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Electromagnetic Radiation and Life: Bioelementological Point of View

A.Kh. Tambiev[1] and A.V. Skalny[2]
[1]*Moscow State University,*
[2]*Institute of Bioelementology, Orenburg State University,*
Russia

1. Introduction

Life on Earth has formed and evolved under exposure to electromagnetic radiation (EMR) of the Sun, which is the most potent natural source of electromagnetic waves. Besides the Sun, electromagnetic oscillations form during lightning strikes. Also, there is geomagnetic background of our planet. Artificial electromagnetic radiation is produced by different electrical equipment, electric motors, radars, transmitters, computers, mobile phones and other devices. It is impossible to imagine modern civilization without television, radio, telemetry, radars and navigation systems. Along with the traditional technical use of almost the whole scale of electromagnetic waves, there expands their practical application in various fields: medicine, biology, agriculture and biotechnology. The Sun radiates its energy almost over the whole scale of electromagnetic waves, including radio, ultra high frequency and optical ranges as well as the range of ionizing radiation. Basically, all the energy of Sun's EMR lies within the wavelength range from 1500 Å to 5 mm, and the share of our planet is an enormous amount of energy equal to 2×10^{17} watts per second. There was emerged a new science electromagnetobiology, which deals with a part of the general problem of biological effects of mild and ultramild physical and chemical factors. It is believed that the action of such factors lies below the threshold for activation of protective biological mechanisms and therefore is capable of cumulating at the subcellular level. Man-made electromagnetic pollution is increasing very rapidly: during the last 45 years, as estimated, electromagnetic pollution has grown 45-50 thousand times. Currently the world is published several thousand articles on electromagnetobiology per year. Standards on electromagnetic safety for man are developed by a number of different national and international organizations. Thus, the biological effects of ultramild agents, particularly EMR, are a fundamental scientific problem with distinct application-oriented character. Apparently, there is no other external factor, which would have such a powerful influence on living objects as electromagnetic waves do. In the biosphere permanently occur periodic electromagnetic processes with frequencies distributed throughout the whole electromagnetic spectrum. It is natural to assume that any part of this spectrum has played a certain role in the evolution of living organisms, affecting their vital processes. For example, a trace effect of low frequency natural electromagnetic fields on living organisms could be fixed in the form of biorhythmic oscillations. Though electromagnetic waves existed on Earth ever, the mankind "has guessed" about the existence of this form of matter only in the second half of the 19th century (D. Maxwell, H. Hertz). Men were first used artificial electromagnetic waves for their purposes only in the late 19th century (A. Popov, G. Marconi). It has long been observed that changes in the static magnetic field are not indifferent to living organisms; these changes always accompany the development of life on Earth. Some believe that, for instance, inversions of poles of the Earth magnetic dipole could cause biological effects of global scale, in particular, cause appearance and disappearance of biological species and life as a whole. According to conception of bioelements and bioelementology as a new integrative approach in life sciences, proposed by A.V.Skalny (2003-2011) the existing of biosystems depends on combination of internal factors (presence of bioelements as blocks of life) and external conditions including electromagnetic fields. Biosphere is an assembly of bioelements and living organisms existing under permanent regulatory influence of physico-chemical factors of terrestrial and cosmic origin. Electromagnetic waves can be powerful agent for production of so called secondary bioelements from primary ones, according to our (Skalny, 2009, 2011a) concept of bioelements. Thus, we suppose that electromagnetic technologies can be an effective instrument of enlargement of biomass with increased "food density", enriched by essential nutrients, for Humankind (Skalny, 2011c). It is also known that some ions participate in magnetoreception. Thus, calcium ions have been shown to be involved in many biological processes: synaptic transmission, excretion of various compounds to the environment, flagellar motility, enzymatic activation, muscular contraction, reproduction, growth and development. Since some proteins, which bind calcium, can bind magnesium too, the cites of binding for the calcium and magnesium may be identical, so that ions of magnesium and calcium may be considered as potential targets of magnetic fields. Around the same way of involving in the processes of life is probable for potassium, sodium, rubidium and lithium. Electromagnetic waves of millimeter scale exist in the spectrum of the Sun, but do not reach the Earth's surface, because absorbed by water vapor. Consequently, this range could not be an environmental factor that participated in evolution running in the biosphere. It was artificially obtained recently in 1965-1966, when Russian scientists under the guidance of academician N.D. Devyatkov and professor M.B. Golant developed generators that produce this kind of waves. From that time these waves started to be applied in medicine and then in biology. There were used mostly the waves of low, non-thermal intensity, when the upper limit of the incident power density was ca. 10 mW/cm2. Thus, as for the quantity of absorbed energy, this range can be attributed to mild or even ultramild influence. Millimeter waves have several important features: strong absorption by molecules of water, a resonant effect, the ability to produce convective mixing of the irradiated liquid. At the same time, the biological effect of millimeter waves is usually cumulative. We have first (Tambiev et al., 1997) identified a significant biological effect of these waves on the photosynthetic organisms: cyanobacteria and microalgae. It was shown in many studies that water retains the information about the history of physical influences on it, which significantly affects the flow of processes in the aquatic environment and creates opportunities for developing new channels of control of chemical, biochemical and biological processes. The experiments showed that millimeter waves can influence on the chemical (mineral) content of cells of the photosynthetic organisms – cyanobacteria and microalgae, which are widespread objects of photobiotehnology. We have managed to significantly increase the synthesis of so-called secondary bioelements by microalgae Spirulina platensis and Spirulina maxima. Under the influence of millimeter wave radiation there is observed increased accumulation of a number of trace elements from the environment: selenium, chromium, zinc, copper, lithium, etc., with dramatic change in elemental composition of the algal cells (Tambiev et al., 2000-2011). Our studies indicate the applicability of electromagnetic millimeter waves for the efficient biosynthesis of secondary bioelements and the necessary building blocks for life maintenance, and finally for increasing the mass and diversity of living matter on the planet, which has an undoubted theoretical and practical importance.

2. Electromagnetic radiation of millimeter range (EHF-radiation) of low intensity and its effect on phototrophic microorganisms

Life on Earth has been formed under the influence and in environments of solar electromagnetic radiation, which is the most powerful external factor affecting the existence of living organisms. The biosphere is constantly full of electromagnetic processes with various wavelengths. It is possible that any part of this spectrum anyhow influences the evolution and vital processes, causing either extinction or adaptation of living species. For example, natural rhythms of electrical oscillations in living organisms may form as a result effected by natural low-frequency electromagnetic fields.

Sun, as we know, is a giant thermonuclear boiler with a temperature of 5780 K at the surface and up to 16 million K in the center. Its spectrum contains lines of more than 70 chemical elements, and it radiates its energy over almost the entire scale of electromagnetic waves.

Every second Sun spends for radiation more than 4 million tons of its mass, which totally amounts about 2×10^{21} million tons. The share of our planet is 2×10^{17} W of electromagnetic energy per second. Electromagnetic oscillations also emerge in discharges of lightning, including little-known ball lightning. Despite the fact that electromagnetic waves existed on our planet ever, scientists began to closely study this form of matter just in the second half of the XIX century (J. Maxwell in 1865; H. Hertz in 1888). All life processes, as it became evident later, are accompanied by generation of electromagnetic waves.

Artificial electromagnetic waves were first used by man for technical purposes in 1895-1896 (A. Popov, G. Marconi). This application has developed to such an extent that now it is impossible to imagine modern civilization without television, computers, radios, power lines, radar and navigation systems, radiophones, electric motors, diagnostic and magnetotherapeutic medical equipment etc.

In medicine, electromagnetic waves are used currently in a broad range from ultra-low frequency to x-rays and gamma radiation: as therapeutic and diagnostic means, for heating tissues, in medical lasers etc. In addition, electromagnetic waves are used in such areas as industrial techniques, agriculture, biotechnology and, in particular, in photobiotehnology, which we will discuss below.

In recent decades, a discipline “electromagnetobiology” is formed as a part of the research on biological effects of weak and ultra-weak physical and chemical factors. It may be suggested that influence of these factors is below the threshold of protective biological mechanisms and is therefore capable of accumulating at subcellular level. At the same time, electromagnetic pollution is accumulating in the environment, and its increase over the last two decades was estimated in some works tens of thousands times as much. This makes the problem a very urgent. Various organizations develop standards for Electromagnetic Safety, investigate connection between low-frequency (mostly) electromagnetic fields and the risk of cardiovascular, oncologic, immune and other diseases, study the problems arising from wide use of mobile phones, etc. Currently, several thousand articles on electromagnetobiology is published in the world annually (Bingi, 2002).

