PHYSICOCHEMICAL, ISOTOPIC, SPECTRAL, AND MICROBIOLOGICAL ANALYSES OF WATER FROM GLACIER MAPPA, CHILEAN ANDES

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ABSTRACT

The physicochemical and microbiological parameters of glacial water sourced from Glacier Mappa, Andes Mountains, Chile are described. The study is performed on isotopic composition in collaboration with Oleg Mosin. Glacier Mappa has located 55 km from Los Andes in the Aconcagua river basin and the sub-basin Rio Blanco. The research of glacier water Mappa has performed at 3.5 km from the source and the temperature t was 10.1^0C. The altitude above sea level is 2130 m.

The water source Glacier Mappa, Chile is characterized by microbiological indicators. Research is conducted for *Coliforms, Escherichia coli, Enterococci,* and *Clostridium perfringens.* The pathogenic micro-organisms in the samples of the glacial water from the source mentioned above are determined by the methods according to Ordinance No. 9/2001, Official State Gazette, issue 30, and decree No. 178/23.07.2004 of Council of Ministers, Bulgaria about the quality of water, intended for drinking and household purposes.

The parameters of the physicochemical composition include 18 indicators and are determined according to Ordinance No. 9/2001 (Protocol No. 12735/17.03.2016 of licensed laboratory Eurotest control, Sofia, Bulgaria).

The physicochemical and microbiological parameters of glacier water Mappa, Chile, correspond to all controlling parameters and are in accordance with Ordinance No. 9/2001 (Official State Gazette, issue 30 about the quality of water, intended for drinking and household purposes).

The natural waters consist of 99.7 mol. % of $H_2{}^{16}O$, which are formed by ^1H and ^{16}O atoms (Jouzel, 2003; Mosin, Ignatov, 2011). The remaining 0.3 mol.% is distributed in isotope varieties (isotopomers) of water molecules, whose deuterium atoms form 6 configurations – $HD^{16}O$, $HD^{17}O$, $HD^{18}O$, $D_2{}^{16}O$, $D_2{}^{17}O$, $D_2{}^{18}O$, as 3 configurations are formed by isotopes of oxygen – $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$.

Studies of International standard SMOW of isotopic shift for D were performed for D/H for water from Glacier Mappa, Chile.

The results of glacier water in Mappa, Chile show a lower content of hydrogen and oxygen isotopes. It is not confirmed the presence of pathogenic micro-organisms. The physicochemical indicators are typical for waters during the ice melting in glacier water (Ca^{2+} 6.0; Mg^{2+} 1.0; Na^+ 5.0; K^+ 0.38; HCO_3^- 73.8 /mg. L^{-1}). Glacier water Mappa is soft with total mineralization – 100.1 mg.L^{-1}.

The water of Glacier Mappa is beneficial for health according to the indicated physicochemical, microbiological, and isotopic parameters.

Keywords: Glacier water, Glacier Mappa, Andes Mountains, Chile, physicochemical and microbiological indicators, isotopic composition.

1. INTRODUCTION

The natural waters consist of 99.7 mol. % of $H_2{}^{16}O$, which are formed by ^1H and ^{16}O atoms. The remaining 0.3 mol.% are – $HD^{16}O$, $HD^{17}O$, $HD^{18}O$, $D_2{}^{16}O$, $D_2{}^{17}O$, $D_2{}^{18}O$, $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$ [1-4]. Jouzel provides the following data. Natural waters formed of ~99.7% of $H_2{}^{16}O$ are also constituted of other stable isotopic molecules, mainly $H_2{}^{18}O$ (~2‰), $H_2{}^{17}O$ (~0.5‰), and $HD^{16}O$ (~0.3‰) [1, 4].

It is accepted that the norm for deuterium atoms in natural waters is 150 ppm [5]. The highest deuterium content in water molecules in glacial waters is measured in Greenland or 124.8 ppm [6]. The lowest is measured in the Antarctic; more precisely, it is 89.0 ppm.

One of the co-authors, Ignat Ignatov and Oleg Mosin 2016 measured the isotopic composition of glacier water in Mappa, Chile. The presence of deuterium is 91.3 ppm. The death of Oleg Mosin in 2016 prevented the results from being published earlier.

Physicochemical analyses are performed of glacier water Mappa. The water is alkaline with pH=7.85. It is particularly important that calcium (Ca^{2+}) and sodium (Na^+) ions have close values. For Ca^{2+} the value is 6.0, and for Na^+ it is 5.0 mg.L^{-1}. Research shows that the balance among the following ions Ca^{2+}, Mg^{2+}, Na^+, K^+, Zn^{2+}, and Mn^{2+} in water and food is observed in areas where people are long-living [7-9]. Magnesium (Mg^{2+}) is a co-factor in enzymatic reactions. It also neutralizes one of the most dangerous radicals – the hydroxyl radical OH [10].

