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EXTREMOPHILES — LINK BETWEEN EARTH AND ASTROBIOLOGY

ABSTRACT: Astrobiology studies the origin, evolution, distribution and future of life in the universe. The most promising worlds in Solar system, beyond Earth, which may harbor life are Mars and Jovian moon Europa. Extremophiles are organisms that thrive on the edge of temperature, hypersalinity, pH extremes, pressure, dryness and so on. In this paper, some extremophile cyanobacteria have been discussed as possible life forms in a scale of astrobiology. Samples were taken from solonetz and solonchak types of soil from the Vojvodina region. The main idea in this paper lies in the fact that high percentage of salt found in solonchak and solonetz gives the possibility of comparison these types of soil with “soil” on Mars, which is also rich in salt.

KEYWORDS: Astrobiology, extremophiles, cyanobacteria, halophiles

1. INTRODUCTION

1.1. *About astrobiology*

Astrobiology studies the origin, evolution, distribution and future of life in the universe. Astrobiology is multidisciplinary and brings a common biological perspective to such diverse fields as astronomy, astrophysics, biochemistry, chemistry, the ecology of extremophiles, geology, molecular biology, microbiology, paleontology, physiology, planetary sciences, space exploration and technology, without omitting law and philosophy (J a v a u x, 2006).

One of the prominent goals of astrobiology is to discover life or signs of life on planets beyond Earth. To approach this goal it will be useful to know

the physical and chemical limits for life on Earth and to understand the underlying biophysical characteristics of life that set these limits (Trent, 2000).

There are many definitions of life, but no one generally accepted. This is because it has become evident that there is such a gradual transition between abiotic structures and indisputable life forms, that any boundary drawn between them must, be based on a criterion that is questionable. Many biologists adhere to the view that the presence of DNA or at least RNA is a suitable criterion for life (Van Loon, 2005).

The most promising worlds, which may harbor living microbes are Mars and Jovian moon Europa (Seckbach and Chela-Flores, 2001). Some authors also suggest possible habitats on other icy satellites, in Venus and on the Saturnian moon Titan (Javaux, 2006).

Mars today is a frozen, dry world. The temperature of the surface ranges from -125 to $+25^{\circ}\text{C}$ (average -65°C), with wide diurnal and seasonal fluctuations. Diurnal surface temperature can span over 100 degrees (Bennett et al., 2002). The high fluctuations between midday and midnight temperatures pertain only to the several mm of the surface (Hornack, 2000). Mars has a very thin atmosphere composed of carbon dioxide (95,3%), nitrogen (2,7%), argon (1,6%) and traces of oxygen (0,15%) and water (0,03%). The average pressure on the surface of Mars is less than 1% of Earth's. Europa, a moon of Jupiter which is slightly smaller than Earth's Moon, is one of the smoothest objects in the solar system. A liquid water ocean thought to be between 50 and 100 kilometers deep surrounds its rocky interior, and is covered by a layer of water ice a few kilometers thick (Bennett et al., 2002). Following the analyses of pictures taken of the surface of Europa, it is assumed that under its heavy ice sheaths this Jovian moon contains a liquid water ocean warmed up by volcanic sources. This water body may contain living organisms similar to those found in various places on Earth (Seckbach and Chela-Flores, 2001).

Martian life may not be on the surface but in hiding in the subsurface of the dusty red Planet. The same subsurface extraterrestrial life could exist on several planets of the solar system and beyond it in the cosmos. Today the surface of Mars is inhospitable because it lacks surface liquid water, but fluids may exist in the warmer interior of the planet. Also, there is an opinion that on Mars, any liquid water would have to be a highly concentrated brine solution. Hence, that means that any present-day Martian microorganisms would be similar to terrestrial halophiles. Even if present-day life is not present on Mars, it is an interesting consideration that ancient bacteria preserved in salt deposits could be retrieved from an era when the climate of Mars was more conducive to life (Landsis, 2001).

