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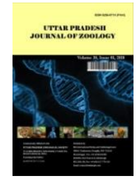

ORIGIN OF LIFE AND LIVING MATTER IN HOT MINERAL WATER AND PROPERTIES OF POLAR MOLECULES IN THE PRIMARY HYDROSPHERE AND HYDROTHERMAL PONDS

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AUTHOR'S CONTRIBUTION
The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The origin of life on Earth dates back to 3.5 billion years ago. There is even evidence that living matter was present 3.8 billion years ago, relatively shortly after the planet was formed around 4.54 billion years ago. Therefore, a valid question arises whether life originated from a series of random events or as a result of consistent patterns. And if such patterns are generated by the laws of Nature, they could hopefully give rise to life also on other planets. Following our previous research on the possibility for the origination of life in hot mineral water, comparative spectral analysis was performed of mineral water samples from Rupite, Bulgaria that are rich in hydrogen carbonate and calcium ions, as well as of cactus juice. Previous experiments were performed with *Bacillus subtilis* in heavy water according to existing evidence that, in the primary hydrosphere, there were more deuterium atoms in water molecules.
Comparative measurements were also done with Black sea water from Varna, Bulgaria. Our studies are based on the hypothesis that constant geothermal activity on land supported the biochemical processes of emerging living organisms, i.e. stromatolites and primary cyanobacteria. This was happening in a hydrosphere much different from the present one. There were volcanic islands and boiling lava was pouring into the primary ocean. Besides, there were hot ponds on these islands.The atmosphere was also different. It contained nitrogen, carbon dioxide, ammonia etc. These gases were being absorbed into the ponds, subsequently enhancing the favorable chemical reactions. Our work also represents the logical development of conclusions by other researchers that cell membranes could not have been formed in the marine environment.

Keywords: Hot mineral water; origin of life, polar molecules; Rupite; Bulgaria.

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1. INTRODUCTION

1.1 Chemical Reactions in the Primary Hydrosphere

Let us consider the following reactions in the primary hydrosphere.

$$CO_2 + 4H_2S + O_2 \rightarrow CH_2O + 4S + 3H_2O \quad (1)\ [1]$$

$$2HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O \quad (2)\ [2,3]$$

The first equation illustrates how some chemosynthetic bacteria have been able to utilize energy from the oxidation of hydrogen sulphide (H_2S) to sulphur (S).

And the second reaction has been taking place during the emergence of stromatolites. These reactions have also been accompanied by:

$$CO_2 + OH^- \rightarrow HCO_3^- \quad (3)\ [4]$$

$$2H_2O + 2\ e^- \rightarrow H_2 + 2OH^- \quad (4)\ [5]$$

An overall analysis of reactions (1), (2) and (3) reveals that the following chemical compounds are polar - H_2S, CH_2O, $CaCO_3$ molecules and HCO_3^- ions are polar, while CO_2 is a gas.

In addition, the reaction:

$$2HCO_3^- + Ca^{2+} \rightarrow Ca\ (HCO_3)_2 \rightarrow CaCO_3 + H_2CO_3 \rightarrow CaCO_3 + CO_2 + H_2O \quad (5)$$

produces layers of $Ca^{2+}CO_3^{2-}$ deposits where polar calcium carbonate molecules are in contact with water molecules along the layer boundary. Thus, carbonate groups (CO_3^{2-}) are attracted to hydrogen atoms of water molecules. Previous molecular simulations of the dynamics of carbonate ions in water and their hydration shells as well as analytical results of Yadav, Chandra demonstrate that bonds between carbonate ions and water hydrogen atoms are stronger than those between water molecules [6]. As a result, structures consisting of a carbonate ion and three water molecules are formed, where hydrogen bonds are between oxygen atoms of carbonate ions and hydrogen atoms of water. That is how layers of carbonate ions interacting with water molecules are formed.

Calcium ions can be attached to carbonate ones in layers of calcium carbonate. They may also be free in the water. Hydrocarbonate ions are mainly dissolved in water. Allen has shown that, according to Niel's equation, the following reaction takes place [7]:

$$CO_2 + 4e^- + 4H^+ \rightarrow CH_2O + H_2O \quad (6)$$

The author has also shown the possibility of the following reactions in the primary hydrosphere [8,9].

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (7)$$

$$2H_2O + 2\ e^- = H_2 + 2OH^- \quad (8)$$

$$H_2O + e^- \rightarrow H^* + OH^- \quad (9)$$

$$H^* + H^* \rightarrow H_2 \quad (10)$$

The ability of water to lose electrons is characterized by its oxidation/reduction potential (ORP, E_H). A study has shown that if reducers such as sulfates, etc., are present, water has negative ORP.

Sasselov et al. consider the possibility that sulfur compounds, and most of all sulphate ions (SO_4^{2-}) to have played a key role in the primary hydrosphere for the emergence of life [10].

Other research has shown that the most effective crystallization of calcium and carbonate ions is observed at approximately 40°C and pH=12.

Besides, Calvin considers the possibility for primary hydrosphere reactions of condensation-dehydration of amino acids and nucleotides in individual blocks of peptides and nucleic acids. Optimal pH for such reactions is pH=9–11 [11].

Let us also examine the reactions:

$$2HCO_3^- + Ca^{2+} \rightarrow Ca\ (HCO_3)_2 \rightarrow CaCO_3 + H_2CO_3 \rightarrow CaCO_3 + CO_2 + H_2O \quad (11)$$

$$CO_2 + 4e^- + 4H^+ \rightarrow CH_2O + H_2O \quad (12)$$

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (13)$$

Bosak et al. indicate that Archaean electron donors included hydrogen sulfide (H_2S), hydrogen (H_2) and ferrous iron. Carbon dioxide (CO_2) and nitrogen (N_2) gasses are electron acceptors [12]. During Archaean, there were insignificant amounts of oxygen (O_2).