Zinc and Manganese improve the properties of antioxidant enzymes in the blood red cells [11]. China also researched the interdependence between longevity zones and metal ions in water and rice. People over the age of 90 took part in scientific studies. People over the age of 100 are classed as centenarians, and over 110 years of age – are supercentenarians (supercentenarians). Calcium had the largest positive correlation among the six metal ions we examined. Magnesium, potassium, zinc, and manganese had a weaker correlation [12]. The antioxidant effects of Zamzam water, Saudi Arabia on rats were studied [13].

The presence of a positive correlation between longevity and the number of calcium ions in water allows us to conduct a more detailed analysis. In Nova Scotia live 210 centenarians per 1 million people. Supercentenarians also live there. The content of Ca^{2+} ions in the water is 6.8 mg.L^{-1} [14]. Druzyak reports that the content of calcium ions in the water in Russia's longevity zones is 8-20 mg.L^{-1} [15]. The content of Ca^{2+} ions in Rosenlaui glacier, Swiss Alps is 8.7 mg.L^{-1} [16].

In Bulgaria for Rodopi municipality, Plovdiv Province, the northern part of Rhodope mountain – the calcium content 1400 m above sea level is 3.5 mg.L^{-1} [8,17].

In the study are applied spectral methods as follows: Non-equilibrium Energy Spectrum (NES) and Differential Non-equilibrium Energy Spectrum (DNES) [18, 19].

The results obtained for the amounts of calcium have their own explanation. Over the past 50-60 years, increasing evidence shows that water hardness affects cardiovascular disease [20-22]. The purpose of the studies of physicochemical, microbiological, and isotopic parameters, as well as NES and DNES spectral analyses, is to show the health benefits of glacier water in Mappa, Chile.

2. METHODS AND MATERIALS

We analyzed the waters of the examined springs in respect of physicochemical composition, in compliance with Ordinance No 9/2001, Official State Gazette, issue 30, and decree No. 178/23.07 004 about the quality of water intended for drinking and household purposes [23-25].

2.1. Methods for physicochemical analysis

Method for determination of color according to Rublyovska Scale – method by Bulgarian State Standard (BDS) 8451: 1977;

Method for determination of smell at 20^0C – method BDS 8451: 1977 using technical device glass mercury thermometer, conditions No. 21;

Method for determination of turbidity – EN ISO 7027, technical device turbidimeter type TURB 355 IR ID No 200807088;

Method for determination of pH – BDS 3424: 1981, technical device pH meter type UB10 ID NoUB10128148;

Method for determination of oxidisability – BDS 3413: 1981;

Method for determination of chlorides – BDS 3414: 1980;

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Method for determination of nitrates – Validated Laboratory Method (VLM) – NO_3 – No. 2, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of nitrites – VLM NO_2-No. 3, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of ammonium ions – VLM-NH_4-No. 1, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of general hardness – BDS ISO 6058;

Method for determination of sulfates – VLM-SO_4-No. 4, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of calcium – BDS ISO 6058;

Method for determination of magnesium – BDS 7211: 1982;

Method for determination of phosphates – VLM-PO_4-No. 5, technical device photometer "NOVA 60 A" ID No 08450505;

Method for determination of manganese – VLM-Mn-No. 7, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of iron – VLM-Fe-No. 6, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of fluorides – VLM-F-No. 8, technical device photometer "NOVA 60 A" ID No. 08450505;

Method for determination of electrical conductivity – BDS EN 27888, technical device – conductivity meter inoLab cond 720 ID No 11081137.

2.2. Nutrient media

1. Nutrient agar (MPA) with contents (in %) – meat water, peptone – 1%, agar – agar – 2%. Endo's Agar (for defining *Escherichia coli* and *coliform* bacteria) with contents (ing.L^{-1}) – peptone – 5,0; tryptone – 5.0; lactose – 10.0; Na_2SO_3 – 1.4; K_2HPO_4 – 3.0; fuchsine– 0,14; agar – agar – 12,0 pH 7.5 – 7.7 (Grabow, du Preez, 1979; Grant, 1997; Pitkänen et al, 2007).
2. Nutrient gelatine (MPD) (for defining *Pseudomonas aeruginosa*) with contents (in %) – Peptic digest of animal tissue; 25 % gelatin; pH = 7.0 - 7.2.
3. Medium for defining enterococci (esculin – bile agar).
4. Medium for defining sulfite-reducing bacteria (Iron Sulfite Modified Agar).
5. Wilson-Blair medium (for defining of sulfite reducing spore-forming anaerobes (*Clostridium perfringens*) with contents (g.L^{-1}) – 3% Nutrient agar; 100 mL 20% solution of Na_2SO_3; 50 mL 20% glucose solution; 10 mL 8% solution of Fe_2SO_4.

