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Mountain Water as Main Longevity Factor in Research of Phenomenon of Longevity in Mountain Areas of Bulgaria

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Abstract. In this paper are submitted data on longevity factors and mountain water in factorial research of phenomenon of longevity in mountainious and field areas of Bulgaria. The dependence was established among various internal and external factors on a phenomenon of longevity – residence area, health status, gender and heredity. It was shown that water is among the most important factors for longevity. Natural waters derived from various Bulgarian water springs were investigated by non-equilibrium energy (NES) and differential non-equilibrium energy spectrum of water (DNES) method. The biological effects of water with varrying content of deuterium are also discussed. 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 reduced deuterium content on 20–30 % has beneficial effects on health. By using IR-spectroscopy were investigated various samples of water with varying contents of deuterium, received from Bulgarian water springs and blood serum of cancer patients as well. 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 maxima in the IR-spectra of various samples of water and human blood serum at -0,1387 eV and wavelength 8,95 μm. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local maxima in IR-spectra are shifted to lower energies relative to the control group. This testifies to the structural changes of water. The obtained results testify to necessity of consumption of clean natural water which quality satisfies mountain water from Bulgarian water springs.

Keywords: deuterium; heavy water; deuterium depleted water; longevity; mountain water; NES; DNES.

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, 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 gives the longevity of immediate direct genetic ancestors. That is why the direct descendants of centenarians generally have the best chances for longevity. O. Burger demonstrate that life expectancy has increased substantially from the 19th to the 20th century and that this cannot be advantageously associated with the human genome [1]. 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. In Russia, the average life expectancy in 2012 has reached 69 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 [2]. Such mutations can reduce the ability of cells to the 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 that finaly results in cell death. Cells taken from the 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. This is most possible that, for this reason, the probability of cancer occurrence increases with age. According to thermodynamics, the process of aging is the process of alignment of the entropy by the human body with that of the environment [3].

Water is the main substance of life. The human body is composed from 50 to 75 % of water. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research. Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water is the maing 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 prolifiration of prokaryotes and eukaryotes in water with different isotopic content [4, 5]. 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 forces, 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 varries from 3 to 60 units [6]. Other important indicator of water quality is its isotopic composition. The natural water consists on 99,7 mol.% of $H_2{}^{16}O$, which molecules are formed by ^1H and ^{16}O atoms [7]. 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 a phenomenon of longevity – residence area, health status, gender, heredity, isotopic composition of water with using non-equilibrium (NES) and differential non-equilibrium (DNES) spectrum of water. The research was carried out under the joint scientific project "NATURE, ECOLOGY AND LONGEVITY" conducted in Bulgaria. In frames of this project 217 people living in the municipalities of Teteven, Yablanitza and Ugarchin, Lovech district (Bulgaria), where is lived the most number of long living people and their siblings, 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 heredity, body weight, food, diseases, positive attitude towards life.

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-spectrrometry, 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 years 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 °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 λ = 580±7 nm. The device measures the angle of evaporation of water drops from 72,3 ° to 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 217 Bulgarian centenarians 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 12 mountain water springs located in Teteven district (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}); Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova); Differential Non-equilibrium Spectrum (DNES).

Statistical processing of experimental data was performed using *t*-criterion of Student (at $p < 0,05$).

Results and discussions

Comparative analysis between longevity of long living people and their siblings.

In frames of the research 54 long living people from Bulgaria over 90 years of age have been studied together with their siblings. The average lifespan of Bulgarian centenarians is 89,1 years, and for their brothers and sisters the average lifespan is 87,8 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 $t = 2,36$ years.

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

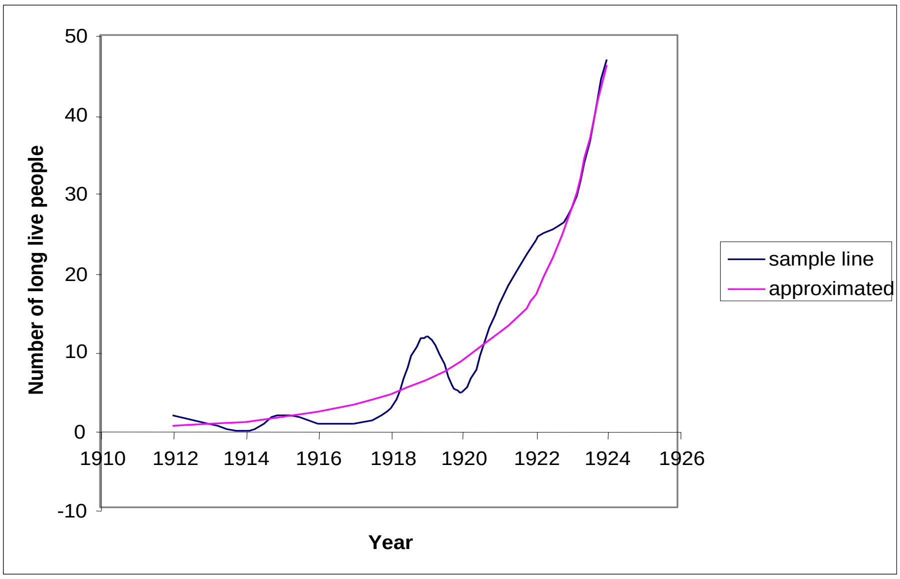

Figure 1. Interrelation between the year of birth of long-living people (age) and their number in Teteven municipality, Bulgaria.

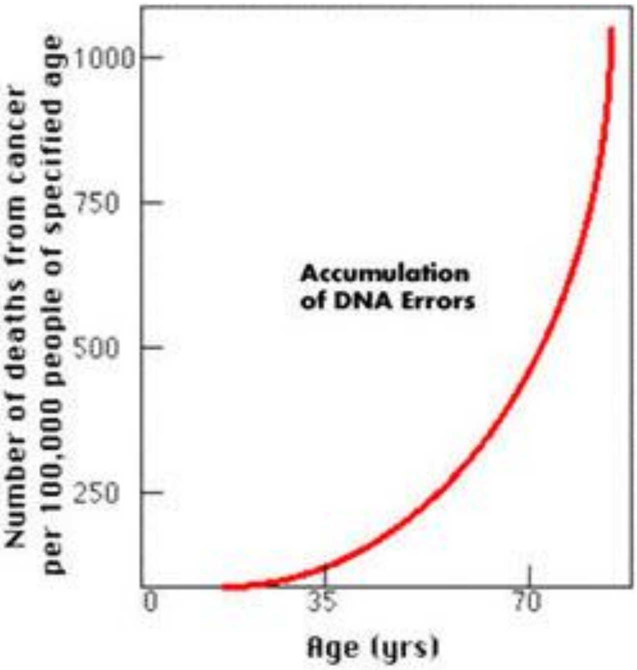

Figure 2. Interrelation between age and the number of cancer patients.

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 [8]. 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.

Empirical evidence on life duration.

Human experience shows that long-living people inhabit mainly high mountainous areas where mountain rivers flow being feeded by mountain springs. In Russia the most number of centenarians live in Russian North and Dagestan region (Russian Federation). One explanation for this is that water in those places contains less deuterium than ordinary drinking water [9].

In 1960-s G. Berdishev from Medical Institute in Tomsk (Russia) studied the phenomenon of longevity of centenarians in Yakutia and Altai regions (Russian Federation) [10]. He linked the longevity of the Yakuts and the Altaians with the consumption of melt water from glaciers formed earlier in Yakutia's mountains than those ones of Greenland. According to the State's statistics most of the Russian centenarians live in Dagestan and Yakutia – 353 and 324 persons per 1 million inhabitants. This number for all Russia is only 8 people for 1 million. In Bulgaria the average number of centenarians makes up 47 per 1 million, while in Teteven Municipality – 139 centenarians per 1 million. In the Bulgarian municipalities the oldest inhabitant in field areas is 93 years old, and the oldest inhabitant in mountainious areas is 102 years old. There are distances of no more than 50 km between these places and the only difference is mountain water and air.

Here are submitted the data for Bulgaria:

- Varna district – centenarians 44 per 1 million, plain and sea regions;
- Pleven district – centenarians 78 per 1 million, plain regions;
- Teteven district – centenarians 279 per 1 million, hills and mountainious regions;
- Bulgaria – centenarians 47 per 1 million.

Analogious 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 water sources are known as moderately warm, with temperatures of 8–10 °C, the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with ordinary tap water, that is believed to have a positive effect on the tissue cells and metabolism. Melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and has a beneficial effect on metabolism [11].

Today are known 14 crystalline modifications of ice, each has its own structure and a character of disposition of hydrogen atoms (Table 1). The natural ice is represented by the hexagonal I_h configuration. 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. In the crystalline structure of natural ice I_h hydrogen bonds are oriented towards the tetrahedron apexes at strictly defined angles equal to 109^o5 (in liquid water this angle is 104^o5) (Fig. 3). 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 vertices – 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. The carcasses of hydrogen bonds allocate H_2O molecules in form of a spatial hexagon network with internal hollow hexagonal channels inside. 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 (anomality of temperature, pressure, density, viscosity, fluidity, etc). According to theoretical calculations, at the melting of the ice breaks about 15% of all hydrogen bonds [12]; by further heating to 40 °C breaks down about half of hydrogen bonds in H_2O associates. In water vapor hydrogen bonds are absent.

Table 1: Ice crystal modifications and their physical characteristics

Modification	Crystal structure	Hydrogen bond lengths, Å	Angles H–O–H in tetragonals, °
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
XII	Tetragonal	4,01	90
XIII	Monoclinic	7,47	90–109,7
XIV	Rhombic	4,08	90

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

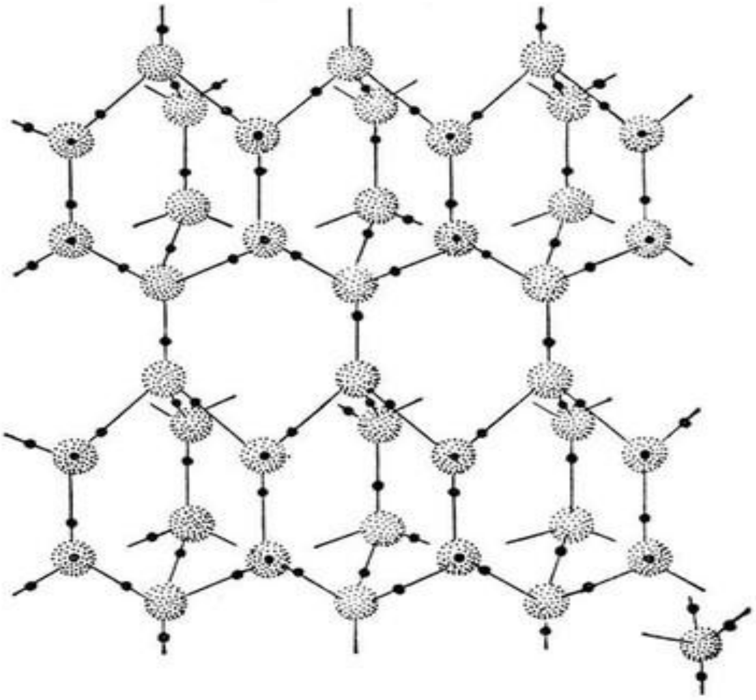

Figure 3. Hydrogen bonding in crystalline structure of natural ice I_h under the temperature at -10 °C and normal pressure at 1 atm. Grey balls – O-atoms, black balls – H-atoms. On the right below is shown the structural unit of crystalline ice structure

Mountain water in springtime is the result of the melting of ice and snow accumulated in the mountains. Natural ice with I_h modification (hexagonal lattice) is usually much cleaner than water, because solubility of all substances (except NH_4F) in ice is extremely low. The growing ice crystal is always striving to create a perfect crystal lattice and therefore displaces impurities. Meltwater has a certain “ice-like” structure, because it preserves the hydrogen bonding between water molecules; as a result it is formed complex intermolecular associates (clusters) – analogues of ice structures, consisting of a larger or smaller number of H_2O molecules (Fig. 4). However, unlike ice crystal, each associate has a very short time of existance, as a result there occurs the constant processes of decay and formation of water associates having very complicated structure [13]. The specificity of intermolecular interactions characteristic for the structure of ice, is kept in melt water, as it is estimated that in the melting of ice crystal is destroyed only 15 % of all hydrogen bonds in the associates. Therefore, the inherent to ice connection of each H_2O molecule with four neighboring H_2O molecules is largely disturbed, although there is obserbed the substantially "blurring" of oxygen lattice framework. Processes of decay and formation of clusters occur with equal probability, that is probably why physical properties of melt water are changed over time, i.g. dielectric permittivity comes to its equilibrium state after 15–20 min, viscosity – in 3–6 days [14]. Further heating of fresh melt water above +37 °C leads to a loss of its biological activity. Storage of meltwater at +22 °C is also accompanied by a gradual decrease in its biological activity; within 16–18 hours it is reduced by 50 %. 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 down of hydrogen bonds. H_2O molecules being out of equilibrium positions begin to fall down 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) during the transition from ice to melt water varies from 4,4 at +1,5 °C to 4,9 at +80 °C.