Radio-wave range				Microwave range (MW)				Optical range			Ionizing radiation	
>1000m	100…1000 m	110…100 m	1…10 m	10…100 cm	1…10 cm	1…10 mm	0.1…1 mm	100…0.76 µm	0.76…0.4 µm	400…10 nm	10…0.01 nm	0.01…0.0001 nm
Myriametric waves	Long waves	Medium wave	Short waves	Decimeter waves	Centimeter waves	Millimeter waves	Submillimeter waves	Infrared radiation (IR)	Visible light	Ultraviolet radiation (UV)	X-radiation	Gamma radiation
This range has long been widely used in various devices for wireless communication (radio, television, etc.). It is traditionally called "radio-wave range". Some parts of it are used in medical devices.				Technical use of microwaves began to develop rapidly in the second half of XX century after invention of radar, radio relay links, satellite communication systems, control systems, mobile phones etc. Microwave electronic devices made it possible to create medical equipment for diagnostics and treatment of many diseases.				These types of radiation are of considerable interest for medical application, especially after the invention of IR, visible and UV lasers, xenon radiators and improved mercury lamps.			Ionizing radiation is the very first type of electromagnetic waves, which have been applied in medicine at the end of XIX century to diagnose and treat cancer.	

Table 1. The scale of electromagnetic waves

In 1965-1966 a team led by academician N.D.Devyatkov has developed generators for medical and biological use on the basis of wide-range backward wave tubes. This made it possible to get an artificial radiation of the millimeter range (wavelength 1-10 mm), called EHF radiation (denoted "extremely high frequency"), and gave the opportunity to explore the effect of millimeter waves of low (non-thermal) intensity on various microorganisms and experimental animals. The interest in such work was supported by the fact that in nature there are no strong sources of these waves since this range is much absorbed by water molecules, which have a large dipole moment. For example, a water layer 1 mm thick degrades the millimeter waves with $\lambda = 8$ mm by 100 times, and with $\lambda = 2$ mm by 10,000 times. Thus these waves present in the spectrum of solar radiation, did not reach the surface of Earth, because they were stopped by water vapor in the atmosphere. And therefore they could not be a factor of evolution.

The biological action of this kind of electromagnetic waves was to be studied with allowance for their peculiarities. In almost all experiments on the effect of millimeter waves on unicellular and multicellular organisms there was used the upper limit of low intensity, not exceeding 10 mW/cm2. Larger intensity produced undesirable thermal effects in biological objects and eliminated the possibility to find previously unknown effects, such as informational ones.

An important point was also the fact that quantum of energy in the EHF range has a magnitude less than the energy of thermal motion of atoms and molecules at room temperature. In the literature this is called "kT problem" because it is unclear how these rays can change the speed of biochemical reactions, i.e. to cause a resonance-like biological effect. If to imagine the position of EHF quantum on energy scale, then it will be located below the energy of hydrogen bonds, the oscillation energy of atoms and molecules, the energy of activation and ionization, but above the energy of rotational motions of atoms and molecules and the energy of magnetic ordering. Hence at room temperature the EHF quanta can affect mainly only the kinetic energy of rotation of polar molecules (Tambiev et al., 2003). In terms of physics this phenomenon is yet have no explanation but only a hypothesis. And this is a very important, just maybe the most important problem of magnetobiology, discussed in literature (Bingi, 2002).

The next important effect of millimeter waves is convective mixing of the irradiated fluid. This is a consequence of high field gradients and high temperature in the irradiated thin surface layer, which can further extend to the entire irradiated volume. The convection can enhance transport of ions, water and various substances through biomembranes.

Effects caused by irradiation of objects with millimeter waves, as a rule, are frequency-dependent. The effective frequencies are called "resonance" or active, and the reasons for their action are not always explainable, – most likely, there are subtle mechanisms of their influence on some parts of cell metabolism. The biological effect can be seen only at these resonant frequencies and disappears when you shift them by 100-200 MHz. Thus, in experiments with microorganisms, diagrams of dependence of the biological effect on the incident power density of electromagnetic waves have extended regions (plateau), where the effect was not manifested until fit to resonant values of power. At the resonant values the effect became observed, but after passing off these values the plateau could extend again until the next resonance values approach. Biological effects caused by the action of millimeter waves, in many cases, have a cumulative nature, which apparently suggests that the manifestation of them involves not only fast, but also relatively slow biochemical processes.

It is important to note that a very small amount of energy absorbed by objects during its irradiation with low-intensity millimeter waves (EHF irradiation) allow us to categorize this physical factor as a weak or even ultra-weak influence, which in recent years attracted much attention (Tambiev et al., 2003).

Since the beginning of practical use of the millimeter waves, the most impressive achievements have been obtained in medicine. More than thirty-five years' experience of

EHF radiation use in clinical practice has shown its high therapeutic efficacy in treatment of various diseases by devices with active wavelengths of 4.9, 5.6, 7.1 mm. The incident power density of the radiation is typically a few tenths of milliwatt or several milliwatts per square centimeter. EHF therapy is well combined with other treatments, as well as frequently used as monotherapy; it increases body functional reserves, improves immune status.

In the mid-1980s we were first published works, which described the effect of EHF radiation on photosynthetic organizms – representatives of a vast world, vital for the existence of the biosphere. As the objects, we chose cyanobacteria of Spirulina genus, which are common targets in photobiotechnology.

Now photobiotechnology is an intensively developing field of science and industry. It uses productive facilities of phototrophic organisms, especially prokaryotic cyanobacteria (blue-green algae), eukaryotic microalgae (green, yellow-green, red, brown etc.), and higher plants. Industrial cultivation of microalgae and cyanobacteria has several advantages, which include the ease of growing in controlled conditions, rapid proliferation and the possibility of quick obtaining of large biomass, whose composition exceeds many natural sources of organic compounds. Particularly, the biomass of cyanobacteria and microalgae contains all essential amino acids, several essential vitamins and other nutrients. In addition, at mass cultivation it is possible to obtain biomass with different chemical composition, thereby conducting controlled biosynthesis.

In cultivators of open type (sunlit) the average productivity of dry biomass of cyanobacteria per day can reach 50-60 g/m2, which is equal to 45-70 tons/ha per year. I.e. the total yield of the relatively low-cost biomass is 10 times as much as the yield of wheat and 15 times as much as the yield of soybeans. The biomass can be used in animal and poultry farming, pisciculture, sericulture, horticulture, crop production, food and feed production, for creating a protein-vitamin supplements and drugs, food dyes, enzymes, vitamins, in cosmetics, in metal-working, microbiological and chemical industries, for obtaining additional energy resources as through bioconversion into methane, alcohols or other products. Besides, direct synthesis of hydrocarbons by algae is a process connected to purification of waste water and protecting the environment.

World production of the dry biomass of cyanobacteria and microalgae for food and feed purposes reaches more than ten thousand tons per year. Only Japanese production of macrophytic algae grown in aquaculture exceeds 1.5 million tons per year.

We studied the possibility to stimulate growth and biomass productivity using millimeter waves on cyanobacteria Spirulina platensis, Spirulina maxima (prokaryotes), and a Black Sea green flagellate Platymonas viridis (eukaryote). These organisms can serve as a non-traditional source of protein, may be the first link in the mariculture food chain. They tolerate wide variations in temperature, light and salinity, and is resistant to chemical pollution. S. platensis and S. maxima, as the most suitable subjects for large-scale production of edible micro-algae, are cultivated in the United States, Japan, Germany, Israel, Mexico, Brazil, India, Taiwan, Italy, China, Thailand and other countries.

As it can be seen from Table 2, protein amounts up to 70% of the cyanobacterial cells (dry weight). Its composition is close to that of egg white and fairly balanced for all essential amino acids. Besides the listed components, the composition includes vitamins B1, B2, B3, B6, B12, E, folic acid, biotin and pantothenic acid. Beta-carotene in spirulina is 20-25 times as much as in carrots. Among the lipids there prevail unsaturated fatty acids and rare gamma-linolenic acid, necessary for humans. Spirulina is relatively rich in potassium, magnesium, iron, phosphorus, calcium. It also contains many essential trace elements such as zinc, manganese, copper, and these minerals are contained in chelate form, friendly for human body. Unlike most of the algae, cell's wall of spirulina is composed of complex carbohydrates, rather than cellulose. Due to this, spirulina is easily digested, and its assimilability equals to 85–95%. Biological value of spirulina puts it on par with such products as meat and soy protein, and is significantly higher than that of such foods as legume and cereal flour.