2.3. Methods for determination of microbiological indicators

1. Methods for evaluating microbiological indicators according to Ordinance No. 9/2001, Official State Gazette, issue 30, and decree No. 178/23.07.2004 about the quality of water, intended for drinking purposes.
2. Method for determination of *Escherichia coli* and coliform bacteria – BDSEN ISO 9308 -1: 2004;
3. Method for determination of enterococci – BDS EN ISO 7899-2;
4. Method for determination of sulfite-reducing spore anaerobes – BDS EN 26461-2: 2004;
5. Method for determination of the total number of aerobic and facultative anaerobic bacteria – BDS EN ISO 6222: 2002;
6. Method for determination of *Pseudomonas aeruginosa* – BDS EN ISO 16266: 2008.
7. Determination of coli – titer by fermentation method – Ginchev's method
8. Determination of coli – bacteria over Endo's medium–membrane method.
9. Determination of sulfite-reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

The number of isolated bacteria was presented in the tested waters' colony-forming units per ml (CFU.mL^{-1}.).

2.4. NES and DNES Spectral Analyses

The methods of Non-equilibrium Energy Spectrum (NES) and Differential Non-equilibrium Energy Spectrum (DNES) for the measurement of hydrogen bonds energy distribution were applied to characterize the effect of EVOfilter technology on processed water. The device invented by A. Antonov [26-30] for spectral analysis with NES and DNES methods is based on an optical principle. Water drop evaporation occurs in a hermetic chamber on a glass plate covered with 350 μm thick BoPET (biaxially-oriented polyethylene terephthalate) foil. The evaporation of water drops was performed at a stable temperature of 22ºC (Fig. 1).

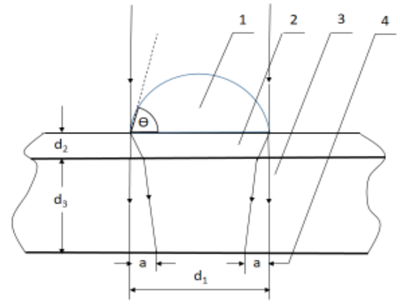

Figure 1. The operating principle of the method for measuring the wetting angle of liquid drops on a hard surface: 1-drop, 2 – thin mylar foil, 3 –glass plate, 4 – refraction ring width. The wetting angle θ is a function of a and d_1.

The device has the following technical features:

- Monochromatic filter with wavelength $\lambda = 580\pm7$ nm;
- Angle of evaporation of water varies from 72.3^0 to 0^0 deg;
- Measured range of energy of hydrogen bonds among water molecules is $\lambda = 8.9–13.8$ μm or E=-0.08– -0.1387 eV.

The energy ($E_{H...O}$) of hydrogen O...H-bonds among H_2O molecules in the water sample is measured in eV. The function f(E) is called the distribution spectrum according to energies. The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation and this is a non-equilibrium energy spectrum (NES) and is measured in eV^{-1}. DNES is defined as the difference,

Δf(E) = f (samples of water) – f (control sample of water),

DNES is measured in eV^{-1}

where *f(*)* denotes the evaluated energy [25-27].

2.5. Methods for research of heavy water (D_2O)

For the preparation of growth media, we used D_2O (99.9 atom%) received from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). Inorganic salts were preliminarily crystallized in D_2O and dried in a vacuum before use. D_2O distilled over $KMnO_4$ with the subsequent control of deuterium content in water by ^{1}H-NMR-spectroscopy on Brucker WM-250 device ("Brucker," Germany) (working frequency – 70 MHz, internal standard – Me_4Si) and Brucker Vertex ("Brucker" Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}) [2, 3, 31].

3. RESULTS AND DISCUSSION

Research is performed on Glacier water Mappa, Chile's physicochemical, microbiological, and isotopic parameters. Analyses are produced using NES and DNES spectral methods. Fig. 2 illustrates Glacier Mappa and Fig. 3 glacier water Mappa.

Figure 1. Glacier Mappa, Chile.

Figure 2. Glacier water Mappa, 3.5 km from the source.

3.1. Physicochemical analysis of Glacier water Mappa, Chile

Physicochemical parameters of the Glacier water Mappa, Chile are presented in Table 1.

Table 1. Physicochemical parameters of the Glacier water Mappa, Chile.