1.2. *Extremophiles and extreme environment conditions*

When biologists ask the question 'What is life?' they are constrained by the range of life forms on Earth. However, when the astrobiologist asks the same question all boundaries are removed. Imagination is the only limitation

to the astrobiologist's thinking. In case of that, extremophiles are only one of possibilities in human imagining of beginning, evolution and distribution of life in the Universe.

The special microorganisms which are able to colonize ecophysiological severe conditions are called "extremophiles". From our anthropocentric point of view these habitats are considered "extreme" although by the microorganisms themselves these places are essentials "oasis" (S e c k b a c h and C h e - l a - F l o r e s, 2001).

Extremophiles thrive on the edge of temperature, hypersalinity, pH, pressure, dryness and desiccation. All three domains of life (Archea, Bacteria and Eukarya) are among the extremophiles (S e c k b a c h and C h e l a - F l o r e s, 2001). Two of these domains are prokaryotes, namely Bacteria and Archea. Extremophiles have been found in a wide range of environments on Earth, wherever there is liquid water. Extremophiles thrive at 3 km depth under the surface, in nuclear reactors, hydrothermal vents and springs, acid mine drainages and acid rivers (Rio Tinto river in Spain), in areas of high heavy metal concentrations, in halite crystals, in polar ice and lakes and in vacuums and under anaerobic conditions. They have been also found in the Dry Valleys of Antarctica and in the Atacama desert of Chili (J a v a u x, 2006).

Classification and examples of extremophiles are given in Table 1.

Tab. 1 — Classification and examples of extremophiles (G r o s s, 1998)

	Type	Definition	Examples
Temperature	hyperthermophile thermophile mesophile psychrophile	growth > 80°C growth 60—80°C 15—60°C < 15°C	<i>Pyrolobus fumarii</i> , 113°C <i>Synechococcus lividis</i> <i>Homo sapiens</i> <i>Psychrobacter</i> , some insects
Radiation			<i>Deinococcus radiodurans</i>
Pressure	barophile piezophile	Weight loving Pressure loving	Unknown For microbe, 130 MPa
Gravity	hypergravity hypogravity	> 1g < 1g	None known None known
Vacuum		tolerates vacuum (space devoid of matter)	tardigrades, insects, microbes, seeds.
Desiccation	xerophiles	anhydrobiotic	<i>Artemia salina</i> ; nematodes, microbes, fungi, lichens
Salinity	halophile	Salt loving (2—5 M NaCl)	<i>Halobacteriaceae</i> , <i>Dunaliella salina</i>
pH	alkaliphile acidophile	pH > 9 low pH loving	<i>Natronobacterium</i> , <i>Bacillus firmus</i> OF4, <i>Spirulina</i> spp. (all pH 10.5) <i>Cyanidium caldarium</i> , <i>Ferroplasma</i> sp. (both pH 0)
Oxygen tension	anaerobe microaerophil aerobe	cannot tolerate O ₂ tolerates some O ₂ requires O ₂	<i>Methanococcus jannaschii</i> <i>Clostridium</i> <i>Homo sapiens</i>
Chemical extremes	Gases Metals	Can tolerate high concentrations of metal (metalotolerant)	<i>Cyanidium caldarium</i> (pure CO ₂) <i>Ferroplasma acidarmanus</i> (Cu, As, Cd, Zn); <i>Ralstonia</i> sp. CH34 (Zn, Co, Cd, Hg, Pb)

1.2.1. Extreme Temperatures

Life on Earth is based on the chemistry of carbon in water. The temperature limits compatible with the existence of life are thus imposed by the essential properties of chemical bonds engaged in this type of chemistry at different temperatures. Two requirements are obligate. Firstly, the covalent bonds between carbon and other atoms involved in the structure of biological molecules should be sufficiently stable to allow the existence of large macromolecules with informational, catalytic properties or both. Secondly, non-covalent links (ionic and hydrogen bonds) should be labile. This is a very important point since only weak bonds can allow specific, fast and reversible interactions of biological molecules and macromolecules. These chemical limits will mainly define the upper and lower temperatures for life. And, it is known that terrestrial organisms can live in the temperature range from -12°C to 113°C (ESA, 1999).

