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Studying the Composition and Properties of Mountain and Melt Water of Bulgaria and Russia as Factors of Longevity. Effects of Calcium, Magnesium, Zinc and Manganese in Water on Organism

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Abstract

This paper demonstrates that mountain and melt water is among the most important factors for longevity. Other factors are hereditary, gender, body weight, food, psychological status, family relationships. Natural waters derived from various Bulgarian water springs, as well as melt water and blood serum of cancer patients between 50 and 70 years old were investigated by IR, NES and DNES-methods. We applied the NES- and DNES-methods for calculation of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules in the samples, as well as the percent distribution of H_2O molecules according to energies of hydrogen bonds within intervals (from -0.08 to -0.1387 eV). As estimation factor was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules, as well as local extremums in DNES- and IR-spectra of various samples of water and human blood serum, detected at $E = -0.1387$ eV and $\lambda = 8.95$ μm . For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in DNES-spectra are shifted to lower energies relative to the control group. The IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at $\lambda = 8.95$ μm . Natural mountain and melt water with unique chemical composition and less deuterium content seems to be one of the most important factors for longevity. In Bulgaria, most long lived people and centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. The similar characteristics possess mountain water from Teteven and other Bulgarian sources. There are new proofs for biophysical and biochemical effects of Ca^{2+} , Mg^{2+} , Zn^{2+} and Mn^{2+} in water.

Keywords: longevity, mountain water, melt water, IR spectroscopy, NES, DNES.

1. Introduction

Water is the main substance of life. The human body is composed from 48 to 54% of water for adult people. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research. Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water is the main

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factor for metabolic processes and aging [1]. Earlier studies conducted by us have demonstrated the role of water, its structure, isotopic composition and physico-chemical (pH, temperature) in the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content [2–4]. These factors and the structure of water are of great importance in biophysical studies. The peculiarities of chemical structure of H₂O molecule create favorable conditions for formation of electrostatic intermolecular van der Waals, dipole-dipole forces and donor-acceptor interaction with transfer of charges between H-atom and O-atoms in H₂O molecules, binding them into water associates (clusters) with the general formula (H₂O)_n where n varies from 3 to 50 units [5].

Other important indicator of water quality is its isotopic composition. The natural water consists on 99.7 mol.% of H₂¹⁶O, which molecules are formed by ¹H and ¹⁶O atoms [6]. The remaining 0.3 mol.% is represented by isotope varieties (isotopomers) of water molecules, wherein deuterium forms 6 configurations of isotopomers – HD¹⁶O, HD¹⁷O, HD¹⁸O, D₂¹⁶O, D₂¹⁷O, D₂¹⁸O, while 3 configuration are formed by isotopomers of oxygen – H₂¹⁶O, H₂¹⁷O, H₂¹⁸O.

In frames of this research 415 people living in the municipalities of Teteven, Yablanitza, Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Pleven district (Bulgaria), where is lived the most of long lived people and their siblings, were studied.

2. Material and Methods

2.1. Preparation of Water Samples with Varying Deuterium Content

For preparation of water samples with varying deuterium content we used D₂O (99.9 atom%) received from the Russian Research Centre “Isotope” (St. Petersburg, Russian Federation). Inorganic salts were preliminary crystallized in D₂O and dried in vacuum before using. D₂O distilled over KMnO₄ with the subsequent control of deuterium content in water by ¹H-NMR-spectroscopy on Bruker WM-250 device (“Bruker”, Germany) (working frequency – 70 MHz, internal standard – Me₄Si) and on Bruker Vertex (“Bruker”, 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).

2.2. Preparation of melt water

The melt water was obtained from Moscow tap water by the freeze-thaw method in a standard procedure: 1.5 l of Moscow tap water was placed in a glass jar with a lid and placed in the refrigerator freezer at -14 °C for 4–5 hours. Then, the first ice crystals were mechanically removed from the mixture, and the jar again was placed in the freezer additionally for 8–10 hours before ¾ of liquid freezes. Thereafter, the liquid brine is decanted and the remaining ice was thawed at room temperature and used for further experiments. The melt water was stored in a glass container in refrigerator. Other experiments were carried out with deuterium depleted water (DDW) with residual deuterium content of 60–100 ppm, purchased from Langway Water Inc. (Moscow, Russia).

2.3. DNES Spectral Analysis

The device for DNES was made by A. Antonov on an optical principle. In this study was used a hermetic camera for evaporation of water drops under stable temperature (+22–24 °C) conditions. The water drops are placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light is monochromatic with filter for yellow color with wavelength $\lambda = 580 \pm 7$ nm. The device measures the angle of evaporation of water drops from 72,3 ° to 0 °. The spectrum of hydrogen bonds among H₂O molecules was measured in the range of -0.08– -0.1387 eV or $\lambda = 8.9–13.8$ μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{\text{H...O}}$) of hydrogen O...H-bonds between H₂O molecules in human blood serum.

2.4. Studying the Bulgarian Centinarians

Interviews have been conducted with 415 Bulgarian centenarians and long lived people and their siblings. Their heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With using DNES method was performed a spectral analysis of 15 mountain water springs located in municipalities Teteven and Kuklen (Bulgaria).

The composition of water samples was studied in the laboratory of “Eurotest Control” (Bulgaria). Statistics methods were attributed to the National Statistical Institute of Bulgaria.

2.5. Studying the human blood serum

1% (v/v) solution of human blood serum was studied with the methods of IR-spectrometry, non-equilibrium (NES) and differential non-equilibrium (DNES) spectral analysis. The specimens were provided by Kalinka Naneva (Municipal Hospital, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases.

2.6. IR-spectroscopy

IR-spectra were registered on 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}) and on Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova).

2.7. Statistical Processing of Experimental Data

Statistical processing of experimental data was performed using the statistical package STATISTISA 6 using the Student's *t*- criterion (at $p < 0.05$).