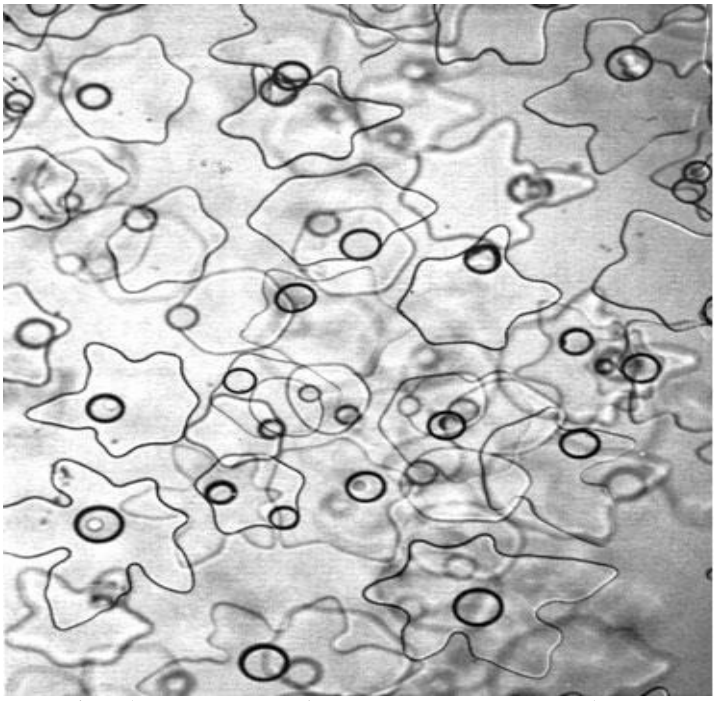

Figure 4. Structure of meltwater according to computer simulations with "smearing" fragments of regular ice structures

Preliminary analyses of water from various water sources show that mountain 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 is approximately 20–30 mg/l, Mg^{2+} – 5–10 mg/l, Ca^{2+} – 25–35 mg/l, the content of SO_4^{2-} – <100 g/l, HCO_3^- 50–100 mg/l, Cl^- – less than 70 mg/l, total rigidity ≤ 5 mEq/l, the total mineralization ≤ 0,3 g/l, pH – 6,5–7,0 at 25 °C (Table 2). 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 that of ordinary portable water. This fact is important because some authors consider the hardness of the water to be among the main factors in cardiovascular diseases [15].

However, mild correlation was further proven that water hardness could not be a decisive factor for human longevity.

Table 2: Composition of melt water

Cations, mg/l	
$K^+ + Na^+$	20–30
Mg^{2+}	5–10
Ca^{2+}	25–35
Anions, mg/l	
SO_4^{2-}	<100
HCO_3^-	50–100
Cl^-	<70
Total rigidity, mEq/l	≤5
Total mineralization, g/l	≤0,3
pH at 25 °C	6,5–7,0

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) [16]. 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 varried 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 of 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.

Clinical evidence on the benefits of deuterium depleted water for health.

When biological objects being exposed to water with different deuterium content, their reaction varies depending on the isotopic composition of water and magnitude of isotope effects determined by the difference of constants of chemical reactions rates k_H/k_D in H_2O and D_2O. The maximum kinetic isotopic effect observed at ordinary temperatures in chemical reactions leading to rupture of bonds involving hydrogen and deuterium lies in the range k_H/k_D = 5–7 for C–H versus C–D, N–D versus N–D, and O–H versus O–D-bonds [17].

Our previous studies have shown that heavy water of high concentration is toxic for the organism, chemical reactions are slower in D_2O, compared with ordinary water, the hydrogen bonds formed with participation of deuterium are somewhat more stronger that those ones formed from hydrogen [18]. In mixtures of D_2O with H_2O with high speed occurs dissociation reactions and isotopic (H-D) exchange resulting in formation of semi-heavy water (HDO): $D_2O + H_2O = HDO$. For this reason deuterium presents in smaller content in aqueous solutions in form of HDO, while in the higher content – in form of D_2O. The chemical structure of D_2O molecule is analogous to that one for H_2O, with small differences in the length of the covalent H–O-bonds and the angles between them. D_2O boils at +101,44 °C, freezes at +3,82 °C, has density of 1,1053 g/cm³ at 20 °C, and the maximum density occurs not at +4 °C as in H_2O, but at +11,2 °C (1,1060 g/cm^3). These effects are reflected in the chemical bond energy, kinetics, and the rate of chemical reactions in D_2O.

The chemical reactions and biochemical processes in the presence of D_2O are somehow slower compared to H_2O. D_2O is less ionized, the dissociation constant of D_2O is smaller, and the solubility of the organic and inorganic substances in D_2O is smaller compared to these ones in H_2O. However, there are also such reactions which rates in D_2O are higher than in H_2O. In general these reactions are catalyzed by D_3O^+ or H_3O^+ ions or OD^- and OH^- ions. According to the theory of a chemical bond the breaking up of covalent C–H bonds can occur faster than C–D bonds, the mobility of D_3O^+ ion is lower on 28,5 % than H_3O^+ ion, and OD^- ion is lower on 39,8 % than OH^- ion, the constant of ionization of D_2O is less than that of H_2O [19].

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 [20]. It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype [21].

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. The cell evidently implements the special adaptive mechanisms promoting functional reorganization of work of the vital systems in the presence of D_2O.

D_2O can cause metabolic disorders, kidney's malfunction, violation of hormonal regulation and coused immunosuppression [22], notwithstanding the strong radioprotective effect of D_2O [23]. Also deuterium induces physiological, morphological and cytological alterations on the cell with forming cells more 2–3 times larger in size in D_2O. At high concentrations of D_2O are suppressed enzymatic reactions, cell growth, mitosis and synthesis of nucleic acids [24]. Thus, the most sensitive to replacement of H^+ on D^+ are the apparatus of biosynthesis of macromolecules and a respiratory chain, i.e., those cellular systems using high mobility of protons and high speed of breaking up of hydrogen bonds. Last fact allows to consider adaptation to D_2O as adaptation to the nonspecific factor affecting simultaneously the functional condition of several numbers of cellular systems: metabolism, ways of assimilation of carbon substrates, biosynthetic processes, and transport function, structure and functions of macromolecules. Evidently cells are able to regulate the D/H ratios, while its changes trigger distinct molecular processes. One possibility to modify intracellular D/H ratios is the activation of the H^+-transport system, which preferentially eliminates H^+, resulting in increased D/H ratios within cells [25].

We have obtained results on growth and adaptation to D_2O of various cells of prokaryotic and eukaryotic organisms. Our studies have shown that animal cells are able to withstand up to 25–30 % D_2O, plants – up to 50–60 % D_2O, and protozoa cells are able to live on ~90 % D_2O. Further increase in the concentration of D_2O for these groups of organisms leads to cellular death. On the contrary, deuterium depleted water with 25–30 % decreased deuterium content has benefitial effects on organism. Experiments on animals and plants demonstrated that after consumption of water with reduced on ~25–30 % deuterium pigs, rats and mice produced a larger number of offspring, upkeep of poultry with 6-day old to puberty on deuterium depleted water leads to accelerated development of the genital organs (size and weight), and strengthen the process of spermatogenesis, wheat ripens earlier and gives higher yields [26]. In addition, deuterium depleted water delays the appearance of the first metastasis nodules on the spot inoculation of cervical cancer and exerts immunomodulatory and radioprotective effect [27].

Radioprotective effects of deuterium depleted water were studied in reports [28] at irradiation of mice's cells by γ-radiation at semimortal dose LD_{50}. Survival level of animals treated with deuterium depleted water for 15 days prior to γ-radiation, was 2,5-fold higher than in control group (dose of 850 R). The surviving experimental group of mice has the number of leukocytes and erythrocytes in the blood remained within the normal range, while in the control group the number of leukocytes and erythrocytes was significantly decreased [29].

Consumption of deuterium depleted water by cancer patients during or after radiation therapy treatments allows restore the composition of blood and relieve nausea [30]. According to

G. Shomlai, the results of clinical trials conducted in 1998–2010 in Hungary showed that the survival rate for patients drinking deuterium depleted water in combination with traditional therapies or after are significantly higher than for patients who only used the chemotherapy or radiation therapy [31].

Biological experiments with deuterium depleted water carried out in Moscow Research Oncological Institute after P.A. Herzen and N.N. Blokhin and Institute of Biomedical Problems, confirmed the inhibitory effects of deuterium depleted water on the process of growth of various tumors, i.g. division of breast adenocarcinoma MCF-7 tumor cells being placed in deuterium depleted water started with a delay of ~5–10 hours [32]. In 60 % of mice with immunosuppressed immunity and transplanted human breast tumor MDA and MCF-7 consumption of deuterium depleted water caused tumor regression. A group of mice with transplanted human prostate tumor PC-3 consumed deuterium depleted water showed the increase in the survival rate by ~40 %; the ratio number of dividing cells in tumors of dead animals in experimental group was 1,5:3,0, and in control group – 3,6:1,0. In this regard special attention deserves two indicators: the delay of metastasis and loss of animal’s weight during experiments. Stimulating action of deuterium depleted water on the immune system of animals has led to delay of development of metastasis by 40 % in comparison with the control group, and weight loss in animals that consumed deuterium depleted water at the end of the experiment was 2 times less. It was also reported that deuterium depleted water may delay the progression of prostate cancer [33] and inhibit human lung carcinoma cell growth by apoptosis [34].

Preliminary experimental results on motility of human sperm, performed by V.I. Lobyshev and A.A. Kirkina [35], indicates that in deuterium depleted water water (4 ppm) motility is on 40 % higher during 5 hours of the registration. However, the effect depends on the initial properties of a sperm sample. These dasta indicated that deuterium content variation in water including deep deuterium depletion produce various nonlinear isotopic effect on key processes in a cell as enzyme action of Na, K-ATPase, regeneration, motility, fertilizing effectiveness and embryo developing. It should be noted that for any deuterium concentration dependence there should be an optimal condition for the best result.

One prominent effect of deuterium depletion is to inhibit fatty synthesis, chain elongation and desaturation. These anabolic reactions utilize acetyl-CoA, as well as hydrogen of water for new fatty acid pools [36]. Fatty acids then are used for new membrane formation in the rapidly proliferating cell. The complex structure and molecular organization of the mammalian fatty acid synthase offer remarkable opportunities with altered morphology and flux handling properties.

The positive influence of drinking deuterium depleted water on blood chemistry included a significant reduction of glucose, cholesterol, erythrocyte sedimentation rates, leukocyte counts and cortisol (stress hormone) levels, while also revealed an increase in antioxidant capacities [37]. These data evidence the significance of deuterium depleted water to increase energy resources even in a healthy cohort, while decreasing risks of psycho-emotional stress, which is known to pose a negative influence on blood biochemistries that often lead to psychosomatic diseases and shorten life. It was also noted the positive impact of water on indicators of saturation the liver tissue by oxygen: the observed increase in pO_2 was ~15 %, i.e., cell respiration increased 1,3 times [38]. On beneficial effect on health of experimental mice evidenced the increased resistance and weight increase compared with the control group [39]. It was also indicated that deuterium depleted water increases the rate of metabolic reactions. It was observed geroprotective (anti-aging), anti-mutagenic and radioprotective effects of deuterium depleted water with reduced on 5 % deuterium content on the development cycle of fruit fly *Drosophila melanogaster*.

The total effects of deuterium depleted water depends on the following parameters – total body mass, total body water, the amount of daily consumption of deuterium depleted water and the degree of its isotope purification. The main impact of deuterium depleted water on the organism is explained by gradual reduction of the deuterium content in the physiological fluids of the body by reactions of isotopic (H-D) exchange. These results indicate that regular using of deuterium depleted water helps improve the functioning of some vital systems [40]. With regular consumption of deuterium depleted water occurs the cleaning of organism from HDO due to the reaction of isotopic (H-D) exchange in physiological fluids, and it was recorded the change of the isotopic composition of urine and Ca^{2+} content as well. Daily consumption of deuterium depleted water allows naturally reduce the content of HDO in the human body due to isotopic (H-D) exchange. It is believed that this process is accompanied by an increase in the functional activity of cells, cell tissues and organs. Thus regular consumption of deuterium depleted water provides a natural way to reduce the content of HDO in the human body to a lower values. It has beneficial effects on metabolism, invigorates the body, and also promotes the rapid recovery after strenuous physical exercise. This testifies the usage of deuterium depleted water for residents of large cities and megapolices.

Clinical trials of deuterium depleted water with a residual content of deuterium 60…100 ppm, showed that it can be recommended as an adjunct in the treatment of patients having metabolic syndrome (hypertension, obesity, impaired glucose metabolism) and diabetes. In addition, it was shown that deuterium depleted water improves the quality of life for patients having renal stone disease (nephrolithiasis) and various disorders in the gastrointestinal tract (colitis and gastritis), cleanses the body of toxins, enhances the action of drugs, promotes weight correction, protects cells from radiation. Deuterium depleted water can be recommended for fast and deep cleaning of the human body from deuterium that is essential for metabolic disturbances. Taking into consideration the dynamics of the distribution of water in the human body, the reaction of isotopic (H/D and $^{16}O/^{18}O$) exchange and the results obtained with deuterium depleted water, it can be expected that the greatest effect the isotopic purification of water will have on the regulatory system and metabolism.

The effectiveness of the influense of deuterium depleted water depends on the following parameters – total body mass, total body water, the amount of daily consumption of deuterium depleted water and the isotopic content of deuterium. The results on the gradual increasing of deuterium content in the human body at regular consumption of deuterium depleted water with varried residual deuterium content are shown in Table 4. This table shows that the content of deuterium in the human body decreases while drinking the deuterium depleted water. Thus, at the consumption of water with a residual deuterium content of 60 ppm deuterium content in the body decreases after 45 days to 117,3 ppm, and at the consumption of water with a residual content of deuterium 100 ppm – to 131 ppm at 1 liter of water consumption per a day, to 122,6 ppm at water consumption of 1,5 liters of water a day. Hence, the regular use of deuterium depleted water provides a natural way to reduce the content of HDO in the human body to a value of ~117 ppm.

