Methods for Research of Mountain and Melt Water as Factor of Longevity. Chemical Composition, NES and DNES Methods for Spectral Analysis. Effects of Calcium, Magnesium, Zinc and Manganese

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Abstract

It was shown that 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 were investigated by IR-spectroscopy (NES and DNES-methods), methods for research of chemical composition of water. It was shown, that the increased content of deuterium leads to physiological, morphological and cytology alterations of the cell, and also renders negative influence on cellular metabolism, while deuterium depleted water with decreased deuterium content on 20-30% has beneficial effects on health. By using spectroscopy methods as ^{1}H-NMR, IR-spectroscopy, NES, DNES, EXAFS-spectroscopy, X-Ray and neurons diffraction were investigated various samples of water. The methods for chemical composition of water were applied in Eurotest laboratory. From Bulgarian water springs and blood serum of people with excellent health and cancer patients between 50 and 70 years old were applied NES, DNES methods and research of chemical composition of water. 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 extremum in the IR-spectra of various samples of water and human blood serum at -0.1387 eV and wavelength – 8.95 μm (Ignatov, Mosin, 2012). For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in IR-spectra are shifted to lower energies relative to the control group. The obtained results testify to necessity of consumption of clean natural water which quality satisfies mountain water from Bulgarian water springs. The computer calculation of polyhedral nanoclusters $(H_2O)_n$, where n = 3–20 are carried out. Based on this data the main structural mathematical models describing water structure (quasicrystalline, continuous, fractal, fractal-clathrate) have been examined and some important physical characteristics were obtained. The authors were applied method for percent distribution of water molecules according energies of hydrogen bonds from (-0.08 to -0.1387 eV) (Ignatov, Mosin, 2013).

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 is 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 60 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 ^{1}H 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. They have the same heredity, but have lived under different conditions. In all municipalities there is a mountainous and a field part. Mountain and melt water were used for drinking.

2. Material and Methods

For preparation of growth media we used D_2O (99.9 atom%) received from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). Inorganic salts were 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 ^{1}H-NMR-spectroscopy on Brucker WM-250 device ("Brucker", Germany) (working frequency – 70 MHz, internal standard – Me_4Si) and 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}). 1% (v/v) solution of human blood serum was studied with the methods of IR-spectrometry, non-equilibrium (NES) and differential non-equilibrium (DNES) spectrum. 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. 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 ^{0}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 $\lambda = 580\pm7$ nm. The device measures the angle of evaporation of water drops from 72,3 ^{0} to 0 ^{0}. The spectrum of hydrogen bonds among H_2O molecules was measured in the range of -0.08– -0.1387 eV or λ = 8.9– 13.8 μm 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 H_2O molecules in human blood serum. 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. 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});

Statistical processing of experimental data was performed using the statistical package STATISTISA 6 using 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).
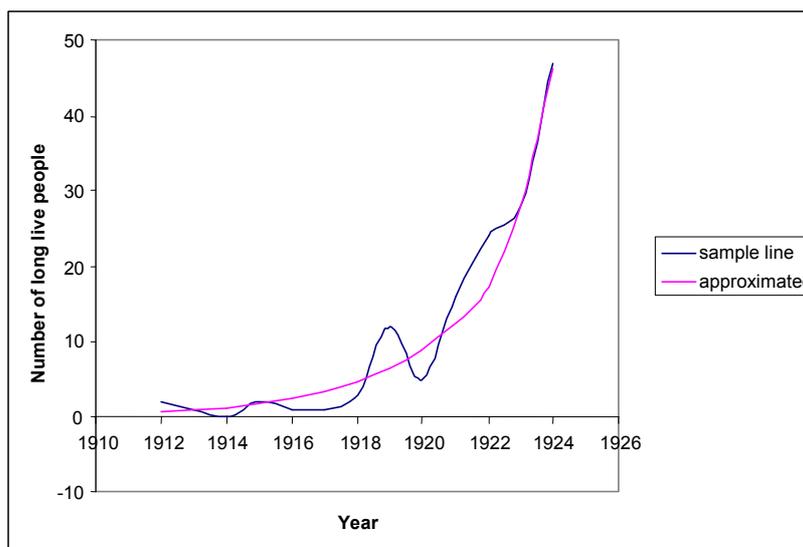
Figure 1. Interrelation between the year of birth of long lived people (age) and their number in Teteven municipality, Bulgaria.
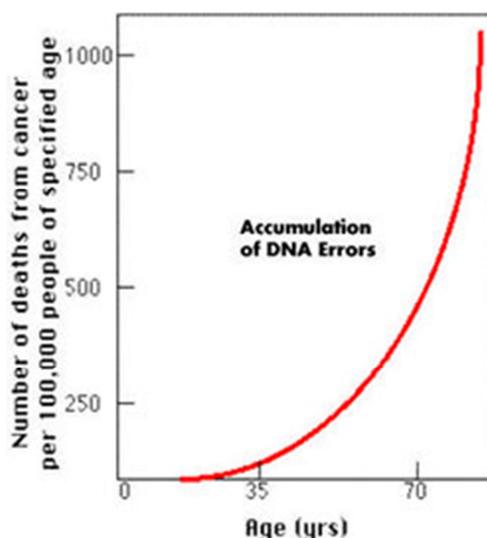

Figure 2. Interrelation between age and the number of cancer patients (Orgel, 1963).

