

Studying the Composition and Properties of Mountain and Melt Water of Bulgaria and Russia as Factors of Longevity

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Abstract

This paper shows that mountain and melt water is among the most important factors for longevity. Other factors are – hereditary, gender, body weight, food, movement, 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. 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 -0.1387 eV and wavelength – $8.95 \mu m$. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in DNES- and IR-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 $\lambda = 8,95 \mu m$. The authors 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 water molecules according to energies of hydrogen bonds from intervals (-0.08 to -0.1387 eV). 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. Similar characteristics possess mountain water from Teteven and other Bulgarian sources

Keywords: deuterium, heavy water, deuterium depleted water, longevity, mountain 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 (Pocock *et al.*, 1981; Howard & Hopps, 1986). 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 factor for metabolic processes and aging (Ignatov, 2012). 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 (Mosin & Ignatov, 2012; Ignatov & Mosin, 2013; Ignatov & Mosin, 2013). These factors and the structure of water are of great importance in biophysical studies. The peculiarities of chemical structure of H_2O 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 H_2O molecules, binding them into water associates (clusters) with the general formula $(H_2O)_n$ where n varies from 3 to 50 units (Saykally, 2005; Ignatov, Mosin, 2013).

Other important indicator of water quality is its isotopic composition. The natural water consists on 99.7 mol.% of $H_2^{16}O$, which molecules are formed by 1H and ^{16}O atoms (Mosin & Ignatov, 2012). The remaining 0.3 mol.% is represented by isotope varieties (isotopomers) of water molecules, wherein deuterium forms 6 configurations of isotopomers – $HD^{16}O$, $HD^{17}O$, $HD^{18}O$, $D_2^{16}O$, $D_2^{17}O$, $D_2^{18}O$, while 3 configuration are formed by isotopomers of oxygen – $H_2^{16}O$, $H_2^{17}O$, $H_2^{18}O$.

In frames of this project 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 D_2O (99.9 atom%) received from the Russian Research Centre “Isotope” (St. Petersburg, Russian Federation). Inorganic salts were preliminary crystallized in D_2O and dried in vacuum before using. D_2O distilled over $KMnO_4$ 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 from 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 μ m using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) 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 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) 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 People 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).

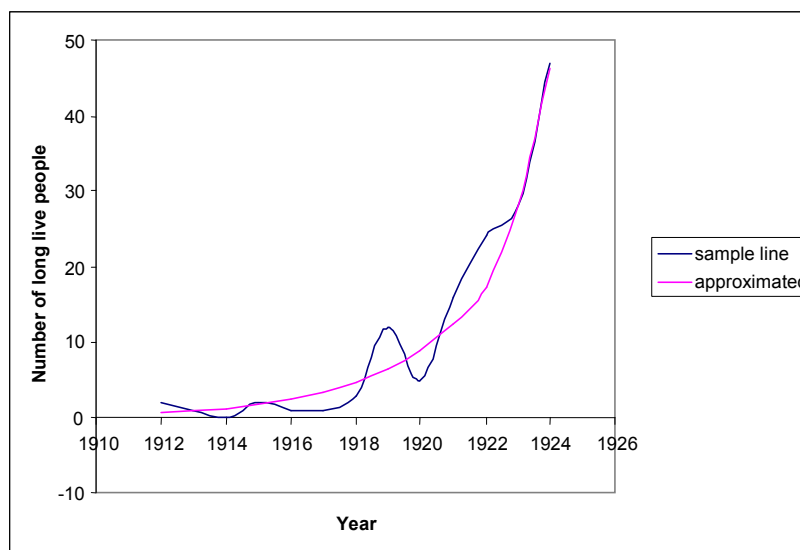


Figure 1: Interrelation between the year of birth of long lived people (age) and their number in Teteven municipality, Bulgaria.