Table 4: Gradual decreasing of deuterium content in the human body over time,
with regular consumption of deuterium depleted water*

Number of days	The residual content of deuterium in water, ppm		
	60	100	100
	Daily consumption of deuterium depleted water, liters		
0	1	1	1,5
1	150,5	150,7	150,8
2	145,5	147,9	146,9
7	136,5	143,6	140,5
14	130,6	138,3	134,7
21	120,8	135,6	129,6
28	120,0	133,9	126,6
35	119,6	132,6	124,5
45	117,3	131,5	122,6

*Notes: The calculation was performed based on the following data:
- daily consumption of deuterium depleted water – 1 or 1,5 liter;
- daily water exchange rate – 2,5 liters;
- deuterium content in the body corresponds to its content in natural water ~ 150 ppm;
- the average volume of water in the body – 45 liters (average body weight ~ 75 kg).

Clinical evidence with human blood serum testing.

It was established experimentally that at 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 [41]. 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. 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 $O\cdots H^{+}–O^{-\delta}$.

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 between H_2O molecules (E) is established:

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

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:

$\theta = \arccos(-1 - 14{,}33E)$, (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 5 shows the average spectrum of deionised water. On the X-axis are sshown 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 as $\bar{E}$ = -0,1067±0,0011 eV.

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature 36,6 °C, is at its maximum. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

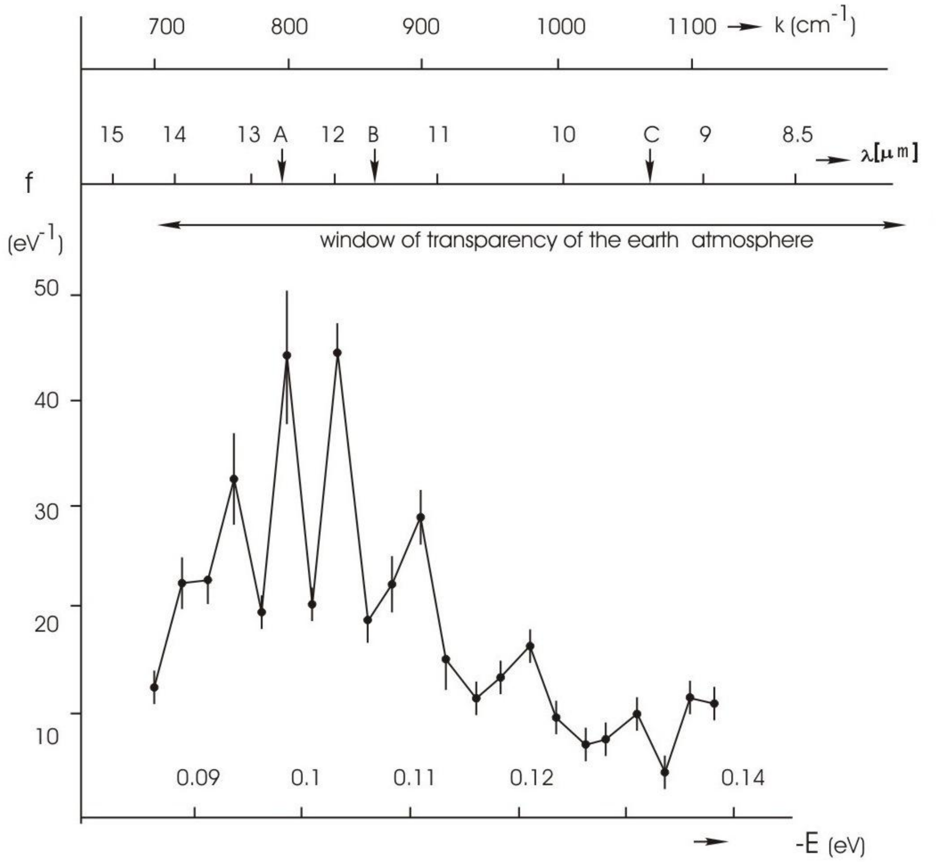

Figure 5. Non-equilibrium (NES) spectrum of water; λ – wavelength; *k* – wave number

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 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 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 Δf(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 *t*-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 bllod serum by IR-spectrometry. The magnitude of the largest local maximum in IR-pectrum 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 maxima 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. The resulting peak at λ = 8.95 μm in IR-spectrum [42] 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 < 0,05 according to Student's t-criterion.

In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water [43]. 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 Rhodope 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.

Table 3 shows the composition of the seven mountain springs in Teteven (Bulgaria) and local maximums in DNES-spectra of water. The local maximums 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 maximum 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 [44]. These results sugest 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. 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 area is cleared in a natural way by zeolite. Therefore, a new parameter is entered into Table 3 – a local maximum of energy at (-0,1362±0,1387 eV). This value 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 maximum 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 3: The composition of mountain water springs in Teteven (Bulgaria) and local maximums in DNES-spectra of water

Sourses	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±10,1	4,2±0,4	15,5±1,6	9,6±0,96	89,9±9,0	7,8±0,2	20,0±1,0
3.Dolna cheshma	94,4±0,94	2,5±0,3	1,10±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	–	–	–	–	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±3,04	0,62±0,10	2,46±0,25	13,0±1,4	17,9±1,8	6,82±0,1	31,6±1,6

Notes:
*Function of distribution of H_2O molecules according to energy f(E).

Another important physical parameter was calculated with using NES and DNES methods – the average energy ($\Delta E_{H...O}$) of H...O-bonds between H_2O compiled –0,1067±0,0011 eV. The most remarkable peculiarity of H...O-bond consists in its relatively low strength; it is 5–10 times weaker than chemical covalent bond. 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 covalent O–H-bonds in H_2O molecule – 109 kcal/mole. With fluctuations of water temperature the average energy of hydrogen H...O-bonds in H_2O 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. The next conclusion that can be drawn from our research is that there is the distribution of energies among individual H_2O molecules.

Heredity, stress, diet, smoking, body mass as additional longevity factors

Research showed that tobacco smoking increases the number of free radicals in the body [45]. The accumulation of free radicals leads to distortion of DNA replication. Evidently free radical-induced damage of DNA molecule plays an essential role in the process of aging. These data show that the average difference between the length of life of centenarians and their brothers is 10 of the 54 studied centenarians, only 3 were long-time smokers (Table 5).

Table 5: Distribution of long living people by gender. Distribution of parents and grandparents of centenarians based on gender

Number of centenarians	Health status	Body mass	Smoking	Gender	Heredity	Positive attitude towards life
54	In good health 48	Normal 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 6: Data for centenarians depending on their way of life

Number of centenarians	Gender 20th and 21st century	Parents and grandparents over 20th and 21st century
54	Female 37 Middle age	Female 15 Middle age 94,5
	Male 17 Middle age	Male 13 Middle age 95,4

Table 6 shows an interesting trend, which, however, requires additional data for statistical analysis. In 2013 and 2014 the number of females was 69% and males – 21 %. The number of parents and grandparents of long living people was 54 % for females and 46 % for males. The only two different factors were stress and probably smoking.

It is known that during the process of aging T-cell generation from the thymus is much reduced [46]. The decline rate of most T-cell and B-cell lymphocytes, which are crucial for the immune system, is faster in males than in the femailes. Furthermore, males 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, are increased in number with age. The increase rate is higher in females than in males.

It should be noted that the process of aging can be limited if food caloricity of diet is being restricted on 40–55 %. In studies with 54 Bulgarian centenarians, all of them have had normal body mass throughout their lives; 48 of them were in excellent health condition, while 6 have various diseases. It is doubtful that these people would have reached longevity without being healthy. All of these studied people have had great physical activity. They live in friendly ecological environment in which the combination of mountain water, physical activity, diet and less stress are optimal for longevity. Further test has been created for the state of muscles, joints and tendons with prognostics for a longer life.

Conclusions

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 centenarians who live in the mountain regions of Bulgaria and Russia and their high average number. Natural water with increased content of deuterium seems to be one of the most important factors for longevity. In Bulgaria, most centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. It worth to note that IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at $\lambda = 8{,}95$ μm. Similar spectral characteristics possess mountain water from Teteven, Bojana 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 drow a vital state statusof 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. Further we are planning to continue this project for the research of phenomenon of human longevity in field area (Pleven region), sea area (Varna region) and mountainious area (Smolyan region) in Bulgaria. Although additional data for parents and grandparents of long-living people are needed, total statistical analysis for all these summary factors will be essential for further scrutinized conclusions.

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Горная вода как главный фактор долголетия в исследовании феномена долголетия в горных районах Болгарии

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Аннотация. В данной работе представлены данные о горной воде как главном факторе долголетия в горных районах Болгарии. Авторами была установлена зависимость между различными внутренними и внешними факторами на феномен долголетия болгарских долгожителей, в том числе качествам потребляемой воды, изотопным составом воды, места жительства, состояния здоровья, пола и наследственности. Было показано, что вода является одним из наиболее важных факторов для долголетия. Природные воды, полученные из различных болгарских родников были исследованы методами неравновесного энергетического (НЭС) и дифференциального неравновесного энергетического (ДНЭС) анализа. Также изучены биологические эффекты воды с различным содержанием дейтерия. Показано, что повышенное содержание дейтерия в воде приводит к физиологическим, морфологическим и цитологическим изменениям в клетках, а также оказывает негативное влияние на клеточный метаболизм, а вода с пониженным содержанием дейтерия на 20–30 % благотворно влияет на здоровье. С помощью ИК-спектроскопии были исследованы различные образцы воды с различным содержанием дейтерия, полученные из различных болгарских водных источников, а также сыворотка крови больных раком. Как фактор оценки измеряли значения средней энергии водородных связей ($\Delta E_{H...O}$) между молекулами H_2O, а также локальные максимумы в ИК-спектрах различных образцов воды и сыворотки крови человека при -0,1387 эВ и длине волны 8,95 мкм. Показано, что для группы людей в критическом состоянии жизни и больных со злокачественными опухолями наибольшие значения локальных максимумов в ИК-спектрах смещаются в сторону меньших энергий по отношению к контрольной группе. Это свидетельствует о структурных изменениях воды. Полученные результаты свидетельствуют о целесообразности потребления чистой природной воды, качеству которой которая удовлетворяет горная вода из болгарских источников.

Ключевые слова: дейтерий; тяжелая вода; протиевая вода; долгожитие; горная вода; ИК-спектроскопия; НЭС; ДНЭС.

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Basic Concepts of Magnetic Water Treatment

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Abstract. This review article outlines an overview of new trends and modern approaches for practical implementation of magnetic water treatment to eliminate scaling salts (carbonate, chloride and sulfate salts of Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} cations) in power heat-exchanger devices and pipe lines. The principles of physical effects of the magnetic field on H_2O molecules as well as the parameters of physico-chemical processes occurring in water and the behavior of the dissolved in water scaling salts subjected to the magnetic treatment are discussed. It is demonstrated that the effect of the magnetic field on water is a complex multifactorial phenomenon resulted in changes of the structure of hydrated ions as well as the physico-chemical properties and behavior of dissolved inorganic salts, changes in the rate of electrochemical coagulation and aggregate stability (clumping and consolidation), formation of multiple nucleation sites on the particles of fine dispersed precipitate consisting of crystals of substantially uniform size. There are also submitted data on constructive features of various magnetic water treatment devices produced by domestic industry, based on the permanent magnets and electromagnets (solenoids), such as hydro magnetic systems (HMS), magnetic transducers (MT) and magnetic activators (MA) of water. It was estimated the efficiency of using the various magnetic water treatment devices in water treatment technologies.

Keywords: magnetic field; magnetic water treatment; scaling salts; power heat industry.

Introduction

As is known, the effect of magnetic field on water bears a complex and multifactorial character that in the final result affects the structure of water and hydrated ions as well as the physico-chemical properties and behavior of dissolved inorganic salts [1]. When being applied to water, the magnetic field therein changes the rates of chemical reactions due to the occurrence of competing reactions of dissolution and precipitation of the dissolved salts, facilitates the formation and decomposition of colloidal complexes, and improves electro-coagulation followed by sedimentation and crystallization of scaling salts of Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} [2].

Hypotheses explaining the mechanism of action of the magnetic field on water are divided into three main groups – colloidal, ionic and water hypotheses.

The first hypotheses assume that under the influence of the magnetic field in the treated water there occurs a spontaneous formation and decay of colloidal complexes of metal cations - Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}, which fragments of decay further forming the cetrtes of nucleation of inorganic salts that accelerates their subsequent sedimentation. It is known that the presence of metal cations (particularly, Fe^{3+}) and microinclusions of ferromagnetic iron particles of Fe_2O_3 in water intensifies the formation of colloidal hydrophobic sols of Fe^{3+} cations with chlorine Cl^- anions and neutral H_2O molecules having the general formula $[xFe_2O_3 \cdot yH_2O \cdot zFe^{3+}] \cdot 3zCl^-$, which may cause the formation of nucleation centers which surface adsorbs calcium Ca^{2+} and magnesium Mg^{2+} cations (forming the basis of the carbonate hardness of water) that leads to the formation of fine crystalline precipitation as a sludge [3]. Thus, the more stable the ion hydrate shell is, the more difficult they converge or deposit on the adsorbent complexes formed on the surfaces between the liquid and solid phases.

The hypotheses of the second group explain the action of the magnetic field on water by polarization of dissolved ions and deformation of their hydration shells by the magnetic field, accompanied by a decrease of hydration - an important factor that contributes to the solubility of the dissolved salts in water, electrolytic dissociation, distribution of various substances between solid and liquid phases, kinetic constants and equilibrium chemical reactions rates in aqueous solutions, which in its turn increases the likelihood of convergence of the hydrated ions and sedimentation processes and crystallization of inorganic salts [4]. In the scientific literature there is experimental evidence that under the influence of the magnetic field there occurs a temporary distortion of hydration shells of the dissolved in water ions that alters their distribution between the solid and the liquid phase [5]. It is assumed that the influence of the magnetic field on the dissolved in water ions of Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} can be also associated with the generation of a weak electric current in a moving stream of water or with the pressure pulsation [6].