Allen suggests that, in addition to CO_2 fixation, light-driven N_2 fixation with an unlimited supply of reductant may have been an immediate benefit for the first cyanobacteria to utilize water as the electron donor.

According to Zavarzin, the following reactions demonstrate an oxidation reduction process in the case of ancient stromatolites [13]:

$$CO_3^{2-} + H_2O \Leftrightarrow HCO_3^- + OH^- \quad (14)$$

$$Ca^{2+} + CO_3^{2-} \Leftrightarrow CaCO_3 \quad (15)$$

Sulphates (SO_4^{2-}) in the water when obtaining S^{2-} have ORP (-120- -180) mV. There is also a release of electrons [14].

All the above reactions were enhanced by the constant release of thermal energy from magma.

1.2 Stromatolites

The most ancient evidence of stromatolites on Earth dates back to 3.5 billion years ago. A relatively short interval of 1.04 billion years was sufficient for non-living matter in water to give rise to organic molecules and living matter.

There are actual findings of ancient stromatolites near hot water. In 2017, the Tara Djokic team demonstrated that the stromatolites in the Dresser Formation were located in an area with hot mineral water [15]. There have also been shallow seas nearby. In 2019, the Baumgartner team explored the Dresser Formation rocks, which are 3.48 billion years old [16].

In 1953, Miller demonstrated experimentally the formation of hydrogen cyanide (HCN) in the primary atmosphere [17]. Hydrogen cyanide (HCN) is a polar molecule. HCN has a boiling point of 26°C. To be preserved in liquid solutions, the necessary temperature is around 80–100°C.

Das et al. showed that, at similar water temperature in the primary hydrosphere, there may have been conditions for thermochemistry and polymerization of HCN [18]. In 1966 Abelson proved that chemical reactions of HCN are not possible in an acidic medium with pH = 4–6. Generation of HCN, CH_2N_2 and $HN(CN)_2$, as well as condensation in separate blocks of amino acids, is possible under alkaline conditions with pH = 9–10 [19]. HCN, CH_2N_2 and $HN(CN)_2$ are polar molecules.

It should be pointed out that geothermal springs might have been the environment for the synthesis of various organic molecules. In 1964, Harada and Fox detected amino acids in aqueous solutions of formaldehyde CH_2O with hydroxylamine NH_2OH and of formaldehyde with hydrazine (N_2H_4), also containing HCN, after heating to +95^{0}C. In the model experiments, reaction products were polymerized into peptide chains which is the important stage towards an inorganic synthesis of proteins [20]. In a HCN–NH_3 aqueous solution, purines and pyrimidines were formed.

The main conclusion from all the above is that the basic molecules taking part in the genesis of life are polar. They are H_2S, NH_3, CH_2O, HCH_3, $CaCO_3$, NCN, CH_2N_2 and $HN(CN)_2$. Water molecules are polar, too.

1.3 Self-Organization and the Second Principle of Thermodynamics Applied to the Origin of Life in Hot Mineral Water

According to Prigogine, increasing chaos and entropy could be able to induce order. Then, entropy decreases and dissipative structures are formed. Dissipative structures could be a well-kept secret of emerging life [21]. The author has analyzed entropy processes in different living organisms. The conclusion is that the most inferior organisms adapt most easily to the surrounding environment. The difference in organism-environment entropies is the lowest for inferior organisms [22,23]. The second law of thermodynamics states those temperatures of systems in contact with one another change towards equilibrium. In the process of equalization, work is done but meanwhile, heat is lost at the expense of increasing entropy. As entropy increases, so does chaos. In hot mineral water, there is a constant heat supply from geothermal activity. The formation of stromatolite layers does not decrease temperature. So, entropy decreases and ordered states are preserved.

Allen has pointed out that research of Dagan et al. had revealed that the earliest cyanobacteria had indeed been filamentous nitrogen-fixers [24,25].

In a study by Panou and Gkelis it was shown that cyanobacteria are connected with hydrogen cyanide, based on their ability to catabolize it by the nitrogenise enzyme, as a part of nitrogen fixation [26]. Nitrogenise can also use hydrogen cyanide instead of its normal substrate, dinitrogen and convert it to methane and ammonia. In this study, we tested whether cyanobacteria are able not only to reduce but also to produce HCN.

The author suggests that there were two processes in the primary hydrosphere. The first one was the formation of layered structures – stromatolites that had an exchange with water. Free electrons for oxidation were obtained by chemical reactions. The second process gave rise to the primary cyanobacteria. The main molecules in both processes are polar which enables structuring and polymerization.

Self-assembled vesicles according to Shapiro are essential components of primitive cells [27]. Cyanide plays a critical role in the origin of life hypotheses that have received strong experimental support from the cyanide-driven synthesis of amino acids, nucleotides, and lipid precursors [28].

Szostak and Chen indicate the physicochemical properties of elementary cells [29].

In 2010, a team of American scientists showed that the speed of relevant chemical reactions increases with temperature [30]. As an example, hydrolysis of polysaccharides accelerates 190 000 times when the temperature increases from 25 to 100 °C. They reviewed the effects of hot water in primary biochemical processes and enzymes.

In 2011, a team of Japanese scientists headed by Sugawara conducted an experiment, which showed how life most probably originated in warm or hot water [31]. They created protocells similar to bubbles in an aqueous solution of organic molecules, DNA and synthetic enzymes. The solution was heated to 95°C. From that temperature close to the boiling temperature of the water, the solution was cooled to 65°C. Thus the formation of protocells with membranes was observed. These protocells were also able to divide. The experiment was a step closer to the creation of artificial cells. It is just another clue that life has probably originated in hot water [22].

Mulkidjanyan, Galperin et al., showed that K^+, Zn^{2+}, Mn^{2+} and PO_4^{3-} ions participate in primary proteins. They are less common in sea water and can be found in waters with hydrothermal activity [32].

Damer and Deamer have concluded that the formation of cell membranes and lipid vesicles is possible in hot mineral water but not in sea water [33].