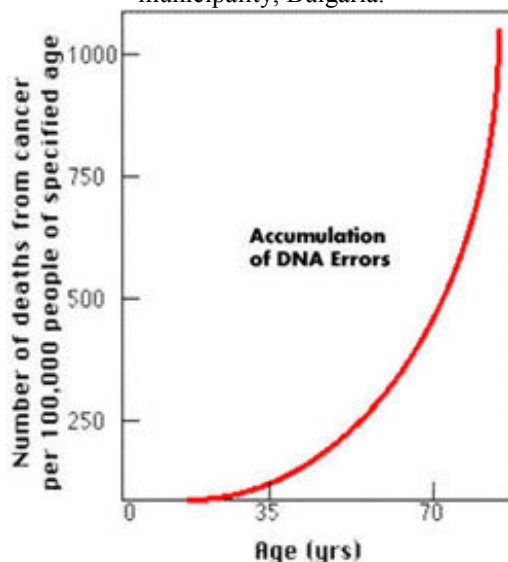


Figure 2: Interrelation between age and the number of cancer patients (Orgel, 1963).

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 (Orgel, 1963). 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.

Here are submitted the data for Bulgaria:

Varna district – centenarians 44 per 1 million of inhabitants, plain and sea regions;

Pleven district – centenarians 78 per 1 million of inhabitants, plain regions;

Teteven district – centenarians 279 per 1 million of inhabitants, hills and mountainous regions;

Bulgaria – centenarians 47 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, Kavkaz water sources 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 (Goncharuk *et al.*, 2013). 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 the process of evaporation of water drops, the wetting angle θ decreases discreetly to 0, and the diameter of water drop basis is only slightly altered, that is a new physical effect (Antonov, 1995; Antonov & Yuskesseliava, 1983). Based on this effect, by means of measurement of the wetting angle within equal intervals of time is determined the function of distribution of H₂O molecules according to the value of $f(\theta)$. The distribution function is denoted as the energy spectrum of the water state. Theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H₂O-molecules (Antonov, 1995). The hydrogen bonding results from interaction between electron-deficient H-atom of one H₂O 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 $O \cdots H^{\delta+} - O^{\delta-}$.

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:

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

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, DNES spectrum is an indicator of structural changes of water as a result of various external factors. The cumulative effect of these factors is not the same for the control sample of water and the water sample being under the influence of this factor.

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 $\Delta f(E)$ 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.

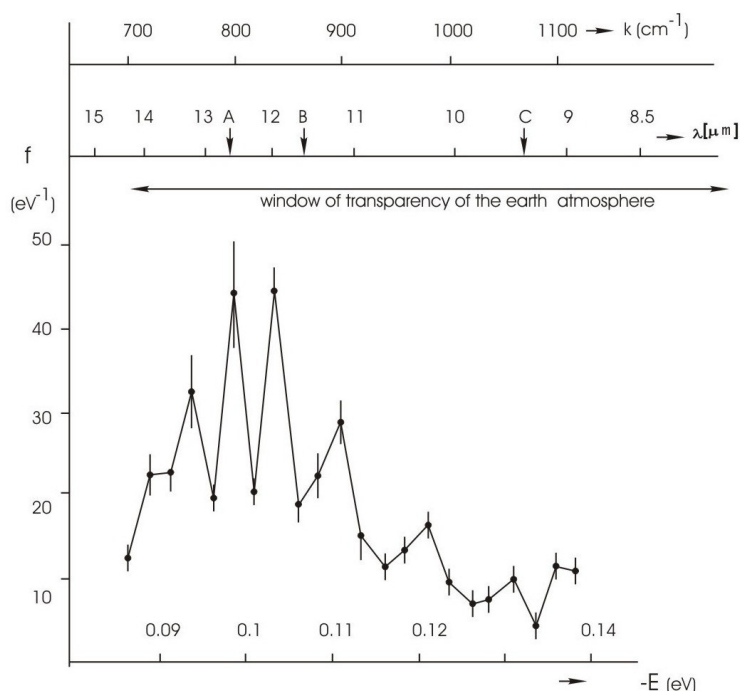


Figure 3: Non-equilibrium (NES) spectrum of water; λ – wavelength; k – wave number

The energy of the hydrogen bonding in the water dimmer is 0.2 eV (~5 kcal/mol), which is larger than the energy of thermal motion of the molecules at the temperature of 300 K. Hydrogen bonds are easily disintegrated and re-formed through an interval of time, which makes water structure quite unstable and changeable (George, 1997). This process leads to structural inhomogeneity of water characterizing it as an associated heterogeneous two-phase liquid with short-range ordering, i.e. with regularity in mutual positioning of atoms and molecules, which reoccurs only at distances comparable to distances between initial atoms, i.e. the first H₂O layer.