The hypotheses of the third group postulate that the magnetic field due to dipole polarization of water molecules directly influences the structure of water associates formed from a variety of H_2O molecules bound to each other via a low energy intermolecular van der Waals forces, dipole-dipole interactions and hydrogen bonding, which may cause the deformation of hydrogen bonds and their partial rupture, as well as the migration of mobile protons H^+ within the associative elements of water and redistribution of H_2O molecules in temporary associatives - clusters with general formula $(H_2O)_n$, where n according to the recent studies can reach tens to several hundreds units [7]. These effects may in combination alter the structure of water that leads to the observed changes in its density, surface tension, viscosity, pH value, and parameters of physical and chemical processes occuring in water under the applied magnetic field, including the dissolution and crystallization of dissolved inorganic salts [8].

There is evidence of the effectiveness of magnetic water treatment in reduction of the concentration of oxygen and carbon dioxide in the magnetic treated water that is explained by the formation of metastable clathrate structures of metal cations as a hexo aqua complex of $[Ca(H_2O_6)]^{2+}$. The complex influence of the magnetic field on the structure of the hydrated cations of scalling salts opens up broad prospects for magnetic water treatment in power heating and related industries, including the water treatment.

There is also evidence pointing out to the bactericidal effect of the magnetic field [9] that is essential for the use of the magnetic water treatment in systems that require a high level of microbial purity.

The above mentioned factors contribute to the use of the magnetic water treatment in the power heat exchange devices and systems that are sensitive to scale - as formed on the inner walls of pipes of heat exchangers the solid deposits of hydrocarbon (calcium carbonates $Ca(HCO_3)_2$ and magnesium carbonates $Mg(HCO_3)_2$, decaying to $CaCO_3$ and $Mg(OH)_2$ when water is heated with the subsequent release of CO_2, as well as sulfate ($CaSO_4$, $MgSO_4$), chloride ($ClCl_2$, $MgCl_2$) and in less degree silicate (SiO_3^{2-}) salts of calcium, magnesium and iron [10]. Limescale reduces the diameter of the pipelines, which leads to the increased flow resistance, which in its turn adversely affects the operation of heat exchange equipment. Since the scale has an extremely low coefficient of thermal conductivity than the metal from which the heating elements were made, water heating consumes much more time. Therefore over time, the energy losses can make the functioning of a heat exchanger on such water ineffective or even impossible. When the thickness of the inner layer of limescale increases, this lead to a violation of water circulation; in boilers this may lead to overheating of the metal and, ultimately, to its destruction. All these factors lead to the need for regular repairs, replacement of piping and plumbing and requires substantial capital investments and additional financial costs to clean out the heat exchange equipment from limescale.

The magnetic water treatment compared with conventional methods of water softening by ion exchange and reverse osmosis technology is simple, economical and environmentally safe. It is effective in the treatment of calcium-carbonate waters, which make up about 80% of all natural waters of the Russian Federation and Bulgaria. That is why the scope of applying of the magnetic water treatment covers the power heating boilers, heat exchangers, boilers, compressors, engine cooling systems and generators, steam generators, network supplying hot and cold water, district heating, piping and other heat transfer equipment etc. The magnetic water treatment reduces corrosion of steel pipes and heat exchange equipment by ~30-50% (depending on the initial composition of water), which makes it possible to increase the lifetime of thermal power equipment, water pipes, and significantly reduce the accident rates [11].

Taking into account the contemporary trends and prospects of using the magnetic water treatment, it is very relevant to develop the new and to improve the existing magnetic water treatment technologies for achievement of higher efficiency and functioning of magnetic water treatment devices for a more complete extraction of the water hardness and increasing resources of heat exchange equipment. The aim of this research was to review the mechanisms of action of the magnetic field on water, the parameters occurring in water physico-chemical processes and the behavior of the dissolved in water salts.

The mechanism of action of the magnetic field on water. The principle of operation of existing water softener magnetic devices is based on complex multifactorial influence of the exposed magnetic field generated by permanent magnets or electromagnets to the dissolved in water hydrated metal cations of Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} and the structure of the hydrates and water associates that leads to a change in the rate of electrochemical coagulation and aggregate stability (clumping and consolidation) of dispersed charged particles in a liquid stream of magnetized water and to formation of multiple nucleation sites on the particles of fine dispersed precipitate consisting of crystals of substantially uniform size [12].

In the process of the magnetic water treatment there are occurred several processes:

- The displacement by the electromagnetic field the balance between the structural components of water and the hydrated ions;
- Increase in the nucleation of dissolved salts on microinclusions of dispersed ferroparticles in a local volume of water;
- Changing in coagulation and sedimentation rates of dispersed particles in the treated by the magnetic field water flux.

As a result, magnesium and calcium salts dissolved in water lose their ability to form a dense deposits - instead of usual calcium carbonate is formed fine crystalline polymorphic $CaCO_3$, which on the structure resembles aragonite – a carbonate mineral with orthorhombic acicular crystals, that is either not released from the treated water as the crystal growth stops at the stage of microcrystals, or is precipitated as a fine sediment accumulating in the sump container.

Anti-scale effect under the magnetic water treatment depends on the composition of the treated water, the magnetic field strength, rate of water movement, the duration of its stay in the magnetic field and other factors. In general, anti-scale effect of the magnetic treatment of water increases with increasing temperature of the treated water; with increasing content of Ca^{2+} and Mg^{2+} cations; with an increase in the pH value of the treated water, as well as with the reducing the total salinity of water.

In theoretical calculations an individual water molecule is considered as the charged dipole. With the flow of water molecules (dipoles) in the magnetic field perpendicular to the magnetic field lines along the axis Y (the vector V) occurs torque F1, F2 (Lorentz force) trying to deploy a molecule in the horizontal plane (Figure 1). When the dipole moves in a horizontal plane, along an Z-axis, in the vertical plane arises a torque. The magnet poles prevent rotation of the dipole molecule; therefore the movement of the dipole perpendicular to the magnetic field lines will be inhibited. This leads to the fact that for a dipole placed between the two poles of the magnet remains only one degree of freedom - the oscillation along X-axis, i.g. the oscillation along the magnetic lines of the applied field. For other coordinates the motion of H_2O dipoles is limited: they became “sandwiched” between the poles of a magnet, making an oscillatory motion about the X-axis. Certain position of the dipoles of H_2O molecules in the magnetic field along the field lines will be maintained, thereby somehow arranging their orientation in the magnetic field. These theoretical calculations are applicable to the description of the behavior in the magnetic field the hydrated metal cations Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}, with the difference that in the magnetic field there occurs polarization of the hydrated cation shells.

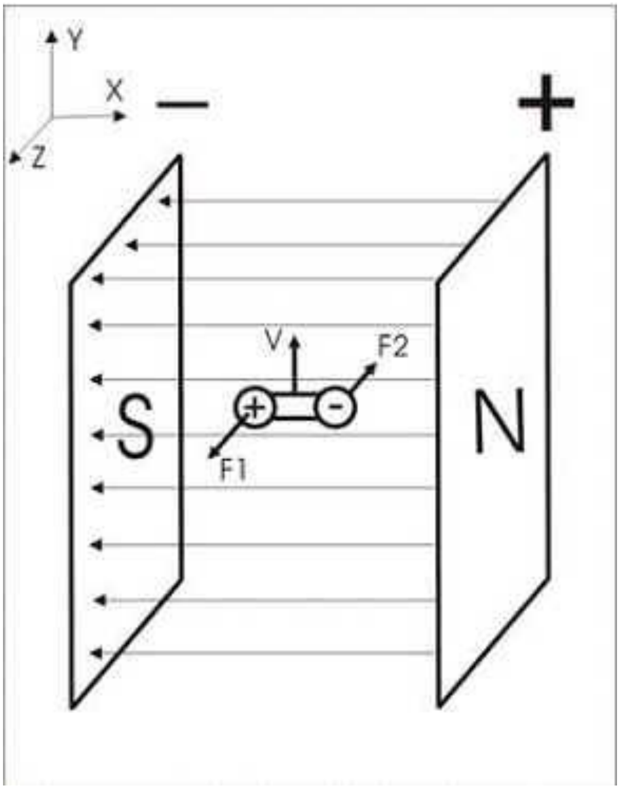


Figure 1. Physical behavior of the dipole in the magnetic field

It was proved experimentally that on the fixed water the magnetic field acts much weaker, because moving in a flux water poccess some electroconductivity; while its moving in the electromagnetic fields are generated small electric currents [13]. Therefore, this method of treatment of moving in a stream water is often designated by the magnetohydrodynamic treatment (MHDT). With the use of modern methods MHDT can achieve effects such those as observed at water treatment, as the increase of pH value of water (to reduce the corrosivity of water flow), creating a local increase in the concentration of ions in the local volume of water (for the conversion of excess content of hardness ions in a finely divided crystalline phase and prevention of salts precipitation on the surface of heat exchange equipment and piping), etc. [14].

Structurally, the majority of magnetic water treatment apparatus are composed of a magneto-cell manufactured in the form of a hollow cylindrical element made from ferromagnetic material with the magnets placed inside by the means of the flange or the threaded connection with the annular air gap cross sectional area, the passage area of which, however, is not smaller than the passage area of the supply and discharge piping, that does not lead to a significant drop in output pressure of the apparatus [15]. As a result of a steady laminar flow of an electrically conductive fluid (water) in the magnetodynamic cell being placed in a uniform transverse magnetic field with induction B_o (Figure 2), the Lorentz force is generated [16], the value of which depends on the charge q of the particle, its velocity u and the magnetic field B.

$$\vec{F}_\Lambda = q \cdot [\vec{u} \times \vec{B}]$$

The Lorentz force is directed perpendicular to the fluid velocity and the lines of the magnetic induction of the electromagnetic field B, whereby the ions and the charged particles in a fluid stream are moving around a circle which plane is perpendicular to the lines of the vector B [17]. Thus, by choosing the required position of the magnetic induction B with respect to the velocity vector of the liquid stream, it can be possible to purposefully affect the cations of Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}, redistributing them in a local volume of an aqueous medium.

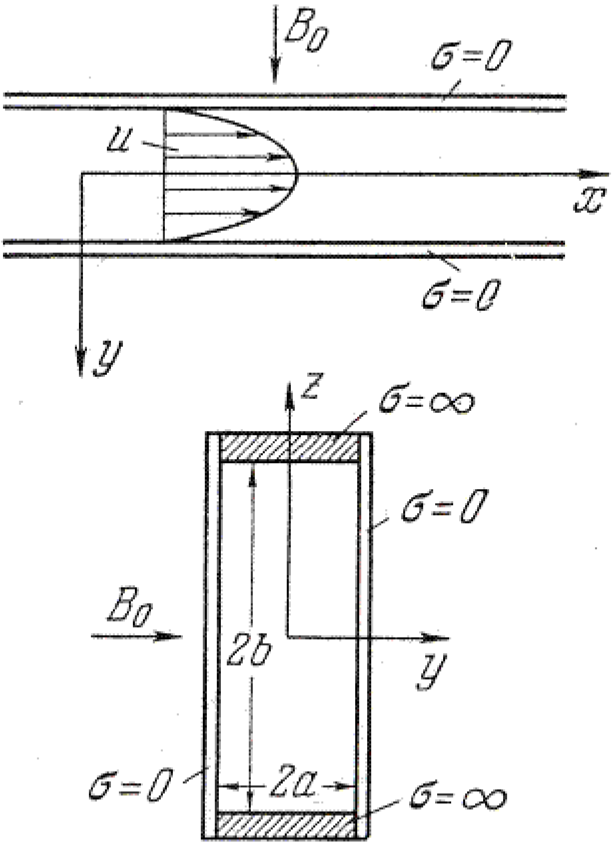


Figure 2. Diagram of flow of water in the MHD cell: σ - conductivity of the cell wall; B_0 - amplitude value of the magnetic field induction vector

According to theoretical calculations, to initiate crystallization of hardness salts within the local volume of the liquid (water) moving through a pipe around the walls of the pipes in the operating air gaps of the magnetic device, is applied such a direction of the magnetic induction B_0, wherein in the middle of the operating air gap is formed the zone with zero induction. For this purpose, the magnets are arranged in the device in such a way that the same magnetic polls are directed towards each other (Figure 3). In this scheme under the action of the Lorentz force in an aqueous medium there occurs a counter flow of anions and cations in the region interacting with the zero value of magnetic induction, which contributes to the creating in this zone the concentrations of interacting ions, which in its turn leads to their precipitation and subsequent formation of nucleation centers of scale-forming salts.

