# Research of Longevity Factors and Mountain Water as a Factor in Teteven, Yablanitsa and Ugarchin Municipalities, Lovech region, Bulgaria

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**Abstract:**

This paper submits data on longevity factors and mountain water in factorial research of the longevity phenomenon in mountainous and field areas in Lovech region, Bulgaria. There are municipalities of Teteven, Yablanitsa and Ugarchin. A dependence was established among various internal and external factors on the phenomenon of longevity – residence area, water, air, health status, body mass, diet, stress, smoking, positive attitude toward life, physical activity, gender and heredity. The research was made with the author methodology of Ignatov. It is shown that mountain water is among the most important factors for longevity. Natural waters derived from various Bulgarian water springs were studied by the NES and DNES-method for spectral analysis. Water was studied with methods for its composition. As an estimation factor was researched the composition of water. Also were studied the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among $H_2O$ molecules, as well as the local maximums in the IR-spectra of various samples of water and human blood serum. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local maximums in IR-spectra were shifted to lower energies relative to the control group with healthy people. For healthy people there is local maximum at -0.1387 eV and wavelength – 8.95 μm. The obtained results testify to the necessity of consumption of clean natural water whose qualities are covered mountain water from Bulgarian water springs. The local maximum at -0.1387 eV and wavelength – 8.95 μm was proven in the spectrum of mountain sources. The report's authors prove that water is one of the most important factors for longevity. The most favorable are the waters with a local maximum in 8.95 μm in the infrared spectrum, analogous to the same local maximum in

the serum of a healthy person.

**Key words:** mountain water, residence area, air, health status, body mass, diet, stress, smoking, positive attitude toward life, physical activity, gender and heredity.


## 1. Introduction

The question of longevity has always been an exciting one for humanity. Aging is a biological process, which leads to reduction of the vital functions of the body, limiting its adaptive capacities, and development of age-related pathologies and ultimately increasing the likelihood of death; all this is a part of the normal ontogeny and is caused by the same processes that lead to increased functional activity of various body systems in earlier periods of life. It is possible that these processes along with other processes (growth and development of the organism, etc.) are programmed in the human genome and biological mechanism of regulation. The question to what extent aging is dependent on heredity is not sufficiently proven in modern science. Like other biological processes, aging is accelerated under the influence of certain exogenous and endogenous factors and occurs in different individuals with different speed, which depends on genetic differences and environmental factors. The best chance for longevity is given the longevity of immediate direct genetic ancestors. That is why the direct descendants of centenarians generally have the best chances for longevity. O. Burger demonstrates that life expectancy has increased substantially from the $19^{th}$ to the $20^{th}$ century and that this cannot be advantageously associated with the human genome (Burger *et al*., 2012). The main factors of longevity are water quality, food and improved advancement of medicine. For example, in Bulgaria the average life expectancy from 1935 to 1939 was 51.75 years, while from 2008 to 2010 it was 73.60 years. From the standpoint of genetics, the process of aging is associated with disruption of the genetic program of the organism and gradual accumulation of errors during the process of DNA replication. Aging may be associated with the accumulation of somatic mutations in the genome and be influenced by free radicals (mainly oxygen and primary products of oxidative metabolism) and ionizing radiation on DNA molecules as well (Woodhead, 1984; Adelman *et al*., 1988; Pryor, 1997). Such mutations can reduce the ability of cells for normal growth and division and be a cause of a large number of various cell responses: inhibition of replication and transcription, impaired cell cycle division, transcriptional mutagenesis, cell aging, finally resulting in cell death. Cells taken from elderly people show a reduction in transcription when transferring information from DNA to RNA. From the standpoint of dynamics, aging is a non-linear biological process, which increases over time. Accordingly, the rate of aging increases with time. The accumulation of errors in the human genome increases exponentially with time and reaches a certain stationary maximum at the end of life. L. Orgel shows that, for this reason, the probability of cancer occurrence increases with age (Orgel, 1963). According to thermodynamics, the process of aging is the process of alignment of the entropy by the human body with that of the environment (Ignatov, Mosin, 2011). Water is the main substance of life. The human body is composed of 50 to 75% of water (Watson, 1980). 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. 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, 2012a; Ignatov & Mosin, 2013a; Ignatov & Mosin, 2013b). These factors and the structure of water are of great importance in biophysical studies. The peculiarities of the chemical structure of the $H_2O$ 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, 2013c). Another important indicator of water quality is its isotopic composition. Natural water consists of 99.7 mol.% of $H_2{}^{16}O$, whose molecules are formed by $^1H$ and $^{16}O$ atoms (Mosin & Ignatov, 2012b). 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 – $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$. This report studies the influence of various internal and external factors on the phenomenon of longevity – residence area, water, air, health status, body mass, diet, stress, smoking, positive attitude toward life, physical activity, gender and heredity. The research was carried out under the joint scientific project "NATURE, ECOLOGY AND LONGEVITY" conducted in Bulgaria. Within the frames of this project 217 people living in the municipalities of Teteven, Yablanitsa and Ugarchin, Lovech district (Bulgaria), where the largest number of long-living people and their siblings have lived, were studied. They have the same heredity, but have lived under different conditions. In all three municipalities there is a mountainous and a field part. Mountain and tap water is used for drinking. Statistical analysis has been conducted for residence area, health status, body mass, smoking, positive attitude toward life, physical activity, gender and heredity.