Humidity, %	4.0-6.0
Ash, %	6.4-9.0
Protein, %	60.0-71.0
Carbohydrates, %	13.0-16.0
Triglycerides, %	6.0-7.0
Ribonucleic acid, %	2.2-3.5
Deoxyribonucleic acid, %	0.65-1.0
Chlorophyll a, %	0.61-0.76
β-carotene	0.15-0.19
Palmitic acid, %	1.65
Linoleic acid, %	1.09
Linolenic acid, %	0.8-2.0

Table 2. Overall chemical composition of S.platensis biomass.

Spirulina (S. platensis, S. maxima) is usually cultivated either in artificial reservoirs with stirring, or in photobioreactors of open or closed type. Biomass of S. maxima is directly extracted and dried from Lake Texcoco (Mexico). In Central Africa, spirulina S. platensis for hundreds of years was collected in the Lake Chad and surrounding area, where it was used by local population for food. Pacific spirulina S. pasiphyca, a strain obtained and cultivated by the company Cianotech in Hawaii, have the highest content of carotenoids in comparison with other species and strains. Growth conditions for the genus Spirulina is very simple: you need carbon dioxide, water, inorganic salts and light. Also an important advantage is the need for highly alkaline environment.

Experiments to study the potential stimulation of growth and yield of biomass using millimeter waves (EHF radiation) were carried out by us on the cultures of S. platensis and P. viridis.

Table 3 shows that the largest statistically significant stimulation of biomass production on the 30th day of growth was observed for S. platensis after a single irradiation for 15 and 30 min, and for P. viridis after a single irradiation for 30 and 60 min. In both cases the wavelength was 8.34 mm, incident power density was 2.2 mW/cm^2.

Exposure time, min.	Age of culture, days				Stimulation of growth on the 30th day	
	I		II			
	A	B	A	B	A	B
10 days						
15	0.15	0.5	0.30•	0.16•		
30	0.20	0.05	0.23	0.18•		
60	0.13	0.05	0.13	0.15		
120	0.12	0.12	0.10	0.16		
360	0.07	0.20	0.043	0.16		
20 days						
15	0.75	0.31	1.40•	0.60•		
30	0.71	0.31	1.07	0.17*		
60	0.67	0.31	1.05	0.54*		
120	1.52	0.27	1.03	0.28*		
360	0.32	0.40	0.26	0.40		
30 days						
15	2.40	0.40	5.20*	0.91•	217	228
30	2.12	0.40	4.16•	0.92*	196	230
60	1.96	0.40	2.08	1.15•	106	288
120	3.24	0.44	2.92	0.49	90	111
360	1.56	0.57	1.44	0.52•	92	91

Note: differences are significant at * - $p < 0.05$; • - $p < 0.01$.

Table 3. Effect of EHF radiation on the biomass production in non-irradiated (I) and irradiated (II) cultures of photosynthetic organisms: S.platensis (A) and P.viridis (B), g/l

Since organisms excreted exometabolites into cultural medium, we proposed a method for measuring reactivity of the medium (RM). It involves the ratio of oxidative (OA) and antioxidant (AOA) activity of the medium. The RM parameters of the cultural medium are closely related to physiological state of the producers in all phases of growth and development. This was shown previously for many species of cyanobacteria, micro- and macrophytic algae, fungi, bacteria, actinomycetes and other objects (Tambiev, 1984).

Figure 1 shows that in irradiated cultures of the cyanobacteria and microalgae OA of the native exometabolites increased and correlated with an increase in biomass. The data obtained earlier on unicellular and macrophytic algae indicated that their normal metabolism is most often accompanied by release of much oxidizing compounds into the medium, thus manifesting OA. On the contrary, metabolic disorders reduce the release of oxidants and induce leak of antioxidants into the medium (which in normal conditions are kept inside the cell and present in the medium just in small amounts), thus manifesting AOA. Therefore, the ratio of oxidants and antioxidants in the medium allow us to monitor physiological status of the cyanobacterial and algal cultures. Figure 1 demonstrates that a single exposure of the cultures by low-intensity millimeter waves in optimal time, which is accompanied by the highest yield of biomass, has the greatest value of OA.

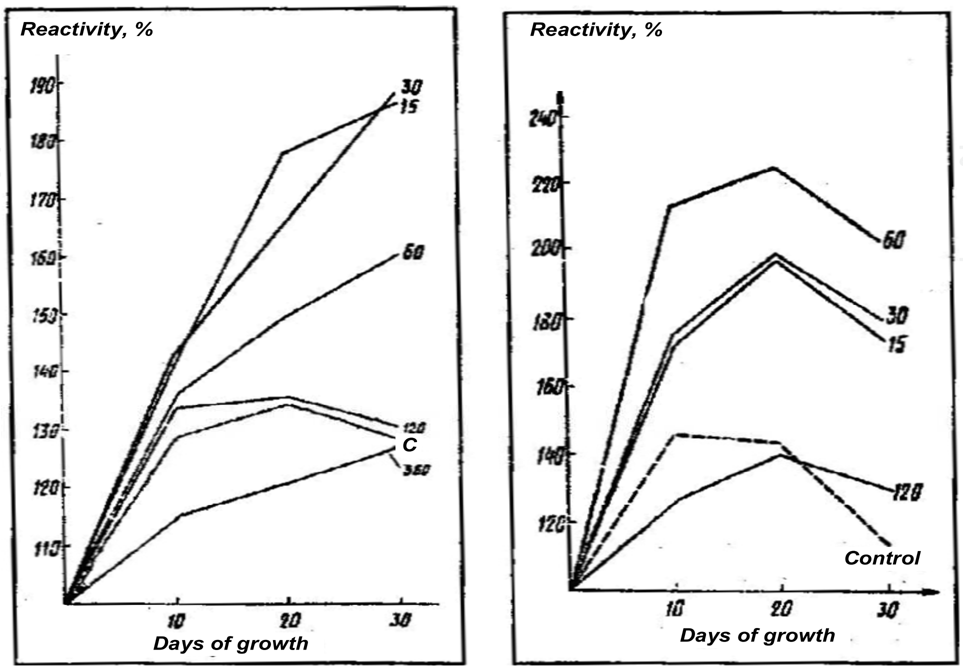


Fig. 1. Effect of EHF radiation on reactivity of cultural medium for S. platensis (left) and P. viridis (right)

Above we have mentioned that millimeter waves possess a resonance effect. We have identified a number of active wavelengths, which caused much more accumulation of S. platensis biomass in comparison with control when irradiated in a liquid medium.

Table 4 shows that at different incident power density and irradiation time equal to 30 min the resonant wavelengths were 7.1, 7.89, and 6.66 mm. During irradiation of S. platensis in the same conditions but at the constant incident power density equal to 1.3 mW/cm2, the greatest effect was observed at the wavelengths 7.1 and 6.25 mm. For culture P. viridis the active wavelengths were 8.34 and 8.30 mm with an increase in biomass of 241 and 218% as compared to control, respectively. At the same time, irradiation at wavelengths 8.25 and 8.20 mm resulted in inhibition of growth. It should be noted that, as before, the changes in RM were correlated with the accumulation of biomass.

In experiments on the same irradiated cultures at wavelength 8.34 mm and incident power density 2.2 mW/cm2, we observed an intensification of photosynthetic process, accompanied by an increase of oxygen and growth of biomass. At the same time, there was increased amount of chlorophyll in cells of the irradiated cultures as compared to control, and the ratio of dark respiration to photosynthesis shifted toward more favorable process for the cells – to photosynthesis.

It was also shown that millimeter waves affect the absorptive-excretory transport of sodium ions, recorded in the medium. This was observed in irradiated cultures earlier than changes in photosynthesis, pigment composition of the cells or growth of biomass.

Experimental conditions	10 days	20 days	30 days		
	Biomass	Biomass	Biomass	pH	RM (% to the control)
Control	0.16	0.49	0.62	9.75	97
8.34 mm, 2.6 mW/cm2	0.21	0.68	0.87	9.84	71
7.1 mm, 1.54 mW/cm2	0.25	0.88	1.35	9.91	158
7.89 mm, 2.38 mW/cm2	0.28	0.95	1.3	9.91	141
7.4 mm, 3.0 mW/cm2	0.25	0.85	1.1	9.93	127
6.66 mm, 3.1 mW/cm2	0.25	0.90	1.4	9.95	180
6.25 mm, 1.64 mW/cm2	0.20	0.77	0.92	9.90	84
6.06 mm, 3.0 mW/cm2	0.17	0.80	1.0	9.83	101

Table 4. Effect of EHF radiation of different frequencies on the increase of S. platensis biomass (liquid medium)

Table 5 shows that after the exposure the excretion of organic compounds (carbohydrate, riboflavin) by S. platensis into the cultural medium was increased, which also manifested the resonance effect; and the biomass of irradiated cultures exceeded that of non-irradiated by 1.6–1.8.