In 2005 R. Saykally (University of California, USA) calculated the possible number of hydrogen bonds and the stability of water clusters depending on the number of H₂O molecules (Figure 4) (Saykally, 2005). It was also estimated the possible number of hydrogen bonds (100) depending on the number of H₂O molecules (250) in clusters (Sykes, 2007). O. Loboda and O.V. Goncharuk provided data about the existence of icosahedral water clusters consisting of 280 H₂O molecules with the average size up to 3 nm (Loboda & Goncharuk, 2010). The ordering of water molecules into associates corresponds to a decrease in the entropy (randomness), or decrease in the overall Gibbs energy ($G = \Delta H - T\Delta S$). This means that the change in enthalpy ΔH minus the change in entropy ΔS (multiplied by the absolute temperature T) is a negative value. These results are consistent with our data on research of DNES spectrum of water on which it may make conclusion about the number of H₂O molecules in water clusters. DNES spectrum of water has energy ranges from -0.08 to -0.14 eV. The spectral range lies in the middle infrared range from 8 to 14 μm ("window" of the atmosphere transparency to electromagnetic radiation). Under these conditions, the relative stability of water clusters depends on external factors, primarily on the temperature. It was shown that the H₂O molecules change their position in clusters depending on the energy of intermolecular H...O hydrogen bonds. The values of the average energy ($E_{H...O}$) of hydrogen bonds between the H₂O molecules in the formation of cluster associates with formula (H₂O)_n compile -0.1067 ± 0.0011 eV. As the energy of hydrogen bonds between H₂O molecules increases up to -0.14 eV, the cluster formation of water becomes "restructuring". In this case, the energy redistribution among the individual H₂O molecules occurs.

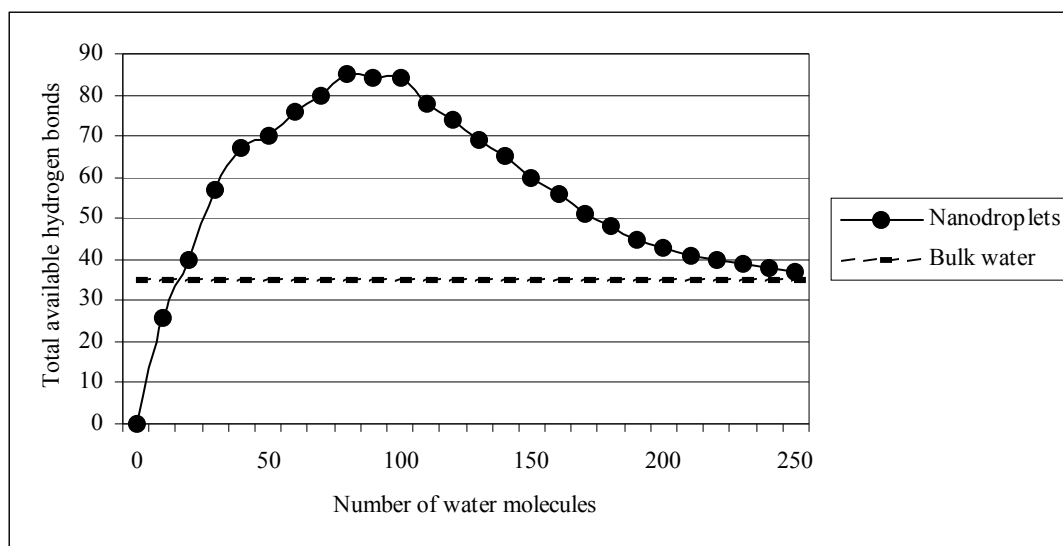


Figure 4: The total number of hydrogen bonds depending on the number of H₂O molecules in clusters.