Controlled parameter	Measuring unit	Maximum Limit Value	Mappa Glacier water
1. pH	pH values	≥ 6,5 and ≤ 9,5	7.85±0.1
2. Electrical conductivity	$\mu S.\ L^{-1}$	2000	132.7 ±4.0
3. Total hardness	$mgekv.L^{-1}$	12	<0.5
4. Color	Chromaticity Values	Acceptable	Acceptable
5. Turbidity	FNU	Acceptable	Acceptable
6. Odour	force	Acceptable	Acceptable
7. Calcium (Ca^{2+})	$mg.L^{-1}$	150	6.0 ±0.6
8. Magnesium (Mg^{2+})	$mg.L^{-1}$	80	1.0 ±0.1
9. Iron (Fe^{2+})(Fe^{3+})	$\mu g.L^{-1}$	200	1.8±0.2
10. Manganese (Mn^{2+})	$\mu g.L^{-1}$	50	1.0±0.1
11. Zinc (Zn^{2+})	$mg.L^{-1}$		0.0015
11. Sodium (Na^{+})	$mg.L^{-1}$	200	5.0±0.5
12. Potassium (K^{+})	$mg.L^{-1}$	-	0.38±0.04
13. Ammonium ion (NH_4^{+})	$mg.L^{-1}$	0.50	<0.05
14. Sulphates (SO_4^{2-})	$mg.L^{-1}$	250	13.9±1.4
15. Nitrates (NO_3^{-})	$mg.L^{-1}$	50	<0.1
16. Nitrites (NO_2^{-})	$mg.L^{-1}$	0.5	<1.0
17. Carbonates (CO_3^{2-})	$mg.L^{-1}$	-	<2.0
18. Hydrocarbonates (HCO_3^{-})	$mg.L^{-1}$	-	73.8±3.7

The research was performed in the Laboratory of Eurotest Control, Sofia, Bulgaria with standards ISO 10304-1:2009; ISO 10523:2008; ISO 11885:2009.
Certificate No.12735/17.03.2016.

3.2. Isotopic analyses of Glacier water Mappa

One of the co-authors, Ignat Ignatov and Oleg Mosin 2016 measured the isotopic composition of glacier water in Mappa, Chile. The studies were performed with different enrichment of heavy water (D_2O) for the facultative methylotrophic bacterium Brevibacterium methylicum [32, 33], and the bacterium Bacillus subtilis [34]. Research for different types of water was done [2, 3]. The presence of deuterium is 91.3 ppm. The death of Oleg Mosin in 2016 prevented the results from being published earlier.

3.3. Spectral analyses of water from Glacier Mappa, Chile

The average energy ($E_{H...O}$) of hydrogen H…O-bonds among individual H_2O molecules in Glacier water Mappa is measured at E=-0.1198±0.006 eV. The result for the control sample (deionized water) is E=-0.1120±0.006 eV. The results obtained with the NES method are recalculated with the DNES method as a difference of the NES (Glacier water Mappa) minus the NES (control sample with deionized water) equaled the DNES spectrum of Glacier water Mappa [35-37]. Thus, the result for Glacier water Mappa recalculated with the DNES method is ΔE=-0.0078 eV. The results show the increase of the values of the energy of hydrogen bonds in Glacier water Mappa regarding the deionized water. This is the effect of stimulation on the human body.

There were studied 10 samples of glacier water Mappa from different places of 100 m during the glacier source. The difference between the results estimated with the Student t-test was significant ($p<0.05$).

3.4. Mathematical model of Glacier water Mappa

The research with the NES method of water drops is received with water from Glacier Mappa (Chile) and deionized water. The mathematical model of glacier water Mappa gives valuable information on the function of distribution of energies f(E) according to the values of (-E) of hydrogen bonds (Table 2 and Fig. 4) [38-41]. These distributions are connected with the restructuring of H_2O molecules with the same energies.

Table 2: Function of distribution of energies f(E) according to the values of (-E) of hydrogen bonds of H_2O molecules in glacier water Mappa (Chile) and deionized water.

-E(eV) x-axis	Water from Glacier Mappa f(E) (eV^{-1})	Deionized water f(E) (eV^{-1})	-E(eV) x-axis	Water from Glacier apa f(E) (eV^{-1})	Deionized water f(E) (eV^{-1})
0.0937	0	0	0.1187	38.2	18.2
0.0962	0	36.4	0.1212	19.1	0
0.0987	0	54.5	0.1237	38.2	36.4
0.1012	19.1	18.2	0.1262	38.2	54.5
0.1037	0	0	0.1287	0	18.2
0.1062	38.2	0	0.1312	0	0
0.1087	0	54.5	0.1337	19.1	0
0.1112	57.2*	0	0.1362	24.2**	0
0.1137	76.3*	18.2	0.1387	57.1**	36.4
0.1162	38.2	0	–	–	–

Notes:

* The result at (-0.1112 eV)(λ=11.15 μm)($\tilde{v}$=897 cm^{-1}); (-0.1137 eV) (λ= 10.91 μm)($\tilde{v}$= 917 cm^{-1}) is connected with improvement of conductivity of Ca^{2+} ions [8, 9].