3. Results and Discussions

3.1. Comparative Analysis between Longevity of Long Lived Centenarians and Their Siblings

In frames of the research 121 long living people from Bulgaria over 90 years of age have been studied together with their 294 siblings. The average lifespan of long lived people and centenarians in mountain areas is 94.1 years. For the average lifespan of long lived people in plain areas the result is 90.6 years. The most adult person from mountain areas is 104 years old and for plain areas is 97 years old. For the brothers and sisters of long live people from mountain areas the average lifespan is 88.5 years. For the brothers and sisters of long live people from plain areas the average lifespan is 86.4 years. The difference in life expectancy of the two groups of people is reliable and is at $p < 0.05$, *t*-Student's criteria at a confidence level of $t = 2.36$. There are distances of no more than 50–70 km between these places and the only difference is mountain water and air.

There have been 21519 residents in Teteven and 142 of them were born before 1924. Figure 1 shows the interrelation between the year of birth of long lived people (age) and their number (Teteven municipality, Bulgaria).

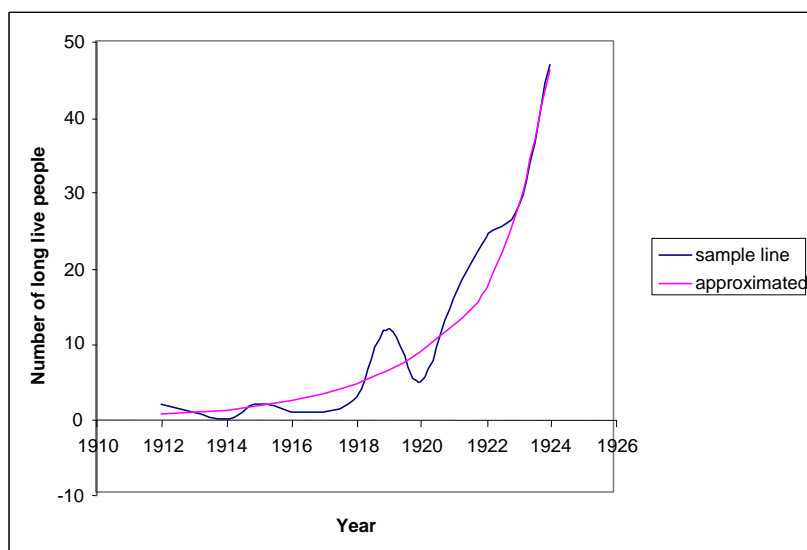


Figure 1. Interrelation between the year of birth of long lived people (age) and their number in Teteven municipality, Bulgaria

It was shown in Figure 1 that the rate of aging increases with time. In 1963 L. Orgel showed that the aging process is associated with the synthesis of abnormal proteins [7]. Figure 2 shows L. Orgel's results on the interrelation between age and number of cancer patients. The accumulation of errors in synthesis of abnormal proteins increases exponentially over time with age. Cells taken from elderly people show the reduced levels of transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of Bulgarian centenarians in the mountainous municipality of Teteven and their age is close to exponential.

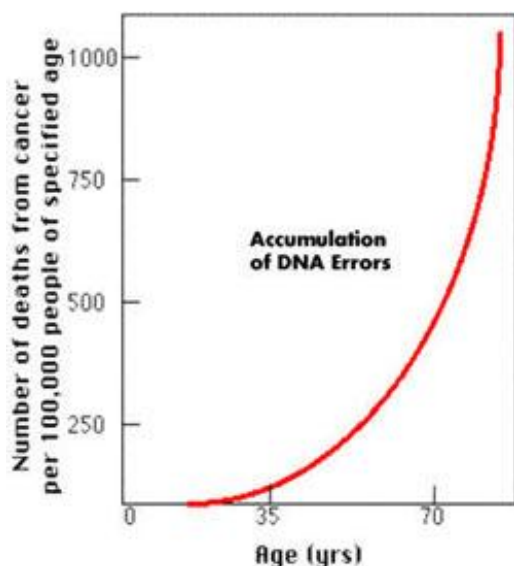


Figure 2. Interrelation between age and the number of cancer patients [7]

Here are submitted the data on longevity for Bulgaria:

- 1) Varna district – 44 centenarians per 1 million of inhabitants, plain and sea regions;
- 2) Pleven district – 78 centenarians per 1 million of inhabitants, plain regions;
- 3) Teteven district – 279 centenarians per 1 million of inhabitants, hills and mountainous regions;
- 4) Bulgaria – 47 centenarians per 1 million of inhabitants.

Analogous situation is observed in the Russian North. According to G. Berdishev, people inhabiting the Russian North – the Yakuts and the Altaians as well as the Buryats, drink mountain water obtained after the melting of ice. Altai and Buryat, Caucasus water sources in Russia are known as moderately warm, with temperatures of 8–10 °C, the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with ordinary tap water that is believed to have a positive effect on the tissue cells and metabolism. Melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and has a beneficial effect on metabolism [8]. In the world are popular the sources with melt water from Canada, Norway, Island and Alaska.

3.2. Clinical Evidence with Human Blood Serum Testing

It was established experimentally that in the process of evaporation of water drops, the wetting angle θ decreases discreetly to zero, and the diameter of water drop basis is only slightly altered, that is a new physical effect [9]. Based on this effect, by means of measurement of the wetting angle within equal intervals of time is determined the function of distribution of H_2O molecules according to the value of $f(\theta)$. The distribution function is denoted as the energy spectrum of the water state. The theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O -molecules. The hydrogen bonding results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen

acceptor) on the neighboring H₂O molecule; the structure of hydrogen bonding may be defined as O...H^{δ+}-O^{δ-}.

For calculation of the function f(E) represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14,33f(\theta)}{[1-(1+bE)^2]^2} \quad (1)$$

where $b = 14.33 \text{ eV}^{-1}$

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H₂O molecules is calculated by the formula:

$$\theta = \arccos(-1 - 14.33E) \quad (2)$$

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used.