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 the main biophysical parameter was investigated the average energy of hydrogen bonds ($\Delta E_{H...O}$) between H₂O 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 ($\Delta E_{H...O}$) at -9.1 ± 1.1 meV and from the second group -1.6 ± 1.1 meV. 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 -0.1387 eV, or at a wavelength of $8.95 \mu\text{m}$. 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 – $8.95 \mu\text{m}$. 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 $9.76 \mu\text{m}$ (Krasnov, Gordetsov, 2009). The resulting peak at $\lambda = 8.95 \mu\text{m}$ in IR-spectrum (Ignatov, 2012) 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 Student's t-test. In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water (Antonov, 1995). 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 H₂O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by DNES at -5.5 ± 1.1 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 -0.11 eV and -0.1387 eV. The value at -0.11 eV is characteristic for the presence of Ca^{2+} . The value at -0.1387 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 -0.1387 eV for shungite and -0.11 eV for zeolite (Mosin & Ignatov, 2013, Ignatov & Mosin, 2014). It should be noted that owing to the unique porous structures both the natural minerals shungite and zeolite are ideal natural water adsorbers 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 (Mosin & Ignatov, 2013c). 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 entered into Tables 1, 2 and 3 – 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 H₂O 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) ~24.1 eV⁻¹. The function of distribution according to energy f(E) for tap water in Teteven is 11.8±0.6 eV⁻¹ (Table 1, 2, 3).

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 differences regarding chemical composition, hardness, local extremum in NES-spectra of water eV⁻¹ at (-0.1362–-0.1387 eV), isotopic composition 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)	100.4	< 150
Magnesium (Mg)	12.65	< 80
Iron (Fe)	0.016	<0.2
Manganese (Mn)	0.0018	<0.2
Zinc (Zn)	0.18	<4.0
Sulfates (SO ₄)	81.8	< 250
Chlorides (Cl)	3.96	< 250
Carbonates (CO ₃)	< 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 Vasilovska Mountain (Teteven municipality, Bulgaria) and local extremum in NES-spectra of water. *Function of distribution of H₂O molecules according to energy f(E)

Indicators	Results of the research (mg/l)	Norm
Sodium (Na)	4.5	< 200
Calcium (Ca)	55.5	< 150
Magnesium (Mg)	2.28	< 80
Iron (Fe)	0.0127	<0.2
Manganese (Mn)	0.0014	<0.2
Zinc (Zn)	0.006	<4.0
Sulfates (SO ₄)	16.9	< 250
Chlorides (Cl)	3.4	< 250
Carbonates (CO ₃)	< 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

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)	3.5	< 150
Magnesium (Mg)	0.63	< 80
Iron (Fe)	0.007	<0.2
Manganese (Mn)	0.002	<0.2
Zinc (Zn)	0.007	<4.0
Sulfates (SO ₄)	26.8	< 250
Chlorides (Cl)	3.00	< 250
Carbonates (CO ₃)	< 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, sulfates (SO₄²⁻) – 19.2, chlorides (Cl⁻) – 9.2, calcium (Ca²⁺) – 50.6, 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 Danubian Plain is 23.2 eV⁻¹ and in Thracian Valley detected at -21.3 eV⁻¹.

In the Danubian Plain and Thracian Valley there are data for 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 and 1300 m attitude in Bulgaria and from Kavkaz, 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)	29.5	< 50
Magnesium (Mg)	1.5	< 10
Iron (Fe)	0.083	–
Manganese (Mn)	0.0017	–
Zinc (Zn)	0.007	–
Sulfates (SO ₄)	21.9	< 100
Chlorides (Cl)	3.2	< 70
Carbonates (CO ₃)	< 2.0	–
Hydrocarbonates (HCO ₃)	69.7	< 100
Other values	Results	
Active reaction (pH)	6.7 normal	6.5-7.0
Electroconductivity	410.9 μS/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