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 ^{0}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

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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 & Yuskesselieva, 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 H_2O molecules according to the value of f(θ). 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 H_2O-molecules (Antonov, 1995). 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 H_2O 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:

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

where b = 14.33 eV^{-1}

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:
$\theta = arcos\ (-1 – 14.33E),$ \qquad (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 **Δf(E) = f (samples of water) – f (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 H_2O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H_2O 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 H_2O molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among H_2O molecules the value of which is calculated:
$\bar{E} = – 0{,}1067 \pm 0{,}0011$ eV \qquad (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 ^0C, 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.
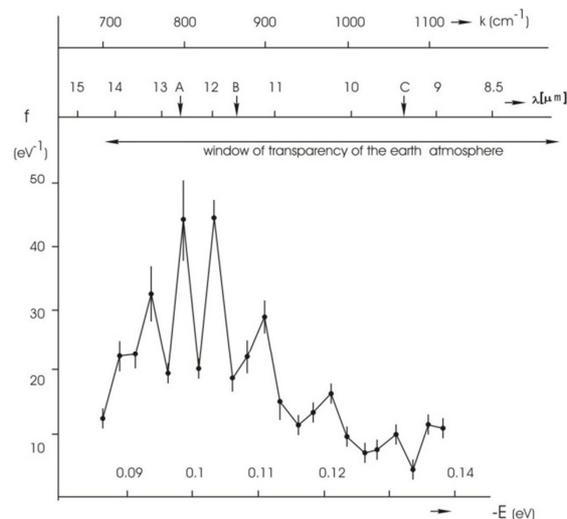
Figure 3. Non-equilibrium (NES) spectrum of water; λ – wavelength; k – wave number

The 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 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 $\Delta f(E) = f$ (samples of water) – f (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 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 posses. IR-spectrum that reflects the structure of water and metabolic processes in the organism. It can be demonstrated by analysis of human blood serum by IR-spectrometry. 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 λ = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35 and 9.76 μm (Krasnov, Gordetsov, 2009). The resulting peak at λ = 8.95 μm in IR-spectrum (Ignatov, 2012) approaching the peak at λ = 8.85 μm monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function f(E) at λ = 8.95 μm 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 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±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 λ = 8.95 μm, 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). These results suggest the restructuring of energy values among H_2O molecules with a statistically reliable increase of local maximums in DNES-spectra. 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 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) ~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 extremum in NES-spectra of water. *Function of distribution of H_2O molecules according to energy f(E).

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

Table 2. The composition of mountain water springs in Vasiliovska Mountain (Teteven municipality, Bulgaria) and local extremum in NES-spectra of water. *Function of distribution of H_2O 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. *Function of distribution of H_2O molecules according to energy f(E).

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

Research shows the results of water composition in field area of Dolni Dabnik. The results are: Hydrocarbonates (HCO_3) – 184.4, Sulfates (SO_4) – 19.2, Chlorides (Cl) – 9.2, Calcium (Ca) – 50.6,   Sodium (Na) – 14.2 mg/l. The hardness is 26.2 dH – very hard water. extremum 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 – 21.3eV^{-1}. In the Danubian Plain and Thracian Valley there

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are data for presence of Nitrites (NO_2), Nitrates (NO_3), Ammonia (NH_4), phosphates (HPO_4) more than norm. The 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. shows 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 human body

The research of local extremum eV^{-1} of function of distribution of water molecules according to energy f(E) at λ = 8.95 μm shows characteristic for inhibiting the growth of cancer cells. In water magnesium (Mg), zinc (Zn) and manganese (Mn) 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 Zn deficiency increased with age, with normal Zn levels observed in about 80% of adult subjects and only in 37% of the nonagenarians. 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 factor are cardiovascular diseases. What are the chemical reactions if there are high concentrations of calcium and magnesium ions?

The reaction of limestone ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) with water to separate the calcium Ca, carbonate CO_3 and sulfate ions SO_4. By increasing the mineralization the content of Ca ions decreases. During the concentration of the solutions they were precipitated. With the increase of carbon dioxide CO_2 and decreasing of pH increases the concentration of calcium ions. Reaction of dolomite ($CaCO_3 \cdot MgCO_3$) with water make the formation of magnesium Mg ions. Magnesium salts are well soluble in water.

