental data, it was established that the water from the investigated spring is low-mineralized, which is why its reaction is nearly neutral. The content of the above ions in the studied spring waters is within the norm except for permanganatometric oxidation. According to the results of the microbiological analysis of the researched spring, it became clear that it is subject to the norms of the drinking water standard of Georgia. It appears that the investigated springs are not contaminated by domestic, wastewater, or sewage sources. A slightly elevated number of mesophilic aerobes and facultative anaerobes was detected in the spring water, which does not pose a risk to human health.. No pathogenic microorganisms were detected in the investigated spring water (including total coliform bacteria, Escherichia coli, Streptococcus faecalis, Pseudomonas aeruginosa, and pathogens such as Salmonella spp). The microbiological contamination levels were found to be within safe limits for human health. Based on the research results, the water from the stomach spring was confirmed to be suitable for drinking.*

***Key words**: Gastric spring, hydrochemistry, microorganism, analysis, substance, threshold concentration, high-sensitivity method, biosphere, infectious diseases, Complexon III.*
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Introduction

The aim was to study the hydrochemical composition and pathogenic microorganisms of the gastric spring water in the towship of Tskaltubo. The relevance of this issue lies in the fact that the content of physiologically active ions was determined for the first time in these waters, for which highly sensitive and well-proven practical methods were selected.

Water is an invaluable treasure and has always been considered the source of life. During rainfall, a large portion of the water seeps into the ground. It first passes through the loose layer of soil, then goes deeper into the earth. It moves through sand, pebbles, and stones, and in this process, the water gets purified. Eventually, it reaches a layer that does not allow water to pass through.

Sometimes, the lower layers of soil are sloped, which prevents the water from accumulating. Instead, it flows through the ground and may eventually rise to the surface. This is how a spring is formed.

A spring is cold and clean. It is a natural outlet of underground water onto the surface of the Earth. People use spring water for drinking.

As water moves through the layers of the Earth's crust, it absorbs various substances, including salts, which results in the formation of a specific composition. The amount of salt in water varies widely. Their total concentration—i.e., mineralization — is relatively low in surface waters but significantly higher in underground waters.

Water is widely and diversely used. Modern humans are capable of influencing the biosphere and utilizing its resources for the development of production and the creation of material well-being. Today, great attention is paid to the rational use and protection of water resources. At present, water is purified and disinfected, after which it is used for drinking and centralized water supply systems.

The presence of calcium and magnesium salts gives natural water its hardness. When water containing CO_2 comes into contact with limestone layers, carbonates are transformed into bicarbonates, which dissolve well in water.

Magnesium and calcium are classified as essential elements for life. The difference in the properties of magnesium and calcium cations is related to the differing density of their positive surface charge.

Magnesium cation is a key activator of enzymatic reactions: it activates oxidative phosphorylation, DNA replication, and the enzymes responsible for the mineralization of bone tissue. Within the intracellular fluid, magnesium cation forms complexes with the anions of ATP and ADP. It is also the central ion in the metal-porphyrin cluster of chlorophyll, playing a vital role in photosynthesis.

The absorption of calcium from food products mainly depends on vitamin D. Unlike magnesium ions, calcium ions are concentrated in the intercellular fluid. Calcium is the main component of bone tissue. When the concentration of calcium ions in blood plasma decreases, hormonal regulation leads to the breakdown of mineral compounds in bone tissue, thereby increasing the calcium ion content in the plasma. The parathyroid hormone stimulates the release of salts from bone tissue.

Calcium ions are involved in the transmission of nerve impulses, muscle contraction, regulation of heart rhythm, and the blood clotting process. Moreover, calcium ions suppress the excitability of the central nervous system, and a decrease in their concentration can lead to convulsions. [3,4].

The development of all sectors of the national economy and the rising standard of living of the population place greater demands on the quality of drinking and household water. The targeted use of water requires in-depth chemical and microbiological analysis, the determination of its physicochemical, organoleptic, and chemical composition, and, based on this, an assessment of its quality.

All of this is closely related to the development and refinement of methods for chemical and bacteriological analysis. The purpose of conducting hydrochemical analysis of water is to teach students the methods of water investigation and treatment.

Surface waters primarily contain oxygen, nitrogen, inert gases, and carbon dioxide. In addition to these, underground mineral waters also contain dissolved methane, hydrogen sulfide, and other gases.

Nitrogen and oxygen are only slightly soluble in water, which is why their concentration in surface waters is low.

The a small town of Tskaltubo, located in western Georgia, is a city of republican subordination. It lies along the banks of the Tskaltubo River. The climate in Tskaltubo is humid subtropical, with mild, warm winters and hot summers. Winds are most common in winter and spring.

The main therapeutic factor is its unique mineral water (slightly radon-containing, nitrogenous, chloride-hydrocarbonate, sulfate, sodium-rich, potassium-magnesium-rich), which has stable physical and chemical properties. The water temperature reaches 33–35°C and is used for baths, inhalation, and spraying procedures.