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

The research of local extremum eV⁻¹ of function of distribution of water molecules according to energy f(E) at $\lambda = 8.95 \mu\text{m}$ shows characteristic for inhibiting the growth of cancer cells. In water magnesium (Mg²⁺), zinc (Zn²⁺) and manganese (Mn²⁺) have influence on enzymes, which are antioxidants (Ignatov, Mosin, 2015). 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 (Lv., J et al., 2011). There was a positive correlation between eSOD activity and age and a negative correlation between eSOD activity and plasma Zn concentrations. An inverse correlation was also found between plasma Zn concentration and age. The prevalence of Zn²⁺ deficiency increased with age, with normal Zn²⁺ levels observed in about 80% of adult subjects 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 increased susceptibility to diseases. Because nutritional factors are involved in improving immune functions, metabolic harmony, and antioxidant defense, some nutritional factors, such as zinc, 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. Intracellular zinc homeostasis is regulated by metallothioneins (MT) via ion release through the reduction of thiol groups in the MT molecule (Mocchegiani, E. 2007). Zinc from water improves antioxidative enzymes in red blood cells (Malhotra, A., Dhawan, D. K., 2008).

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 deficiency has been shown to lead to increased oxidative stress (Begona, M. et al, 2000). Defenses against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), beta-carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase (selenium), catalase (iron), and superoxide dismutase (copper, zinc, manganese) and proteins such as ceruloplasmin (copper). The extent of tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system (Machlin, L. J., Bendich, A, 1988). The norm in water for zinc and manganese from World Health Organization (WHO) is less than 20 µg. For the Sodium (Na) the norm from WHO is less than 20 mg.

There were results of USA and Canada for the concentration of calcium (Ca) in water. From statistical information the most centenarians in Canada per million are in Nova Scotia (210 per 1 million). In the water from Nova Scotia calcium is 6.8 mg/l. The results of Nikolay Druzhyak were from different places in Russia. He shows that in the places with centenarians the calcium is between 8 and 20 mg/l. In the report we were shown the dependence of antioxidant effects and longevity. Risk factors are cardiovascular diseases.

The following reactions occur in water if there are high concentrations of calcium and magnesium ions: The reaction of limestone (CaCO₃) and gypsum (CaSO₄·2H₂O) with water to separate the calcium Ca²⁺, carbonate CO₃²⁻ and sulfate ions SO₄²⁻. By increasing the mineralization the content of Ca²⁺ ions decreases. During the concentration of the solutions they were precipitated. With the increase of carbon dioxide CO₂ and decreasing of pH increases the concentration of Ca²⁺. Reaction of dolomite (CaCO₃, MgCO₃) with water makes the formation of magnesium Mg²⁺ ions. Magnesium salts are well soluble in water. Hydrocarbonates (HCO₃⁻) and carbonates (CO₃²⁻) ions are formed by reaction of the karst rocks, carbon dioxide and water. There was separation of carbonic acid (H₂CO₃). There was observation of antioxidant effects of water on rats (Abdullah, A.M, 2012). In Zamzam water there is Ca²⁺ – 299.7; Mg²⁺ – 18.9; Zn²⁺ – 0.001 mg/l.

3.5. Deuterium in mountain and melt water. IR-spectra of D₂O-solutions

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 (Pocock *et al.*, 1981; Rubenowitz *et al.*, 1999; Marque *et al.*, 2003). 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

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) (Lis *et al.*, 2008). 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 $\delta = +5,0$ D,%, SMOW (Mediterranean Sea) to $\delta = -105$ D,%, SMOW (Volga River). The natural waters of CIS countries are characterized by negative deviations from SMOW standard to $(1.0-1.5) \cdot 10^{-5}$, in some places up to $(6.0-6.7) \cdot 10^{-5}$, but there are observed positive deviations at $2.0 \cdot 10^{-5}$. Content of the lightest isotopomer – H_2^{16}O 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. 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 . On Figure 9 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 extremum 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%.