Hydrocarbonates (HCO_3) and carbonates (CO_3) ions are formed by reaction of the karst rocks, carbon dioxide and water. There was separation of carbonic acid (H_2CO_3). There was observation of antioxidant effects of water on rats (Abdullah, A.M, 2012). In Zamzam water there is Ca – 299.7, Mg – 18.9, Zn – 0.001 mg/l.

3.5. Nature of Hydrogen Bonds in Liquids and Crystals

The peculiarities of chemical structure of H_2O molecule and weak bonds caused by electrostatic forces and donor-acceptor interaction between hydrogen and oxygen atoms in H_2O molecules create favorable conditions for formation of directed intermolecular hydrogen bonds (O–H…O) with neighboring H_2O molecules, binding them into complex intermolecular associates which composition represented by general formula $(H_2O)_n$, where n can vary from 3 to 50 (Keutsch & Saykally, 2011). The hydrogen bond – a form of association between the electronegative O oxygen atom and a H hydrogen atom, covalently bound to another electronegative O oxygen atom, is of vital importance in the chemistry of intermolecular interactions, based on weak electrostatic forces and donor-acceptor interactions with charge-transfer (Pauling, 1960). It 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 H_2O molecule; the structure of hydrogen bonding, therefore may be defined as $O…H^{\delta+}–O^{\delta-}$. As the result, the electron of the H-atom due to its relatively weak bond with the proton easily shifts to the electronegative O-atom. The O-atom with increased electron density becomes partly negatively charged – δ^- , while the H-atom on the opposite side of the molecule becomes positively charged – δ^+ that leads to the polarization of $O^{\delta-} – H^{\delta+}$ covalent bond. In this process the proton becomes almost bared, and due to the electrostatic attraction forces are provided good conditions for convergence of O…O or O…H atoms, leading to the chemical exchange of a proton in the reaction O–H...O ↔ O...H–O. Although this interaction is essentially compensated by mutual repulsion of the molecules' nuclei and electrons, the effect of the electrostatic forces and donor-acceptor interactions for H_2O molecule compiles 5–10 kcal per 1 mole of substance. It is explained by negligible small atomic radius of hydrogen and shortage of inner electron shells, which enables the neighboring H_2O molecule to approach the hydrogen atom of another molecule at very close distance without experiencing any strong electrostatic repulsion. The H_2O molecule has four sites of hydrogen bonding – two uncompensated positive charges at hydrogen atoms and two negative charges at the oxygen atom. Their mutual disposition is characterized by direction from the centre of regular tetrahedron (nucleus of oxygen atom) towards its vertexes. This allows to one H_2O molecule in condensed state to form up to 4 classical hydrogen bonds, two of which are donor bonds and the other two – acceptor ones (taking into consideration bifurcate ("two-forked") hydrogen bond – 5) (Pasichnyk *et al.,* 2008).

A hydrogen bond according to Bernal–Fowler rules (Bernal & Fowler, 1933) is characterized by the following parameters:
a) an oxygen atom of each H_2O molecule is bound with four neighboring hydrogen atoms: by covalent bonding with two own hydrogen atoms, and by hydrogen bonding – with two neighboring hydrogen atoms
(as in the crystalline structure of ice); each hydrogen atom in its turn is bound with oxygen atom of neighbour H_2O molecule.
b) on the line of oxygen atom – there can be disposed only one proton H^+;
c) the proton, which takes part in hydrogen bonding situated between two oxygen atoms, therefore has two equilibrium positions: it can be located near its oxygen atom at approximate distance of 1 Å, and near the neighboring oxygen atom at the distance of 1.7 Å as well, hence both a usual dimmer HO–H...OH₂ and an ion pair HO...H–OH₂ may be formed during hydrogen bonding, i.e. the hydrogen bond is part electrostatic (~90%)

and part (~10%) covalent (Isaacs et al., 2000). The state of "a proton near the neighboring oxygen" is typical for the interphase boundary, i.e. near water-solid body or water–gas surfaces.

d) the hydrogen bonding of a triad O–H...O possess direction of the shorter O–H ($\rightarrow$) covalent bond; the donor hydrogen bond tends to point directly at the acceptor electron pair (this direction means that the hydrogen atom being donated to the oxygen atom acceptor on another H_2O molecule).