## 2. Experimental part. Material and methods

The objects of the study were various prokaryotic and eukaryotic cells obtained from the State Research Institute of Genetics and Selection of Industrial Microorganisms (Moscow, Russia). Experiments were also carried out with the samples of natural mountain water from various Bulgarian springs and human blood serum. 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 $^1H$-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 we used a hermetic camera for evaporation of water drops under stable temperature (+22–24 $^0C$) conditions.

The water drops are placed on a water-proof transparent pad, which consists of thin myalar 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 $72,3^0$ to $0^0$. The spectrum of hydrogen bonds among $H_2O$ molecules was measured in the range of 0.08–0.1387 eV or $\lambda = 8.9$–13.8 μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between $H_2O$ molecules in human blood serum. Interviews have been conducted with 217 Bulgarian long-living people and their siblings. Their water consumption, heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With DNES method we performed a spectral analysis of 12 mountain water springs located in Teteven district (Bulgaria). The composition of the 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$^{-1}$); Non-equilibrium Spectrum (NES) and Differential Non-equilibrium Spectrum (DNES). Statistical processing of experimental data was performed using t-criterion of Student (at $p < 0.05$).

## 3. Results and discussions

### 3.1. Comparative analysis between longevity of long living people and their siblings. DNA replication and aging

54 long-living people over 90 years old have been studied together with their siblings. The average life span of long-living people is 92.1, and of their brothers and sisters it is 74.1. The difference in life expectancy of the two groups is reliable and it is $p < 0.05$, with Student's criteria at a confidence level of $t = 2,36$. There are 21 519 residents in Teteven and 142 of them have been born before 1924. Fig. 1 shows the interrelation between the years of birth of long-living people (age) and their number, Teteven municipality, Bulgaria.
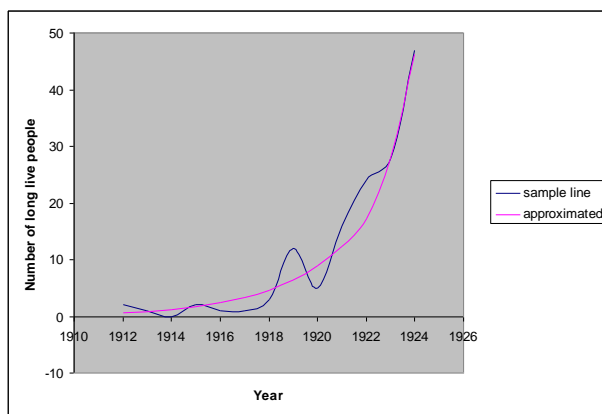


Fig. 1. Interrelation between the year of birth of long-living people (age) and their number, Teteven municipality, Bulgaria.
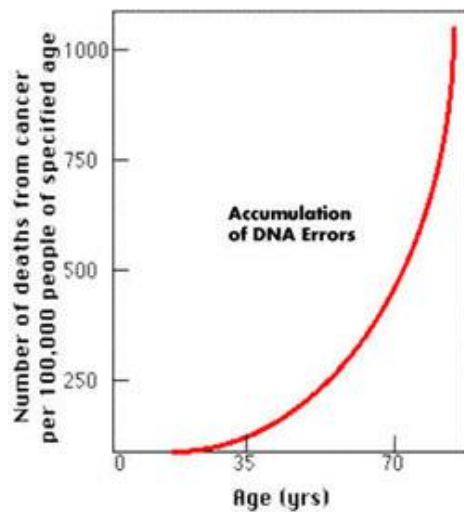
Fig. 2. Interrelation between age and number of cancer patients (Orgel, 1963)