Experimental conditions	Biomass, g/l	Carbohydrates, mg/g dry biomass	Riboflavin, µg/g dry biomass
Control	0.488	266.3	20.5
Irradiation:			
λ = 7,89 mm	0.720	291.0	25.6
λ = 6,66 mm	0.736	315.0	30.7
P = 0.8 mW/cm2			
Control	0.420	619.0	29.7
Irradiation:			
λ = 7,89 mm	0.700	960.0	35.7
λ = 6,66 mm	0.660	827.2	26.5
P = 2.2 mW/cm2			

Table 5. Effect of EHF radiation on excretion of organic compounds by S. platensis (age 40 days)

Some published works studied the influence of millimeter waves of low intensity on non-photosynthetic organisms. Here is a summary (Table 6) from our review (Tambiev et al., 2002), which also includes our works conducted on phototrophic organisms and published before the review.

Active wavelengths (mm)	Organisms	Effects of exposure
Prokaryotes		
Bacteria		
6,0–6,7; 7,1; 5,6; 5,95–7,2	*Escherichia coli*	Stimulation of growth; increasing resistance to dehydration
6,0–6,7; 8,0; 5,95–7,2	*Staphylococcus aureus, Staphylococcus sp.*	Weakening of pigmentation, reduction of colony size
5,95–7,2	*Bacillus mucilaginosus*	Activation of biosynthetic processes
5,95–7,2; 6,2	*Bacillus firmus*	Increase of enzymatic activity and biomass accumulation
8,0	*Salmonella typhimurium*	Activation of free radical reactions
4,04	*Rhodobacter sphaeroides*	Increased yield of triplet states of reaction centers; increase in lifetime of the triplet
4,04	*Halobacterium halobium*	Acceleration of proton transport
6,96; 4,16; 7,1; 4,46	*Photobacterium leiognathi*	Bioluminescence quenching or activation
Actinomycetes		
5,95–7,2	*Streptomyces spheroides*	Increased biosynthesis of enzymes
5,6; 4,6	*Streptomyces xanthochromogenes*	Increase of colony growth rate
5,95–7,2	*Nocardia sp.*	Increased biosynthesis of enzymes
Cyanobacteria		
6,06; 6,25; 6,66; 7,1; 7,89; 8,34; 4,6; 5,6	*Spirulina platensis, Spirulina maxima*	Stimulation of growth; intensification of photosynthesis
8,34	*Anacystis nidulans, Anabaena variabilis, Plectonema boryanum, Fremyella diplosiphon*	Stimulation of growth
Eukaryotes		
Mold fungi		
5,95 – 7,2	*Aspergillus oryzae*	Increase in biomass accumulation and biosynthesis of enzymes
5,95 – 7,2	*Aspergillus awamory*	Weakening of pigmentation; decreased formation of conidia
Yeast-like fungi		
5,95 – 7,2	*Endomyces fibuliger*	Stimulation of yeast cells formation, increased activity of glucoamylase and alpha amylase

Mycophilous fungi		
5,95 – 7,2	*Dacthilyum dendraides*	Increase in protease activity
Yeast		
8,5; 7,17 – 7,21; 5,95 – 7,2	*Saccharomyces cerevisiae*	Stimulation of growth and biosynthetic activity
5,95 – 7,2; 6,035	*Saccharomyces carlsbergensis (pastorianus)*	Accelerating development; temporary discreteness
Algae		
7,1; 5,6	*Scenedesmus quadricauda*	Reducing toxicity of the medium
6,06; 7,1; 8,34	*Platymonas viridis*	Stimulation of growth, intensification of photosynthesis

Table 6. Effects of EHF radiation on different taxonomic groups of microorganisms

There is a certain similarity in effects of single exposure by millimeter waves on photosynthetic and non-photosynthetic organisms. The former were already mentioned above; the latter include bacteria, actinomycetes, fungi, yeast-like fungi, mycophilous fungi, and yeast. Both groups of objects demonstrated intensification of growth and biomass yield, more expressed, as a rule, in photosynthesizing objects. Also, there were observed (1) changes of cell membrane permeability; (2) dependence of the effect on the incident power density; (3) the resonance effect at certain wavelengths; (4) growth inhibition or no effect when removing from the resonant wavelengths; (5) changes in synthesis of biologically active compounds; (6) individual taxonomic sensitivity to resonant wavelengths; (7) absence of mutagenic effect of the irradiation; (8) prolongation (in some cases) of the stimulatory effect on subsequent passages of the cultures.

The identified differences are: (1) non-photosynthetic microorganisms, especially yeast, in some cases needed repeated exposure to obtain distinct stimulatory effect, while photosynthetic organisms were irradiated only once; (2) in non-photosynthetic microorganisms, especially yeast and fungi, morphological changes in cells were observed, which never happened in photosynthetic organisms; (3) the range of resonant wavelengths in the photosynthetic organisms was shifted toward 10 mm; (4) in some cases there was a bactericidal effect of millimeter waves on the non-photosynthetic microorganisms.

Besides the above-mentioned physiological characteristics, we have first studied the effect of nonthermal low-intensity EHF radiation on mineral composition of cyanobacterial cells and on the possibility of the accumulation of certain trace elements in them.

Trace elements are chemical elements contained in human or animal body in very small quantities, 10^{-3}–10^{-12} % by weight, and this low concentration range in living cells and tissues is virtually the only defining attribute of them. Life, as we know, has been originated in the ocean with its extreme diverse but simultaneously definite chemical composition.

The biological evolution (Skalny, 2011b) has led to a sharp increase in mass and diversity of the living substance on the planet, including formation of new chemical compounds and molecules, the novel (secondary) bioelements (in cells).

There appeared the biosphere as an open thermodynamic system in which some secondary bioelements can disappear and others can appear, while the set of primary bioelements – progenitors of life – is likely to remain mainly stable (Skalny, 2011a; Skalny, Skalnaya, 2011).

Simple bioelements produced four fundamental components of cellular life, which, according to J.D.Marth (2008), divided into 68 molecular building blocks ("building blocks of life"). I.e., the simplest bioelements formed more complicated, macromolecular bioelements (Skalny, 2011a; Skalny, Skalnaya, 2011).

Following this logic, we proposed to subdivide bioelements into simple (atoms, ions and water as the universal solvent), and complicated ones, consisting of the above-mentioned 68 molecules (8 of them are nucleosides, which compose DNA and RNA, 20 are natural amino acids necessary for protein synthesis, at least 32 glycans, 8 types of lipids (Marth, 2008)).

The simplest (H, C, O, N, P, S, vital chemical elements, evolutionarily selected by cells from the environment to supply biological functions) (a) and derivative (the 68 molecules, water, oxygen etc.) (b) bioelements we propose to call primary bioelements: respectively, simple (a) and complicated (b). Primary bioelements are, in essence, pre-biological elements or "prebiotic" (Ferris 1999). Other bioelements are likely secondary, because for their formation the primary bioelements were "selected" by cells from the extracellular environment in the process of evolution for performing specific regulatory functions. It is very important for understanding the biological role of chemical elements, which is determined not so much by a chemical element as such, as by its chemical species in the body. I.e., the talk about a specific role of a chemical element in living organism has no biological meaning. The biological meaning is in its chemical variety (Skalny, 2009, 2011a; Skalny, Skalnaya, 2011).

Among the most important properties of the primary single-celled organisms were chemoreception and selective permeability, which helped them adapting to changes in the environmental concentration of various elements, and thus not to die but to adjust to new conditions.

Primary bioelements once produced the first protocell – LUCA (Hoenigsberg, 2003) or probiont (Galimov, 2004) – a hypothetical primary organism which originated all the modern diversity of life on Earth. This organism contained, inter alia, the macromolecules (pro-proteins and pro-DNA) and acquired the ability to reproduce itself (Galimov, 2004).

Primary bioelements existed long before the emergence of life. They had the highest resistance to external conditions due to its simplicity (Skalny, 2009, 2011a; Skalny, Skalnaya, 2011). Bioelements are components and legacy of the "primordial soup", from separate ions to H2O, CO2, O2, glucose and other sugars, amino acids and "proto-RNA". Electric discharges, electromagnetic fields, ultraviolet and visible light, gases – these are the conditions (environment) in which bioelements can unite and "turn in the living" (Skalny, 2009, 2011a; Skalny, Skalnaya, 2011).

Some of the bioelements became components of active centres in enzymes, dramatically accelerating the evolution of life due to formation of metabolic pathways. In the course of evolution, the developed elemental composition common to cells of different species of living organisms has become their most important feature.