The most remarkable peculiarity of hydrogen bond consists in its relatively low strength; it is 5–10 times weaker than chemical covalent bond (Pimentel & McClellan, 1960). In respect of energy hydrogen bond has an intermediate position between covalent bonds and intermolecular van der Waals forces, based on dipole-dipole interactions, holding the neutral molecules together in gasses or liquefied or solidified gasses. Hydrogen bonding produces interatomic distances shorter than the sum of van der Waals radii, and usually involves a limited number of interaction partners. These characteristics become more substantial when acceptors bind H-atoms from more electronegative donors. Hydrogen bonds hold H_2O molecules on 15% closer than if water was a simple liquid with van der Waals interactions. The hydrogen bond energy compiles 5–10 kcal/mole, while the energy of O–H covalent bonds in H_2O molecule – 109 kcal/mole (Arunan *et al.,* 2011). The values of the average energy ($\Delta E_{H...O}$) of hydrogen H…O-bonds between H_2O molecules make up 0.1067 ± 0.0011 eV (Antonov & Galabova, 1992). With fluctuations of water temperature the average energy of hydrogen H...O-bonds in of water molecule associates changes. That is why hydrogen bonds in liquid state are relatively weak and unstable: it is thought that they can easily form and disappear as the result of temperature fluctuations (Ignatov & Mosin, 2013).

Another key feature of hydrogen bond consists in its cooperatively coupling. Hydrogen bonding leads to the formation of the next hydrogen bond and redistribution of electrons, which in its turn promotes the formation of the following hydrogen bond, which length increasing with distance. Cooperative hydrogen bonding increases the O–H bond length, at the same time causing a reduction in the H…O and O…O distances (Goryainov, 2012). The protons held by individual H_2O molecules may switch partners in an ordered manner within hydrogen networks (Bartha *et al*., 2003). As the result, aqueous solutions may undergo autoprotolysis, i.e. the H^+ proton is released from H_2O molecule and then transferred and accepted by the neighboring H_2O molecule resulting in formation of hydronium ions as H_3O^+, $H_5O_2^+$, $H_7O_3^+$, $H_9O_4^+$, etc. This leads to the fact, that water should be considered as associated liquid composed from a set of individual H_2O molecules, linked together by hydrogen bonds and weak intermolecular van der Waals forces (Liu *et al.,* 1996). The simplest example of such associate can be a dimmer of water: $(H_2O)_2 = H_2O^{..}HOH$

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 H_2O layer. As it is known, a liquid in contrast to a solid body, is a dynamic system: its atoms, ions or molecules, keeping short-range order in mutual disposition, participate in thermal motion, the character of which is much more complicated than that of crystals. For example H_2O molecules in liquid state under normal conditions (1 atm, 22 ^{0}C) are quiet mobile and can oscillate around their rotation axes, as well as to perform the random and directed shifts. This enabled for some individual molecules due to cooperative interactions to "jump up" from one place to another in an elementary volume of water. Random motion of molecules in liquids causes continuous changes in the distances between them. The statistical character of ordered arrangement of molecules in liquids results in fluctuations – continuously occurring deviations not only from average density, but from average orientation as well, because molecules in liquids are capable to form groups, in which a particular orientation prevails. Thus, the smaller these deviations are, the more frequently they occur in liquids. The energy of hydrogen bonds (E) is measured in electron-volts (eV) and is designated by the spectrum of energy distribution. The water spectrum characterized by a non-equilibrium process of water droplets evaporation, thus the term "non-equilibrium energy spectrum of water" (NES) is applied.

The difference $\Delta f(E) = f$ (samples of water) – f (control sample of water) is called the "differential non-equilibrium energy spectrum of water" (DNES). DNES is a measure of changes in the structure of water as a result of external influences. The cumulative effect of all other factors is the same for the control sample of water and the water sample, which is under the influence of this impact.
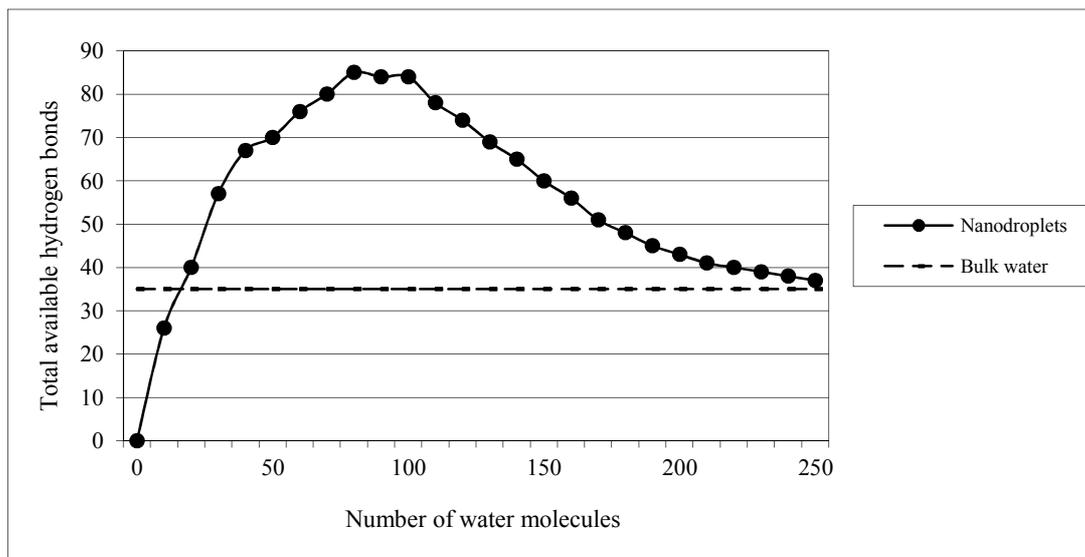
Figure 4. The total number of hydrogen bonds depending on the number of water molecules in clusters.