The difference $\Delta f(E) = f(\text{samples of water}) - f(\text{control sample of water})$ – is called the “differential non-equilibrium energy spectrum of water” (DNES).

Thus, the DNES spectrum is an indicator of structural changes in water, because the energy of hydrogen bonds in water samples differ due to the different number of hydrogen bonds in water samples, which may result from the fact that different waters have different structures and composition and various intermolecular interactions – various associative elements etc. [10]. The redistribution of H₂O molecules in water samples according to the energy is a statistical process of dynamics.

Figure 3 shows the average NES-spectrum of deionised water. On the X-axis are shown three scales. The energies of hydrogen bonds among H₂O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H₂O molecules according to energies f(E), measured in unit eV⁻¹. For DNES spectrum the function is Δf(E) in unit eV⁻¹. Arrow A designates the energy of hydrogen bonds among H₂O molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among H₂O molecules the value of which is calculated:

$$\bar{E} = -0.1067 \pm 0.0011 \text{ eV} \quad (3)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature 36.6 °C, is at its maximum. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

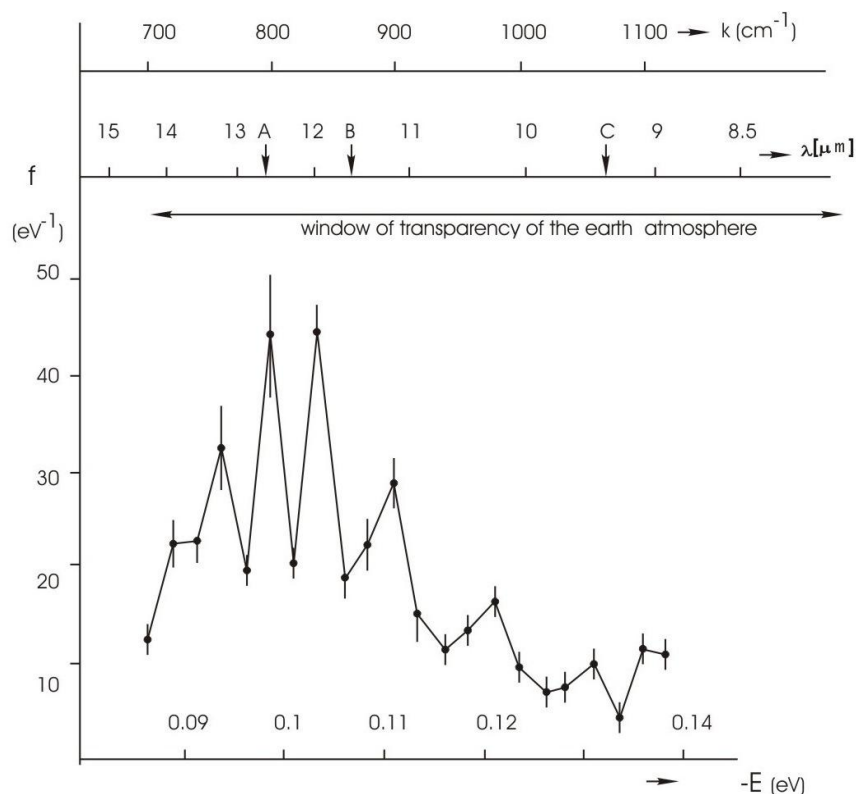


Figure 3. Non-equilibrium (NES) spectrum of deionized water (chemical purity – 99.99 %; pH – 6,5–7,5; total mineralization – 200 mg/l; electric conductivity – 10 $\mu\text{S}/\text{cm}$): the horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV); the vertical axis – the energy distribution function – f (eV^{-1}); k – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm)

The study of the IR spectrum of water in the composition of physiologic fluids (urine, blood, serum) can also provide data on metabolic processes in the human body and longevity, because the IR-spectrum reflects the metabolic processes. Authors have conducted studies of a 1% (v/v) solution of blood serum by spectral analysis of non-equilibrium energy (NES) spectrum and differential equilibrium energy (DNES) spectrum on two groups of people between 50 and 70 years of age. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. As a main biophysical parameter was investigated the average energy of hydrogen bonds ($\Delta E_{\text{H}\dots\text{O}}$) among H_2O molecules in the blood serum. The result was obtained as a difference between the NES-spectrum of 1% solution of blood serum and NES-spectrum of deionized water control sample – DNES-spectrum, measured as the difference $\Delta f(E) = f(\text{samples of water}) - f(\text{control sample of water})$. The DNES-spectrum obtained from the first group has a local maximum energy ($\Delta E_{\text{H}\dots\text{O}}$) at -9.1 ± 1.1 meV and from the second group -1.6 ± 1.1 meV. The results between the two groups have a statistical difference in Student's criterion at $p < 0.05$. For the control group of healthy people the value of the largest local maximum in the DNES-spectrum was detected at $E = -0.1387$ eV, or at a wavelength of $\lambda = 8.95$ μm . For the group of people in a critical state and the patients with malignant tumors, the analogous values of the largest local maximums of the DNES-spectrum shifted to lower energies compared with the control group of people. Water in the human body possesses IR-spectrum that reflects the metabolic processes in the organism. It can be demonstrated by analysis of human blood serum by IR-spectroscopy. The magnitude of the largest local maximum in IR-spectrum of blood serum from healthy people of control group observed at -0.1387 eV at a wavelength – 8.95 μm . For a group of people in critical health condition and patients with malignant tumors the greatest values of local extremum in the IR-spectrum are shifted to lower energies relative to the control group. In IR-spectrum of human blood serum are detected local maxima at $\lambda = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35$ and 9.76 μm [11]. The resulting peak at $\lambda = 8.95$ μm in IR-spectrum