Thus, bioelements can be subdivided into primary, i.e. those which could exist before the origin of life, and secondary, i.e. those which have formed as production of living organisms. This division is necessary for us to better understand the nature and role of bioelements. For example, the fact that life is a self-sustaining process that can produce "raw material" for new living structures. This agrees with the theory of natural self-organization of pre-biological processes by M.Eigen (1971) and ideas of I.Prigogine (1980) about self-organization in open systems (Skalny, 2009, 2011a; Skalny, Skalnaya, 2011).

Based on the ideas of V.P.Kaznacheyev (Kaznacheev, Spirin, 1991), we can assume bioelements the internal condition (medium) for the existence of biological systems, while electromagnetic components – the external condition (environment). Biosphere is an assembly of bioelements and living organisms existing under permanent regulatory influence of physico-chemical factors of terrestrial and cosmic origin.

Trace elements are important part of bioelements. They are not incidental ingredients of tissues, cells and fluids of living things, but components of a naturally developed very ancient and complex physiological system involved in regulating vital functions of the organisms at all stages of their life (Skalny, Rudakov, 2004; Skalny, 2011a). The definition of trace elements is based on three fundamental principles (Avtsyn et al., 1991): selective absorption of them; their selective concentration in certain organisms, organs, tissues or organelles of some cells; selective elimination. Interaction of these mechanisms is just what maintains the trace element homeostasis. In their biological effects and physico-chemical properties, trace elements can be quite dissimilar to one another and the same way different from macro elements, but their main feature remains the extremely small concentration in cells.

The biological role of chemical elements has come under intensive studying in the second half of the twentieth century. There was opened the essentiality of about 20 chemical elements to living organisms, deepened the knowledge of toxic and carcinogenic properties of a number of trace elements, created tens of thousands of drugs and dietary supplements containing trace elements, and food products fortified with them. But “the lack of multidisciplinary approach has been the Achilles heel of biological trace element research” (Iyengar, 1989).

According to the biological role for mammalians and humans, chemical elements are divided into vital (or essential: calcium, phosphorus, potassium, chlorine, sodium, zinc, manganese, molybdenum, iodine, selenium, sulfur, magnesium, iron, copper, cobalt), probably essential (fluorine, silicon, titanium, vanadium, chromium, nickel, arsenic, bromine, strontium, cadmium) and elements with little-known or unknown role (lithium, boron, aluminum, germanium, zirconium, tin, cesium, mercury, bismuth, thorium , beryllium, scandium, gallium, rubidium, silver, antimony, barium, lead, radium, uranium) (Skalny, Rudakov, 2004; Skalny, 2011a). New facts, accumulating from year to year, provide prerequisites for moving certain trace elements from the third group into the second, and from the second into the first. Although this process is not fast, it happens permanently. Among 92 naturally occurring chemical elements 81 are found in human body.

According to the current definition, bioelementology is a science studying formation of bioelements, their metabolism, physiological and pathochemical mechanisms of their participation in the regulation of vital functions at either cellular or population and biosphere levels of organization of the living matter (Skalny, Rudakov, 2004).

“Bioelement” is the main term of bioelementology. In literature we found term “bioelement”. Different authors are using it mainly as a synonym to “chemical element” which “plays some biological role” or “exists in the living body”. Why we named elements “chemical” but not “physical”? Only following tradition, because chemistry as science formed much earlier than nuclear physics and physics of elementary particles. The elements have chemical and even more physical properties, but it is nonsense to separate special “biological elements”. Active use of the term "bioelement" by A.V.Skalny and his followers in recent years in scientific articles (Skalny, Rudakov, 2005; Skalny, 2009), books (Skalny, Rudakov, 2004; Oberleas et al., 2008; Skalny et al., 2009), in textbooks (Borisova et al., 2008; Toxicological Chemistry, 2010) and in reports at international scientific meetings showed that part of scientists, especially chemists, feel a certain rejection of it because of association with the term "chemical element." Nevertheless, it is difficult to find another term which would be more apt and apparently satisfying most scientists. In fact, “element” is a multivocal word. We comfortably use expressions “electrical element”, “heating element”, or “data element”. So, “bioelement” can be a similar case.

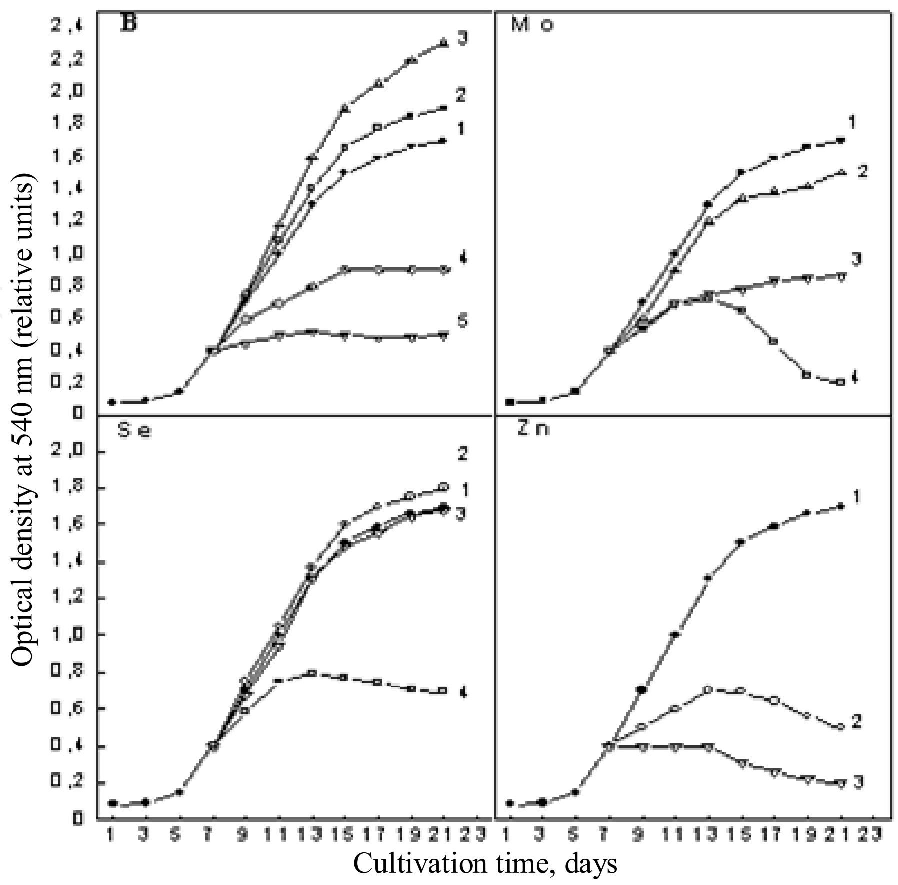
Atoms, atomic nuclei, elementary particles and fields that bind them, which have independent significance at the physicochemical stage of evolution, after being included in biological molecules lose this self-importance and play their role in the ensemble, called bioelement, where everything is interdependent, more complicated and at the same time more vulnerable to external influence. Since the general conditions of biological evolution (the composition of biosphere), are continuously changing, a set of bioelements in a living organism can also change. This distinguishes them from chemical elements as objects of physicochemical stage, which remain identical to themselves along the course of evolution. So, bioelement is the elemental functioning unit of living matter, which is a biologically active complex of chemical elements as atoms, ions and nanoparticles with organic compounds of exogenous (primary) or biogenous (secondary) origin under the influence of electromagnetic fields (Skalny, Skalnaya, 2011).

Bioelements can continuously form from ionic compounds when they enter the cell. Inside the cell, biopolymers and their complexes create a complicated, coordinated and regulated system for transformation of substances. Cell is the main place of natural birth of secondary bioelements and their destruction (Skalny, Skalnaya, 2011).

We were interested in the possibility of production (biosynthesis) of secondary bioelements through increased accumulation of various trace elements, primarily essential, in cells of cyanobacteria, and in the influence of physical and chemical factors on this process. The physical factor was the exposure to millimeter waves of low nonthermal intensity; the chemical factor was the fortification of cultural medium with gradually increasing concentrations of selected trace elements.

First we have studied the ability of cyanobacteria S. platensis, S. maxima and Nostoc commune to accumulate elevated concentrations of As, Mo, Se, Zn. We added salts of these elements into the cultural medium during the logarithmic phase on the 7th day of cultures’ growth. It is known that for most mineral components there is more or less wide plateau of concentrations not affecting growth of microorganisms. At the same time, a number of elements, e.g. Se, Cu, Zn, are characterized by very narrow boundary between the concentrations which are necessary for growth and which are toxic. For different groups of microorganisms the inhibitory effect of high concentrations of elements is also different.