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 H_2O molecules (Figure 4) (Saykally, 2005). It was also estimated the possible number of hydrogen bonds (100) depending on the number of H_2O molecules (250) in clusters (Sykes, 2007). O. Loboda and O.V. Goncharuk provided data about the existence of icosahedral water clusters consisting of 280 H_2O 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 H_2O 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 H_2O 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 H_2O molecules in the formation of cluster associates with formula $(H_2O)_n$ compile -0.1067 ± 0.0011 eV. As the energy of hydrogen bonds between H_2O molecules increases up to -0.14 eV, the cluster formation of water becomes "restructuring". In this case, the energy redistribution among the individual H_2O molecules occurs.

All these data indicate that the water is a complex associated non-equilibrium liquid consisting of associative groups containing according to the present data, from 3 to 20 individual H_2O molecules (Tokmachev *et al*., 2010). Associates can be perceived as unstable groups (dimers, trimers, tetramers, pentamers, hexamers etc.) in which H_2O molecules are linked by van der Waals forces, dipole-dipole and other charge-transfer interactions, including hydrogen bonding. At room temperature, the degree of association of H_2O molecules may vary from 2 to 6. In 1993 K. Jordan (USA) (Tsai & Jordan, 1993) calculated the possible structural modifications of small water clusters consisting of six H_2O molecules (Figure 5a–c). Subsequently, it was shown that H_2O molecules capable of hydrogen bonding by forming the structures representing topological 1D rings and 2D chains composed from numerous H_2O molecules. Interpreting the experimental data, they are considered as pretty stable elements of the structure. According to computer simulations, clusters are able to interact with each other through the exposed protons on the outer surfaces of hydrogen bonds to form new clusters of more complex composition.
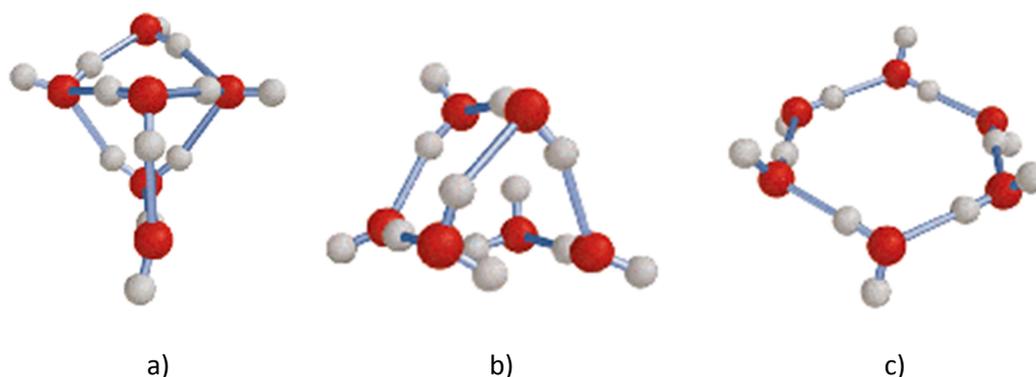
a)	b)	c)

Figure 5. Calculation of small water cluster structures (a–c) with general formula $(H_2O)_n$, where n = 6 (Tsai & Jordan, 1993).

In 2000 it was deciphered the structure of the trimmer water, and in 2003 – tetramer, pentamer and the water hexamer (Wang & Jordan, 2003).

3.5. Ice crystals and melt water

The further important feature is that the hydrogen bonds are spatially oriented. As each H_2O molecule has four sites of hydrogen bond formation (two non-shared electron pairs at an oxygen atom and two uncompensated positive charges at a hydrogen atom), one H_2O molecule in a condensed state is capable to form hydrogen bonds with four H_2O molecules (two donor and two acceptor) (Figure 6), which results in forming a tetrahedron crystal structure clearly observed in ice crystals.
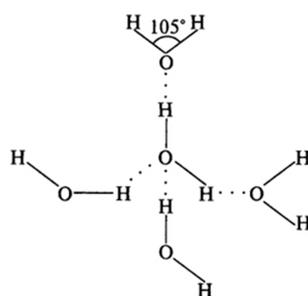

Figure 6. Hydrogen bonding between four individual H_2O molecules. It is shown the value of the angle between the covalent H–O–H bond in H_2O molecule.