The rate of aging increases with time. In 1963 Orgel showed that the aging process is associated with the synthesis of proteins. Fig. 2 shows Orgel's results about the interrelation between age and number of cancer patients. The accumulation of errors in the synthesis of proteins increases exponentially with age. Cells taken from elderly people show reduced transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of centenarians in the mountainous municipality of Teteven and their age is close to exponential.

### 3.2. Empirical evidence on life duration; impact of mountain water

Statistical evidence shows that long-living people inhabit mainly high mountain areas. In the Russian North there are more centenarians compared to other parts of Russia. From 1960 to 1965 the scientist Berdishev from the Tomsk Medical Institute studied centenarians in Yakutia and Altai. He linked the longevity of the Yakuts and the Altaians with the use of water from glaciers. It is well known that most of the Russian centenarians live in Dagestan and Yakutia – 353 and 324 persons per million inhabitants, respectively. This figure for overall Russia is only 8 people. In Bulgaria the number of centenarians is 47 per million. Bulgaria has third place in Europe for centenarians per million after Spain and France. In Teteven Municipality the centenarians are 279 per million. In Teteven, Yablanitsa and Ugarchin municipalities the oldest inhabitant of the field areas is 94 years old, and the oldest inhabitant of the mountain areas is 102. This is for distances of no more than 50 km between field and mountain areas, and the main differentiating factors are mountain air and water.

Data for Bulgaria:

Varna district – centenarians 44 per million, plain and sea

Pleven district – centenarians 78 per million, plain

Teteven district – centenarians 279 per million, hills and mountain

Bulgaria – centenarians 47 per million

According to Berdishev, defrosted water has a beneficial effect on cellular metabolism. Besides, these people as well as the Buryats, drink mountain water obtained from the melting of ice. The temperature of this water is 10-15°C. Analyses of water across the planet show that mountain water contains the smallest amounts of deuterium atoms in water molecules. In winter and early spring deuterium content is reduced. This water also contains ions of Ca, Mg. Na, Fe and $SO_4$. Mountain water in springtime is the result of the melting of ice and snow from the mountains. Natural ice modification $I_h$ (hexagonal lattice) is much cleaner than the water. The growing crystal of ice always strives to create the perfect crystal lattice and removes impurities. Melted water has a markedly ice-like structure and the clusters of water molecules are better preserved in it due to the presence not only of hydrogen, but also of covalent links. Each cluster exists for a short time and there is a continuous destruction of water clusters and the formation of new ones. Water analyses of different mountain springs from Bulgaria and Russia indicate that mountain water contains 3-5% lesser amount of deuterium atoms as HDO compared to river water. In natural waters the deuterium content is uneven: from 0,02−0,03 mol.% for river and sea water, to 0.015 mol.% for Antarctic ice water. Deuterium is a heavier isotope of hydrogen. The concentration of deuterium atoms is less than 1% in the water of human body. The study of the water spectrum in the human body can also answer the question of longevity. Some authors consider the hardness of water as a factor in cardiovascular disease. A mild correlation has been proven, but we do not consider water hardness to be decisive for human longevity.

### 3.3. Clinical evidence with blood serum testing

In 1983 Antonov and Yuskesselieva discovered a new physical effect. They showed experimentally that in the process of evaporation of water drops, the wetting angle θ decreases discreetly to 0, and the diameter of the drop base is only slightly altered. Based on this, through measurement of the wetting angle at equal intervals of time, the function of distribution according to value of last f (θ) is determined. The function is called spectrum of the state of water. A theoretical research of Luzar, Svetina and Zeksh has established a relation between the surface tension of water and the energy of hydrogen bonds among the water molecules (Fig. 3).
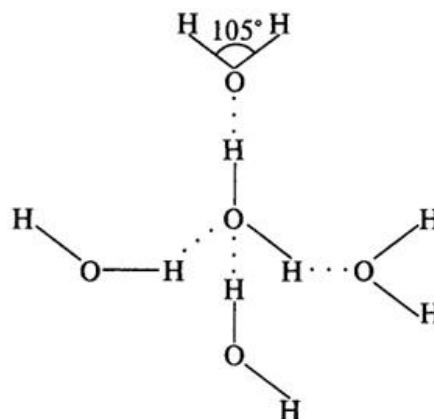


Fig. 3. Hydrogen bonding between four individual $H_2O$ molecules. The value of the angle between the covalent H–O–H bond in the $H_2O$ molecule is shown.