detected by us [12] approaching the peak at $\lambda = 8.85 \mu\text{m}$ monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function $f(E)$ at $\lambda = 8.95 \mu\text{m}$ compiles 75.3 eV, and in a group of people in critical condition – 24.1 eV. The level of reliability of the results is $p < 0.05$ according to the Student's t-test. In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water [13]. There was a decrease of the spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the spectrum of human blood serum of terminally ill people relative to that of healthy people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by DNES at $-5.5 \pm 1.1 \text{ meV}$, the difference in age was of 20–25 years in relation to the control group. It should be noted that most of Bulgarian centenarians inhabit the Rhodopes Mountains areas. Among to the DNES-spectrum of mountain waters similar to the DNES-spectrum of blood serum of healthy people at $\lambda = 8.95 \mu\text{m}$, was the DNES-spectrum of water in the Rhodopes. The mountain waters from Teteven, Boyana and other Bulgarian provinces have similar parameters. Tables 1, 2 and 3 show the composition of mountain springs in Teteven and Kuklen (Bulgaria) and local extremums in NES-spectra of water. The local extremums were detected at $E = -0.11 \text{ eV}$ and $E = -0.1387 \text{ eV}$. The value at $E = -0.11 \text{ eV}$ is characteristic for the presence of Ca^{2+} . The value at $E = -0.1380 \text{ eV}$ is characteristic for inhibiting the growth of cancer cells. Experiments conducted by A. Antonov with cancer cells of mice demonstrated a reduction of this local extremum to a negative value. Analysis by the DNES-method of aqueous solutions of natural mineral sorbents – shungite (carbonaceous mineral from Zazhoginskoe deposit in Karelia, Russia) and zeolite (microporous crystalline aluminosilicate mineral from Most village, Bulgaria) showed the presence of a local maximum at $E = -0.1387 \text{ eV}$ for shungite and $E = -0.11 \text{ eV}$ for zeolite [14]. It should be noted that owing to the unique porous structures both the natural minerals shungite and zeolite are ideal natural water adsorbents effectively removing from water organochlorine compounds, phenols, dioxins, heavy metals, radionuclides, and color, and gives the water a good organoleptic qualities, additionally saturating it with micro-and macro-elements [15]. It is worth to note that in Bulgaria the main mineral deposits of Bulgarian zeolites are located in the Rhodope Mountains, whereat has lived the greatest number of Bulgarian centenarians. It is thought that water in these areas is cleared in a natural way by zeolite. Therefore, a new parameter is researched – a local extremum of energy at (-0.1362– -0.1387 eV). This value was determined by the NES-spectrum as function of distribution of individual H_2O molecules according to energy $f(E)$. The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local extremum of $f(E) \sim 24.1 \text{ eV}^{-1}$. The function of distribution according to energy $f(E)$ for tap water in Teteven is $11.8 \pm 0.6 \text{ eV}^{-1}$.

3.3. Composition of water in the mountain area in Teteven municipality in Stara Planina Mountain and Kuklen municipality, Rhodopes Mountain

The statistical data shows that the difference between the age of long lived people in mountain and plain areas is 3.7 years. The analyses of water sources show the differences regarding chemical composition, hardness, local extremum in NES-spectra of water eV^{-1} at (-0.1362– -0.1387 eV), isotopic shifts of D/H in water.

Tables 1, 2 and 3 show the chemical composition of mountain springs in Teteven and Kuklen (Bulgaria) and local extremums in NES-spectra of water.

Table 1. The composition of mountain water springs in Zlatishko-Tetevenska Mountain (Teteven municipality, Bulgaria) and local extremums in NES-spectra of water

Indicators	Results of the research (mg/l)	Norm
Sodium (Na ⁺)	0.96	<200
Calcium (Ca ²⁺)	100.4	<150
Magnesium (Mg ²⁺)	12.65	<80
Iron (Fe)	0.016	<0.2
Manganese (Mn ²⁺)	0.0018	<0.2
Zinc (Zn ²⁺)	0.18	<4.0
Sulfates (SO ₄ ²⁻)	81.8	<250
Chlorides (Cl ⁻)	3.96	<250
Carbonates (CO ₃ ²⁻)	<2.0	–
Hydrocarbonates (HCO ₃ ⁻)	184.0	–
Other values	Results	
Active reaction (pH)	7.9 alkaline	6.5–9.5
Electroconductivity	536.8 μS/cm	<2000
Hardness of water	16.5 dH hard	<33.7
Local extremum* eV ⁻¹ at (-0.1362--0.1387 eV)	36.9	>24.1

*Function of distribution of H₂O molecules according to energy f(E)

Table 2. The composition of mountain water springs in Vasiliovka Mountain (Teteven municipality, Bulgaria) and local extremum in NES-spectra of water.

Indicators	Results of the research (mg/l)	Norm
Sodium (Na ⁺)	4.5	<200
Calcium (Ca ²⁺)	55.5	<150
Magnesium (Mg ²⁺)	2.28	<80
Iron (Fe)	0.0127	<0.2
Manganese (Mn ²⁺)	0.0014	<0.2
Zinc (Zn ²⁺)	0.006	<4.0
Sulfates (SO ₄ ²⁻)	16.9	<250
Chlorides (Cl ⁻)	3.4	< 250
Carbonates (CO ₃ ²⁻)	< 2.0	–
Hydrocarbonates (HCO ₃ ⁻)	118.0	–
Other values	Results	
Active reaction (pH)	7.4 alkaline	6.5–9.5
Electroconductivity	285.0 μS/cm	<2000
Hardness of water	7.9 dH slightly hard	<33.7
Local extremum* eV ⁻¹ at (-0.1362--0.1387 eV)	40.1	>24.1

*Function of distribution of H₂O molecules according to energy f(E)

Table 3. The composition of mountain water spring Eco Hotel Zdravetz, Rhodopes Mountain (Kuklen municipality, Bulgaria) and local extremum in NES-spectra of water