At present time 14 crystalline modifications of ice are known, each of them has its own structure and a character of disposition of hydrogen atoms (Table 5). Crystals of all ice modifications are made up from H_2O molecules, linked by hydrogen bonds into a 3D carcass, consisting of individual tetrahedrons, formed by four H_2O molecules (Figure 7). In the crystalline structure of natural ice I_h hydrogen bonds are oriented towards the tetrahedron apexes at strictly defined angles equal to 109°5 (in liquid water this angle is $104^0 5$) (Mosin & Ignatov, 2013). In ice structures I_c, VII and VIII this tetrahedron is nearly the same as a regular 4 triangular tetrahedron. In ice structures II, III, V and VI the tetrahedrons are noticeably distorted. In ice structures VI, VII and VIII two intercrossing systems of hydrogen bonds are distinguished. In the centre of the tetrahedron is located an oxygen atom, at each of the two verticess – H-atom, which electron take part in formation of covalent bond with an electron pair of O-atom. The rest two vertices of the tetrahedron are occupied by two pairs of non - shared electrons of O-atom not participating in formation of molecular bonds.

Table 5. Ice Crystal modifications and their physical characteristics

Modification	Crystal structure	Hydrogen bond lengths, Å	Angles H–O–H in tetragonals, [0]
I_h	Hexagonal	2.76	109.5
I_c	Cubic	2.76	109.5
II	Trigonal	2.75–2.84	80–128
III	Tetragonal	2.76–2.8	87–141
IV	Rhombic	2.78–2.88	70.1–109
V	Monoclinic	2.76–2,87	84–135
VI	Tetragonal	2.79–2.82	76–128
VII	Cubic	2.86	109.5
VIII	Cubic	2.86	109.5
IX	Tetragonal	2.76–2.8	87–141
X	Cubic	2.78	109.5
XI	Hexagonal	4.50	90.0
XII	Tetragonal	4.01	90.0
XIII	Monoclinic	7.47	90–109.7
XIV	rhombic	4.08	90.0

Keys: I_h – natural hexagonal ice; I_c – cubic ice.

The carcasses of hydrogen bonds allocate H_2O molecules in form of a spatial hexagon network with internal hollow hexagonal channels. In the nodes of this network O-atoms are orderly organized (crystalline state), forming regular hexagons, while H-atoms have various positions along the bonds (amorphous state). When ice melts, its network structure is destroyed: H_2O molecules begin to fall down into the network hollows, resulting in a denser structure of the liquid – this explains why water is heavier than ice. The hydrogen bonding explains other anomalies of water (anomalies of temperature, pressure, density, viscosity, fluidity etc. According to theoretical calculations, at the melting of the ice breaks about 15% of all hydrogen bonds (Mosin & Ignatov, 2011); by further heating to 40^0C breaks down about half of hydrogen bonds in water associates. In the water vapor hydrogen bonds are absent.

The main difference between the structure of ice and water is more diffuse arrangement of the atoms in the lattice and disturbance of long-range order. Thermal oscillations (fluctuations) lead to bending and breaking of hydrogen bonds. Those out of the equilibrium positions H_2O molecules fall into the adjacent structural voids and for a time held up there, as cavities correspond to the relative minimum of potential energy. This leads to an increase in the coordination number, and the formation of lattice defects. The coordination number
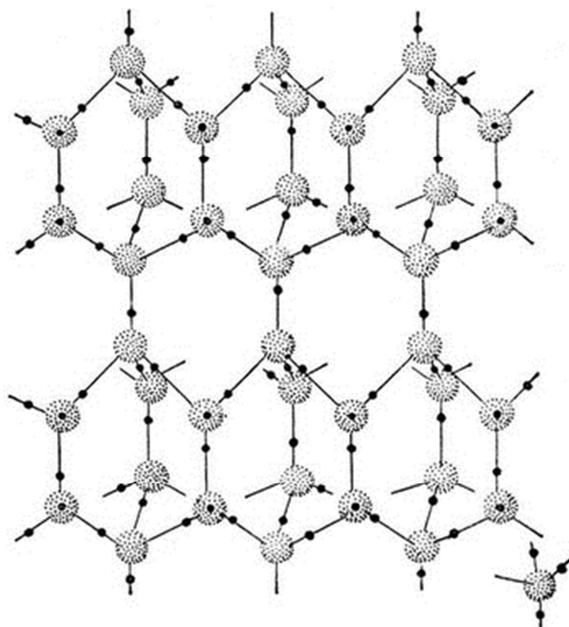(the number of nearest neighbors) varies from 4.4 at 1.5^0C to 4.9 at 80^0C.