The results of the experiments studying toxicity of four trace elements on S. platensis are shown in Fig.2.



Concentration of the salts in the medium (mmol/l): 1 = control (Zarrouk medium);
B – 2 = 50, 3 = 100, 4 = 200, 5 = 250; Mo – 2 = 40, 3 = 60, 4 = 80;
Se – 2 = 0.06, 3 = 0.03, 4 = 0.6; Zn – 2 = 0.03, 3 = 0.06

Fig. 2. Effect of B, Mo, Se, Zn, introduced into the cultural medium in high concentrations, on the growth of S. platensis

Figure 2 shows a significant difference between the elements, which by the degree of toxicity for the cultures of S. platensis and N. comunne can be arranged in series: Zn> Se> Mo> B. Resistance of cyanobacteria to elevated content of boron and molybdenum in the medium is apparently due to the ability of these elements to complexation. As for selenium, its introduction into the medium of S. platensis in concentrations above 0.6 mmol/l caused a color change to tile-red, which was due to adaptation of the cyanobacteria to high concentrations of selenium in the medium. As it is known from the literature, the excessive amounts of selenium accumulated by the cells are transferred from organic to inorganic form. And as for zinc, even its introduction into the medium in concentration 0.03 mmol/l significantly inhibited growth of the culture, shortened the logarithmic growth phase, and made the maximum density of the cells' suspension in stationary phase of growth not to exceed half of the control value. It is known that zinc ions, showing toxicity when introduced in concentration 0.06 mmol/l in our experiments, affect the entire complex of light and dark reactions of photosynthesis in inhibiting manner.

We proposed the concept of optimum concentration of a trace element, introduced into the cultural medium: this is the concentration that causes maximum possible accumulation of the trace element in the cells without leading to pronounced decrease in the yield of biomass. When determining the optimal concentrations, we used the criterion equal to the product of intracellular trace element content by the yield of biomass at the given concentration of the trace element in the medium. The maximum value of the criterion corresponds to the optimal concentration of the element in the medium. The optimal concentrations are specific to certain trace elements and to the objects under study. In some cases, apparently, it is more sensible to say about a range of optimal concentrations (Tambiev et al., 2003).

S. platensis		*N. commune*	
Optimal concentrations (mmol/l)	Content in cells (μg/g)	Optimal concentrations (mmol/l)	Content in cells and sheath (μg/g)
B			
150.0	907.2 ± 56.8	1.5	14448.9 ± 423.4
Mo			
20.0	5912.0 ± 295.5	0.25	11206.1 ± 560.7
Se			
0.3	450.0 ± 30.5	0.25	6288.6 ± 310.1
Zn			
0.03	455.5 ± 28.1	0.03	1500.9 ± 75.1

Table 7. The inclusion of B, Mo, Se, Zn in cells of S. platensis, N. commune after their introduction into the medium in optimal concentrations

Table 7 shows that optimal concentrations are higher for less toxic B, Mo, and considerably lower for Se, Zn, which are more toxic for the cyanobacteria. The objects differ in optimal concentrations and intracellular accumulation of the trace elements due to their individual sensitivity and to the fact that, unlike S. platensis, N. commune has a thick mucous sheath, which can also accumulate certain trace elements in considerable amounts.

The first time we were able to establish the effect of individual trace elements, introduced into the cultural medium in high concentrations, on the total mineral composition of cyanobacterial cells. The data are presented in Table 8.

Elements, added to the medium	Species	The observed changes	
		Macro elements	Trace elements
B	*S. platensis*	-	Zn
	N. commune	-	Ca
Mo	*S. platensis*	-	Fe, Mn, Zn
	N. commune	-	Fe, Mn, Zn
Se	*S. platensis*	Ca, Mg	Fe, Zn
Zn	*S. platensis*	Na, K*, Mg, Ca	Fe, Mn, Cu
	N. commune	Na, Mg, Ca	Mn, B

* Note: For intracellular K it was a decrease, for all other elements – an increase.

Table 8. Change in mineral composition of cyanobacteria after adding optimal concentrations of the trace elements into the cultural medium

Table 8 shows that the most significant changes in mineral composition of the cyanobacterial cells happened after introduction of zinc, which is most toxic for cyanobacteria among the investigated trace elements. The least changes were observed after introduction of boron, which is least toxic. Molybdenum and selenium were placed at intermediate positions. These data seem to us very important. We can make the assumption that toxicity of certain trace elements in comparison with others is largely determined by larger or smaller shifts in the overall mineral composition of cells.

We have managed to obtain the greatest possible accumulation of some essential trace elements by cell cultures of S. platensis and S. maxima. This could considerably increase the value and applicability of spirulina biomass in human health care nutrition. The work has begun from registering a patent (Tambiev et al., 1997) for a method of obtaining selenium-containing preparation of spirulina biomass enriched with selenium in organic form with pronounced antioxidant properties. The content of selenium in the cells was determined by atomic-adsorption and fluorometric methods, the total elemental composition of the cell – by developed complex of ICP-AES methods (Sedykh et al., 2005; Skalny et al., 2009).

The possibility to obtain spirulina biomass enriched with selenium in organic form, which has increased bioavailability, has promptly caused the appearance of works extending this direction. In some works a spirulina preparation enriched with bioavailable selenium in combination with reduced glutathione was administered to rats, allowing normalization of intestinal permeability, impaired after systemic anaphylaxis. In other studies it was shown that effective regulation of homeostasis was possible by addition of selenium-rich spirulina to rats' feed. Also there were reports about successful use of selenium-enriched spirulina and yeast as a dietary supplement for patients with non-specific ulcerative colitis and for patients with coronary heart disease (Mazo et al., 2001; Notova et al., 2006).

The optimal concentration of selenium in the cultural medium for S. platensis was taken as 0.3 mmol/l (Table 7), since with it there was no sharp decline in biomass growth, and the cells actively captured selenium. It was revealed that the best way to introduce selenium is to add it into the medium at the logarithmic growth phase of cyanobacteria (5-10 days). There was found clear correlation between selenium content in the cells and in the cultural medium.

There were also shown notable changes in concentrations of certain elements in the cells of S. platensis after the introduction of selenium into the medium (Table 8). When comparing cultures of S. platensis and S. maxima, it was shown that in the presence of sodium selenite in the medium S. maxima accumulates 1.7 as much selenium as S. platensis.

We have separated the biomass of S. platensis, enriched with selenium, molybdenum and zinc, into the following fractions: (1) the sediment, which includes cell wall fragments obtained after ultrasonic cell disruption, amphiphilic proteins and polysaccharides; (2) the chloroform fraction, which includes hydrophobic proteins and lipids; and (3) the water-methanol fraction containing low molecular compounds, hydrophilic cytoplasmic proteins and monosaccharides. The balance of cellular fractions was accurate enough: in total for selenium 95.5%, for molybdenum 91.2% and for zinc 97.4%.

A significant portion of molybdenum, accumulated by cells of S. platensis and N. commune, was found in the chloroform fraction and less than 30% of it contained in the sediment. Over

70% of selenium accumulated by both cultures was found in the sediment and less than 20% – in the chloroform fraction. Similar to selenium, much of the accumulated zinc was also found in the sediment, and about 30% was in the chloroform fraction.

Subsequently we determined optimal concentrations of copper when adding copper sulfate, nitrate and acetate to the medium of S. platensis and S. maxima. Copper sulfate was appeared to be the most toxic: it strongly inhibited growth of S. platensis at the concentration 1.25 mg/l. Copper from nitrate began to markedly incorporates into cells (80 μg/g dry biomass) at the concentration 0.125 mg/l. Acetate at the concentration 0.5 mg/l gave the greatest incorporation of copper into the cells (323 μg/g dry biomass vs. 11.35 μg/g in the control).

Toxicity of copper nitrate and copper acetate did not differ significantly from each other. Nevertheless, in case of copper nitrate (0.5 mg/l), we observed a lengthening of trichomes in S.platensis by 50%, while in case of copper acetate (0.5 mg/l) – by 100% as compared to control.

We have examined the influence of EHF radiation on the accumulation of certain trace elements by cyanobacterial cells and on overall mineral composition of the cells. We introduced zinc sulfate in concentration 0.06 mmol/l into cultural medium of S. platensis at the logarithmic growth phase. Without irradiation its accumulation in the cells was somewhat more than 1000 μg/g dry biomass, while at 0.09 and 0.12 mmol/l growth of the culture was virtually stopped due to high toxicity of zinc (Table 9, upper part).