Another therapeutic factor is the microclimate of the karst caves, which has a beneficial effect on conditions such as hypertension, hypotension, bronchial asthma, and others. In the lowland areas, a significant part of the land is occupied by various subtropical podzolic soils. [5].

Significant microorganisms in the formation of water chemical composition, which are used as food in water, are mainly organic and convert it into mineral substances, therefore, it is of interest to microbiological examination of the source, well and grid waters, since groundwater contaminated with fecal masses of water, which are used by humans for various purposes, can cause intestinal infections, diuretic tract and genitals Injury, bacteremia, poisoning and others. A large portion of surface waters—and in some cases, underground waters as well—are polluted. Water pollution is a general anthropogenic change that affects water quality, contaminates it, and poses a threat to humans, animals, and ecosystems across the globe. In villages where there is no centralized water supply system, residents obtain drinking water from wells or natural springs. If these wells are flooded with runoff water, contaminated water may mix with them, increasing the risk of infection among the population. Drinking water contaminated with microorganisms and chemical substances is one of the main causes of intestinal and infectious diseases. This is why various infectious diseases are especially common in Georgia during the spring [6, 7, 8].

Experiment.

Thus, for the first time, the following parameters were determined in the gastric mineral waters of the a small town of Tskaltubo: pH, water hardness, Mg^{2+}, Ca^{2+}, total iron, HCO_3^-, SO_4^{2-}, Br^-, total arsenic, and Cl^- ions, permanganate oxidizability, dissolved oxygen, free carbon dioxide, BOD_5, and the content of biogenic elements—using chemical and photometric methods.

Table N 1. The analysis results are presented in Table #1.

Hydrochemical Analysis Results of the Gastric Spring Water in the a small town of Tskaltubo

N	Local (Regional) Names of Spring Waters	pH	mg-eq./l	mg/L												
			Water hardness	Ca^{2+}	Mg^{2+}	Common iron	HCO_3^-	Cl^-	Br^-	SO_4^{2-}	Open oxygen	SBM5	Oxidation of permanganate 02	Free CO_2	Common arsenic	Dry balance
1	Gastric	6,62	1.40	17,6	6,35	0,28	0,74	36,92	0,18	0,022	3,65	36.16	15,99	0,50	0,026	1.22

Experimental Section. Methodology for Determining Chemical Elements in Water

The analyses were carried out at the Hydrochemistry Laboratory of Akaki Tsereteli State University in Kutaisi. Methods approved in hydrochemical practice were used for the analysis [9, 10, 11, 12]. The acidity (pH) level was measured using the potentiometric method (potentiometer pH 673-M)).

The content of calcium and magnesium, as well as the total hardness of the water samples, was determined using the complexometric method (titrant: 0.01N Complexon III). To determine the concentration of magnesium ions, Eriochrome Black T was used as an indicator, and the recommended pH range was created using an ammonia buffer solution. For the determination of calcium ions, Murexide was used as an indicator, and the alkaline medium was established using 2N sodium hydroxide.

Total iron in the water was determined using the photometric method after preliminary oxidation in an alkaline medium (photometric reagent: sulfosalicylic acid) (instrument: UN-5100B UV/VIS spectrophotometer).

Hydrocarbonates were determined using the acidimetric method (titrant: 0.1–0.01 N; indicator: methyl orange). Sulfate ions and dry balance were defined by the classical gravimetric method, the sedimentary form is $BaSO_4$.

Sulfate ions and dry residue were determined using the classical gravimetric method, with the precipitate forming a specific solid phase. To determine chlorides, both the mercurimetric method (titrant: 0.01 N; indicator: diphenylcarbazone) and the argentometric method (titrant: 0.01 N $AgNO_3$; indicator: potassium chromate) were used.

Total arsenic was determined using the iodometric method, with 0.05 N iodine solution as the titrant and starch as the indicator.

Free carbon dioxide was determined using the alkalimetric method. The titrant was 0.1–0.01 N. $NaOH$. A small amount of Rochelle salt was added to the water sample before titration, and phenolphthalein was used as the indicator.

Oxidizability was determined by the permanganatometric method (oxidizing agent: 0.01 N $KMnO_4$ in an acidic medium; titrant: 0.01 N $H_2C_2O_4$)).

The dissolved oxygen content and BOD_5 were determined using the iodometric method (titrant: 0.02 N $Na_2S_2O_3$). In an alkaline environment, dissolved oxygen $Mn(OH)_2$ oxidizes manganese(II) to a tetravalent manganese compound, which, upon acidification of the solution and in the presence of excess oxygen KI , forms free iodine I_2 [5]

Bromide ions were determined using the iodometric method. Sodium hydrogen phosphate, sodium hypochlorite, and sodium formate were added to the water sample. The analytical mixture was heated and then cooled. Potassium iodide, sulfuric acid, and ammonium molybdate were then added. The titration was carried out using 0.01 N $Na_2S_2O_3$. titrant, with starch as the indicator. Bromide ions are oxidized by sodium hypochlorite to bromate ions.