1.4 Purpose of the Present Study

Analyses of water molecules clustering in hot spring water were conducted because such water was the medium for the first sediments with hydrocarbonate and calcium ions on their boundaries. Interactions with and within such a neighboring medium gave rise to the process allowing some already formed structures to self-organize and preserve themselves. Such structures were inactive chemical exchange with water as the surrounding environment.

The present work includes a new comparative analysis of cactus juice and hot mineral water from Rupite, Bulgaria. A new comparative analysis was also conducted of sea water and jelly-fish extract from Chalkida, Evia island, Greece.

The purpose was to test the idea that the processes in the primary hydrosphere were taking place with polar molecules, including water as a solvent. Their interactions lead to formation of more stable structures. Moreover, cluster structuring of water molecules probably enhanced the inclusion of inorganic and organic substances into even more complex cluster formations.

2. MATERIALS AND METHODS

2.1 Investigated Samples

During the interval from 2010 to 2021, water samples from Rupite-1 hydrothermal spring, Bulgaria and the Black sea water (Varna, Bulgaria) were investigated. Sediments from a hot spring and a pond in Rupite, Bulgaria and sea salt from the Black Sea were studied with Fourier-transform IR spectroscopy. Extracts from *Echinopsis pachanoi* cactus plant and Jelly-fish *Aurelia aurita* jelly-fish were taken as model systems and were investigated with Fourier-transform IR spectroscopy and DNES spectral analysis.

In 2021, a new series of measurements was performed on: mineral water (Rupite-2 hydrothermal spring, Bulgaria), seawater (Chalkida, Evia island, Greece), extract from *Echinopsis pachanoi* cactus plant and *Cotylorhiza Tuberculata* jelly-fish.

2.2 IR-Spectroscopy

2.2.1 Brucker vertex fourier-IR spectrometer

IR-spectra of water samples were measured on Brucker Vertex ("Brucker", Germany) Fourier-IR spectrometer (spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; spectral resolution – 0.5 cm^{-1}; the accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1});

2.2.2 Thermo nicolet avatar 360 fourier-transform IR spectrometer

The Avatar Nicolet 360 spectrometer has entry level FT-IR spectrometer that offers a packaged solution for the entire FT-IR process, from receiving the sample to successfully communicating the results. It's the ease of use and reliability make the Avatar ideal for both industrial and educational laboratories.

2.3 NES and DNES Spectral Analysis

The following methods have been successfully applied by many authors for investigation of molecular clusters in water over the past few decades - far-infrared (FIR) [34], vibration-rotation-tunneling ('VRT) [35], EXAFS- and X-Ray spectroscopy [36], ^{1}H-NMR [37], neutron diffraction [38] and the SCC-DFTB method [39]. The present work includes studies of such clusters with NES and DNES spectral analysis [40].

NES and DNES spectral analysis was performed with an optical device invented by Antonov [41,42]. Evaporation of water drops took place on a mylar foil pad supported by a glass plate in a hermetic chamber.

Its characteristics were:

1. Monochromatic light with wavelength λ=580±7 nm (yellow color in the visible spectrum);
2. Angle of evaporation of water drops: from 72.3^0 to 0^0;
3. Temperature range: (+22–24 ^{0}C);
4. Energy range of hydrogen bonds between water molecules: E=(-0.0800)–(-0.1387) eV (corresponding to λ=8.9–13.8 μm of electromagnetic radiation).

The energy ($E_{O...H}$) of hydrogen O...H-bonds between H_2O molecules in water samples was measured in eV. The function f(E) is called *the energy distribution spectrum*. It was determined with the non-equilibrium process of water droplet evaporation. That is why the method is called the Non-equilibrium energy spectrum (NES) [43].

Fig. 1 shows the schematics of the method for measurement of the wetting angle of liquid drops on a hard surface.

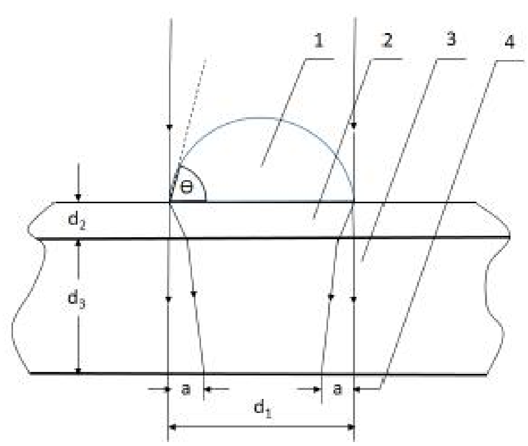

Fig. 1. Operation principle of the method for measurement of the wetting angle of liquid drops on a hard surface
1-drop, 2 – thin mylar foil, 3 –glass plate, 4 – refraction ring width (a). The wetting angle θ is a function of a and d_3

The relation between f(θ) and the energy of hydrogen bonds between water molecules is expressed as:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2} \tag{16}$$

where E is the energy measured in electron volts (eV) and the dimension of f (E) is eV^{-1}.

In addition, the difference:

$$\Delta f(E) = f(sample) - f(control) \tag{17}$$

is called Differential non-equilibrium energy spectrum (DNES) [44]. DNES is a measure of modification of water structure as a result of a certain varied experimental factor. The overall effect of all other uncontrolled factors is the same for the control and the sample.

The dimension of DNES is eV^{-1}.

Ignatov and Mosin have developed a model of clusters in water composed of 8 to 20 water molecules held together by hydrogen bonds [45].

Fig. 2 illustrates a cluster with 20 water molecules [46]. It displays 10 out of 40 hydrogen atoms with covalent bonds only, being located outside the cluster core, while all 20 oxygen and the other 30 hydrogen atoms participate in both covalent and hydrogen bonds within the cluster core. Oxygen atoms exert the strongest attraction of electrons in water molecules and that is why the latter are polars. In other words, oxygen atoms are negatively charged, while hydrogen atoms are positively charged, thus turning water into a universal solvent.