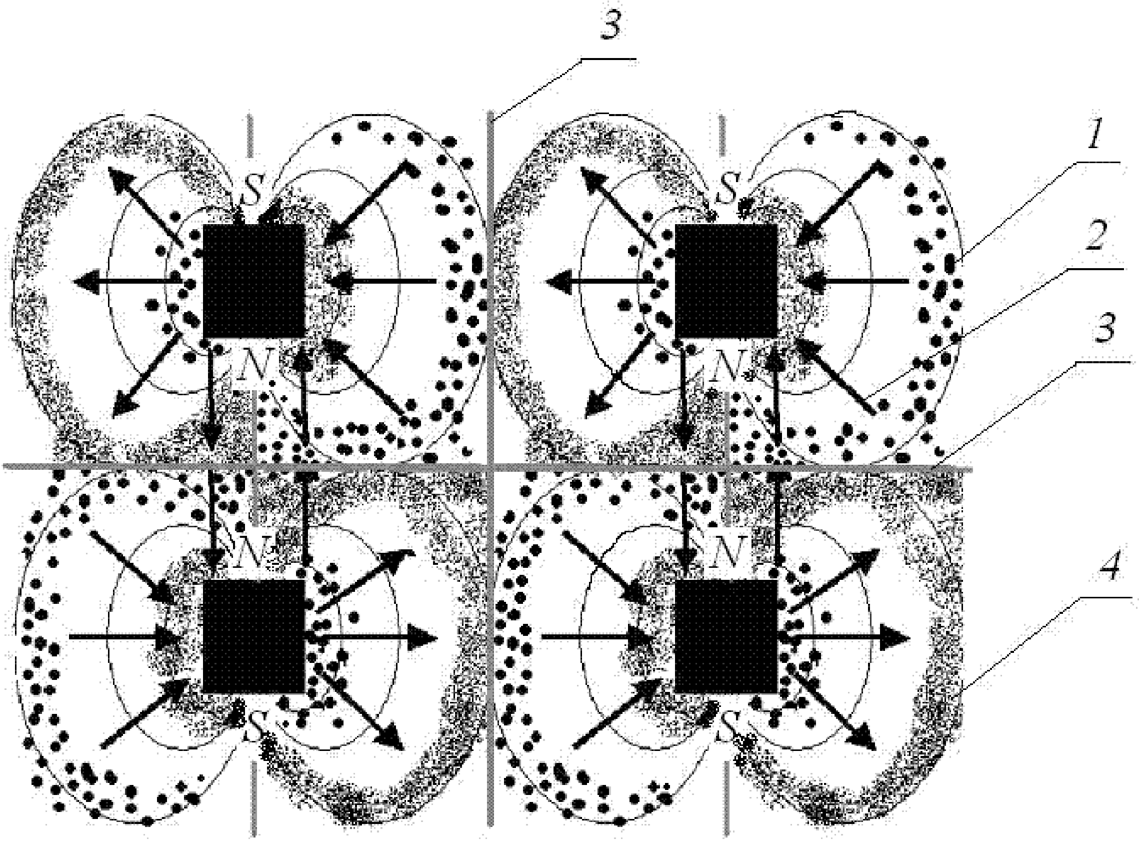


Figure 3. Scheme of layout of magnets, lines of induction, Lorentz force vectors and ions in MHDT. 1 - anions, 2 - the direction of the induced currents, 3 - zones with zero induction, 4 – cations

When designing magnetic devices it need be specified the type of device performance, the amplitude of the magnetic field in the gap and the corresponding magnetic field strength, the velocity of water in the operating air gap, the passage time of water through the core unit, the composition of the ferromagnet (apparatus with electromagnets/solenoids), magnetic alloy and dimensions of the magnet [18].

Design of magnetic water treatment devices. Domestic industry produces two types of devices for the magnetic water treatment (DMW) – based on permanent magnet made from hard magnetic ferrites (Table 1, Table. 2) and operates on AC solenoid electromagnets (Table 3) (solenoids with ferromagnets), generating an alternating magnetic field. These devices subdivided into hydromagnetic systems (HMS), magnetic transducers (hydromultipoles) - MSP, MWS, MMT and activators of water series AMP, MPAV, AIM, KEMA for domestic and industrial usage. Most of them are similar in design and principle of operation (Figure 4 and Figure 5). Both these devices are mounted to the pipeline by means of threaded or flanged connections.

Installing of threaded magnetic water treatment devices is carried out in the following cases:

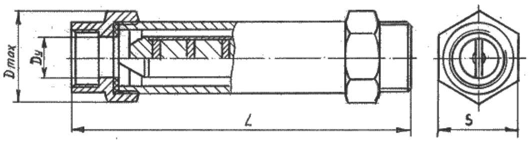
- If the system has a pump unit, the selection carries out on the performance of the pump;
- When setting the threaded device the distance to the object to be protected must be in a range from 1 m to 5 m;
- If there is a water meter, the unit is set up not less than 1 meter after the water meter along the flow of water;
- It is necessary to install the device in a relaxing (laminar) flow of water, i.e. before the pump or more than 15 m after it;
- If possible to set up the unit before the pump to protect it;
- To protect the divice from sludge it is required to set up the device before the magnetomechanical filter;

- Selection of magnetofilter is carried out by the connection diameter;

Installation of flange magnetic water treatment devices is carried out in the following cases:

- The unit is installed at least not far from 3 meters and within 30 meters from the protected equipment;
- The unit is set up in laminar flow of water, or before the pump or more than 15 m after it;
- If possible to set the unit to the pump to protect it;
- In front of the divice is set sludge filter set (not necessarily magnetomechanical);
- Selection of magnetofilter is carried out by connection diameter.

Table 1: Specifications of domestic magnetic water treatment devices (screw connection) based on permanent magnets

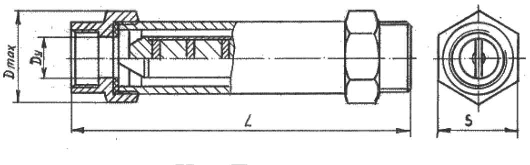


Key Features:

- Nominal diameter (mm): 10; 15; 20; 25; 32
- Nominal pressure (MPa): 1

Parameter	Apparatus model				
	AMP 10 PZ	AMP 15 PZ	AMP 20PZ	AMP25 PZ	AMP32 PZ
The amplitude peak value of the magnetic induction (B_o) on the surface of the working area, mT	180				
The number of working areas	5				
Nominal water flow, min./norm./max, m^3/h	0,15/0,5/0,71	0,35/1,15/1,65	0,65/1,9/2,9	1,0/3,0/4,5	1,6/4,8/7,4
Nominal diameter, mm	10	15	20	25	32
Compound, inch	1/2	1/2	3/4	1	$1^1/_4$
Maximum working pressure, MPa	1				
Operating temperature range, °C	5–120				
Dimensions, (L×D_y), mm	108×32	124×34	148×41	172×50	150×56
Weight, kg	0,5	0,75	0,8	1,2	1,8

Table 2: Specifications of domestic industrial magnetic water treatment devices (flange connection) based on permanent magnets

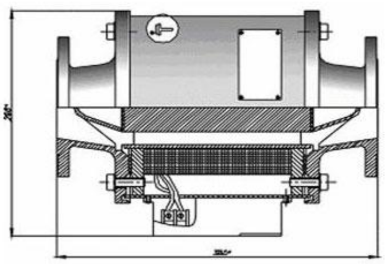


Key Features:

- Nominal diameter (mm): 32; 40; 50; 65; 80; 100; 125
- Nominal pressure (MPa): 10

Parameter	Apparatus model						
	AMP 32FZ	AMP 40FZ	AMP 50FZ	AMP 65FZ	AMP 80FZ	AMP 100FZ	AMP 125FZ
The amplitude peak value of the magnetic induction (B_o) on the surface of the working area, mT	180						
The number of working areas	5						
Nominal water flow, min./norm./max, m^3/h	1,6/4,8/7,4	2,5/7,5/11,5	4,8/11,8/18	6,6/20/30,5	10/30,5/46	15,7/47/72	20/75/112,5
Nominal diameter, mm	32	40	50	65	80	100	125
Maximum working pressure, MPa	10						
Operating temperature range, °C	5–120						
Dimensions, (L×D_y), mm	280×145	326×160	398×180	418×195	460×215	540×245	568×280
Weight, kg	8,0	13,5	19,0	24,0	32,0	45,0	56,0

Table 3: Specifications of domestic magnetic water treatment devices based on the electromagnets (solenoids)



Key Features:

- Nominal diameter (mm): 80; 100; 200; 600
- Nominal pressure (MPa): 1,6

Parameter	Apparatus model			
	AMO-25UHL	AMO-100UHL	AMO-200UHL	AMO-600UHL
Voltage, V	220			
Frequency, Hz	60			
Performance for treated water m^3/h	25	100	200	600
The magnetic field strength, kA/m	200			
Temperatures of water, °C	60	40	50	70
Working water pressure, MPa	1,6			
Electromagnet power consumption, kW	0,35	0,5	0,5	1,8

The dimensions of the electromagnet, mm	260×410	440×835	520×950	755×1100
Overall dimensions of the power supply, mm	250×350×250			
Electromagnet weight, kg	40	200	330	1000
Weight of power supply unit, kg	8.0			

a)

b)

Figure 4. Types of domestic devices for magnetic water treatment based on permanent magnets: a) - with flange compounds; b – with screw compounds

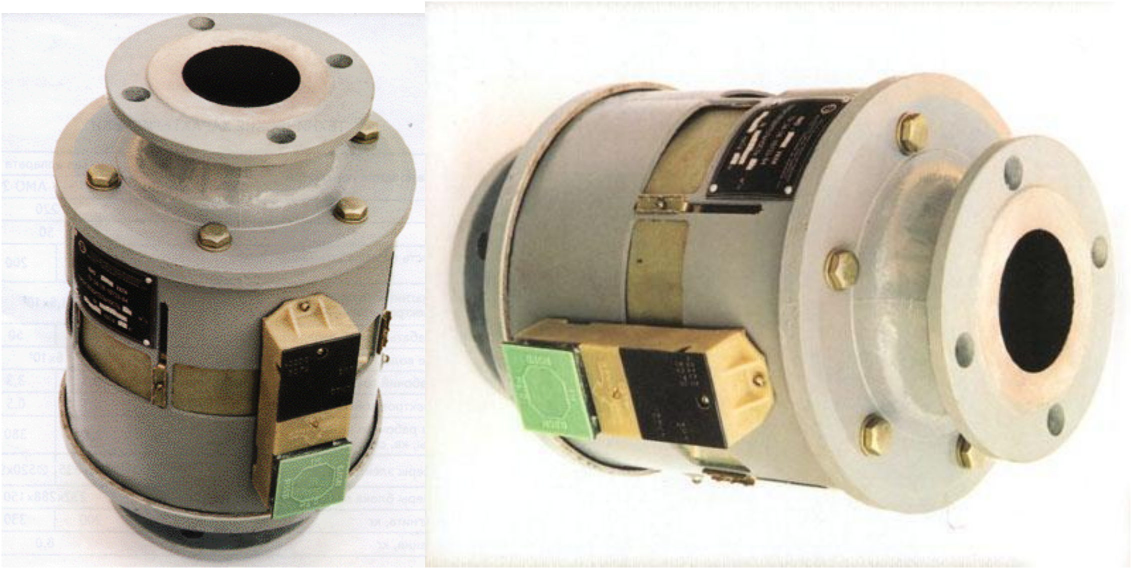


Figure 5. Domestic apparatus for magnetic water treatment based on electromagnets AMO-25 UHL (“SIBKOTLMASH”, Russia)

In magnetic devices operating on electromagnets (solenoids), water is exposed by continuously adjustable influence of the magnetic field of different strength and alternating direction of the magnetic induction, wherein electromagnets can be located both inside and outside the unit. The electromagnet consists of a coil-winding and the magnetic circuit formed by the core, the rings of the coil and the casing. Between the core and the coil is formed an annular gap for the passage of an influx of the treated water. The magnetic field crosses twice the water flow in a direction perpendicular to its motion. The control unit provides a half-wave rectification of AC to DC. To install the electromagnet in the pipeline there are provided special electric adapters. The unit itself must be installed as close as possible to the protected equipment. If the system has a centrifugal pump, the magnetic treatment unit is mounted after the pump.

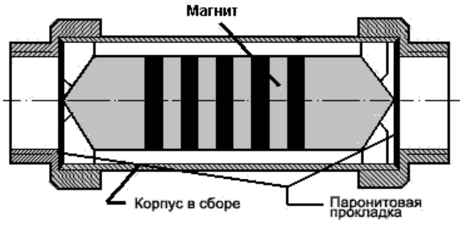
In the constructions of the second type of magnetic devices are applied permanent magnets based on modern powdered magnetic carriers - ferromagnetic ferrite, barium and rare earth alloy magnetic materials of rare earth metals neodymium (Nd), samarium (Sm) with zirconium (Zr), iron (Fe), copper (Cu), titanium (Ti), cobalt (Co) and boron (B). Recent types on neodymium (Nd), iron (Fe), titanium (Ti) and boron (B) are preferable because they possess long service life, the magnetization 1500-2400 kA/m, a residual induction of 1,2-1,3 Tesla, the energy of the magnetic field 280-320 kJ/m^3 (Table 4) and do not lose their properties when heated to 150 ^{o}C.

Table 4: Main physical parameters of rare-earth permanent magnets

Magnet composition	Residual induction, T	Magnetization, kA/m	Magnetic field energy, kJ/m^3
Sm–Zr–Fe–Co–Cu	1,0–1,1	1500–2400	180–220
Nd–Fe–Co–Ti–Cu–B	1,2–1,3	1500–2400	280–320

The permanent magnets oriented in a certain way in a unit are arranged coaxially within the cylindrical body of the magnetic element made from stainless steel of marking 12X18H10T, which ends are provided with tapered centering tips elements connected by argon-arc welding [19]. The main element of the magnetic transducer (magnetic dinamic cell) is a multi-pole magnet of cylindrical shape that creates a symmetrical magnetic field, the axial and radial components of which under the transition from one pole to another pole of the magnet change in the opposite direction. Due to the location of the magnets, creating a high-gradient magnetic field transverse with respect to the water flow, is achived the maximum efficiency of the magnetic field on the ions of dissolved in water scale-forming salts. As a result, crystallization of scale-forming salts does not occur on the walls of the heat exchangers, but in the bulk water as a fine suspension (dredge), which is removed by blowing a stream of water in special sump collectors installed in heating systems after DMW-device, as well as in hot water magistrales. Hydromagnetic water treatment systems (HMS) are varried favorably on techno-economic characteristics. The optimal interval velocity of the water flow for HMS makes up 0,5-4,0 m/s, the optimal pressure – 0,8-1,0 MPa (Table 5) [20]. Operating costs of such devices usually make up 10 years. These devices can be installed in both industrial and domestic conditions: magistral lines, feeding water into the water mains, boilers, flow heaters, steam and water boilers, water heating systems of various technological equipment (compressor stations, electric cars, and other thermal equipment). Although HMS are designed for water flow rates from 0,08 to 1100 m^3/h and for the pipe's diameter of 15-325 mm, however, there have been experience in creating the magnetic devices for power plants with pipe sizes of 2000 to 4000 mm [21].