** The result at (0.1362 eV)(λ=11.15 μm)($\tilde{v}$=897 cm^{-1}); (-0.1387 eV) (λ=8.95 μm)($\tilde{v}$=1119 cm^{-1}) is characteristic for inhibiting the growth of tumor cells [8, 9].

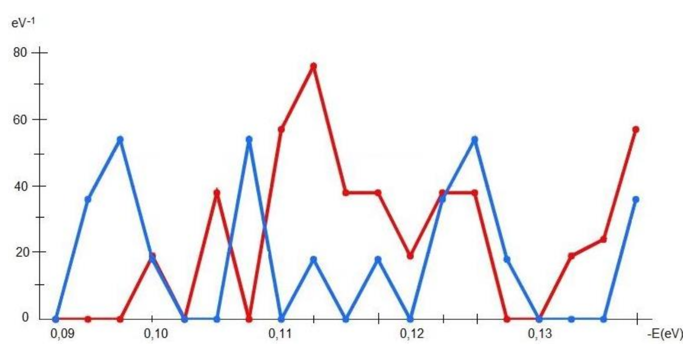

Figure 4. The function of distribution of energies f(E) according to the values of (-E) of hydrogen bonds of H_2O molecules in glacier water Mappa (Chile) and deionized water

Conclusions from the mathematical model of water from Glacier Mappa:

The experimental data obtained testified to the following conclusion from the mathematical models of Glacier water Mappa according to the mathematical model of deionized water. The distribution (%, $(-E_{value})/(-E_{total\ value})$ of water from Glacier Mappa and deionized water is different. However, for the value E = -0.1387 eV or λ = 8.95 μm there is the big local extremum corresponding to the re-structuring of hydrogen bonds among H_2O molecules. This difference may indicate the different number of hydrogen bonds in water samples, as well as their physical parameters (pH, ORP- oxidation/reduction potential), resulting in a different distribution of H_2O molecules and different values of H_2O molecules with ratios of $(-E_{value})/(-E_{total\ value})$. Particularly, the statistical re-structuring of H_2O molecules in water samples was observed according to the energies. The experimental data may prove that water from Glacier Mappa stipulates the restructuring of H_2O molecules on the molecular level, and may be used for prophylaxis against the development of tumor cells. For the value E = -0.11 eV, the result is connected with the improved conductivity of nervous tissues from Ca^{2+} ions.

3.5. Comparative analysis of the function of distribution of energies f(E) according to the values of (-E) of hydrogen bonds

Table 3 illustrates the comparative analysis of the function of distribution of energies f(E) according to the values of (-E) of hydrogen bonds.

Table 3. Comparative analysis of the function of distribution of energies f(E) according to the values of (-E) of hydrogen bonds.

Type of Water	Value eV^{-1} of Local Extremum at (-0.1362– -0.1387)	Value eV^{-1} of Local Extremum at (-0.1362– -0.1387)
Deionized water	36.4±1.8	36.4±1.8
Danubian Plain, village of Sadovetz, Pleven region, Bulgaria	25.1±1.3	23.2±1.7
Mountain water from Vasiliovska mountain, Bulgaria Stara planina	57.2±3.0	44.9±2.2
Northern Rhodope	53.3±3.0	59.3±3.0
Glasier Rosenlaui, zwitzerland	89.0±4.5	70.1±3.5
Glasier Mappa, Chile	93.5±4.7	81.3±4.1

Notes:

*The values (E= -0.1112;-0.1137 eV) correspond to the wavelengths (λ=10.91; 11.91μm).

**The values (E= -0.1362; -0.1387 eV) correspond to the wavelengths (λ=8.95; 9.10 μm).

The results show that glacier water Mappa has the highest parameters of all types of water – deionized, tap, plain, and mountain water. All minerals are in the norm and glacier water Mappa benefits human health.

3.6. Microbiological research of Glacier water Mappa, Chile

The results from the studies for determination of the total number of mesophilic aerobic and facultative anaerobic bacteria [42-45] in Glacier water Mappa, Chile are illustrated in Table 4.

Table 4. Total number of mesophilic aerobic and facultative anaerobic bacteria in the studied glacier water Mappa, Chile

Examined water source	Indicator, $CFU.mL^{-1}$
Glacier water Mappa, Chile with t=10.1°C, 3.5 km from the source	0

Research with glacier water Mappa, Chile is conducted for *Coliforms, Escherichia coli, Enterococci,* and *Clostridium perfringens* (Table 5)[42-45]. The pathogenic micro-organisms in the samples of the glacial water from the source mentioned above are determined by the methods according to Ordinance No. 9/2001, Official State Gazette, issue 30, and decree No. 178/23.07.2004 of Council of Ministers, Bulgaria about the quality of water, intended for drinking and household purposes.