1.2.1.1. High Temperatures

The thermophiles and hyperthermophiles are microorganisms living at high temperature. Thermophiles are found in hot waters, geothermal areas and sun-heated soils. In the hot springs of Yellowstone National Park several species, including the bacterium *Thermus aquaticus*, grow at temperatures higher than 70°C . Hyperthermophilic Archea and bacteria have been cultured from production fluids at temperature up to 110°C from oil reservoirs 3 km below the bed of North Sea (Seckbach, 2000). *Pyrolobus fumarii* inhabits submarine hydrothermal vents at temperature for over 100°C , and it has a highest temperature tolerance of 113°C among all forms of life (Blöchl et al., 1997). An older publication suggested extremely high values for archaean temperature limits of cells isolated from submarine vents or “black smokers” (Baross & Deming, 1983). Those archaean cells were reported to grow at 250°C at 265 atmospheres. It is interesting to note that the most deeply rooted organisms are thermophiles, both in Bacteria and Archea, suggesting that the earliest common ancestors might have been thermophilic microorganisms (Hornec, 2000).

1.2.1.2. Low Temperatures

Life is extremely diverse in the ocean at temperatures of 2°C . Living organisms, especially microorganisms, are also present in the frozen soils of arctic and alpine environments (Russell, 1992). Though, their optimal growth temperatures are ordinarily well above the temperature of the site of isolation. Those organisms with optimal growth temperatures below 15°C and minimal growth temperatures below 0°C are psychrophiles. And those capable of growth at 0°C but with optimal growth temperatures above 15°C are psychrotrophs. Psychrotrophs usually outnumber psychrophiles in a given biotope, because they can benefit more efficiently from ephemeral “warm” conditions (ESA, 1999; Cavicchioli, 2006).

1.2.2. High-Salt Environments

A group of well-studied extremophiles are the salt-loving organisms known as extreme halophiles (Galinski & Tyn dall, 1992). Monovalent and divalent salts are essential for terrestrial life (K^+ , Na^+ , Mg^{++} , Zn^{++} , Fe^{++} , Mn^{++}) because they are required as co-catalysts in many enzymatic activities. All organisms are salt-dependent, in that sense. But, the tolerated salt concentrations are usually quite low ($< 0.5\%$) because high salt concentrations disturb the networks of ionic interactions that shape macromolecules and hold together macromolecular complexes. The extreme halophiles, have managed to thrive in hypersaline biotopes (salines, salted lakes) up to 250—300 g/l NaCl. They are so dependent on such high salt concentrations that they cannot grow at concentrations below 10% NaCl (ESA, 1999). Halophiles may grow in hypersaline water bodies such as the Great Salt Lake (Utah) or in the Dead Sea (Israel) and in saltern evaporation ponds. Halophiles have been also observed in underground salt deposits. Many of these environments contain microorganisms that may have survived millions of years in a dormant state (Seckbach, 2000; Roberts, 2005).

1.2.3. Acidic and Alkaline Environments

The chemistry of life on Earth is optimised for neutral pH. Some microorganisms have been able to adapt to extreme pH conditions, from pH 0 (extremely acidic) to pH 12.5 (extremely alkaline), although maintaining their intracellular pH between pH 4 and 9 (ESA, 1999). Acidophilic microorganisms are found in all three domains of life. These organisms grow at pH levels of 0 to 4. Among the acidophiles are sulfur bacteria, Archea and phototrophic hot spring protest like the thermoacidophilic algae *Cyanidium caldarium*, *Dunaliella acidophilum* and diatoms. Hyperthermophilic archaean *Pyrolobus fumari* lives at pH range of 4 to 6,5 (Blöch et al., 1997; Madigan & Oren, 1999). Many bacteria and a few archaea, live at the other extreme of the pH range, from pH 9 up to pH 12, they are called the alkaliphiles (Grant & Horikoshi, 1992). They are present everywhere on Earth. Some of them, which have been discovered in soda lakes rich in carbonates, are also halophiles (haloalkaliphiles). Most alkaliphiles are mesophiles or medium thermophilic, but there are hyperthermophilic alkaliphiles, like *Thermococcus alkaliphilus* (Keller et al., 1995).