Experimental conditions	Zn content in the cells and suspension density	Zn concentration in the cultural medium (mol/l)			
		0.03	0.06	0.09	0.12
Zn adding	Zn, μg/g	455±22.7	1015±50.5	-	-
	D_{540}	*0.5±0.01*	*0.20±0.01*	-	-
EHF irradiation + Zn adding	Zn, μg/g	450.2±32.0	1102.2±50.9	5417.0±570.5	11269.8±810.2
	D_{540}	*1.25±0.02*	*0.88±0.02*	*0.63±0.01*	*0.54±0.02*

Note: age of culture = 21 days; Zn content in the control (Zarrouk medium) = 63.8 ± 10.7 μg/g; D = 1.7 ± 0.1; yield of biomass = 1.01 ± 0.04 g/l dry weight.

Table 9. The inclusion of Zn in S. platensis cells (μg/g) after adding different concentrations of ZnSO4 into the cultural medium and after previous exposure to EHF irradiation

Single EHF irradiation (wavelength 7.1 mm, incident power density 1.5 mW/cm2) of S. platensis before introduction of zinc sulfate into the medium significantly reduced its toxicity. At zinc concentrations in the medium 0.09 and 0.12 mmol/l, which previously stopped the growth, now the biomass on the 21st day reached 50% and 40%, respectively, with significant accumulation of zinc in the cells (Table 9, lower part).

Experiments have shown that EHF radiation also affected mineral composition of S. platensis and N. commune cells.

Figure 3 demonstrates that in the cells of S. platensis the amount of sodium, potassium and magnesium increased. For a number of other studied elements there was observed only a tendency to increase that may be due to low incident power density in the experiment. We have previously stated that EHF radiation causes a stimulating effect on growth and yield of biomass of cyanobacteria. As it can be seen, after irradiation of N. commune there was observed an increase in the amount of not only intracellular sodium and potassium, but also boron, which can bind to the polysaccharide sheath of the cells.

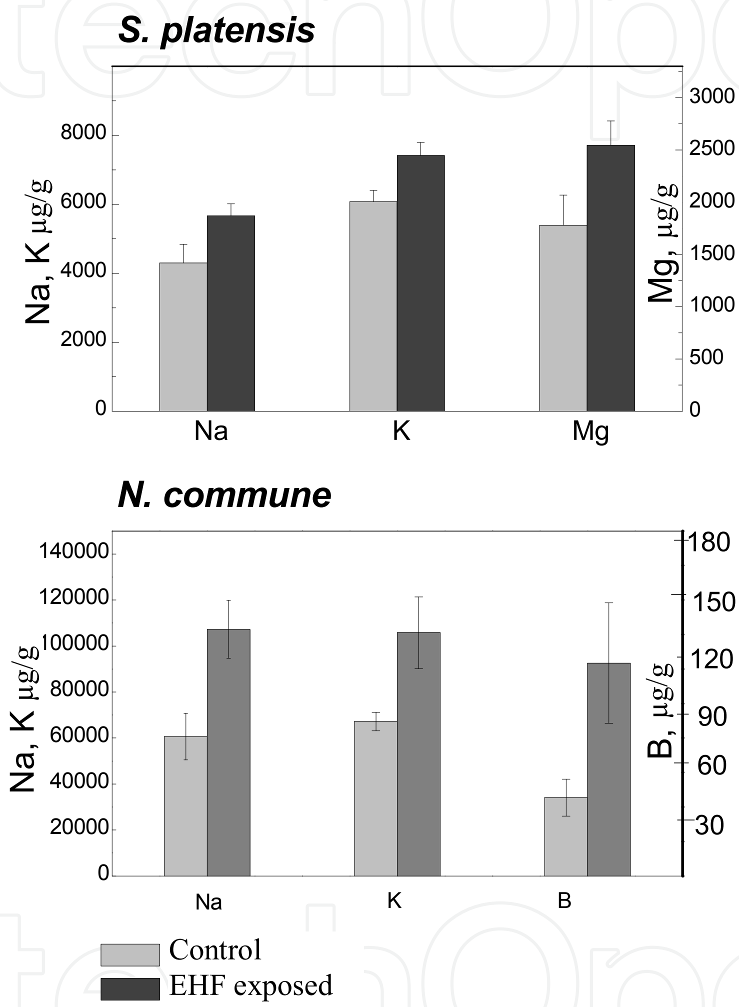


Fig. 3. Effect of EHF radiation on mineral composition of S. platensis, N. commune cells

The cyanobacterium N. commune is a food species, widely spread from the Far North, e.g. Taimyr wetlands and Yakutia, up to the South – Mongolia, Java, India etc. Nutritional properties of this genus are known from the VI century AD. Currently species of this genus, including N. commune, are used for food besides the mentioned places also in China, Japan and the Philippines. China in the 1990s planned to organize mass cultivation of this species.

We have studied chemical composition of N. commune biomass, including the total content of components, amino acids, vitamins, fatty acids, inter alia essential ones, and the content of 13 macro and trace elements.

Parameter	Content	Determination method
The overall composition (% of dry biomass)		
Humidity	93.9	Gravimetric method
Dry matter	6.1	Gravimetric method
Free amino acids	0.167	Amino acid analyzer
Protein	23.5	Lowry assay
Carbohydrates	36.5	Phenol-sulfuric method
Lipids	2.2	Folch method
Vitamins (μg/g)		
β-Carotene	350.5	Gas-liquid chromatography (HPLC Jasco, Japan)
Thiamin (vitamin B1)	2.8	Fluorescence spectrofluorimetry (Perkin Elmer MPF-43A, UK)
Riboflavin (vitamin B2)	5.3	Fluorescence spectrofluorimetry (Perkin Elmer MPF-43A, UK)
Nicotinamide (vitamin B3)	38.4	Spectrophotometry
Pantothenic acid (vitamin B5)	5.27	Method with β-naphthoquinone-4-sulfonate (Folin reagent)
Pyridoxine (vitamin B6)	1.5	Gas-liquid chromatography (HPLC Jasco, Japan)
Inositol (vitamin B8)	2772.7	Method with rhodizonic acid
Folic acid (vitamin B9)	0.0066	Spectrophotometry
Cobalamin (vitamin B12)	15.4	Spectrophotometry
Biotin (Vitamin H)	2.69	Microbiological method with lactic-acid bacteria
Tocopherol (vitamin E)	3.0	Fluorescence spectrophotometry
Naphthoquinones (vitamin K)	25.5	Thin layer chromatography
Macro and trace elements (μg/g)		
Calcium	1687.0	Atomic-emission spectrometry with inductively coupled plasma (ICP-AES)
Phosphorus	3719.0	
Magnesium	2727.0	
Iron	704.7	
Zinc	43.38	
Copper	3.379	
Manganese	55.11	
Chrome	3.303	
Sodium	12890.0	
Potassium	11120.0	

Pigments (μg/mg)		
Phycocyanin	9.8	Spectrophotometry
Chlorophyll	18.4	
Carotenoids	8.28	
Fatty acids (μg/mg)		
Linoleic *	2.29	Gas chromatography with mass spectrometric detector
γ-Linolenic *	0.32	
Oleic	1.03	
Palmitic	0.86	
Amino acids		
Alanine, arginine, aspartic acid, glutamic acid, cystine, glycine, histidine, isoleucine, leucine*, lysine*, methionine*, phenylalanine*, praline*, serine, threonine, tryptophan*, tyrosine*, valine*		Amino acid analyzer

* - Essential fatty acids and essential amino acids

Table 10. Chemical and mineral composition of N. commune biomass

Also, we studied accumulation of important essential trace elements, namely boron, molybdenum, selenium, zinc, by N. commune cells, and determined the optimal concentrations for them (Table 7). It was also shown that introduction of these trace elements into the cultural medium in optimal and higher concentrations causes shifts in mineral composition of the N. commune cells (Table 8).

Currently there is an interest in studying the biological properties of vanadium compounds, which potentially can be used in treatment of diabetes and cancer, as confirmed by clinical studies. It was noted that the effects of insulin on carbohydrate and lipid exchange can be simulated by vanadium compounds. Therefore vanadium compounds having high hypoglycemic activity and low toxicity are actively investigated.

We studied effects of VOSO4 (vanadyl sulfate) and NaVO3 (sodium vanadate), possessing high biological activity and insulin-like action. The compounds were introduced into cultural medium of S. platensis and S. maxima in elevated concentrations, on growth and yield of biomass. We determined dynamics of vanadium accumulation by both cultures and the optimal concentration for obtaining valuable biomass enriched with vanadium in organic form.