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 appear 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) $\nu_1; \nu_2; \nu_3 = 2671; 1178.4; 2787.7$ 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, cm^{-1} and 710–645 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.

In the IR-spectrum of liquid water the absorbance band is considerably broadened and shifted relative to the corresponding bands in the spectrum of water vapor. Their position depends on the temperature (Ignatov & Mosin, 2013b). The temperature dependence of individual spectral bands of liquid water is very complex (Zelmann, 1995). 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) (Yukhnevitch, 1973). 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 $^\circ\text{C}$, the absorbance of L_1 increasing with increasing temperature, while L_2 absorbance decreases but broadens with reduced wave number with increasing temperature (Brubach et al., 2005). 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 (Max & Chapados, 2009). 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 (4–17500 cm^{-1}) visible region and the ultraviolet region – from 200 nm^{-1} to ionization limit at 98 nm^{-1} (Walrafen, 1972). In the middle visible region at 4–7500 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 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.

Results of IR-spectroscopy with a 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. These results are shown in Figure 5. 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 H₂O and D₂O

Main vibrations of liquid H ₂ O and ² H ₂ O				
Vibration(s)	H ₂ O (t = +25 °C)		D ₂ O (t = +25 °C)	
	ν , cm ⁻¹	E_0 , M ⁻¹ cm ⁻¹	ν , cm ⁻¹	E_0 , M ⁻¹ 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 H₂O monomers to H₄O₂ dimer and H₆O₃ trimer absorption maximum of valent stretching vibrations of the O-H bond is shifted toward lower frequencies ($\nu_3 = 3490 \text{ cm}^{-1}$ and $\nu_1 = 3280 \text{ cm}^{-1}$) (Eisenberg & Kauzmann, 1969) 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 [(H₂O)₂]. These frequencies are significantly lower than the valence modes of ν_1 and ν_3 vibrations of isolated H₂O 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 H₂O 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 H₂O 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 H₂O molecules with each other, as well as with cations and anions.

ANALYSIS

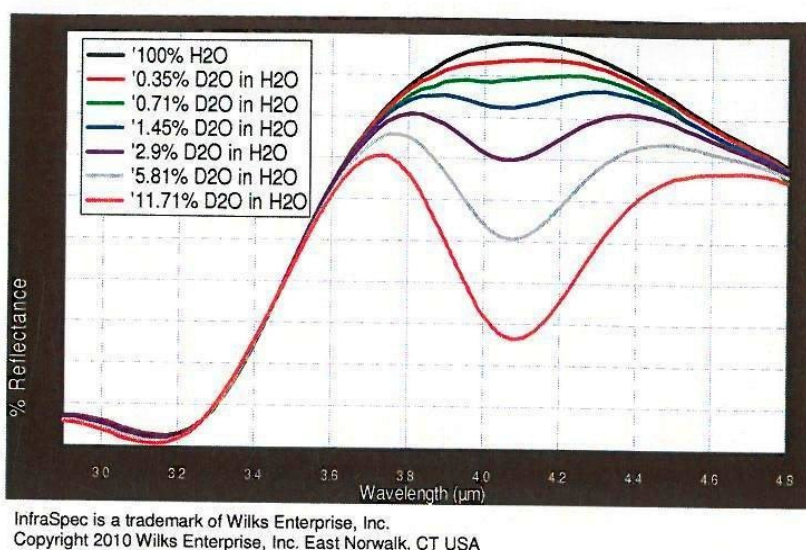


Figure 5: The typical IR-spectra of water with varying content of deuterium

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 D₂O (Cleland, 1976). It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype (Thomson, 1960; Katz, 1960; Den'ko, 1970; Török *et al.*, 2000).

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 (Mosin *et al.*, 2012; Mosin *et al.*, 2013; Mosin & Ignatov, 2012; Mosin & Ignatov, 2013). 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 project 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. Research conducted by us 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 of 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 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 there is optimal composition of mountain and melt water from areas where were lived the long live people and centenarians. There are new proofs for biophysical and biochemical effects of calcium, magnesium, zinc and manganese.

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