1.2.4. High-Pressure Environments

The barophilic Archaea and bacteria are present in deep subterranean locations as far as 4 km below the continental crust and on the ocean floor (pressure 1100 bar) (Seckbach, 2000). The extreme pressure limit for life on Earth is unknown, environments of above 1100 bar have not been explored. Though, it might be quite high, because macromolecules and cellular consti-

tments apparently only begin to denature at pressure of 4—5000 bar (ESA, 1999). Recent space travel to the Moon and back to Earth has shown that bacteria can tolerate long periods (at least 2.5 years) of vacuum. These observations confirm the extent of the diversity of microorganisms (S e c k b a c h, 2000).

1.3. *Extremophiles and Extraterrestrial Life*

Among early microorganisms, Cyanobacteria played a major role, inventing oxygenic photosynthesis and causing the most profound alteration in history of our planet. A few decades ago S a g a n (1961) proposed an extravagant planetary engineering plan. He suggested seeding the atmosphere of hostile planet with cyanobacteria for making these planets habitable for life. F r i e d m a n n and O c a m p o - F r i e d m a n n (1995) followed this idea of Sagan's and also proposed propagating Mars with cyanobacteria for extraterrestrial terraforming (S e c k b a c h, 2000). S v i r ě v (2005) define practical use of cyanobacteria in astrobiology, with term “astrobiotechnology”.

The only criteria that is required without an exception is that every ecological niche which supports life has the presence of water in liquid form for some portion of the year. Mars is a salt-rich environment. Any present-day water would likely be a saturated salt solution, with a lower vapor pressure and a lower melt temperature than pure water (L a n d i s, 2001). Conditions for life on present-day Mars do not exist, but it may be possible that halobacteria may still be retrieved in salt deposits on Mars and cultivated in a suitable medium for growth. It is verified that bacteria on Earth can survive in salt deposits that are over 650 million years old. It is reasonable to extrapolate that possibly bacteria could survive in salt deposits for over a billion years. If this were to be true, then bacteria might be retrieved and cultured from an era dating to the time that Mars had a warm climate with liquid water, approximately 3.5 billion years ago (L a n d i s, 2001). Although the number of extreme conditions on Mars surpass all natural combinations of extreme conditions on Earth, it can be drawn a parallel between, in this case, salt rich soils on Earth and environment on Mars, which is also salt rich.

Because Mars is a salt-rich environment some halophiles might survive there. Here we are considering, basically, salinity as the reason why we should, in some cases, identify these two environments on Earth and on Mars. Vojvodina has regions that are salt rich and there are found many different strains of cyanobacteria (G a n t a r et al., 1991; S v i r ě v, 1992). In this paper are given some data of cyanobacterial strains sampled and cultivated from regions mentioned above. This data can serve for the further investigations in this field, and help us to understand better life and conditions under it can be obtained, also to understand connection between extremophiles, Earth and space.

The aim of this paper is to try in some way to connect extraterrestrial extreme environments (in this case on the Mars), with extreme environments in our region.

2. MATERIAL AND METHODS

Analysis of cyanobacterial appearance and isolation and determination of cyanobacterial strains have been performed on the samples of salt soils belonging to solonetz and solonchak types. The samples were taken from different depths (0—1 cm, 1—30 cm and 30—60 cm) during spring, summer and autumn 1996.