Table 5. Microbiological results according to Ordinance No. 9/2001, Official State Gazette, issue 30 of glacier water Mappa

Indicators	Measuring unit	Maximum limit value	Glacier water Mappa, Chile with t=10.1°C, 3.5 km from the source
Coliforms	$CFU.\ L^{-1}$	0/100	0/100
Escherichia coli	$CFU.\ L^{-1}$	0/100	0/100
Enterococci	$CFU.\ L^{-1}$	0/100	0/100
Suphite-reducing anaerobes *(Clostridium perfringens)*	$CFU.\ L^{-1}$	0/100	0/100
Pseudomonas aeruginosa	$CFU.\ L^{-1}$	0/250	0/250

CONCLUSIONS

From the research of glacier water Mappa valid the following conclusions:

The variety of ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, CO_3^{2-}), chemical-physical parameters (pH, electroconductivity) and the decreased content of deuterium in studied water samples renders beneficial effects of this type of water on human health.

Mountain and glacier water Mappa have indirect antioxidant activity over enzymes in the human body containing ions of magnesium (Mg^{2+}), manganese (Mn^{2+}), and zinc (Zn^{2+}).

Glacier water Mappa is very specific with equal quality of Calcium (Ca^{+2}) – 6 mg. L^{-1} and Sodium (Na^+) – 5 mg. L^{-1}. They are beneficial for the nervous and cardiovascular systems. In Nova Scotia (Canada) and the Caucasus mountains (Russia), calcium content varies from 6 to 8 mg. L-1and these areas are where many centenarians live.

The water is excellent for the cardiovascular system. Glacier water Mappa is soft with total mineralization – 100.1 $mg.L^{-1}$.

The pH factor of Glacier water Mappa is 7.85. Water is alkaline and optimal for biochemical processes in the human body. In an alkaline medium, there is a decrease in tumor cells.

According to the analysis of various water samples by the NES and DNES methods can be drawn the main conclusions below:

1. The energy of hydrogen bonds of water in the samples differed because of the different number of hydrogen bonds in the water samples, which may result from the fact that different waters have different structures and various intermolecular interactions – various associative elements with different structure, clusters of formula $(H_2O)_n$ with different n, connected into the molecular associates;
2. Due to the different energies of hydrogen bonds, the surface tension of water samples was increasing or decreasing. For Glacier water Mappa there is increasing of surface tension according to the control samples. This effect is connected with the preservation of energy in the human body;
3. The experimental data obtained with Glacier water Mappa confirmed the following conclusion from the mathematical models of Glacier water Mappa compared to mathematical models of tap water. There is local big structuring of hydrogen bonds for the value E = -0.1387 eV or λ = 8.95 μm. This fact may prove that Glacier water Mappa stipulates the restructuring of H_2O molecules, and on a molecular level, it is very useful for the prophylaxis against the development of tumor cells.
   - For the value E = -0.11 eV the result is connected with the improved conductivity of nervous tissues from Ca^{2+} ions.
   - The hydrogen bond network may be stabilized with metal cations and anions in glacier Mappa. In ice, the hydrogen bonds have an energy of 4.6 kcal/mol or -0.1995 eV. For liquid water the energy of hydrogen bonds makes up 1–3 kcal/mol or 0.043–0.13 eV.

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The article is in memory of Oleg Mosin (1966-2016).