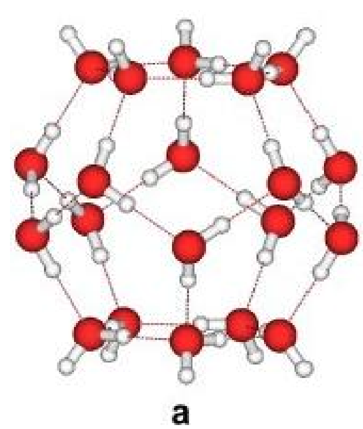

Fig. 2. Cluster of 20 water molecules

2.4 Oxidation Reduction Potential

The device – HANNA Instruments HI221 meter equipped with Sensorex sensors was used for the measurement of Oxidation Reduction Potential (ORP) in mV, and pH..

The Range of HANNA Instruments HI221 meter is:

pH - (2.00-16.00 ±0.01)

ORP (±699.9±0.01 – ±2000±0.1) mV

3. RESULTS AND DISCUSSION

3.1 Cluster Structures of Water Molecules and Dependence of Polarity

Russel and William point to diffusion taking place in hydrothermal jets of the primary ocean. Ions moving from volumes of higher concentration to volumes of lower concentration give rise to electrical gradient [47]. In 2020 Ignatov performed ORP measurements of water samples from Rupite, Bulgaria [8].

Here are up-to-date results from 2021 for mineral water from spring Rupite 2 (Table 1 and Fig. 3).

The presence of free electrons in the hot mineral water enables the occurrence of an electrical gradient with the calcium ions information of stromatolites. The electrical charge takes part in exchange processes when structuring the living matter.

3.2 Spectral Analysis of Water from Rupite, Sea Water, Jelly-Fish and Cactus Juice

In 2010 Ignatov and in 2012 Ignatov and Mosin conducted spectral analyses of hot mineral water of Rupite, Bulgaria, sea water, a jelly-fish from Black Sea and cactus juice [48]. The following results are achieved (Table 2).

Table 1. Results of temperature (°C) and Oxidation Reduction Potential (ORP) of mineral water from Rupite 2, Bulgaria

Temperature (° C)	Oxidation Reduction Potential (ORP) (mV)
25	52
30	41
35	33
40	27
45	23
50	-25
55	-37
60	-41
65	-49
70	-55

The received results in 2010 are from the springs in Rupite are with t=73.4 °C . The quantity of hydrogen carbonate ions (HCO_3^-) then is 1320-1488 , and of calcium (Ca^{2+}) is 29-36 mg/dm^3 [49]. In 2020 the quantity of hydrocarbon ions (HCO_3^-) is 1495-1526, and of calcium (Ca^{2+}) is 31.6-40.2 mg/dm^3 with t= 73.4°C [50,51].

In 2021 is conducted a physicochemical examination of two ponds in Rupite with.

For the first pond Rupite 1 with t= 55.4°C the amount of hydrocarbon ions (HCO_3^-) is 1285, and of calcium (Ca^{2+}) is 10 mg/dm^3. The amount of the sulfates (SO_4^{2-}) is 107 and of the sulfur (S) is 44 mg/dm^3.

For the second pond Rupite 1 with t= 61.3°C the amount of hydrocarbon ions (HCO_3^-) is 1280, and of calcium (Ca^{2+}) is 25 mg/dm^3. The amount of the sulfates (SO_4^{2-}) is 105 and of the sulfur (S) is 40 mg/dm^3.

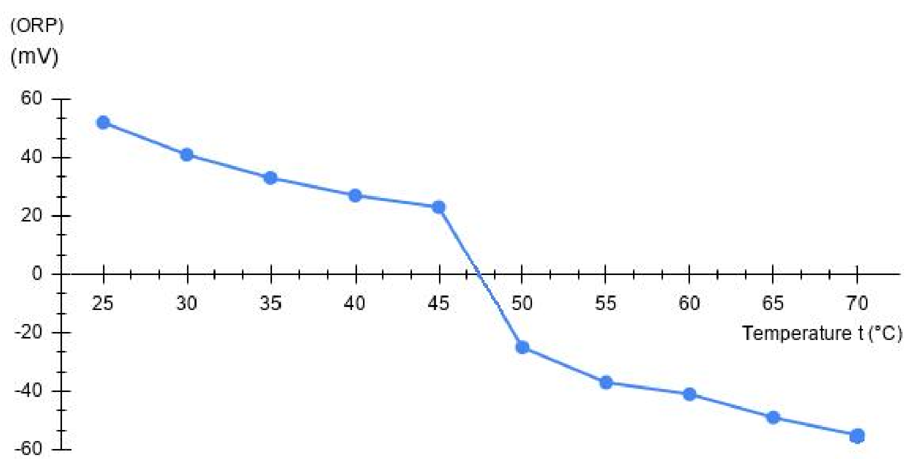

Fig. 3. Results of temperature (°C) and Oxidation Reduction Potential (ORP) of mineral water from Rupite 2, Bulgaria

This reaction from sulfates to sulfur gives ORP (-120 – -180) mV and is the source of free electrons for structuring primordial stromatolites [10]. In the ponds of Rupite 1 and Rupite 2 there is increasing of pH from 6.85-7.18 to 8.22-8.32. There is increasing of pH value in the ponds with 1.14-1.37. These shows structuring of hydroxyl groups (OH^-). The reaction

$$2H_2O + 2\,e^- \rightarrow H_2 + 2OH^- \quad (18)$$

plays role for the structuring of the stromatolites.

The results from 2010 are in Table 2. New research is performed using NES method. Results obtained in 2010 and 2021 are shown in Table 3 and Fig. 4.

Fig. 4 shows results with method NES for water spectrums from mineral ponds Rupite 1 and Rupite 2.