Table 5: Specifications of hydromagnetic systems



Key features:

- Nominal diameter (mm): 15; 20; 25
- Nominal pressure (MPa): 0,8

Model	Pipe diameter, mm (inch)	Nominal pressure, Mpa	Productivity, m^3/h
HMS-15	15(1/2")	0,8	1,5
HMS-20	20(3/4")	0,8	2,0
HMS-25F	25(1")	0,8	7,0
HMS-35F	35(1")	0,8	20,0

In recent time are also used the apparatus of pulsed magnetic field, the distribution of which in the space is characterized by an electric frequency modulation and pulse intervals composed of microseconds, capable of generating a strong induction at 5-100 T and superstrong magnetic fields with the the magnetic induction being more than 100 T. For this purpose are used mainly helical coils made of durable steel and bronze. For formation of superstrong constant magnetic fields with greater induction are used superconducting electromagnets [22].

Devices based on permanent magnets are favorably differend from the magnetic devices based on electromagnets (solenoids), because during their operation there are not any problems associated with the consumption of electricity and, therefore, with the repair from electrical breakdown of electromagnet coils (Table 6, Table. 7). These devices can be installed in both industrial and domestic conditions: lines, feeding water into the water mains, boilers, water heating systems of various technological equipment. The main disadvantage of these devices is that the permanent magnets on the base of barium ferrite are demagnetized on 40-50% after 5 years. For industrial purposes it is recommended to use the magnetic devices based on electromagnets, as AMO-25UHL, AMO-100UHL, AMO-200UHL and AMO-600UHL.

When designing magnetic devices is setting up an apparatus type, its capability, the magnetic field in the operating air gap and the corresponding magnetic field strength, the velocity of water in the operating air gap, the time of passage of water, the core unit, the composition of the ferromagnet (machines with electromagnets), magnetic alloy and dimensions of the magnet (machines with permanent magnets).

Table 6: Magnetic activators AMV. Household series (with screw connections)

№	Model	Capacity, m^3/h		
		Minimal	Average	Maximum
1	Magnetic activator AMB D_y 10	0,1	0,5	0,9
2	Magnetic activator AMB D_y 15	0,2	1,35	2,5
3	Magnetic activator AMB D_y 20	0,5	2,25	4,0
4	Magnetic activator AMB D_y 25	1,0	4,00	7,0

Table 7: Magnetic activators AMV. Industrial series (with flanged connection)

№	Model	Capacity, m^3/h		
		Minimal	Average	Maximum
1	Magnetic activator AMB D_y 32	1,8	5,9	10,0
2	Magnetic activator AMB D_y 40	2,5	7,7	13,0
3	Magnetic activator AMB D_y 50	3,3	11,7	20,0
4	Magnetic activator AMB D_y 65	5,0	20,0	35,0
5	Magnetic activator AMB D_y 80	8,0	26,5	45,0
6	Magnetic activator AMB D_y 100	12,0	51,0	90,0
7	Magnetic activator AMB D_y 125	20,0	85,0	170,0
8	Magnetic activator AMB D_y 150	30,0	130,0	260,0
9	Magnetic activator AMB D_y 175	45,0	170,0	345,0
10	Magnetic activator AMB D_y 200	55,0	215,0	435,0
11	Magnetic activator AMB D_y 250	100,0	400,0	700,0

Apparatus for magnetic water treatment may be used for household and industrial purposes to prevent scaling; to reduce the effect of scaling in pipes of hot and cold water magistrales, in heating elements of the boiler equipment, heat exchangers, steam generators, cooling equipment, etc.; to prevent corrosion in pipes of local hot and cold water magistrales; for purification of water (for example, after chlorination); in this case, the deposition rate of scale-forming salts is increased by 2-3 fold with sumps requiring smaller capacity; increase of the filtration systems for chemical water treatment - filtration cycle is increased by 1,5 times while reducing the consumption of chemical reagents and purification of heat exchange units [23]. The magnetic water treatment devices may be used alone or as part of any installations patterns of apparatus tend to scale formation during operation - water treatment systems in dwellings, cottages, children and health care facilities, water treatment in the food industry, etc. The use of these devices is the most efficient for treatment of water with a carbonate hardness predominance to 4 mg-Eq/l, and the total hardness to 6 mg-Eq/l with a total mineralization level of 500 mg/l.

Requirements regulating the working conditions of the magnetic water treatment devices consist in the following factors:

- Temperature of water heating in the apparatus should be no higher than 95 °C;
- Content of $Fe^{2+}$and Fe^{3+} ions in the treated water should be no less than 0,3 mg/l;
- The total content of sulfates and chlorides of Ca^{2+} and Mg^{2+} ($CaSO_4$, $CaCl_2$, $MgSO_4$, $MgCl_2$) should be not more than 50 mg/l;
- Carbonate hardness ($Ca(HCO_3)_2$, $Mg(HCO_3)_2$), - not more than 9 mEq/l;
- Content of dissolved oxygen in water - less than 3 mg/l;
- The velocity of the water flow in the apparatus - 1-3 m/s.

According to the Russian building regulation norms (SNIP 11-35-76, “Boiler”), the magnetic water treatment for thermal equipment and boilers is advisable to carry out, if the content of Fe^{2+} and Fe^{3+} ions in water does not exceed 0,3 mg/l, oxygen content - 3 mg/l, the constant hardness of water ($CaSO_4$, $CaCl_2$, $MgSO_4$, $MgCl_2$) - 50 mg/l, carbonate hardness ($Ca(HCO_3)_2$, $Mg(HCO_3)_2$) is not greater than 9 mEq/l and the water heating temperature must not exceed 95 °C. For power boilers and cast iron sectional boilers the using of the magnetic water treatment technology is possible if the carbonate hardness is less than 10 mEq/l, the content of Fe^{2+} and Fe^{3+} in water – 0,3 mg/l, on condition that water enters from the tap or surface source. Some productions, however, establish more stringent regulation to the water purification, until its deep softening (0,035-0,05 mEq/l) for water-tube boilers (15-25 atm) – 0,15 mEq/l; fire-tube boilers (5-15 atm) – 0,35 mEq/l; high pressure boilers (50-100 atm) – 0,035 mEq/l.

Conclusions

On the basis of this research can be made the following conclusions:

- The magnetic water treatment affects both an influence on the water, the mechanical impurities and scale-forming salts and ions and on the nature of the physical and chemical processes of dissolution and crystallization;
- In water exposed after magnetic treatment is possible the change of the hydration of ions, salts solubility, pH value, which results in changing the rate of corrosion processes.

Thus, magnetic water treatment causes a variety of related physical and chemical effects. Indisputable advantages of magnetic treatment in contrast to the traditional schemes of water softening by using ion exchange and reverse osmosis is the simplicity of the technological scheme, environment safety and economy. Besides magnetic water treatment method requires no chemical reagents, and is therefore environmentally friendly.

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Основные принципы магнитной обработки воды

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Аннотация. В данной статье приводится обзор современных методов и подходов практической реализации магнитной обработки воды для устранения солей жесткости (карбонатные, хлоридные и сульфатные соли Ca^{2+}, Mg^{2+}, Fe^{2+} и Fe^{3+}) в теплообменной аппаратуре и трубопроводах. Рассмотрены комплексные многофакторные принципы физического воздействия магнитного поля на молекулы H_2O, параметры протекающих в воде физико-химических процессов и поведение растворенных в воде солей жесткости, подвергающихся магнитной обработке. Показано, что влияния магнитного поля на воду является комплексным многофакторным феноментом, результатом которого являются изменения структуры гидратированных ионов, а также физико-химических свойств и поведения растворенных в воде неорганических солей с последующей их агрегацией и преципитацией, образование многочисленных центров кристаллизации на частицах тонкодисперстного осадка из солей, состоящих из микрокристаллов почти одинаковой формы. Также приведены данные по конструктивным особенностям различных аппаратов для магнитной обработки воды, выпускаемы отечественной промышленностью, основанные на постоянных магнитах и электромагнитах (соленоидах): гидромагнитные системы (ГМС), магнитные преобразователи (МП) и магнитные активаторы воды. Показана эффективность использования различных аппаратов магнитной обработки воды в технологиях водоподготовки.

Ключевые слова: магнитное поле; магнитная обработка воды; соли жесткости; теплоэнергетика.

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Crucial Processes' Interaction During the Renewal of Articular Cartilage: the Mathematical Modeling

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Abstract. The article is an attempt to explain the renewal of the articular cartilage in normalcy and osteoarthritis development by principles of mathematical modeling. Such models help to develop advanced methods of prevention, detection and treatment of osteoarthritis including molecular biotechnologies based on tissue engineering conception. We used histological images to perform structural analysis to discover the signs of active system and its states. Received data are useful to develop research protocols in cartilage tissue engineering.

Keywords: articular cartilage; osteoarthritis; cartilage tissue engineering; mathematical modeling.

Introduction

Osteoarthritis is a wide-spread degenerative disease of joints associated with a large social and economic burden. Its incidence rates are greater than 100/100,000 person-years (rates from Fallon Community Health Plan in Massachusetts (USA), Dutch Institute for Public Health (RIVM)) [1]. Ten percent of people who are older than 55 suffer knee osteoarthritis, and 2,5% become disabled. So, the restoration of damaged and lost tissues of the articular cartilage is one of the most important problems of modern regenerative medicine [2, 3].

Biological and social reasons of this are well observed [4, 5]. There are initially low cartilage regenerative capability, increasing age and quality of life, as consequence, quick ageing of population with active lifestyle, “traumatic” plague in connection with technology expansion to all areas of professional activity, and extremism challenges.

Classical approaches assume defects substituting by auto- or homological material or stimulating own regenerative ability of cartilage. Such approaches have a number of irremovable limitations and do not guarantee full cartilage restoration for a long time. The satisfactory solution seems to be within the physical-chemical biology and current molecular tissue engineering technologies [6, 7, 8]. Such approach combines advanced bio-compatible chondroinductive materials with controlling mechanisms of all processes needed to remodel articular cartilage, similar to native one. Tissue engineering together with regenerative medicine, based on stem cells usage, is interdisciplinary scientific area has being developed for a bit more than 25 years. It is based on engineering principles and techniques and uses the latest achievements of material science, chemistry, biology and bioinformatics for biological substitutes, which restore, maintain and improve injured tissue functions. These engineering constructions should be biomimetic, have proper physical-chemical properties and, ideally, should be replaced by organism’s self-tissue within some reasonable period [3, 5]. The development and implementation of such materials requires from a researcher to understand interaction of key processes, underlying articular cartilage matrix remodeling.

There are different approaches to simulate articular cartilage as a biological system. Some researchers handle it as molecular-biological system. Article [9] introduced mathematical model of interactions in system “chondrocytes – (pro-/anti-) inflammatory cytokines”, which had properly described cellular response to some signal molecules. Another common approach was applied in the paper [10]. It introduced 3D diffusion model of locomotion elements destruction and, in particular, articular cartilage, which is described as a mechanical system.

This articles implements active systems theory approach in cartilage tissue remodeling. During the quantitative morphological analysis, we try to discover meaningful measures, which qualify active system states.

Modeling the articular cartilage

We start from point of view about articular cartilage to be an active system, which response to controlling actions might be determined adequately. Therefore, such system modeling problem reduces to finding dependencies that reflect principles of its functioning. However, taking into account the current level of our visions of cartilage renewal, such problem becomes unsolvable because of great variety of evolution factors which affect this organ. For example, more than one hundred genes are directly involved in osteoarthritis development. That is why articular cartilage could not be handled as a black box – the number of probable system state indicators is vast, and there is no way to get values of most of them non-invasively. Thus, we introduce articular cartilage as an active system (fig. 1).