Figure 7. Hydrogen bonding in crystalline structure of natural ice I_h under the temperature -10^0C and pressure 1 atm. Grey balls – oxygen atoms, black balls – hydrogen atoms. On the right below shown the structural unit of crystalline ice structure.
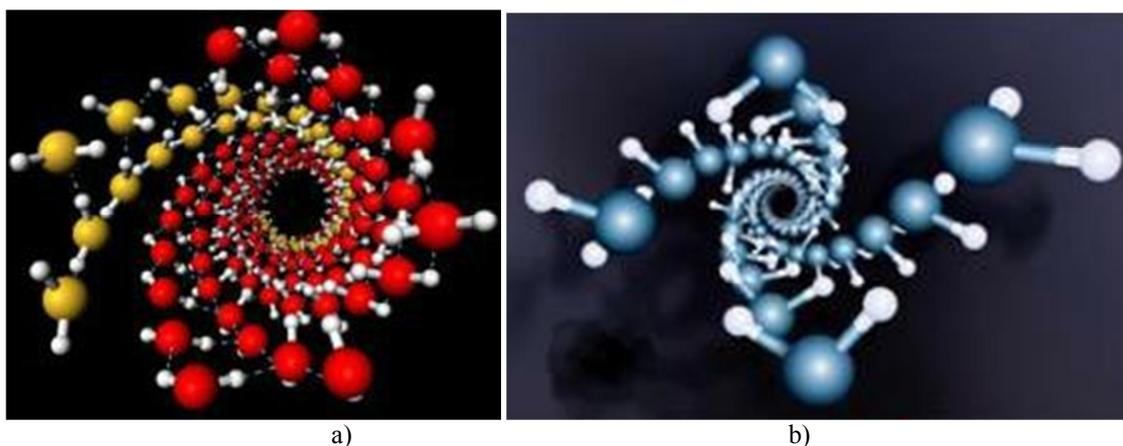
a)   b)

Figure 8. Structures of water crystals in carbon nanotubes according to computer simulations (Bai *et al*., 2006) (diameter of nanotubes 1.35–1.90 nm; pressure 10–40000 atm; temperature -23°C): a) – a general view of the crystal structure of water in nanotubes, b) – the inner wall of the structure of water.
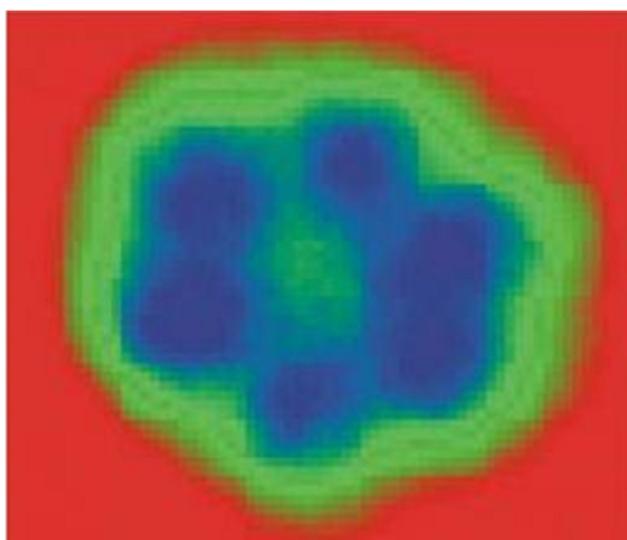

Figure 9. Image of hexamer nanocrystal of ice water (average size ~1 nm) by a scanning tunneling microscope after freezing water to 17 K on the hydrophobic metallic plates of Cu and Ag (Michaelides & Morgenstern, 2007).

Reports about evidence of existence of crystal structures in water have become more frequent in scientific literature nowadays (Michaelides & Morgenstern, 2007). As computer modeling showed, H_2O molecules being placed in carbon nanotubes under high pressure and low temperatures formed crystalline nanostructures like DNA double helix (Bai *et al*., 2006). In modeling experiment water got frozen inside carbon nanotubes with diameter 1.35–1.90 nm with pressure 10–40000 atm and temperature -23°C. Hydrogen bonds in an ice crystal got distorted leading to the formation of a double-walled helix. The inner wall of this structure represents a four–fold twisted helix, while the outer consists of four double helixes, resembling a helix of DNA molecule (Figure 8). While being frozen at 17 K on hydrophobic surfaces of Cu, Ag and their salts, water became crystallized into two-dimensional ice hexamer nanocrystal, consisting of six attached together H_2O molecules (Figure 9).