Indicators	Results of the research (mg/l)	Norm
Sodium (Na ⁺)	7.6	<200
Calcium (Ca ²⁺)	3.5	<150
Magnesium (Mg ²⁺)	0.63	<80
Iron (Fe)	0.007	<0.2
Manganese (Mn ²⁺)	0.002	<0.2
Zinc (Zn ²⁺)	0.007	<4.0
Sulfates (SO ₄ ²⁻)	26.8	<250
Chlorides (Cl ⁻)	3.00	<250
Carbonates (CO ₃ ²⁻)	<2.0	–
Hydrocarbonates (HCO ₃ ⁻)	21.3	–
Other values	Results	
Active reaction (pH)	5.93 normal	6.5–9.5
Electroconductivity	536.8 μS/cm	<2000
Hardness of water	1.4 dH soft	<33.7
Local extremum* eV ⁻¹ at (-0.1362--0.1387 eV)	59.3	>24.1

*Function of distribution of H₂O molecules according to energy f(E)

The research shows the results of water composition in field area of Dolni Dabnik. The results are: hydrocarbonates (HCO₃⁻) – 184.4 mg/l, sulfates (SO₄²⁻) – 19.2 mg/l, chlorides (Cl⁻) – 9.2 mg/l, calcium (Ca²⁺) – 50.6 mg/l, sodium (Na⁺) – 14.2 mg/l. The hardness makes up 26.2 dH – it is very hard water. The maximum peak in NES-spectra of water (eV⁻¹) at (-0.1362--0.1387 eV) in water of Danubian Plain is 23.2 eV⁻¹ and in Thracian Valley is detected at -21.3 eV⁻¹. In water samples from the Danubian Plain and Thracian Valley there are data for the presence of nitrites (NO₂), nitrates (NO₃), ammonia (NH₄), phosphates (HPO₄) more than norm.

Table 4 shows optimal chemical composition of water, hardness, local extremum eV⁻¹ at (-0.1362--0.1387 eV), hardness and total mineralization of water as middle result of different studies. The areas are between 600 m and 1300 m attitude in Bulgaria and from Caucasus, Russia. In these areas are living long lived people.

Table 4. Optimal chemical composition of water, hardness, local extremum eV⁻¹ at (-0.1362--0.1387 eV) and total mineralization of water

Indicators	Results of melt and mountain water (Bulgaria) (mg/l)	Results of melt water (Russia) (mg/l)
Sodium(Na ⁺) + Potassium (K ⁺)	6.1	<30
Calcium (Ca ²⁺)	29.5	<50
Magnesium (Mg ²⁺)	1.5	<10
Iron (Fe)	0.083	–
Manganese (Mn ²⁺)	0.0017	–
Zinc (Zn ²⁺)	0.007	–
Sulfates (SO ₄ ²⁻)	21.9	<100
Chlorides (Cl ⁻)	3.2	<70
Carbonates (CO ₃ ²⁻)	<2.0	–
Hydrocarbonates (HCO ₃ ⁻)	69.7	<100

Other values	Results	
Active reaction (pH)	6.7 normal	6.5-7.0
Electroconductivity	410.9 $\mu\text{S}/\text{cm}$	<2000
Hardness of water	4.65 dH Moderately soft	<33.7
Total mineralization (g/l)	0.132	<0.3
Local extremum* eV^{-1} at (-0.1362-- 0.1387 eV)	49.7	>24.1

*Function of distribution of H_2O molecules according to energy $f(E)$

3.4. Effects of Calcium, Magnesium, Zinc and Manganese in water on biophysical and biochemical processes in the human body

The research into distribution of local extremums (eV^{-1}) in spectra of various water samples as a function of distribution of H_2O molecules according to energy $f(E)$ at $\lambda = 8.95 \mu\text{m}$ shows the analogue extremum at analogous values of $f(E)$, E and λ , which was detected in water with Ca^{2+} ions earlier demonstrated inhibiting the growth of cancer cells. Magnesium (Mg^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}) ions dissolved in water have influence on enzymes, which are antioxidants [16]. The research of China team was categorized three groups of elements from the rice and drinking water according to their effect on longevity: Sr, Ca, Al, Mo, and Se, which were positively correlated with longevity: Fe, Mn, Zn, Cr, P, Mg, and K, which had a weak effect on local longevity, and Cu and Ba, which had a negative effect on longevity [17]. There was a positive correlation between the eSOD activity and the age and a negative correlation between the eSOD activity and concentration of Zn^{2+} in plasma. An inverse correlation was also found between the content of Zn^{2+} ions in plasma relative to the age. The prevalence of Zn^{2+} deficiency is increased with age; with normal Zn^{2+} levels it is observed in about 80% of adult people and only in 37 % of the non-agenarians. Aging is an inevitable biological process that is associated with gradual and spontaneous biochemical and physiological changes and the increased susceptibility to diseases. Because the nutritional factors are involved in improving the immune functions, metabolic balance, and antioxidant defense, some nutritional factors, such as Zn, may modify susceptibility to disease and promote healthy aging. *In vitro* (human lymphocytes exposed to endotoxins) and *in vivo* (old or young mice fed with low zinc dietary intake) studies revealed that zinc is important for immune efficiency (innate and adaptive), antioxidant activity (superoxide dismutase), and cell differentiation *via* clusterin/apolipoprotein J. The intracellular Zn homeostasis is regulated by metallothioneins (MT) *via* an ion release through the reduction of thiol groups in the MT molecule [18]. Zinc in composition of water improves the antioxidative enzymes in red blood cells [19].

The magnesium deficiency and oxidative stress have both been identified as pathogenic factors in aging and in several age-related diseases. The link between these two factors is unclear in humans although, in experimental animals, severe Mg^{2+} deficiency has been shown to lead to the increased oxidative stress [20]. The antioxidants against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), β -carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, Mn) and proteins such as ceruloplasmin (Co). The extent of the tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system [21]. The norm in water for Zn^{2+} and Mg^{2+} according to the World Health Organization (WHO) should be less than 20 μg . For the Na^+ content the norm according to the WHO is less than 20 mg.