With the use of formula (1) and the condition for a mechanical equilibrium of the drop at the time of evaporation the following expression is found:

**f(E) = b × f(θ)/(1 - (1 + bE)²)^(1/2) (1)**

**b = 14,33 eV⁻¹**

It gives the connection between the energy spectrum of water f(E) and state spectrum f(θ). The relation between the wetting angle θ and the energy of the bond among water molecules E is represented in the following way:

**θ = arcos (-1-14,33E) (2)**

The energy is measured in electronvolts (eV). Because the energy spectrum of water is received as a result of non-equilibrium process of evaporation of water drops, the term non-equilibrium energy of water is used (NES). NES can help determine the average value of bond energy among the water molecules.

The difference: **Δf(E) = f (sample) – f (control sample) (3)**

is called differential non-equilibrium energy of water (DNES).

Fig. 4 shows the average spectrum of deionised water. On the X-axis there are few scales. The energies of hydrogen bonds among the water molecules are calculated in eV. The function of distribution according to energies f(E) in unit eV⁻¹ is written on the Y-axis. For DNES the function is Δf(E) in unit eV⁻¹.
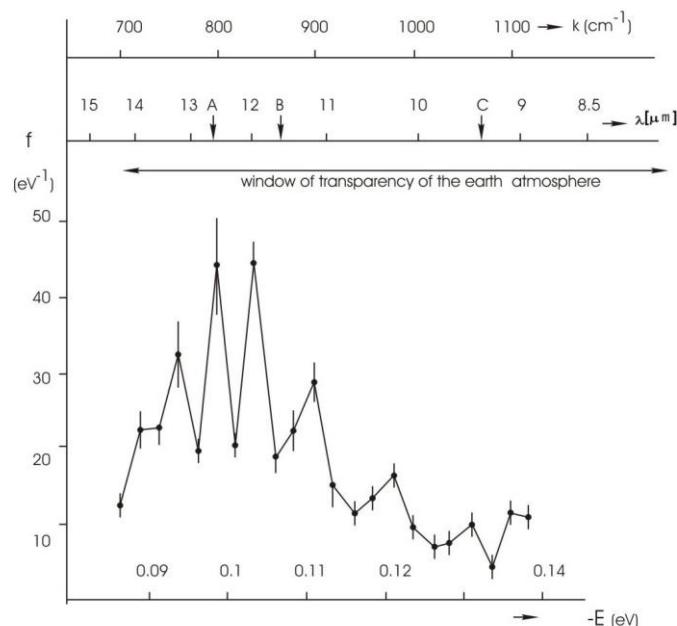


Fig. 4. NES-spectrum of deionized water (chemical purity 99.99%, pH = 6.5–7.5, total mineralization 200 mg/l, electric conductivity 10 μS/cm). The horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV). The vertical axis – energy distribution function – f (eV⁻¹). k – the vibration

frequency of the H–O–H atoms (cm$^{-1}$); λ – wavelength (mm)

Arrow A designates the energy of hydrogen bond among the water molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among the water molecules according to formula (1) through which the value is determined:

$$\bar{E} = -\ 0{,}1067 \pm 0{,}0011\ eV\ (4)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature 36,6ºC, 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 our planet toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