There is one additional local extremum at -0.1212 eV for cactus juice and Rupite 2.

Table 2. Results from 2010 with characteristics of spectra of water of various origin obtained by NES-method*

-E, eV			λ, μm	k, cm^{-1}
Cactus juice	Mineral water from Rupite Village (Bulgaria)	Seawater		
0.1112	0.1112	–	11.15	897
0.1187	0.1187	–	10.45	957
0.1262	0.1262	–	9.83	1017
0.1287	0.1287	–	9.64	1037
0.1362	–	0,1362	9.10	1099
0.1387	0.1387	–	8.95	1117

Table 3. Results with method NES for water spectrums from mineral ponds Rupite 1 and Rupite 2 and cactus juice

-E(eV)	Cactus 2010 f (E) (eV^{-1})	Water Rupite 1 2010 f (E) (eV^{-1})	Water Rupite 2 2021 f (E) (eV^{-1})	-E(eV)	Cactus 2010 f (E) (eV^{-1})	Water Rupite 1 2010 f (E) (eV^{-1})	Water Rupite 2 2021 f (E) (eV^{-1})
0.0937	0	19.5	0	0.1187	45.5	43.9	54.5
0.0962	0	0	0	0.1212	36.4	19.5	27.3
0.0987	0	39.0	9.1	0.1237	0	0	9.1
0.1012	0	6	0	0.1262	45.5	39.0	45.5
0.1037	0	29.3	0	0.1287	45.5	34.2	40.9
0.1062	0	0	18.2	0.1312	27.3	14.6	21.7
0.1087	0	4.9	9.1	0.1337	18.2	14.6	18.2
0.1112	45.5	34.1	45.5	0.1362	54.5	21.4	18.2
0.1137	9.1	19.5	9.1	0.1387	72.7	29.3	36.4
0.1162	0	34.1	36.4	–	–	–	–

*The note: *The function of the distribution of energies Δf among individual H_2O molecules was measured in reciprocal electron volts (eV^{-1}). It is shown at which values of the spectrum -E (eV) are observed the biggest local maximums of this function; λ – wave length; κ – wave number*

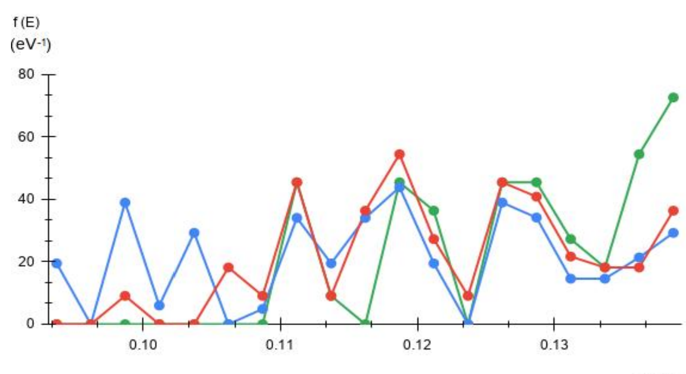

Fig. 4. Results with method NES for water spectrums from mineral ponds Rupite 1 and Rupite 2

Using the method of Fourier spectral analysis are received results as follows, presented in Figs. 5, 6, 7.

Figs. 6 and 7 indicate results with sediments from Rupite and sea salt. Achieved are the following distinctions in the spectrum – 9.23, 9.69, 11.44 and 14.02 μm.

The spectral analyses of jelly-fish and sea salt from Chalkida, Evia Island, Greece show the following local extremums. The results are presented in Fig. 6.

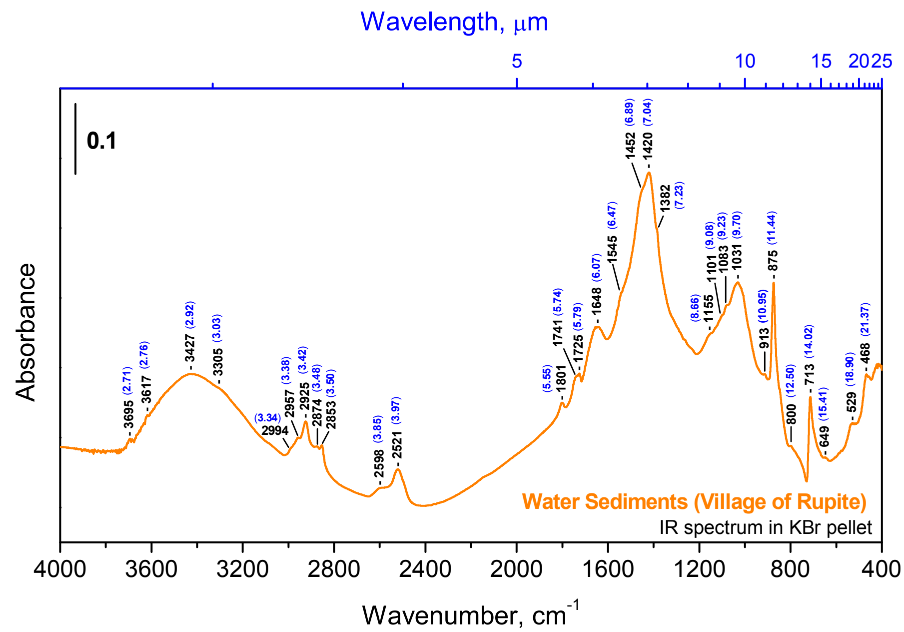

Fig. 5. Analyses and comparative analyses of jelly-fish and sea salt, Aegean Sea, Greece