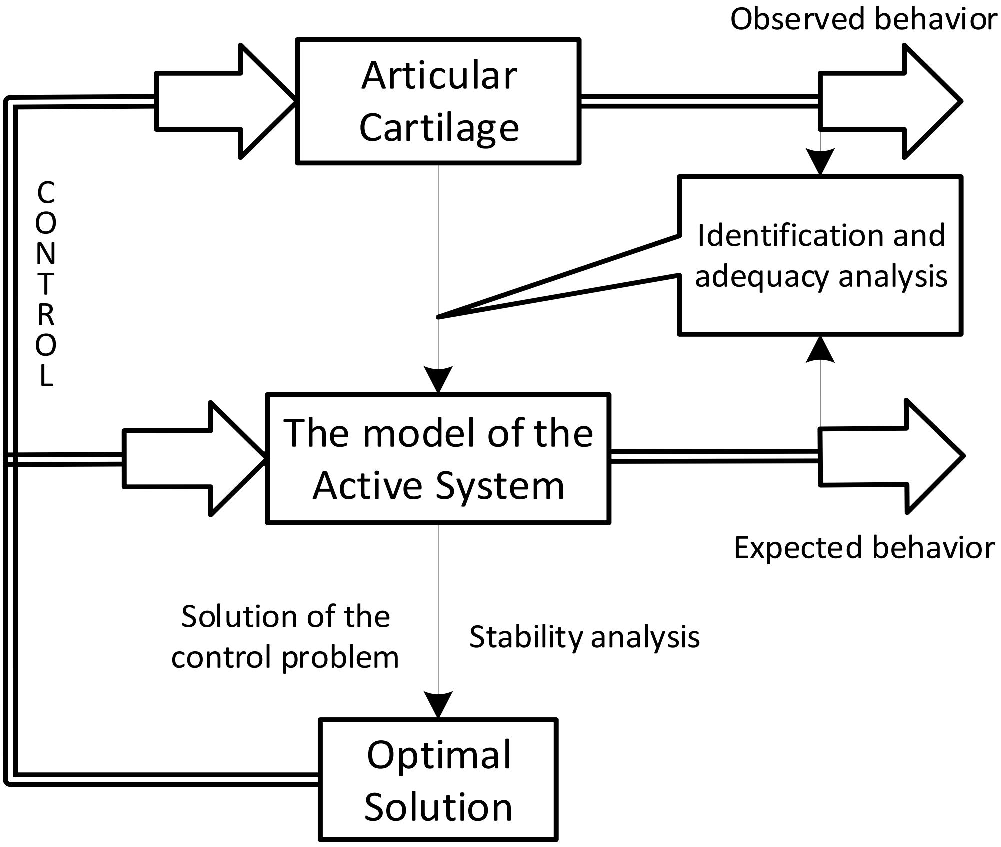

Fig. 1. Steps to the articular cartilage model

Living cells reactions on controlling actions are well studied already. It is difficult to determine or predict the intensity of such reactions, but proposed approach does not require accurate data from this point of view.

In the next section, we consider articular cartilage and its endogenous processes to determine active system components, structure and feasible set of states. We also construct a number of measures, which could be useful for understanding the work of the system.

Articular cartilage endogenesis

Articular cartilage is an amazingly complex of biomaterials. It has high rigidity, compression, strength, stability and amortization indices. Extracellular matrix of cartilage contains about 60% of collagen and 25% of proteoglycans. Another 15% is a widest range of matrix proteins. Collagen net provides cartilage structure sustainability [11].

Chondrocytes maintain physiological regulation and biological consistency of cartilage, although they are widely spaced. Articular cartilage suffers wide range of loads, including shifting, squashing and stretching, because of its location on the surface of the joint. These loads are distributed across all cartilage matrix and are absorbed by its biomolecules. At nanoscale level mechanical loads scatters and then transmits to chondrocytes, which in their turn translate these signals into biochemical signaling molecules [4, 12]. These molecules then start anabolic and catabolic processes. Therefore, chondrocytes are the most suitable candidates to be principals of an active system.

Although osteoarthritis is a chronic inflammatory disease, involving homotypic structural alterations, such alterations are caused by specific decay-accelerating factors. Chondrocytes have independent abilities to initiate and carry on response to cartilage tissue injury. All osteoarthritis course instantiated by two consequent stages: when chondrocyte is trying to restore injured cartilage tissue (1), and when extracellular matrix is obliterated by enzymes produced by chondrocytes (2). During second stage the matrix synthesis inhibits and, in consequence, articular cartilage erosion occurs.

All these considerations allow us to construct a set of the feasible system states $A=\{a_h, a_r, a_d\}$, where a_h is stationary state (non-injured cartilage), a_r is cartilage remodeling state (synthesis processes are prevalent), a_d is tissue degradation state.

Extracellular matrix regeneration rate is strict – it is delicate balance between synthesis and destruction. Osteoarthritis means broken balance with catabolic processes prevalence. In normal and pathologic conditions cartilage matrix homeostasis depends on autocrine and paracrine control mechanisms. These mechanisms regulate anabolic and catabolic ways of control of chondrocytes quantity and extracellular matrix volume.

Chondrocytes produce structural molecules of articular cartilage, such as collagen and proteoglycans, which help to form cartilage tissue. Simultaneously cells produce different metalloproteinases, which regulate the composition of cartilage tissue. On molecular scale such regulation is based on growth factors such a transforming growth factor β (TGF-β), insulin-like growth factor 1 (IGF-1) and bone morphogenetic proteins 2 and 7 (BMP-2, BMP-7). These factors stimulate chondrocytes to produce structural macromolecules (anabolic pathways). At the same time cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) stimulate chondrocytes to secret proteinases, which cause extracellular matrix degradation (catabolic pathways) [13, 14].

The entire process can be divided into two stages. At the first stage trigger impact on the articular cartilage activates T-lymphocytes and synovial macrophages. At the second stage with the help of chondrocyte receptors IL1R1 they activate the transcription factors NF-kB of the corresponding genes with IL-1β [15].

On molecular genetic level, this process is the result of certain genes' expression and suppression. Such genes belong to a limited set and are responsible for cell cycle, metabolism and intercellular communications in cartilage tissue. Therefore, the set of feasible messages U contains some kind of elements $u_1^+, u_2^+, u_3^+, \ldots, u_1^-, u_2^-, u_3^-, \ldots$, where $u_1^+, u_2^+, u_3^+, \ldots$ stimulate chondrocyte to secrete growth factors (TGF-β, IGF-1, OP-1, physical loads [7]), and $u_1^-, u_2^-, u_3^-, \ldots$ cause matrix degradation (chondrocytes are affected by cytokines IL-1, IL-6, TNF-α, etc.).

The next step to representing the articular cartilage as an active system is to construct the functional $\Phi(\eta, y)$, where $\eta \in U, y \in A$. This leads to necessity to find a way to estimate control efficiency. The ideal physiological indicator is mechanoreceptors signals, which is transmitted into the brain. If such signals are different from the expected ones, an organism suffers pain, which is clinical sign of osteoarthritis. Unfortunately, such indicators are impossible to be used because of the current diagnostic techniques. Therefore, we need indicators that are more suitable.

Search for informative indicators

Currently, expert assessments of histological material and MRI examinations are the 'gold standard' of articular cartilage diagnostics. This approach does not presuppose any kind of numeric values. Therefore, we propose the following approach.

The current state of the specific cartilages is judged by different parameters. Tissue density arrangement is very promising numeric indicator. We have determined the value of density in different zones of cartilage. Specific agent toluidine blue has been used for staining the articular cartilage samples of 5-7 μm depth. It easily revealed extracellular matrix proportionally the concentration of glycosaminoglycans [16]. In such case digital image pixels' brightness inversely relates to extracellular matrix density in corresponding area. Articular cartilage tissue has natural inhomogeneity, which can cause unexpected measurement fluctuations. To compensate this we measured average brightness in rectangular areas of histologic image with equivalent size 50 x 80 μm.

Fig. 2 illustrates one-dimensional distribution of extracellular matrix density $D(\tau)$ in samples of healthy (continuous line) and injured (dashed line) cartilages. The axis of abscissas contains values of the parameter $\tau \in [0,1]$, which indicates current point relative position between bone marrow ($\tau = 0$) and synovial fluid. The ordinate axis contains extracellular matrix density values.

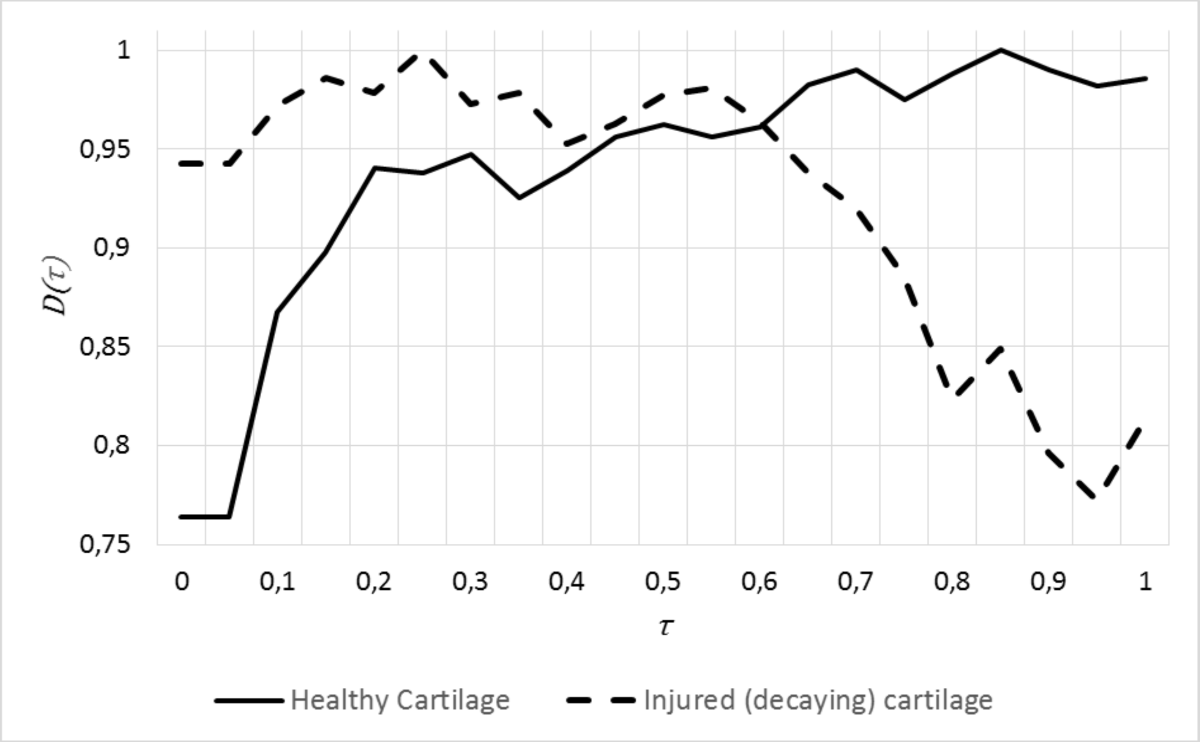

Fig. 2. The distribution of representative extracellular matrix density

These graphs show that slight variety and high value of extracellular matrix density are the good characteristics of healthy cartilage. Accordantly, the reduced density with its additional decreasing at articular surface is typical for injured cartilage. Density values at left part of the graph are lower because of plenty of chondrocytes in cartilage area adjacent to subcartilaginous structures. In consequence of reduced chondrocytes life cycle and physical stress, injured cartilage becomes thin, extracellular matrix density goes down and erosion occurs.

Fig. 2 also demonstrates density distribution of completely healthy cartilage and cartilage with an explicit pathology. In fact, the problem of cartilages differentiation on the basis of certain indicators is extremely complicated. We processed digital photographs of microscopic slides derived from cartilages of six certain dogs. The total quantity of samples was 30. Expert conclusion about status of cartilage and the measurements of tissue density in different sample areas using specialized software were conducted in parallel independent regimen. Finally, we got 74 different density distribution data sets.

We calculated values of eight different indicators for every data set (Table 1).

Table 1: Indicators used to designate articular cartilage processes

Indicator	Evaluation formula	Process or phenomenon reflected by indicator
Cartilage matrix density mean value	$\overline{D} = \sum_{1}^{20} D\left(\frac{i\tau}{20}\right) \Big/ 20$	Current balance of cartilage matrix synthesis and decay
Cartilage matrix volume	$\int_{0}^{1} D(\tau) d\tau$	Prevail cartilage loads volumes
Measured density values variance	$\frac{1}{20}\sum_{1}^{20}\left(D(\tau)-\overline{D}\right)^2$	Differences of rates of cartilage matrix synthesis and decay
Maximum deviation from 1	$\max_{\tau}(1-D(\tau))$	Maximum matrix decay intensity

The position of the point of maximum	$\tau_{\max} = \max \tau : D(\tau) = 1$	Cartilage matrix synthesis and decay balance
The position of beginning point of falling density	$\tau_f : \forall \tau > \tau_f \; D(\tau) < D(\tau_f)$	Balance point of diffusion flows of subcartilaginous structures and synovial fluid.
The position of point of cartilage matrix volumes equality	$\tau_0 : \int_0^{\tau_0} D(\tau)d\tau = \int_{\tau_0}^{1} D(\tau)d\tau$	Dynamic balance of matrix synthesis and decay
Coordinates difference of beginning points of falling density and volumes equality	$\tau_f - \tau_0$	Matrix remodeling process acceleration or deceleration

Hereafter we plan to use these indicators for searching of informative conditions enabling us to differentiate and classify samples. Every examined cartilage is in one of feasible active system states, which belong to set *A*. We believe that informative conditions can be found using statistically different indicators for elements of *A*. Therefore, we calculate these indicators values for samples belonging to each of three separated groups. Obtained series were tested with Wilcoxon-Mann-Whitney criteria. Results are shown in Table 2.

Obviously, the series differentiate slightly by most of indicators. However, the point of maximum density position is different for healthy and regenerating cartilage ($p = 0{,}1311$). Moreover, the position of the point of cartilage matrix volumes equality for certain cartilages difference is statistically confident.

Table 2: Series concurrence probability in groups of cartilages is in certain remodeling states for distinct indicators

Indicator	**p-value of Wilcoxon test for series corresponding to cartilages in the state of…**		
	regeneration and homeostasis	**homeostasis and degradation**	**regeneration and degradation**
Cartilage matrix density mean value	0,6265	0,3778	0,6265
Cartilage matrix volume	0,7843	0,5430	0,8314
Measured density values variance	0,7843	0,5034	0,2478
Maximum deviation from 1	0,8552	0,6926	0,5430
The position of the point of maximum	0,1311	0,2736	0,5090
The position of beginning point of falling density	0,7578	0,8484	0,4042
The position of point of cartilage matrix volumes equality	0,0194	0,2871	0,4202
Coordinates difference of beginning points of falling density and volumes equality	0,9394	0,5430	0,6926

The point of maximum position (p = 0,2736) along with the point of cartilage matrix volumes equality (p = 0,2871) allow to differ degrading and healthy cartilage. The variance values of measured density (p = 0,24777) are significantly different for regenerating and decayed cartilage.