Solonchak soil has been found on locality near Horgoš. Solonchak belongs to halomorphic and salt-accumulative soils. The name itself points at the type of soil which contains a lot of salt. The salt storage zone is usually the largest in surface layers, but can be found on different depths. Carbonate and bicarbonate solonchaks dominate in Vojvodina. Examined solonchaks contain 1—2% of salt, but concentration reaches up to 20% in surface rinds (Ćirić, 1989). Solonetz, like solonchak, belongs to halomorphic soils but in the class of alkaline eluvial-iluvial soils. Solonetz was examined at village Kumane in Banat. If looked from outside, the soil presents mosaic made of meso-ridges and depressions. Meso-ridges are micro altitudes, 30—50 cm higher than depressions. Salt is rinsed from meso-ridges and steppe vegetation is developed. Salt is sedimented in depressions and form white layer on the surface. Usually vegetation is not developed in depressions. In a rainy season this type of soil, especially depressions, is covered by water. Because of the water proof B horizon the water cannot get through deeper layers.

Quantification of cyanobacteria has been carried out in the following way: 3g of soil were added to 50 ml of nitrogen-free (BG-11) medium (Ripka et al., 1979) and mixed on rotary shaker for 15 min at 120 RPM. One ml of soil suspension was then filtered through a Sartorius filter (pore size 0,45 mm) and washed with 10 ml of BG-11. The filter was placed on solid BG-11 medium and incubated under cool white light at a photon fluence rate of 20 mmol m² s⁻¹. Dark green colonies on the filter surface were counted after 20 d and numbers of cyanobacterial colonies are expressed per g of dry soil. Individual colonies were transferred to a liquid medium (BG-11). Care was taken to select morphologically different colonies which were apparently free from bacteria and fungi. Axenic cultures were obtained by isolating individual migrating hormogonia following gamma irradiation (1 x 10⁸ and 2 x 10⁸ rads) (Kraus, 1966) and antibiotic treatment (Vara et al., 1979). The axenic or unialgal cultures were maintained in BG-11 medium under cool-white lights at a photon fluence rate of 20 μmol m² s⁻¹ at 24°C. Cultures of different ages were microscopically examined both in liquid and solid media.

3. RESULTS

3.1. *Number of cyanobacteria*

The amount of nitrogen-fixing cyanobacteria in the surface layer of solonchak was 39040 ind.g⁻¹, and in the surface layers of solonetz was 17533 ind.g⁻¹ in absolutely dry soil in 1996 (Fig. 1). Solonetz is found with a higher

number of nitrogen-fixing cyanobacteria in depressions than on meso-ridges. This specifically refers on rainy seasons of year when depressions are filled with water. If we compare occurrence of cyanobacteria and total amount of algae in solonchak and solonetz, the percentage of cyanobacteria was 69.35% and 48.94% respectively.

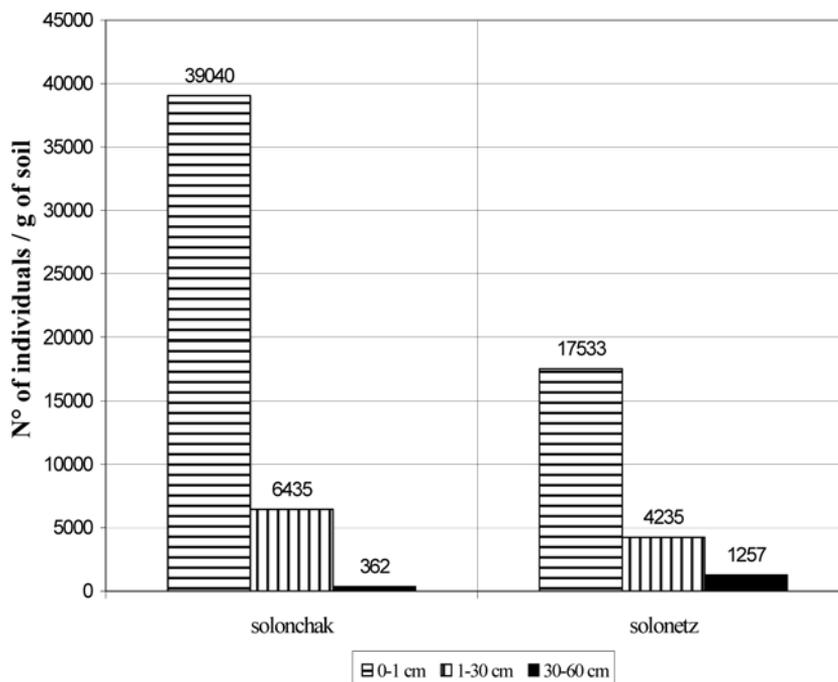


Fig. 1 — Number of nitrogen-fixing cyanobacteria in solonchak and solonetz, on different depths sampled in 1996-yearly average