3.5. Deuterium 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 Ca^{2+}, Mg^{2+}, Na^+, HCO_3^- and SO_4^{2+}. The content of K^+ and N^+ cations in the melt water are <30 mg/l, Mg^{2+} – <10 mg/l, Ca^{2+} – <50 mg/l, the content of SO_4^{2-} – <100 g/l, HCO_3^- <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 ^0C (Table 4). The degree of natural purification of melt water from impurities makes up ~50–60%. The concentration of salts of rigidity – Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, heavy metals and organochlorine compounds, as well as heavy isotopes, including deuterium in melt water is less that of ordinary

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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. 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 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 ^{18}O in sea water: D/H = $(155.76\pm0.05)\cdot10^{-6}$ (155.76 ppm) and ^{18}O/^{16}O = $(2005.20\pm0.45)\cdot10^{-6}$ (2005 ppm) (Lis *et al*., 2008). For SLAP standard isotopic shifts for D and ^{18}O in seawater: D/H = $89\cdot10^{-6}$ (89 ppm) and for a pair of ^{18}O/^{16}O = $1894\cdot10^{-6}$ (1894 ppm). In surface waters, the ratio D/H = $\sim(1.32–1.51)\cdot10^{-4}$, while in the coastal seawater – $\sim(1.55–1.56)\cdot10^{-4}$. 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 $(1.0–1.5)\cdot10^{-5}$, in some places up to $(6.0–6.7)\cdot10^{-5}$, but there are observed positive deviations at $2.0\cdot10^{-5}$. 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.%).

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 10 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%.

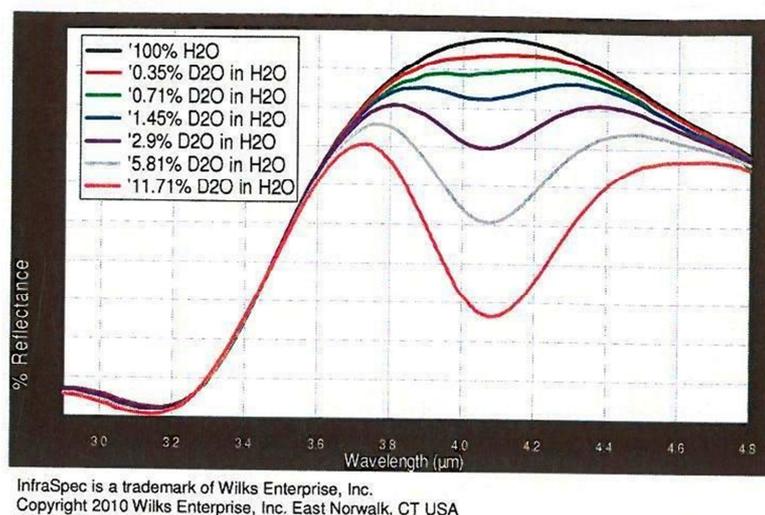Figure 10. The typical IR-spectra of water with varying content of deuterium

At further transition from H_2O monomers to H_4O_2 dimmer and H_6O_3 trimmer absorption maximum of valent stretching vibrations of the O-H bond is shifted toward lower frequencies (v_3 = 3490 cm^{-1} and v_1 = 3280 cm^{-1}) (Eisenberg & Kauzmann, 1969) and the bending frequency increased (v_2 = 1644 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 dimmer [(H$_2$O)$_2$]. These frequencies are significantly lower than the valence modes of v_1 and v_3 vibrations of isolated H_2O molecules at 3657 and 3756

cm^{-1} respectively). The absorbtion 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 fluctuations by another fluctuation when they accidentally overlap each other). The absorption band at 1620 cm^{-1} is attributed to the deformation mode of the dimmer. 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 a 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, i.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.

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

Main vibrations of liquid H_2O and ^2H_2O				
Vibration(s)	H_2O (t = 25 ^{0}C)		D_2O (t = 25 ^{0}C)	
	v, cm^{-1}	E_0, M^{-1} cm^{-1}	v, cm^{-1}	E_0, M^{-1} cm^{-1}
Spinning v_1 + deformation v_2	780-1645	21.65	1210	17.10
Composite $v_1 + v_2$	2150	3.46	1555	1.88
Valence symmetrical v_1, valence asymmetrical v_3, and overtone $2v_2$	3290-3450	100.65	2510	69.70

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 (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 D_2O, 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 D_2O.

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. In all municipalities there is a mountainous and a field part. Mountain and melt water were used for drinking. 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, spectrum, 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 λ = 8,95 μm. 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 H_2O molecules and the distribution function of H_2O 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. Thus, the phenomenon of longevity is a complex multifactorial phenomenon involving both genetic (internal) and phenotypic (external) characteristics of the organism in its adaptation the environment. 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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