All these results show the possibility to discover certain cartilage regeneration states (stationary matrix regeneration, synthesis prevalence and matrix growth, decay prevalence and matrix loss) using spatial distribution of some quantitative morphologic indicators in cartilage tissue.

Conclusion

This paper describes the first step to developing the model of articular cartilage. The main obstacle which does not allow to finish it is the unsolved problem of cartilage samples differentiation using conditions based on quantitative morphometric indicators. Nevertheless, we expect to solve this problem after revealing more informative indicators of cartilage tissue remodeling. By now, we examined only some small part of them.

Certainly, such indicators will be found in the design of molecular, molecular-genetic research and metabolomics. Such markers will lead to developing research protocols which describe not only the current cartilage state, but also the prognosis of articular cartilage remodeling due to application of modern tissue engineering constructions.

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Взаимодействие ключевых процессов при обновлении матрикса суставного хряща: математическая модель

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Аннотация. Авторы предприняли попытку объяснить закономерности регенерации и обновления суставного хряща с позиций теории активных систем. Такое понимание, и основанное на нем математическое моделирование процессов, происходящих в суставном хряще, является важнейшим шагом на пути к формированию новых методов профилактики, диагностики и лечения заболеваний суставов, прежде всего – остеоартроза. На основе структурного анализа гистологических изображений выявлены признаки здорового и дегенерирующего хряща, в результате чего получены индикаторы, которые необходимо достигать при использовании тканеинженерных конструкций.

Ключевые слова: суставной хрящ; тканевая инженерия; остеоартроз; математическое моделирование.

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Effect of Different Cultural Condition on the Growth of *Fusarium moniliforme* Causing Bakanae Disease

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Abstract. In this study, *Fusarium moniliforme* causal organism of Bakanae disease has been isolated from infected rice seeds variety Pusa Basmati-1121 by using blotter technique. The effects of temperature, pH and carbon source on radial growth rate were assessed on potato dextrose broth medium. Precise characterisation of the growth conditions for such a fungal pathogen has an evident interest to understand and to prevent spoilage of rice crops. Study was carried out to check the effect of temperature (15–50 °C), pH (2-10), and different carbon sources (glucose, dextrose, sucrose, rice husk and sugarcane bagasse) on the growth *Fusarium moliniforme*. Optimum temperature and pH for growth was 20 °C and 5.0 with maximum dry mycelium weight and sporulation i.e. 2.168 gm 1.806 million spores / 100ml respectively. Maximum growth was observed when rice husk was used as sole carbon source (2.432 gm and 1.68 million spore/ 100 ml) however maximum sporulation (0.984 million spore/ 100 ml) was achieved when sugarcane bagasse was used as sole carbon source.

Keywords: *Fusarium moniliforme;* Basmati rice; Bakanae disease.

Introduction

Rice is the fastener diet for more than two billion people in Asia and for a few hundreds of millions in Africa and Latin America [1, 2]. One fifth of the total world area under cereals is comes under rice cultivation. The human population is rapidly approaching seven billion and more than one half depend on rice as their food staple [3]. In India, rice is grown in different agro climatic region ranging from Kashmir to Kanyakumari as upland, middle and low land rice. Rice is grown wide areas in India, especially is western Uttar Pradesh and Uttaranchal. The rice crop is highly sensitive and a potential host for several insect pests [4]. Total area coverage under rice is approximately 42.4 million hectare.

Rice crop suffers from the attack of various type of diseases caused by diverse type of pathogen. Out of 43 fungal diseases of rice 15 are worth coming (Table 1). Out of which foot rot / Bakanae is most important in basmati variety 1121. Foot rot of rice or Bakanae disease commonly known as foolish seedling disease caused by the fungus *Gibberella fujikuroi (Fusarium* *moniliforme* anamorph)[5]. Fusarium species are the important pathogen cause significant looses in quality and concomitant with mycotoxins [6].

Table 1: Major diseases of rice and their causative organisms

S. No.	Disease	Casual organism
1	Black kernel	*Curvularia lunata*
2	Blast (leaf, neck [rotten neck], nodal and collar)	*Pyricularia grisea = Pyricularia oryzae*
3	Brown spot	*Bipolaris oryzae*
4	Downy mildew	*Sclerophthora macrospora*
5	Eyespot	*Drechslera gigantea*
6	False smut	*Ustilaginoidea virens*
7	Kernel smut	*Tilletia barclayana = Neovossia horrida*
8	Narrow brown leaf spot	*Cercospora janseana = Cercospora oryzae*
9	Pecky rice (kernel spotting)	*Curvularia* spp. *Fusarium spp. Microdochium oryzae Sarocladium oryzae*
10	Root rots	*Fusarium* spp. *Pythium* spp. *Pythium dissotocum*
11	Seedling blight	*Curvularia* spp. *Fusarium* spp. *Rhizoctonia solani* *Sclerotium rolfsii*
12	Sheath blight	*Rhizoctonia solani*
13	Sheath rot	*Sarocladium oryzae= Acrocylindrium oryzae*
14	Sheath spot	*Rhizoctonia oryzae*
15	Bakanae	*Fusarium moniliforme*

Fusarium moniliforme is an ubiquitous fungus distributed worldwide. Environmental factors such as temperature, water activity and pH have a great influence on fungal development. The fungus affects rice crop in Asia, Africa, and North America. In epidemic cases yield losses may reach up to 20% or more. A 2003 publication from the International Rice Research Institute estimated that outbreaks of bakanae caused crop losses that were 20% to 50% in Japan, 15% in Thailand and 3.7% in India [7]. Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum size have great influence on the growth of pathogen [8]. Present work depicts the role of different pH, temperature and media to understand ecological survival of pathogen which will be helpful in management strategy in the field.

Materials and methods

Isolation, Purification and Identification of *Fusarium moniliforme*

Isolates of *F. moniliforme* were isolated from diseased rice seed variety PB-1121 from Laboratory of Microbiology and Plant Pathology, Centre of Excellence for Sanitary and Phtyo Sanitary (SPS), Certification, Research and Training, Department of Plant Pathology during November 2013. Discolored seeds were placed on sterilized blotter plates and incubated at 20 ± 5°C in the dark / light for 14 days. Fungi were characterized based on their cultural, morphological and spore characteristics and identified by consulting various taxonomic monographs [9, 10, and 11]. A single micro conidial culture was prepared from each isolate. Studies of the following physiological aspects of *F. oxysporum* isolates were conducted in laboratory.

Optimization of Culture Conditions on Growth and Sporulation of *Fusarium moniliformae*

Effect of Temperature

The fungal strain *Fusarium moniliforme* was inoculated into potato dextrose broth and grown at range of temperatures varying from 10 °C to 50 °C for 12 days. Dry mycelium weight and sporulation at each temperature was determined [12].

Dry Mycelium Weight

The culture broth was centrifuged at 14,000 rpm for 20 min and the supernatant fluid was filtered through a filter paper (Whatman No.1). The mycelial biomass yield was estimated by washing with de ionized water and dried at 50°C for 48 h. The mean dry weight of the mycelium was determined as described by Prasad and Chaudhary [13, 4].

Sporulation

Sporulation was calculated with the help of haemocytometer using formula [14]–

Number of spores / 100 mL = V/NX100

N = Average number of spores per square of the four corner square of haemocytometer counted.

V = Volume of haemocytometer (0.256 x10-5) cc

Length of the spores was measured by calibrated ocular micrometer under compound microscope (10 x 45 x of magnification).

Effect of pH

To study the effect of pH, different pH values ranging from 2.0 - 10.0 were used after adjusting pH of the medium by using digital pH meter. Flasks of different pH were inoculated with fungi and incubated at 25 0 C for 12 days. Dry mycelium weight and sporulation were count after 4, 8 and 12 days of incubation.

Effect of carbon source

Glucose, starch, dextrose, sucrose, sugar cane bagasse and rice husk were used as carbon sources. Carbon sources were added separately into basal medium ($MgSO_4$- 0.45 g/l, KH_2PO_4 -5 g/l, NH_4NO_3- 1.85 g/l, $ZnSO4.7H_2O$ -0.2 g/l , $CaCl_2.H_20$- 0.1 g/l ,$CuSO_4$- 0.02 g/l, $CoCl_2$-0.02g/m, Na_2MoO_4-0.02g/l, $Na_2B_4O_7$-0.02 g/l $MnSO_4$- 0.02 g/l) at 10 g (w/v) and growth and sporulation rate was determined.

Result and discussion

Identification of *Fusarium moniliforme*:

On the basis of colony morphology and characteristics of macro and micro conidia, fungal isolates were identified as *Fusarium*. On further microscopic study, isolate was identified as *F. moniliforme* on the basis of micro conidia, produced on phialides, catenate, hyaline, oblong, 5-12 x 1.5-2.5 um, with (0-1) septum. Macroconidia produced in acervuli, hyaline, long fusoid, tapered to the ends, straight or curved, 25-60 x 2.5-4 um with 3-7 septa.

Amongst the fungal isolates*, Fusarium moniliforme* which is causal organism of Bakanae, was selected for further studies.

Effects of Temperature

Different temperature ranges (15-50 ^{o}C) were arranged in different BOD incubators. The *F. moniliforme* was inoculated into potato dextrose broth flasks. The flasks were incubated for 12 days and microbial bio mass and sporulation was recorded every day. Results are shown in Table 2.

Table: 2 Effect of different temperature on the growth of *Fusarium moniliforme*

S. No.	Temp ° C	Dry mycelium weight (in gm)			Spores in millions/100mL medium
		After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	
1	15	0.055	0.099	1.282	0.088
2	20	0.479	1.569	2.168	1.806
3	25	0.305	1.516	1.446	1.421
4	30	0.281	0.411	1.002	0.980
5	35	0.110	0.286	0.311	0.056
6	40	0.006	0.014	0.021	1.39
7	45	0.00	0.00	0.00	0.00
8	50	0.00	0.00	0.00	0.00

Chi and Hansen (1964) [15] reported that *F. solani* isolates grew well at higher temperature of 28 °C. The fungus grew at the temperature range of 15– 30 ° C. However, growth of the fungus was drastically reduced below 15°C and started to decline above 30°C and become zero at 45 °C, as these temperatures did not favor for growth of the fungus. It was observed that at 20 °C, fungus attained the maximum growth and sporulation (2.168 gm and 1.806) while at 25°C, it was (1.446 gm and 1.421) after 12 days of inoculation. These studies are in confirmation with Anjaneya Reddy (2002) [16] who reported that growth of 40 isolates of *F. udum* differed in their temperature requirement which varied from 20°C to 35°C. The aim of this work was to study the effect of temperatures ensure the elimination of *F. moniliforme*. Results are in confirmation with Imran Khan *et al.,* (2011) [17] showed the *F. oxysporum* f.sp. *ciceri* grew highest at 25 °C.

Effect of pH

Table 3: Effect of Different pH on The Growth and Sporulation of *Fusarium moniliforme*

S. No.	pH	Dry mycelium weight (in gm)			Spores in millions/100mL medium
		After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	
1	2.0	0.046	0.082	0.282	0.33
2	3.0	0.123	0.196	0.388	0.81
3	4.0	0.205	0.665	1.134	1.34
4	5.0	0.488	1.411	2.432	1.68
5	6.0	0.410	0.997	2.289	1.56
6	7.0	0.346	0.801	1.971	1.39
7	8.0	0.141	0.621	0.879	0.93
8	9.0	0.068	0.191	0.226	0.18
9	10.0	0.002	0.048	0.032	0.00

Effect of pH are in confirmation with the findings of Jamaria (1972) [18] who reported that as the pH decreases or increases from the optimum, the rate of amount of growth gradually decreases. Gangadhara, *et al* (2010) [19] studied effect of pH levels on growth of *F. oxysporum* f. sp. *vanillae* isolates. Mean of the dry mycelium weight of the fungus and sporulation on different pH levels was calculated and shown in Table 3. Results showed that *Fusarium moniliforme* grew maximum in pH 5.0 (dry mycelial weight 2.289 gm and sporulation count 1.56). At high acidic range of pH, fungi showed very poor growth of mycelium. Growth of fungi increased with increase in pH up to pH 5 and then decrease in growth was observed. Imran Khan *et al.,* (2011) [17] showed optimum pH for growth of *F. oxysporum* f.sp. *ciceri* ranged from 6.5 to 7.0.

Table 4: Effect of Carbon source on the growth and sporulation of *Fusarium moniliforme*

S. No.	Carbon Source	Dry mycelium weight (in gm)			Spores in millions/100mL medium
		After 4 days of incubation	After 8 days of incubation	After 12 days of incubation	
1	Glucose	0.328	0.594	1.211	0.471
2	Starch	0.098	0.154	0.784	0.410
3	Dextrose	0.487	0.925	1.842	0.805
4	Sucrose	0.371	0.858	1.011	0.614
5	Sugarcane bagasse	0.294	0.520	1.220	0.984
6	Rice husk	0.657	1.381	2.754	0.826

Various carbon sources viz. sucrose, glucose and starch and agricultural residue such as rice husk and sugarcane bagasse were tested as sole carbon source for the growth of pathogen. Maximum growth was observed in medium containing rice husk (2.754 gm) after 12 days of incubation (Table-4) while maximum sporulations were achieved in medium containing sugarcane bagasse (0.984). Medium containing Dextrose and sucrose also showed effect on the growth and sporulation on *Fusarium moniliforme*. These results were found in proximity with the research findings of Kuhad *et al* 1998, found maximum growth and xylansae production when inoculated in medium containing wheat bran as sole carbon source.

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