REFERENCES

1. J. Jouzel (2003) Water stable isotopes: Atmospheric composition and applications in polar ice core studies. *Treatise on geochemistry* **4**, 213-243.
2. Mosin, O. V, Ignatov, I. (2011) Separation of heavy isotopes Deuterium (D) and Tritium (T) and Oxygen (^{18}O) in water treatment. *Clean Water: Problems and Decisions* **3-4**, 69-78.
3. Ignatov, I., Mosin, O.V. (2016) Deuterium, Heavy Water and Origin of Life. *LAP LAMBERT Academic Publishing*, 1-500.
4. Masson-Delmotte, V., Jouzel, J. (2021) Air-ice interface: Polar ice (2021) *Paleoclimatology* **899**, 145-149.
5. Rodin, S., Rebellato, Lundin, A., Zubarev, R. A. (2018) Isotopic resonance at 370 ppm deuterium negatively affects the kinetics of luciferin oxidation by luciferase. *Scientific Reports* **8**, 16249.
6. Hut, G. (1985) *Consultants' Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations; IAEA: Vienna, Austria*.
7. Genda, H. (2016) Origin of Earth's oceans: An assessment of the total amount, history, and water supply. *Geochem. J*. **50**, 27-42.
8. Ignatov, I. (2018) Research of the factors of health and longevity of the population in Bulgaria. *Bulgarian Journal of Public Health* **10**, 52-65.
9. Ignatov. I., Angelcheva, M., Angushev, I. (2021) Doses of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Mn^{2+} and Zn^{2+} in mountain spring and mineral waters in hydration reaction during sports training and recovery; Combination with vitamins. *Journal of Pharmaceutical Research International* **33**, 76-85.
10. Juszczyk G. (2021) Chronic Stress and Oxidative Stress as Common Factors of the Pathogenesis of Depression and Alzheimer's Disease: The Role of Antioxidants in Prevention and Treatment. *Antioxidants* **10** 1433.
11. Varesi et al. (2022) The Role of Antioxidants in the Interplay between Oxidative Stress and Senescence. *Antioxidants* **11**, 1224.
12. Liv, J., Wang, W., Krafft, T., Li, Y., Zhang, F., Yuan, F. (2011) Effects of several environmental factors on longevity and health of the human population of Zhongxiang, Hubei, China, *Biol. Trace Elem. Res.* **143**, 702:716
13. Abdullah, A.M., Abdelsalam, E., Abdullah, B., Khaled, A. (2012) Antioxidant effects of Zamzam water in normal rats and those under induced-oxidant stress, *Journal of Medicinal Plants Research* **6**, 5507-5512.
14. Morr, S., Cuartas, E., Alwattar, B., Lane J. M. (2006) How much Calcium is in your drinking water? A survey of Calcium concentrations in bottled and tap water and their significance for medical treatment and drug administration. *HSS Journal* **2**, 130-135.
15. Druzyak, N. G. (2007) Method of mineralizing drinking water from the distillate. Patent RU2417953C2.
16. Ignatov, I, Mosin, O. V., Bauer, E. (2015) Vortex Power spring water: Physical-chemical qualities of this water compared to the mountain and meltwater from Bulgaria, Russia and Glacier Rosenlaui from the Swiss Alps. *Advances in Physics Theories and Applications* **45**, 6-29.
17. Ignatov, I., Mosin, O. V. (2016) Effects of Zinc and Manganese in mountain and glacier water for predominant antioxidant effects. *Journal of Medicine, Physiology and Biophysics* **27**, 11-29.
18. Antonov, A. (1995) Research of the non-equilibrium processes in the area of allocated systems, *Dissertation thesis for degree "Doctor of physical sciences"*, Blagoevgrad, Sofia 1-254.
19. Todorov, *S.,* Damianova, *A.,* Sirviev, *I.,* Antonov, A., Galabova, T. *(2008)* Water energy spectrum method and investigation of the variations of the H-bond structure of natural waters. *Comptes Rendus de l'Académie Bulgare des Sciences* ***61****, 857-862.*
20. Kubis, M. (1985) Relation of water hardness to the occurrence of acute myocardial infarct. *Acta Univ Palacki Olomuc Fac Med* **111**, 321–324.
21. Sengupta, P. (2013) Potential Health Impacts of Hard Water. *Int. J. Prev. Med*. **4**, 866-875.
22. Kozisek, F. (2020) Regulations for calcium, magnesium or hardness in drinking water in the European Union member states. *Regulatory Toxicology and Pharmacology* **112**, 1044589.
23. Methods for evaluating microbiological indicators according to Ordinance No. 9 / 2001, Official State Gazette, issue 30, and decree No. 178 / 23.07.2004 regarding the quality of water intended for drinking and household purposes.
24. Ignatov, I. (2020) Standards for researching and registering drinking mineral and mountain spring waters in Bulgaria. *Asian Journal of Chemical Sciences* **9**, 12-18.
25. Valcheva, N., Ignatov, I. (2022) Microbiological effects of combinations of colloidal Silver (Ag), Copper (Cu), and Zinc (Zn), nanoparticles for wastewater. *Uttar Pradesh Journal of Zoology* **42**, 45-50.