The interesting results on the concentration of Ca^{2+} in water were obtained in USA and Canada. According to the statistical information the most number of centenarians in Canada per 1 million of population is observed in Nova Scotia (210 of centenarians per 1 million). In water from Nova Scotia the Ca^{2+} content makes up 6.8 mg/l. N. Druzhyak, Russia showed that in the places wherein live the most number of centenarians the Ca^{2+} content in water was 8–20 mg/l. The only risk factor regarding the increased Ca^{2+} content in water is cardiovascular diseases [22].

The following reactions occur in water if there are high concentrations of Ca^{2+} and Mg^{2+} ions: the reaction of limestone (CaCO_3) and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) with water to separate the calcium (Ca^{2+}), carbonates (CO_3^{2-}) and sulfate (SO_4^{2-}) ions. By increasing the mineralization of water the content of Ca^{2+} ions decreases. During the concentration of the solutions Ca^{2+} ions are precipitated. With the increase of carbon dioxide (CO_2) in water and decreasing of the pH value the content of

Ca²⁺ increases. The reaction of interaction of dolomite (CaCO₃·MgCO₃) with water makes the formation of Mg²⁺ ions. Hydrocarbonates (HCO₃⁻) and carbonates (CO₃²⁻) ions are formed by reaction of interaction of karst rocks, CO₂ and water.

3.5. The measurement of deuterium content in mountain and melt water

Preliminary analyses of water from various water sources show that mountain and melt water as the result of natural isotope purification contains less amount of deuterium. This water also contains ions of Ca²⁺, Mg²⁺, Na⁺, HCO₃⁻ and SO₄²⁺. The content of K⁺ and N⁺ cations in the melt water are <30 mg/l, Mg²⁺ – <10 mg/l, Ca²⁺ – <50 mg/l, the content of SO₄²⁻ – <100 g/l, HCO₃⁻ <100 mg/l, Cl⁻ – less than 70 mg/l, total rigidity ≤ 5 mEq/l, the total mineralization ≤ 0.3 g/l, pH – 6.5–7.0 at 25 °C (Table 5). The degree of natural purification of melt water from impurities makes up ~50–60%. The concentration of salts of rigidity – Ca²⁺, Mg²⁺, Fe²⁺, Fe³⁺, heavy metals and organochlorine compounds, as well as heavy isotopes, including deuterium in melt water is less that of ordinary portable water. This fact is important because some authors consider the hardness of the water to be among the main factors in cardiovascular diseases. However, mild correlation was further proven that water hardness could not be a decisive factor for human longevity.

Table 5. Chemical composition of melt water obtained from tap water by the freeze-thaw method

Cations, mg/l	
K ⁺ + Na ⁺	20–25
Mg ²⁺	5–10
Ca ²⁺	25–30
Anions, mg/l	
SO ₄ ²⁻	<90
HCO ₃ ⁻	50–100
Cl ⁻	<50
Other physical characteristics	
Total rigidity, mEq/l	≤5
Total mineralization, g/l	≤0,2
pH at t = +25 °C	6.5–7.0
Deuterium content, ppm	~129.5

The analyses of water from various sources of Russia and Bulgaria show that the mountain water contains on average ~2–4% less deuterium in form of HDO, than the river water and sea water. In natural waters, the deuterium content is distributed irregularly: from 0.02–0.03 mol.% for river and sea water, to 0.015 mol.% for water of Antarctic ice – the most purified from deuterium natural water containing deuterium in 1.5 times less than that of seawater. According to the international SMOW standard isotopic shifts for D and ¹⁸O in sea water: D/H = (155.76±0.05)·10⁻⁶ (155.76 ppm) and ¹⁸O/¹⁶O = (2005.20±0.45)·10⁻⁶ (2005 ppm) [23]. For SLAP standard isotopic shifts for D and ¹⁸O in seawater: D/H = 89·10⁻⁶ (89 ppm) and for a pair of ¹⁸O/¹⁶O = 1894·10⁻⁶ (1894 ppm). In surface waters, the ratio D/H = ~(1.32–1.51)·10⁻⁴, while in the coastal seawater – ~(1.55–1.56)·10⁻⁴. Waters of other underground and surface water sources contain varied amounts of deuterium (isotopic shifts) – from δ = +5,0 D,%, SMOW (Mediterranean Sea) to to δ = -105 D,%, SMOW (Volga River). The natural waters of CIS countries are characterized by negative deviations from SMOW standard to (1.0–1.5)·10⁻⁵, in some places up to (6.0–6.7)·10⁻⁵, but there are observed positive deviations at 2.0·10⁻⁵. The content of the lightest isotopomer – H₂¹⁶O in water corresponding to SMOW standard is 997.0325 g/kg (99.73 mol.%), and for SLAP standard – 997.3179 g/kg (99.76 mol.%).

The thawed snow and glacial water in the mountains and some other regions of the Earth also contain less deuterium than ordinary drinking water. On average, 1 ton of river water contains 150–200 g deuterium [24]. The average ratio of H/D in nature makes up approximately 1:5700. According to the calculations, the human body throughout life receives about 80 tons of water

containing in its composition 10–12 kg of deuterium and associated amount of heavy isotope ^{18}O . That is why it is so important to purify water from heavy isotopes of D and ^{18}O .