Studies by Ignatov (2012) were performed on a 1% solution of blood serum with the method of spectral analysis of a Non-equilibrium energy spectrum (NES) and Differential non-equilibrium energy spectrum (DNES). Empirical blood serum samples were provided by Kalinka Naneva Municipal Hospital, Teteven, Bulgaria. The samples were divided into 2 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 suffering from malignant tumors. The average energy of hydrogen bonds among water molecules in the blood serum was examined as a biophysical parameter. The result was obtained as a difference between the spectrum of 1% solution of blood serum (NES) and a control sample with deionized water (NES). This spectrum is DNES. The first group obtained the result -9.1±1,1 meV, and the second – -1.6±1,1 meV. There is a statistical difference between the two groups of results according to the t-criterion of Student at level p <0,05. For the control group of healthy people the value of the spectrum of the largest local maximum is 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 values of the spectrum of the largest local maximums shift to lower energies compared with the control group. A study of blood serum was performed by the method of infrared spectral analysis (Krasnov, Gordetsov, 2009). The following local maximums in the spectrum of absorption were received: 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35 and 9.76 μm. The resulting local maximum at 8.95 (Ignatov, 2012) is close to the one obtained by Russian scientists at 8.85 μm. In the control group of healthy people, the function of distribution according to energy f(E) at 8.95 μm has an average value of 75.3 reciprocal eV (eV$^{-1}$). In the group of people in critical condition this value is 24.1 reciprocal eV. The confidence level of the obtained results is p <0.05 by the t-criterion of Student. The resulting local maximum at 8.62 μm of heavy water is close to the one obtained by Russian scientists at 8.58 μm. In 1992 Antonov performed experiments with impact on tumor cells of a mouse in water. There was a decrease of the spectrum compared with the control sample of cells from a healthy mouse. Decrease was also observed in the spectrum of the blood serum of terminally ill people to that of healthy people. With the age increase of long-living blood relatives, the function of distribution according to energies at -0.1387 eV decreases. With this group a result was obtained at DNES – -5.5±1,1 meV at an age difference of 20-25 years compared with the control group. Most of the long-living people in Bulgaria inhabit the Rhodope Mountains. One of the mountain waters with a spectrum similar to the spectrum of the blood serum of

healthy people in 8.95 μm is the water in the Rhodopes. The mountain waters from Teteven, Boyana, etc. have similar parameters.

### 3.4. Results from mountain sources from Teteven

Table 1 shows the composition of the seven mountain springs in Teteven. The local maximums have been pointed out at -0.11 and -0.1387 eV. The value at -0.11 eV is characteristic of the presence of Ca ions. The value at -0.1362 – - 0.1387 eV is characteristic of inhibiting the development of cancer cells. Experiments conducted by Antonov with cancer tissue of mice demonstrated a reduction of this local maximum to a negative in DNES spectrum. The sample is with cancer tissue of mice in deionized water and control sample is the same deionized water. Analysis with an aqueous solution of shungite and zeolite showed the presence of a local maximum at about -0.1387 eV for shungite and -0.11 eV for zeolite. (Fig. 5).
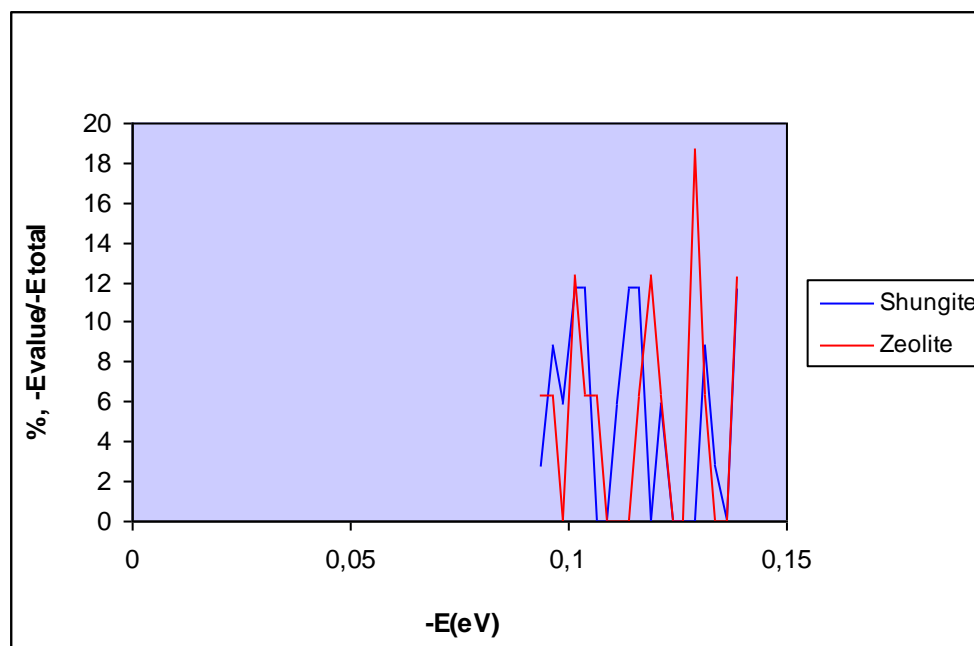