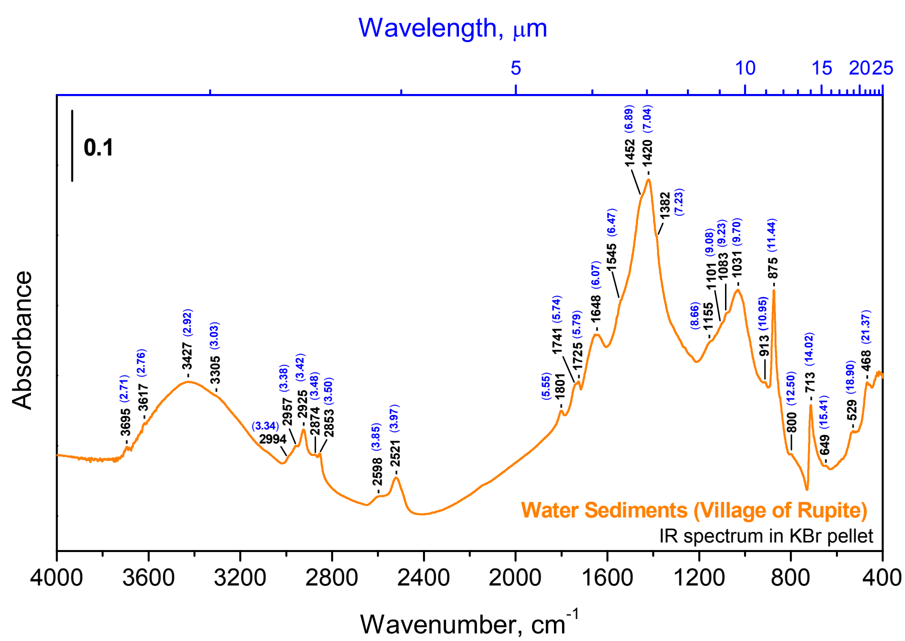

Fig. 6. Spectral analysis of sediments from a spring in Rupite 1

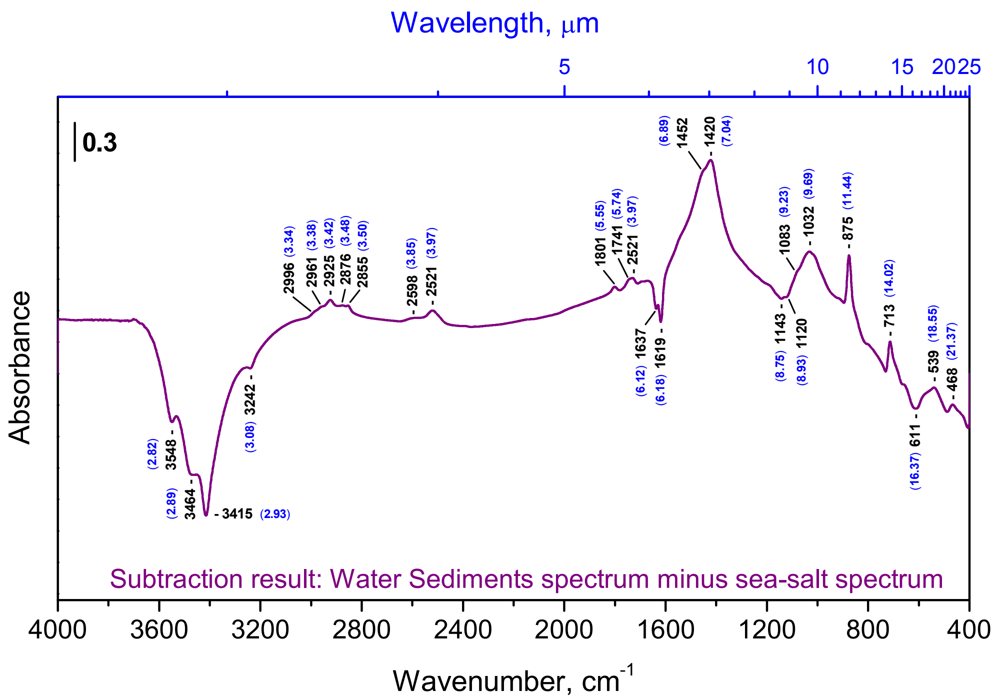

Fig. 7. Comparative analysis between sediments and sea salt

For jelly-fish we have local extremums as follows:

8.98; 10.18 μm

For sea salt:

8.58; 8.78; 8.90 μm

For sediments of Rupite:

8.66; 9.08; 9.23; 9.69; 10.98; 11.44;12.50; 14.02 μm

The study reveals how in the sediments from hot mineral water of Rupite, Bulgaria we have 8 local extremums (Fig. 6).

The sea salt of Aegean Sea water in Chalkida has three local extremums. There are two in the jelly-fish. Two of them are very close in value – 8.90 and 8.98 μm.

Using spectral analysis the following values are achieved for blue-green algae of Rupite, in μm.

The spectrum of Rupite and algae shows a larger number of chemical bonds in comparison with the ones from jelly-fish and sea salt. It is a prerequisite for more stable living structures in the hot mineral water.

There are the photos of Alexander Ignatov from Ripite (a,b,c,d), and extinct volcano Kozhuh (d) and jellyfish from Chakida (e) (Fig. 8).

3.3 The Primary Organisms are a Reflection of the Environment they Live in

Results with spectral analyses indicate that the ancient organisms have spectra similar to the ones of the water they inhabit. Examples of these are the jelly-fish and sea salt from Chalkida. Good illustrations are also algae, cactus and hot mineral water of Rupite, Bulgaria. Locations with warm and hot mineral waters and cyanobacteria in Bulgaria are Rupite [50] and Varna [51]. Rupite has been once a bottom of an extinct volcano. Stromatolites are found in Varna. Strains of the genus *Bacillus* are proved to exist at these places. *Bacillu*s may take part in the formation of a layer of present-day stromatolites. Study of Strains of genus Bacillus, as well as algae, is conducted for Bulgaria [52,53,54].

The research indicates evidence that if there is the presence of calcium and hydrocarbon ions, as well as *Bacillus licheniformis*, there is the sedimentation of carbonate minerals. By changes of Mg/Ca molar ratios, pH increases [55]. It is a process of formation of microbialites (which include stromatolites). The presence of bivalent Calcium

(Ca^{2+}) and Magnesium (Mg^{2+}) ions in warm and hot mineral water takes part in the activation of cortex-lytic enzymes during the germination of *Bacillus subtilis* [56].