26. Antonov, A., Yuskesselieva, L. (1983) Method for determination of structural changes in liquids. *Author's certificate of invention* 43821.
27. Antonov, A. (1984) An optical method version for determination of the welling angle of liquids, *Comptes Rendus de l'Académie Bulgare des Sciences* **37** 1199.
28. Todorova, L., Antonov, A. (2000) Note on the Drop Evaporation Method for Study of Water Hydrogen Bond Distribution: I. An Application to Filtration. *Comptes Rendus de l'Académie Bulgare des Sciences*, **53,** 7-43.
29. Gramatikov, *P.,* Antonov, A., Gramatikova, M. (1992) A study of water systems' properties and structure variations under the stimulus of outside influences, *Fresenius' Journal of Analytical Chemistry* **343**, 134–135.
30. Antonov, A., Yuskesselieva, L., Teodosieva, I. (1989) Influence of ions on the structure of water under conditions far away from equilibrium. *Physiologie* **26**, 255-260.
31. Mosin, O. V., Ignatov, I. (2013) Microbiological Synthesis of H-2-Labeled Phenylalanine, Alanine, Valine, and Leucine/Isoleucine with Different Degrees of Deuterium Enrichment by the Gram-Positive Facultative Methylotrophic Bacterium Brevibacterium Methylicum. *International Journal of Biomedicine* **3**, 132-138.
32. Mosin, O. V., Shvetz, V. I., Skladnev, D. A., Ignatov, I. (2012) Studying of microbic synthesis of deuterium-labeled L-phenylalanine by facultative methylotrophic bacterium Brevibacterium methylicum on media with different content of heavy water. *Russian Journal of Biopharmaceuticals* **4**, 11–22
33. Mosin, O. V., Shvetz, V. I., Skladnev, D. A., Ignatov, I. (2013) Microbial synthesis of ^{2}H-labelled L-phenylalanine with different levels of isotopic enrichment by a facultative methylotrophic bacterium Brevibacterium methylicum with RuMP assimilation of carbon. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry* **7**, 247-258.
34. Mosin, O. V., Shvetz, V. I., Skladnev, D. A., Ignatov, I. (2013) Microbiological synthesis of [^{2}H]-inosine with a high degree of isotopic enrichment by the gram-positive chemoheterotrophic bacterium Bacillus subtilis. *Applied Biochemistry and Microbiology* **49**, 233-243.
35. Mehandjiev, D., Ignatov, I., Neshev, N., Huether, F., Gluhchev, G., Drossinakis, Ch. (2022) Formation of clusters in water and their distribution according to the number of water molecules. *Bulgarian Chemical Communications* **54**, 211-216.
36. Ignatov, I., Huether, F., Neshev, N., Kiselova-Kaneva, Y., Popova, T. P., Bankova, R., Valcheva, N., Ignatov, A. I., Angelcheva, M., Angushev, I., Baiti, S. (2022) Research of water molecules cluster structuring during *Haberlea rhodopensis* Friv. hydration, Plants **11**, 2655.
37. Mehandjiev, D., Ignatov, I., Neshev, N., Vassileva, P., Gluhchev, G., Huether, F., Drossinakis, Ch. (2023) History-dependent Hydrogen Bonds energy distributions in NaCl aqueous solutions undergoing osmosis and diffusion through a ceramic barrier. *Journal of Chemical Technology and Metallurgy* **58**, 340-346.
38. Smith, J., Cappa, C., Wilson, K., Cohen, R., Geisler, P., Saykally, R. (2005) Unified description of temperature-dependent hydrogen-bond rearrangements in liquid water. *PNAS* **102**, 14171.
39. Ignatov, I., Mosin, O. V. (2013) Structural mathematical models describing water clusters. *Journal of Mathematical Theory and Modeling* **3**, 72-87.
40. Ignatov, I., Gluhchev, G., Neshev, N., Mehandjiev, D. (2021) Structuring of water clusters depending on the energy of hydrogen bonds in electrochemically activated waters Anolyte and Catholyte. *Bulgarian Chemical Communications* **53**, 234-239.
41. Neshev N, Ignatov I, Toshkova R, Drossinakis Ch, Gluhchev, G. (2022) Hydrogen bonds energy distribution and information-theoretic analysis of blood serum from hamsters with experimental Graffi tumor. *Libri Oncologici* **50**, No. 52-61.
42. Popova, T. P., Petrova, T. E., Petrichev, M., Valyova, M. (2019) Action of activated waters on plants after adverse chemical effects, imitating acid rain. *Bulgarian Journal of Agricultural Sciences* **25**, 638-645**.**
43. Popova, T. P., Petrova, T. E., Kaleva, M. D., Karadzhov, S. D. (2018) Comparative study of the effect of electrochemically activated water solutions on pseudomonas aeruginosa. *Acta Microbiologica Bulgarica* **34**, 160-164.
44. Popova, T.P., Ignatov, I., Petrova, T.E., Kaleva, M.D., Huether, F., Karadzhov, S.D. (2022) Antimicrobial activity in vitro of cream from plant extracts and nanosilver, and clinical research in vivo on veterinary clinical cases. *Cosmetics* **9**, 122.
45. Popova, T. P., Ignatov, I.,Valcheva, N., Ignatov, A. I. (2022) Research of zeolite and zeolite water from Rhodope Mountains, Bulgaria. *Journal of the Turkish Chemical Society Section A: Chemistry* **9**, 901-908.