3.2. Taxonomical diversity of Cyanobacterial strains

Fifteen strains of cyanobacteria are isolated during our investigation. Only one belongs to genus *Tolypothrix* (Fig. 2), and the rest fourteen belong to genus *Nostoc* (Fig. 3) (Tab. 1). All strains are morphologically described (stage), dimensions of vegetative cells, dimensions and percentage of heterocysts are obtained.

Tab. 1 — Morphological characteristics of isolated strains of nitrogen-fixing Cyanobacteria

Number of strain	Strain	Cyano-bacteria	Soil	Vegetative cells length x width (mm)	Heterocysts length x width (mm)	Hetero-cysts (%)	Aserial stage
1	NS AFCC 1	<i>Nostoc</i> sp.	Solonetz	3.31 x 3.31	4.78 x 3.77	8.67	+
2	NS AFCC 21	<i>Nostoc</i> sp.	Solonetz	3.03 x 3.07	5.18 x 5.10	3.06	+
3	NS AFCC 22	<i>Nostoc</i> sp.	Solonetz	3.61 x 3.35	4.93 x 4.83	14.45	+
4	NS AFCC 23	<i>Nostoc</i> sp.	Solonetz	2.55 x 2.75	4.95 x 3.82	5.98	+
5	NS AFCC 58	<i>Nostoc</i> sp.	Solonchak	2.93 x 2.55	3.43 x 2.75	6.86	+
6	NS AFCC 301	<i>Nostoc</i> sp.	Solonetz	4.96 x 4.39	5.34 x 5.04	7.16	+
7	NS AFCC 302	<i>Nostoc</i> sp.	Solonetz	3.00 x 3.79	3.79 x 3.82	6.72	+
8	NS AFCC 303	<i>Nostoc</i> sp.	Solonetz	3.91 x 3.67	3.98 x 4.30	12.40	+
9	NS AFCC 304	<i>Nostoc</i> sp.	Solonetz	3.30 x 3.48	4.34 x 4.66	8.36	+
10	NS AFCC 308	<i>Nostoc</i> sp.	Solonetz	3.34 x 3.27	3.58 x 3.62	9.57	+
11	NS AFCC 310	<i>Nostoc</i> sp.	Solonetz	3.64 x 4.18	3.94 x 4.16	6.98	+
12	NS AFCC 312	<i>Nostoc</i> sp.	Solonetz	4.02 x 4.20	4.16 x 3.86	—	+
13	NS AFCC 68	<i>Nostoc</i> sp.	Solonetz	2.55 x 3.40	3.22 x 3.52	9.09	+
14	NS AFCC 69	<i>Nostoc</i> sp.	Solonetz	2.80 x 3.82	2.93 x 4.46	10.14	+
15	NS AFCC 67	<i>Tolypothrix tenuis</i> f. <i>terrestris</i>	Solonetz	5.00 x 6.54	3.39 x 4.65	7.76	—

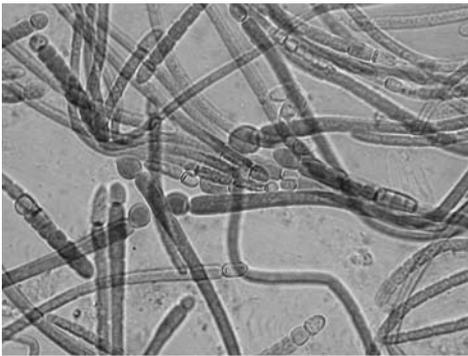


Fig. 2 — *Tolypothrix* sp.