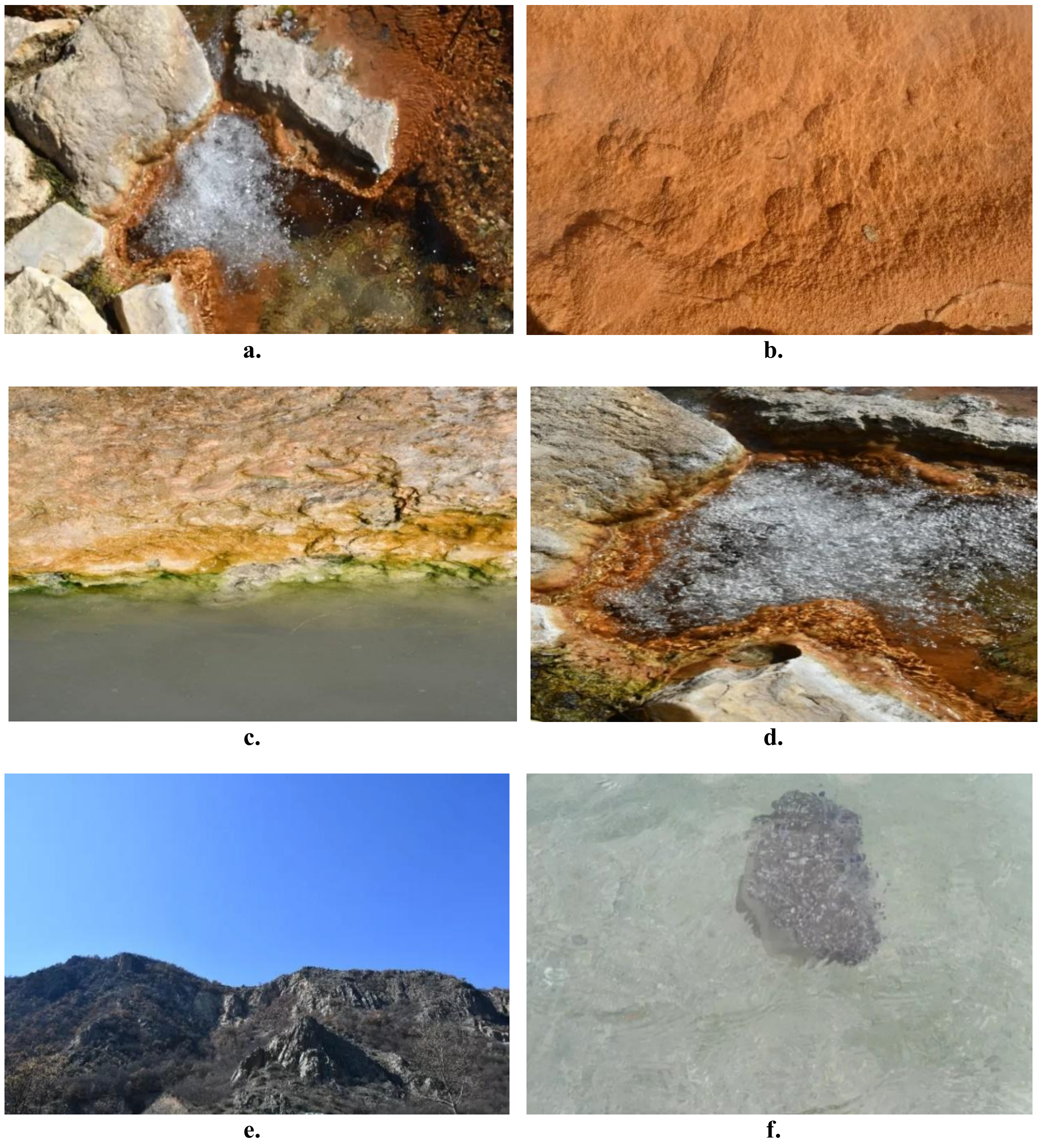

Fig. 8. Photos of Alexander Ignatov from Ripite (a,b,c,d), and extinct volcano Kozhuh (d) and jellyfish from Chakida (e)

A team of Kamburova finds in Rupite the specific bacteria *Anoxybacillusrupiences sp. Nov* [57,58]. The water in Rupite contains cyanobacteria [59]. Other locations, where specific bacteria and archaea are found – Yellowstone National Park [60], Kamchatka [61,62], water rich in arsenic [63].

Such microbiological examinations prove that the primary organisms shape evolutionary the environment they inhabit.

More deuterium was present in the ancient hydrosphere.

3.4 Polar Molecules set up an Arrangement of Organic Molecules in Living Cell Structuring

Considered in the article polar molecules provide the possibility to seek self-organization during the formation of hydrogen and chemical bonds. Hydrogen chains of organic molecules can be established. To self-preserve they can bend and self-structure. We looked at a model with 20 water molecules in a water cluster. There are 20 free hydrogen atoms, which can connect to polar molecules. The number of water molecules can be smaller, for example 8, to also connect to polar molecules.

Berdyugina suggests a model of $COOH-CH_2-....-CH_2-CH_2-CH_2$ that can be a lipid structure of the cell [64].

Chen demonstrates how the protocells are self-organizing lipids in spheres.

According to Chen and Szostak, a simpler model of a cell at the beginning of evolution can be considered, and a part of the model is vesicles [65].

In the early 60's of XX century the American scientist Fox performed the following experiment. An anhydrous mixture of amino acids gets heated to 170°C. 18 out of 23 amino acids were formed. These amino acids are found today in present-day organisms. When rinsing the hot mixture with water or some solutions, structures were formed with a diameter of several nanometers. These artificial protein-like compounds were called proteinoids. Proteinoids have the characteristics of proteins with a two-layer shell. Upon a change of osmotic pressure they tend to divide themselves. Fox successfully combined amino acids into short irregular chains - non-matrix synthesis of polypeptides. He described self-organizing structures similar to coacervates and called them microspheres.