The local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state; the substitution with deuterium changes the vibrational-rotational transitions in H_2O molecule that is why it appears other local maximums in IR-spectra. In the water vapor state, the vibrations involve combinations of symmetric stretch (ν_1), asymmetric stretch (ν_3) and bending (ν_2) of the covalent bonds with absorption intensity (H_2O) $\nu_1; \nu_2; \nu_3 = 2671; 1178.4; 2787.7 \text{ cm}^{-1}$. For liquid water absorption bands are observed in other regions of the IR-spectrum, the most intense of which are located at $2100, \text{ cm}^{-1}$ and $710\text{--}645 \text{ cm}^{-1}$. For D_2O molecule these ratio compiles $2723.7, 1403.5$ and 3707.5 cm^{-1} , while for HDO molecule – $2671.6, 1178.4$ and 2787.7 cm^{-1} . HDO (50 mole% H_2O + 50 mole% $^2\text{H}_2\text{O}$; ~50 % HDO, ~25 % H_2O , ~25 % D_2O) has local maxima in IR-spectra at 3415 cm^{-1} , 2495 cm^{-1} , 1850 cm^{-1} and 1450 cm^{-1} assigned to OH^- -stretch, OD^- -stretch, as well as combination of bending and libration and HDO bending respectively. The local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state because at changing the atomic mass of hydrogen and deuterium atoms in the water molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same; with the substitution with deuterium the vibrational-rotational transitions are changed, that is why it appears other local maximums in IR-spectra. The result is reliable regarding the content of deuterium in natural waters from 0.015–0.03%.

In the IR-spectrum of liquid water absorbance band considerably broadened and shifted relative to the corresponding bands in the spectrum of water vapor. Their position depends on the temperature [25]. However, the temperature dependence of individual spectral bands of liquid water is very complex [26]. Furthermore, the complexity of the IR-spectrum in the area of OH^- stretching vibration can be explained by the existence of different types of H_2O associations, manifestation of overtones and composite frequencies of OH^- groups in the hydrogen bonds, and the tunneling effect of the proton (for relay mechanism) [27]. Such complexity makes it difficult to interpret the spectrum and partly explains the discrepancy in the literature available on this subject.

In liquid water and ice the IR-spectra are far more complex than those ones of the vapor due to vibrational overtones and combinations with librations (restricted rotations, e.g. rocking motions). These librations are due to the restrictions imposed by hydrogen bonding (minor L_1 band at 395.5 cm^{-1} ; major L_2 band at 686.3 cm^{-1} ; for liquid water at $0 \text{ }^\circ\text{C}$, the absorbance of L_1 increasing with increasing temperature, while L_2 absorbance decreases but broadens with reduced wave number with increasing temperature [28]. The IR spectra of liquid water usually contain three absorbance bands, which can be identified on absorption band of the stretching vibration of OH^- group; absorption band of the first overtone of the bending vibration of the molecule HDO and absorption band of stretching vibration of OD^- group. Hydroxyl group OH^- is able to absorb much infrared radiation in the infrared region of the IR-spectrum. Because of its polarity, these groups typically react with each other or with other polar groups to form intra-and intermolecular hydrogen bonds. The hydroxyl groups, which are not involved in formation of hydrogen bonds, usually produce the narrow bands in IR spectrum, while the associated groups – broad intense absorbance bands at lower frequencies. The magnitude of the frequency shift is determined by the strength of the hydrogen bond. Complication of the IR spectrum in the area of OH^- stretching vibrations can be explained by the existence of different types of associations of H_2O molecules, a manifestation of overtones and combination frequencies of OH^- groups in hydrogen bonding, as well as the proton tunneling effect (on the relay mechanism).

An assignment of main absorption bands in the IR-spectrum of liquid water is given in (Table 6). The IR spectrum of H_2O molecule was examined in detail from the microwave till the middle ($4\text{--}17500 \text{ cm}^{-1}$) visible region and the ultraviolet region – from 200 nm^{-1} to ionization limit at 98 nm^{-1} [29]. In the middle visible region at $4\text{--}7500 \text{ cm}^{-1}$ are located rotational spectrum and the bands corresponding to the vibrational-rotational transitions in the ground electronic state. In the ultraviolet region (200 nm^{-1} to 98 nm^{-1}) are located bands corresponding to transitions from the excited electronic states close to the ionization limit in the electronic ground state. The intermediate region of the IR-spectrum – from 570 nm to 200 nm corresponds to transitions to higher vibrational levels of the ground electronic state.

The results of IR-spectroscopy with device Infra Spec VFA-IR show that at 4.1 μm , even at low concentrations of deuterium of 0.35 and 0.71%, there is observed a decline in the local maximums relative to the local maximum of 100% pure water (the local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state because at changing the atomic mass of hydrogen and deuterium atoms in the water molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same; with the substitution with deuterium the vibrational-rotational transitions are changed, that is why it appears other local maximums in IR-spectra. The result is reliable regarding the content of deuterium in natural waters from 0.015–0.03%.

Table 6. The assignment of main frequencies in IR-spectra of H_2O and D_2O

Main vibrations of liquid H_2O and $^2\text{H}_2\text{O}$				
Vibration(s)	H_2O (t = +25 °C)		D_2O (t = +25 °C)	
	ν , cm^{-1}	E_0 , $\text{M}^{-1} \text{cm}^{-1}$	ν , cm^{-1}	E_0 , $\text{M}^{-1} \text{cm}^{-1}$
Spinning ν_1 + deformation ν_2	780-1645	21.65	1210	17.10
Composite $\nu_1 + \nu_2$	2150	3.46	1555	1.88
Valence symmetrical ν_1 , valence asymmetrical ν_3 , and overtone $2\nu_2$	3290-3450	100.65	2510	69.70