Fig. 5. The distribution (%, (-Evalue)/(-Etotal value) of water molecules in water solution of shungite/zeolite according to energies of hydrogen bonds (-Evalue) to total result of hydrogen bonds energy

In Bulgaria the main zeolite deposits are located in the Rhodope Mountains. The greatest number of centenarians in Bulgaria is also in the Rhodopes. Further scientific research in this area is needed. There is a new parameter in the table. This is the local maximum at (-0.1362– -0.1387 eV) (Ignatov, Mosin, 2012). This value is in the spectrum NES as function of distribution according to energy f(E). The norm has a statistically reliable result for blood serum for the control group with people with cancer and the local maximum of f(E) is 24.1 eV$^{-1}$ . The function of distribution according to energy f(E) for Teteven tap water is 23.8 ±1.2   eV$^{-1}$. Table 1 shows the local maximums of different mountain sources, ions of $Ca^{2+}$, $Na^+$, $Mg^{2+}$, $Fe^{2+}$, $SO_4^{2-}$ and PH.

| Sources | $Ca^{2+}$ | $Na^+$ | $Mg^{2+}$ | $Fe^{2+}$ | $SO_4^{2-}$ | pH | local maximum* at (-0.1362 – -0.1387) |
|---------|-----------|--------|-----------|-----------|-------------|-----|------------------------------|
|  | mg/ dm$^3$ norm (<150) | mg/ dm$^3$ norm (<200) | mg/ dm$^3$ norm (<80) | mg/ dm$^3$ norm (<200) | mg/ dm$^3$ norm (<250) | norm (6.5-9.5) | eV$^{-1}$ norm (>24.1) |
| 1. Klindiovo | 89.9 ±9.0 | 4.1 ±0.4 | 6.98 ±0.7 | 40.2 ±4.0 | 17.7 ±1.8 | 8.0 ±0.1 | 47.1 ±2.4 |
| 2.Gorna cheshma | 103.6 ±10.4 | 4.2 ±0.4 | 15.5 ±1.6 | 9.6 ±0.96 | 89.9 ±9.0 |  | 30.0 ±1.0 |
| 3.Dolna cheshma | 94.4 ±0.94 | 2.5 ±0.3 | 12.10 ±1.21 | 9.0 ±0.9 | 15.99 ±1.60 | 7.9 ±0.1 | 31.6 ±1.6 |
| 4. Sonda | 113.6 ±11.4 | 7.3 ±0.7 | 15.99 ±1.60 | 5.00 ±0.5 | 57.2 ± 5.7 | 7.3 ±0.1 | 48.8 ±2.4 |
| 5.Vila Cherven | x | x | x | x | 13.3 ±1.3 | 7.5 ±0.1 | 44.4 ±2.2 |
| 6. Gechovoto | 66,0 ±6.0 | 1.46 ±0.15 | 2,1 ±0.2 | 11,4 ±1.1 | 15,9 ±1.6 | 7.94 ±0.1 | 44.4 ±2.2 |
| 7.Ignatov izvor | 40.44 ±4.04 | 0.6240 ±0624 | 2.46 ±0.25 | 13.0 ±1.4 | 17.9 ±1.8 | 6.82 ±0.1 | 31.6 ±1.6 |

Table. 1 Mountain sources in Teteven and its ions and pH; *function of distribution according to energy f(E); function of distribution according to energy f(E) for tap water in Teteven is 23.8±1.2 eV$^{-1}$

### 3.5. Heredity, stress, diet, tobacco smoking, body mass, air and longevity

This study shows that the average difference between the length of life of long-living people and their brothers and sisters is 18 years. Long-living people and their brothers and sisters have common heredity. They were lived in different conditions and brother and sisters were influence on different negative factors for longevity – smoking, stress, overweight, diseases, and low physical activity. Have been studied 54 long-living people over 90 years old together with their brothers and sisters. The average life span of long-living people is 92.1, and of their siblings it is 74.1. Table 2 shows the data for long-living people as way of life. The difference in life expectancy of the two groups is reliable and it is $p < 0.05$, with Student's

criteria at a confidence level of t = 2,36. Scientific studies have shown that tobacco smoking increases the number of free radicals in the body (Pryor, 1997). The increase of free radicals leads to a deterioration of DNA replication. Adelman, Saul and Ames have shown in their research that free radical-induced DNA damage may play a central role in the aging process. Of the 54 analyzed centenarians, only 3 have smoked for several years.