The proteinoid microspheres divide themselves with the increase of the alkalinity of the medium.

It should be noted, that geothermal sources might be used for the synthesis of various organic molecules. Thus, amino acids were detected in solutions of formaldehyde CH_2O with hydroxylamine NH_2OH, formaldehyde with hydrazine (N_2H_4) in water solutions with HCN, after heating of a reactionary mixture to +95 ^{0}C (Harada , Fox, 1964) [66]. In model experiments reaction products were polymerized into peptide chains that are the important stage towards the inorganic synthesis of protein. In a reactionary mixture with a $HCN–NH_3$ solution in water were formed purines and pyrimidines. In other experiments amino acid mixtures were subjected to the influence of temperatures from +60 ^{0}C up to +170 ^{0}C with the formation of short protein-like molecules resembling early evolutionary forms of proteins subsequently designated as thermal proteinoids.

Under certain conditions in a hot mixture of proteinoids in water solutions are formed elementary structures like proteinoid microspheres with diameter 5–10 μm (Nakashima, 1987) [67].

To study the polarity of electric charge is applied gas electric discharge with color coronal spectral analyses (Ignatov, Mosin, 2012) [68,69].

In the ancient atmosphere there was strong gas discharge activity. The experiments of Miller are conducted with gas discharge. It is quite realistic from the ancient atmosphere for the gas discharge to reach the hydrosphere.

The experiments are carried out in a hostafan electrode full of water. Electrical parameters are U=12 kV and ν=12 kHz. The water comes from Rupite, and its volume is 120 ml. The water is heated to a boiling point temperature and then cooled. When applying the gas discharge structures are formed ~1.2–1.3 mm.

The structures are showing in Fig. 9:

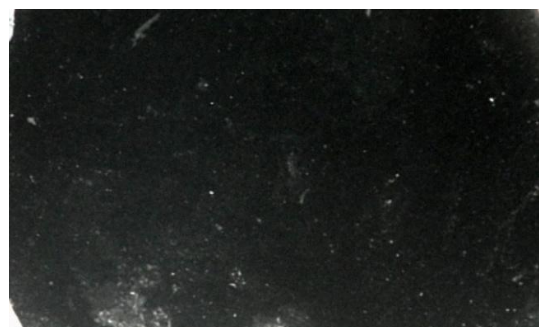

Fig. 9. The organized structure in water placed on an electrode, which is heated to the boiling point temperature in an electric field of high frequency and voltage

Mosin and Ignatov perform experiments with different deuterium saturation [70,71]. Electropherograms of proteins isolated from hydrolysates of total proteins of microbial biomass of *B. subtilis* grown on D_2O also showed differences in the qualitative composition of total protein obtained on D_2O (Fig. 10).

The experimental data demonstrates how changes are observed in the cell growth in different types of water. In the heavy water there are changes in the following parameters – the 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. Research shows how microorganisms adapt easily to the conditions of the surrounding environment [72]. In the ancient atmosphere, there was the presence of more deuterium atoms. The hydrogen bonds in deuterium atoms are more stable. It is a condition for the preservation of living structures. Overview analyses are performed of chemical and hydrogen bonds among molecules during the emergence of life [73,74,75].

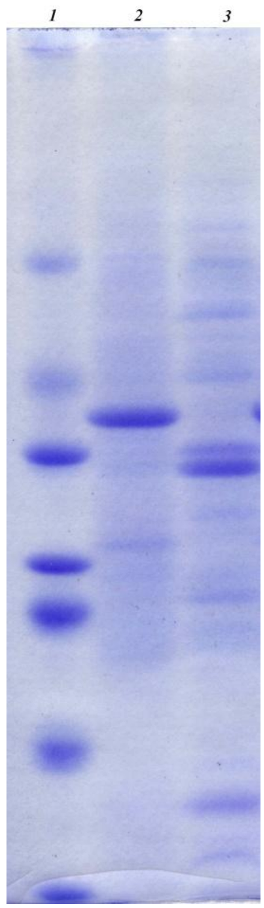

Fig. 10. Electropherograms of proteins isolated from hydrolysates of the total biomass of *B. subtilis*: 1 – a standard set of proteins; 2 – a sample obtained from the protonated medium; 3 – - a sample obtained from D_2O-medium

4. CONCLUSIONS

The following in-depth analyses are made for the emergence of life and living matter in hot mineral water:

1. Considered are interactions of polar molecules in the primary hydrosphere with the polar molecules of the water. It is reviewed the formation of hydrogen bonds and stable structures.
2. Analyzed are processes in the most ancient organisms stromatolites and cyanobacteria. Demonstrated are chemical processes for the presence of electrons in the water. These electrons participate in oxidation-reduction processes. Current studies are shown in warm and hot mineral water with bacteria genus Bacillus. They take an active part in the modern evolution in stromatolites formation.
3. New spectral and comparative analyses are carried out of cactus juice, the water of Rupite, Bulgaria, seawater, and jelly-fish of Chalkida, island Evia, Greece. Applied are physicochemical analyses and methods of IR-spectroscopy.
4. Considered are thermodynamic and entropy processes for the preservation of living structures at a constant geothermal activity. Established are properties of warm and hot mineral water during the primary processes of life emergence.
5. Indicated are experimental results with gas discharge and heavy water as models for the origin of life in the primary hydrosphere [69, 76].

DECLARATION

1. I, the alone corresponding author, am authorized to submit this manuscript.
2. Submission of the manuscript represents that it has not been published previously and is not considered for publication elsewhere.
3. The manuscript, or any part thereof, is in no way a violation of any existing original or derivative copyright.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. In addition, the research has not been funded by any production company an is a result ofauthors' personal efforts.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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