At further transition from H_2O monomers to H_4O_2 dimer and H_6O_3 trimer absorption maximum of valent stretching vibrations of the O-H bond is shifted toward lower frequencies ($\nu_3 = 3490 \text{ cm}^{-1}$ and $\nu_1 = 3280 \text{ cm}^{-1}$) [30] and the bending frequency increased ($\nu_2 = 1644 \text{ cm}^{-1}$) because of hydrogen bonding. The increased strength of hydrogen bonding typically shifts the stretch vibration to lower frequencies (red-shift) with greatly increased intensity in the infrared due to the increased dipoles. In contrast, for the deformation vibrations of the H–O–H, it is observed a shift towards higher frequencies. Absorption bands at 3546 and 3691 cm^{-1} were attributed to the stretching modes of the dimer [$(\text{H}_2\text{O})_2$]. These frequencies are significantly lower than the valence modes of ν_1 and ν_3 vibrations of isolated H_2O molecules at 3657 and 3756 cm^{-1} respectively). The absorption band at 3250 cm^{-1} represents overtones of deformation vibrations. Among frequencies between 3250 and 3420 cm^{-1} is possible Fermi resonance (this resonance is a single substitution of intensity of one fluctuation by another fluctuation when they accidentally overlap each other). The absorption band at 1620 cm^{-1} is attributed to the deformation mode of the dimer. This frequency is slightly higher than the deformation mode of the isolated H_2O molecule (1596 cm^{-1}). A shift of the band of deformation vibration of water in the direction of high frequencies at the transition from a liquid to a solid state is attributed by the appearance of additional force, preventing O–H bond bending. Deformation absorption band in IR-spectrum of water has a frequency at 1645 cm^{-1} and very weak temperature dependence. It changes little in the transition to the individual H_2O molecule at a frequency of 1595 cm^{-1} . This frequency is found to be sufficiently stable, while all other frequencies are greatly affected by temperature changes, the dissolution of the salts and phase transitions. It is believed that the persistence of deformation oscillations is stipulated by processes of intermolecular interactions, e.g. by the change in bond angle as a result of interaction of H_2O molecules with each other, as well as with cations and anions.

Thus the study of the characteristics of the IR spectrum of water allows to answer the question not only on the physical parameters of the molecule and the covalent bonds at isotopic substitution with deuterium, but also to make a certain conclusion on associative environment in water. The latter fact is important in the study of structural and functional properties of water associates and its isotopomers at the isotopic substitution with deuterium. The substitution of H with D affects the stability and geometry of hydrogen bonds in an apparently rather complex way and may, through the changes in the hydrogen bond zero-point vibration energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of DNA and proteins in D_2O [31] (Cleland, 1976). It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype [32, 33].

Our experiments demonstrated that the effects of deuterium on the cell possess a complex multifactor character connected to changes of physiological parameters – magnitude of the lag-period, time of cellular generation, outputs of biomass, a ratio of amino acids, protein, carbohydrates and fatty acids synthesized in D₂O, and with an evolutionary level of organization of investigated object as well [34, 35]. The cell evidently implements the special adaptive mechanisms promoting functional reorganization of work of the vital systems in the presence of D₂O.

4. Conclusion

In frames of this research 415 people living in the municipalities of Teteven, Yablanitza, Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Pleven district (Bulgaria), where is lived the most number of long lived people and their siblings, were studied. They have the same heredity, but have lived under different conditions. The research shows that the direct relationship of man and nature – clean air, natural food from eco-farms and physical activity explains the difference between the larger number of long lived people and centenarians who live in the mountain regions of Bulgaria and Russia and their high average number. Natural mountain and melt water with chemical composition, less deuterium seems to be one of the most important factors for longevity. In Bulgaria, most long lived people and centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. It worth to note that IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at $\lambda = 8.95 \mu\text{m}$. Similar spectral characteristics possess mountain water from Teteven and other Bulgarian sources. Thus, the phenomenon of longevity is a complex phenomenon involving both genetic and phenotypic characteristics of the organism to external factors and environment – free radicals, radiation, heavy isotopes, as well as the structure and the isotopic composition of drink water. Other longevity factors are living area, health status, body mass, gender and heredity. Studying the human blood serum by NES and DNES-methods show that by measuring the average energy of hydrogen bonds among H₂O molecules and the distribution function of H₂O molecules on energies it is possible to show a vital state status of a person and associated life expectancy. These data indicate that water in the human body has the IR-spectrum resembling the IR-spectrum of human blood serum. On the characteristics of the IR-spectrum of water exerts an influence also the presence of deuterium. In the research was studied an optimal composition of mountain and melt water from areas where were lived the long live people and centenarians. There are also new proofs for biophysical and biochemical effects of calcium, magnesium, zinc and manganese in water.

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Изучение состава и свойств горной и талой воды Болгарии и России как факторов долголетия. Эффекты катионов кальция, магния, цинка и марганца в воде на организм человека

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Аннотация

Показано, что горная и талая вода являются важными факторами долголетия. К другим факторам относятся наследственность, пол, масса тела, питание, психологический статус, семейные отношения. Природные воды, полученные из различных болгарских родников, а также талая вода и сыворотка крови больных раком людей 50-70 лет были исследованы с помощью ИК-, НЭС- и ДНЭС-методов. Мы применили НЭС- и ДНЭС-методы для расчета средней энергии водородных связей ($\Delta E_{H...O}$) между молекулами H_2O в образцах, а также распределение молекул H_2O процентов по энергиям водородных связей в интервале энергий (от -0.08 до -0.1387 эВ). Как фактор оценки измеряли значения средней энергии водородных связей ($\Delta E_{H...O}$) между молекулами H_2O , а также локальных экстремумов в ДНЭС- и ИК-спектрах различных образцов воды и сыворотки крови человека, детектируемые при $E = -0,1387$ эВ и $\lambda = 8,95$ мкм. Для группы людей в критическом состоянии жизни и больных со злокачественными опухолями наибольшие значения локальных экстремумов в ДНЭС-спектрах смещены в сторону меньших энергий по сравнению с контрольной группой. ИК-спектр горной воды наиболее близок к ИК-спектру сыворотки крови группы здоровых людей с локальным максимумом $\lambda = 8.95$ μm . Природная горные и талая вода с уникальным химическим составом элементов и меньшим содержанием дейтерия рассматривается нами как один из самых важных факторов долголетия. В Болгарии, наибольшее количество долгожителей живут в горах Родопы, в то время как в России – в Дагестане и Якутии. Аналогичные характеристики имеет горная вода из Тетевен и других болгарских источников. Получены новые доказательства биофизических и биохимических эффектов Ca^{2+} , Mg^{2+} , Zn^{2+} и Mn^{2+} в воде.

Ключевые слова: долголетие, горная вода, талая вода, ИК-спектроскопия, НЭС, ДНЭС.

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