| Number of centenarians | Health status | Body mass | Physical activity | Smoking | Gender | Heredity | Positive attitude towards life |
|---|---|---|---|---|---|---|---|
| 54 | In good health 48 | Normal 54 | 54 | Abstainers 51 | Female 37 | Parents and grandparents over 90 18 | 54 |
| | With diseases 6 | Above normal 0 | | Smokers 3 | Male 17 | No heredity 36 | |

Table 2a. Data for long-living people: way of life

| Number of centenarians | Gender 20th and 21st century | Parents and grandparents over 90 19th and 20st century Gender** |
|---|---|---|
| 54* (Female* and Male*) | Female* 37 Middle age 96.4 | Female** 15 Middle age 94.5 |
| 28** (Female** and Male**) | Male* 17 Middle age 91.8 | Male** 13 Middle age 95.4 |

Table 2b. Distribution of long-living people by gender; distribution of parents and grandparents of centenarians based on gender

Table 2b shows an interesting trend, but it requires additional data for statistical analysis. In 2013 and 2014 for long-living people the percentage of women was 69% and of men 21%. The percentage of parents and

grandparents of long- living people is 54% for women and 46% for men. The two differentiating factors are stress and probably smoking. With aging, T-cell generation from the thymus is much reduced (Tsukamoto, 2009). The decline rate of most T-cell and B-cell lymphocytes, which is crucial for the immune system, was faster for men. Furthermore, men showed a quicker decline in the two cytokines IL-6 and IL-10 in relation to age. Two types of immune system cells which annihilate external attackers, CD4 T-cells and natural killer (NK) cells, increased in number with age. Here the increase rate was higher for women than for men. According to experimental data, aging can be limited if food calories are reduced by 40-55%. It has been proven that obese rats live longer than slimmer ones (Weindruch, 1986). In our study with 54 centenarians, all of them have had normal body weight throughout their lives. 48 of them are in excellent clinical health, and 6 have diseases. It is doubtful that these people would have reached longevity without being healthy. All have performed regular physical activities. They live in an ecological environment in which the combination of mountain water and air, physical activity, diet, and limited stress are optimal for longevity. A test has been created for the state of muscles, joints and tendons with prognostics for a longer life (De Brito, 2012). In the mountain areas of the municipalities Teteven, Yablanitsa and Ugarchin the air has more oxygen and negative ionization. Near the sea the air also has negative ionization. Also the basic food of the people, who are living near the sea, is fish. Fish food has positive effect on cholesterol like negative factor for cardiovascular diseases. This is one of explanations for the number of centenarians of sea areas.

## 4. Conclusion

Our research shows that the composition of water and its spectrum influence the vital processes occurring in the human cells and can cause premature aging. The composition of the water, which we are drinking, and deuterium atoms affect the spectrum and hence the vital functions (Ignatov, Mosin, 2012). In our opinion, the direct relationship of man with nature – in particular the consumption of pure mountain water, accompanied by regular physical activity and a positive attitude towards life – can explain the difference between the larger number of centenarians who live in the mountain areas and the average number. Additional beneficial factors for longevity such as clean air with increased oxygen content and vitalized food from eco-farms will need further research. In this report we show some evidence that mountain water is one of the most important factors for longevity. We examine the possibility to delay the aging process by delaying errors in transcription and replication of DNA in the process of protein synthesis depending on the water we drink. The most favorable are the waters with a local maximum in 8.95 μm in the infrared spectrum, analogous to the same local maximum in the serum of healthy persons. There are results with the following factors for longevity: health status, body mass, smoking habit, gender, heredity, nervous tension (stress), and a positive attitude towards life. The project will be continued with research in the field area (Pleven region), sea area (Varna region) and in a second mountain area (Smolyan region) in Bulgaria. We need additional data for parents and grandparents of long-living people. Also we will make additional analyses of different types of water. Additional statistical analysis for all factors will be material to draw final conclusions.

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