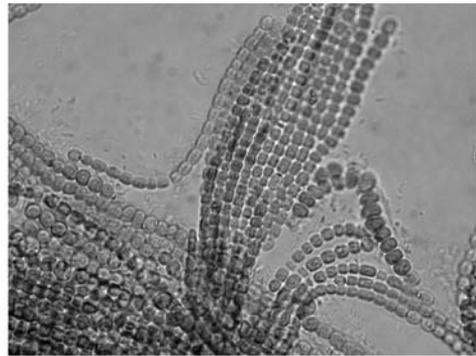


Fig. 3 — *Nostoc* sp.

4. DISCUSSION

Gantar et al. (1991), examined occurrence nitrogen-fixing cyanobacteria in surface layer of three types of soil in Vojvodina: black meadow, solonetz and chernozem. Results of their studies showed that higher amount of nitrogen-fixing cyanobacteria were found in solonetz, with average value in one year of 4000 ind.g⁻¹. Together with our results, this is the indicative fact that large amount of cyanobacteria is present in salt soils, and their presence is re-

gistered beneath the surface (in the range of 30—60 cm). That would be, in the case of Mars, of great significance because of the reason that intensive UV radiation is absorbed on the surface and by that its interior is sheltered from the big temperature fluctuations that are dominant at the Mars surface.

The thesis that water exists on Mars with a lower vapor pressure and a lower melt temperature than pure water goes with the facts that saturated solution of K_2CO_3 , for example, will depress the freezing point of water to below 236 K. Multicomponent aqueous salt solutions can have freezing temperatures as low as 210 K and water in micron-scale pores between grains of regolith would have even lower freezing point due to capillary-pore effects (L a n d i s, 2001), which could more or less successfully bridge over, on first look, insurmountable obstacle between water in liquid state and low temperatures on Mars.

Initiated examination can serve for further, larger examinations in this directions, and also for investigations connected with isolation of extreme cyanobacteria determined genes, responsible for their “extremeness” and possibilities of forming a new species by genetic engineering which will be useful in Mars terraformation. Various microbes on Earth developed a strategy to cope with a combination of extreme conditions found in their habitats, such as the cyanobacterium *Chroococidiopsis* which survives a large variety of extreme conditions of dryness, acidity, salt and high as well as low temperatures (W h i t t o n, 2000).

In the case that life in the Earth surroundings does not exist itself, the terraforming with Earth appropriate cyanobacterial extremophiles is still possible.

5. CONCLUSION

Despite the fact that the conditions in extreme salt-rich environments on Earth are differed than that on Mars it is reasonable to assume that from enormous diversity of strains there will be at last few of them that might serve like a good analog for possible life on Mars, prior to the cyanobacteria have been found in almost all extreme environments on Earth.

This work sets inside frames in which the link between Earth and astrobiology could be proposed, or Earth and one of life possibilities beyond its boundaries.

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ЕКСТРЕМОФИЛИ — ВЕЗА ИЗМЕЂУ ПЛАНЕТЕ ЗЕМЉЕ И АСТРОБИОЛОГИЈЕ

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Резиме

Астробиологија се бави проучавањем настанка, развића, распрострањености и будућности живота у свемиру. Места на којима је највећа вероватноћа да постоји живот ван Земље, а унутар Сунчевог система, су Марс и Јупитеров сателит Европа. Екстремофили су организми који живе и опстају у условима екстремних температура, притисака, рН вредности, екстремно сланих станишта, итд. У овом раду разматране су екстремофилне цијанобактерије као могући облици живота у астробиолошким размерама. Узорковање је обављено у Војводини, а узорци су узети са земљишта солоњец и солончак. Чињеница да солоњец и солончак садрже висок проценат соли, даје шансу поређења ова два типа земљишта са животним условима на Марсу, такође окарактерисаним високим процентом соли, што је и основна идеја овог рада.