Biogenic substances were determined by the photometric method: NO_2^- membrane reagent, NO_3^- sodium salicylate, NH4+ – nesler reactivation, PO_4^{3-} ammonium fosformolibdate.

NO_2^- - The photometric determination using the Gris reagent in the acid area is based on a reaction to the formation of reddish-brick azole as a result of the interaction of sulfanylic acid, nitrite ion, and alpha-naphtilamine.

NO_3^- - It was determined by the photocolorimetric method using sodium salicylate. The method is based on the interaction between nitrate ions and sodium salicylate ions, with the coherence of sulfuric acid, during which the yellow coloring produced is directly proportional to the concentration of nitrate ion.

The determination of $NH4^+$ is based on the interaction between ammonium ion and nesler reagent (mercury tetra iodine) in the alkaline area in which the yellow coloration produced is directly proportional to the concentration of ammonium ion.

PO_4^{3-} was defined by a photocolorimetric method based on the interaction of orthophosphoric acid and ammonium molybdate in the acid area, in which the blue coloration produced is directly proportional to phosphate concentration[6].

Sanitary-microbiological examinations of the water samples were conducted at the Microbiology Laboratory of Akaki Tsereteli State University in Kutaisi and the testing laboratory of LLC "Microbiologist." Using modern methods, the following were analyzed: mesophilic aerobes and facultative anaerobes, total coliform bacteria, ***Escherichia coli*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, and pathogens including *Salmonella* spp**. The analysis results are presented in Table 2.

Table 2. Microbioligial Analysis of Gastric Spring Water

#	Highlights	Units of measurement	Normative No more than	Exam result	Testing method
1.	**Mesophilic Aerobes and Facultative Anaerobs**	**mL/ml 37^{oC} 22oC**	**20 100**	**38 117**	მმ.2.1.4.1184-03
2.	**Common Coliform Bacteria**	**in 300 ml**	**Is not allowed**	**Was not detected**	ISO 9308-3:1998
3.	**Escherichia coli**	**in 300 ml**	**Is not allowed**	**Was not detected**	ISO9 308-3:1998
4.	**Streptococcus faecalis**	**in 300 ml**	**Is not allowed**	**Was not detected**	ISO 7899-2:1984
5.	**Pseudomonas aeruginosa**	**in 300 ml**	**Is not allowed**	**Was not detected**	ISO 8360-1:1988
6.	**Pathogens, including salmonella - Salmonella spp**	**100ml**	**Is not allowed**	**Was not detected**	ISO 19250:2010

Based on the analytical results of the investigated spring, it became clear that the water meets the standards required by the Georgian drinking water regulations. The results indicate that the examined springs are not contaminated by domestic wastewater or sewage. A slightly elevated number of mesophilic aerobes and facultative anaerobes was detected in the spring water. No pathogenic microorganisms were found (**including total coliform bacteria, Escherichia coli, Streptococcus faecalis, Pseudomonas aeruginosa, and pathogens such as Salmonella spp.).** According to the results of the microbiological study, the stomach spring water was confirmed to be suitable for drinking.

Conclusion: Thus, water pollution is a problem that affects almost every country in the world, including Georgia. This issue has a significant impact not only on the ecological and social conditions of the country but also on its economic situation. For this purpose, we examined the hydrochemical and microbiological status of the gastric spring water in the a small town of Tskaltubo. The conducted analysis of the spring water shows that the levels of magnesium, calcium, total iron, hydrocarbonates, sulfates, total arsenic, bromide and chloride ions, free carbon dioxide, dry residue, oxygen, BOD_5, and the main microbiological indicators are within the acceptable limits and comply with the standards of drinking water quality in Georgia. Biogenic substances were not detected in the analyzed spring water. However, the permanganate oxidizability exceeds the standard limit—15.99 mg/L O_2. A slightly elevated number of mesophilic aerobes and facultative anaerobes was also detected in the spring water; however, this does not pose a threat to human health. No pathogenic microorganisms were found. Based on the analysis results, the spring is not contaminated by domestic, wastewater, or sewage discharges. Compared to the Georgian Technical Regulation on Drinking Water Quality, the particularly high value of permanganate oxidizability may be explained by the presence of organic substances and inorganic compounds with reducing properties (such as sulfites and iron(II) salts). Determining the exact composition and concentration of these compounds—especially the organic ones—is a complex task. Despite this, the water is still considered suitable for drinking. I believe that this study will contribute to addressing the problem of water pollution, which in turn will help improve public health, individual well-being, and the socio-economic and ecological conditions in Georgia.

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