We have shown that S. platensis and S. maxima accumulates vanadium in form of either vanadyl cation (+4) or vanadate anion (+5) more effectively when the cultural medium is enriched with vanadate. Thus, when vanadium concentration in the medium was 2.0 g/l (vanadate), its accumulation in S. platensis cells was 3050 ± 280 μg/g, while if the medium was enriched to the same concentration by vanadyl, than the accumulation in the cells was 1550 ± 165 μg/g. An increase of vanadium concentration in the medium over 2.0 g/l led to a sharp slowdown of the vanadium accumulation by cells. These data are shown in Fig. 4, 5.

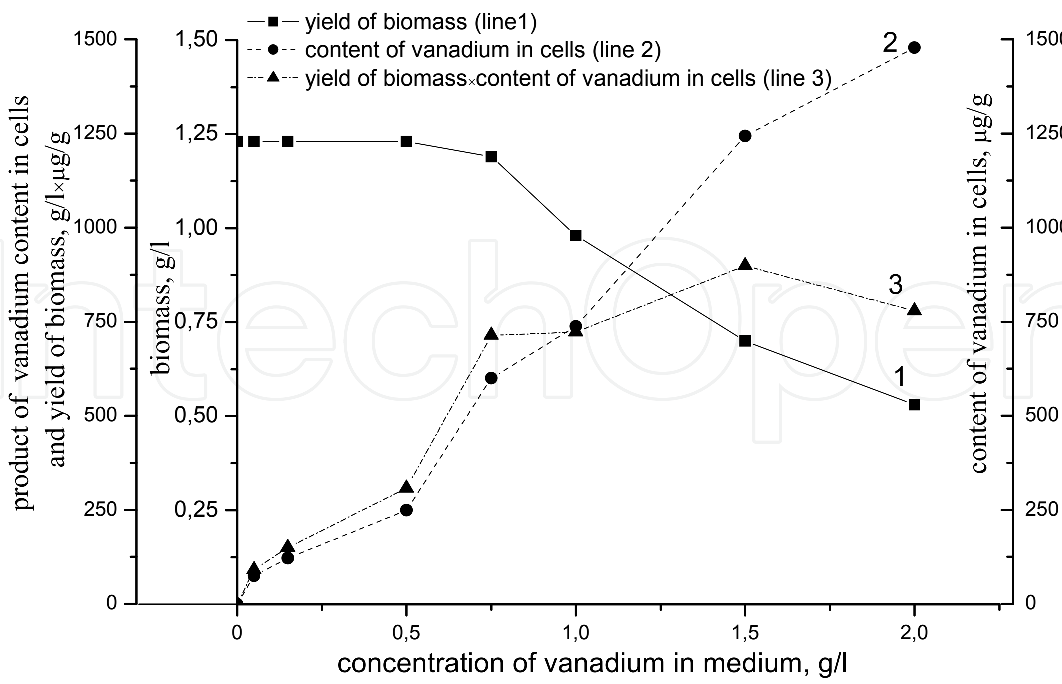


Fig. 4. Content of vanadium in S. platensis cells and yield of biomass depending on concentration of vanadium in the form of vanadyl cation in cultural medium and the value of optimal concentration

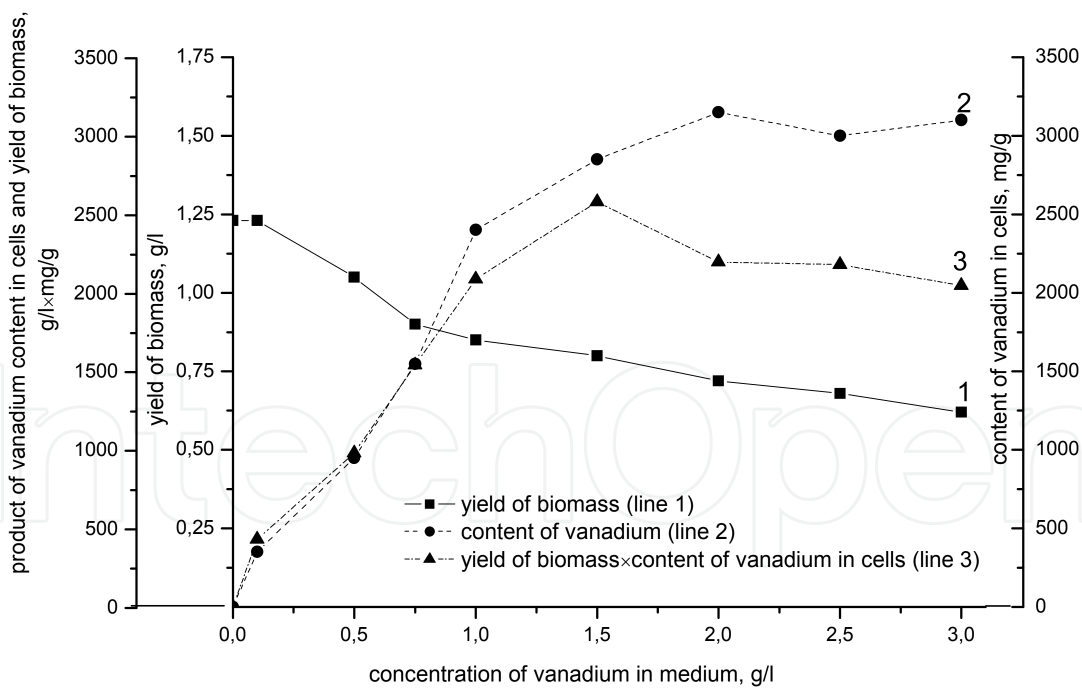


Fig. 5. Content of vanadium in S. platensis cells and yield of biomass depending on concentration of vanadium in the form of vanadate anion in cultural medium and the value of optimal concentration

The optimal concentration of vanadium introduced into the medium in the form of vanadyl was taken as 1.5 g/l, and intracellular accumulation was 1550 ± 75 µg/g (S.platensis) and

1245 ±105 μg/g (S.maxima). The optimal concentrations of vanadium introduced into the medium in the form of vanadate were somewhat different, being for S. platensis 1.5 g/l with intracellular accumulation 3180 ± 185 μg/g, and for S. maxima 1.0 g/l with intracellular accumulation 2650 ± 206 μg/g. It was shown that S. maxima is more resistant to high concentrations of vanadium and stores it in the cells in higher amounts compared with S. platensis (Vasilieva et al., 2011).

The introduction of these forms of vanadium into the growth medium of both cultures caused similar changes in mineral composition of the cells. This apparently indicates that both forms affect the same aspects of metabolism of the cyanobacteria. After adding 2 g/l vanadyl sulfate into the medium, there was observed a decrease in calcium concentration, an increase in concentrations of iron, magnesium, manganese, and a significant increase in boron, chromium and zinc.

At present, there is a significant amount of data suggesting that lithium may be an essential element for humans. Low level of lithium in drinking water leads to an increase in mental illness and suicide, increased crime and drug addiction. Lithium salts are used in medicine for prevention and treatment of mental illness; they promote cell regeneration after injury resulted from the disease. Today, lithium is used, in addition, in dermatology and in treatment of cancer. Some data indicate that organic compounds of lithium are more effective and less toxic than inorganic ones.

We have studied the ability of S. platensis and S. maxima to accumulate lithium in organic form, the dynamics of this process, and the effect of elevated lithium concentrations on mineral composition of cells. The element was introduced as LiCl into the cultural medium of both cultures in the middle of the exponential growth phase.

Relatively small concentration of lithium in the medium caused an increase in biomass of both cultures by 15-17% as compared with the control. A further increase in concentration cancelled the stimulating effect; the first signs of growth inhibition in S. maxima were observed at lithium concentration in the medium 0.75 g/l, in S. platensis – at 0.5 g/l.

Figures 6 and 7 shows that further increase of lithium concentration in the medium to 2.0 g/l resulted in biomass reduction by 41-44% for S. platensis and by 33-36% for S. maxima. The data demonstrates a somewhat greater stability of S. maxima culture to high concentrations of this element. However under the same cultivation conditions cells of both cultures accumulated similar amounts of lithium. The optimal concentration of lithium in cultural medium we took as 1.5 g/l. At this concentration there were no visible morphological changes in cells, while intracellular lithium content in S. platensis and S. maxima became 853 ± 103 and 805 ± 78 μg/g, respectively.

As it can be also seen from the Figures 6-7, the upper limit of the optimal concentrations of lithium was 2.0 g/l. At this concentration there was observed somewhat reduction in biomass, but there was high lithium content in the cells: 1470 ± 189 (S. platensis) and 1508 ± 137 (S. maxima) μg/g. However, by the 17th-18th day of growth in the cultural medium dense clumps of interwoven trichomes began to form, cell morphology altered, trichomes varied in size, multiple fractures and inflations appeared on them, trichomes sticked together in structureless mass, spherical or filamentous forms.

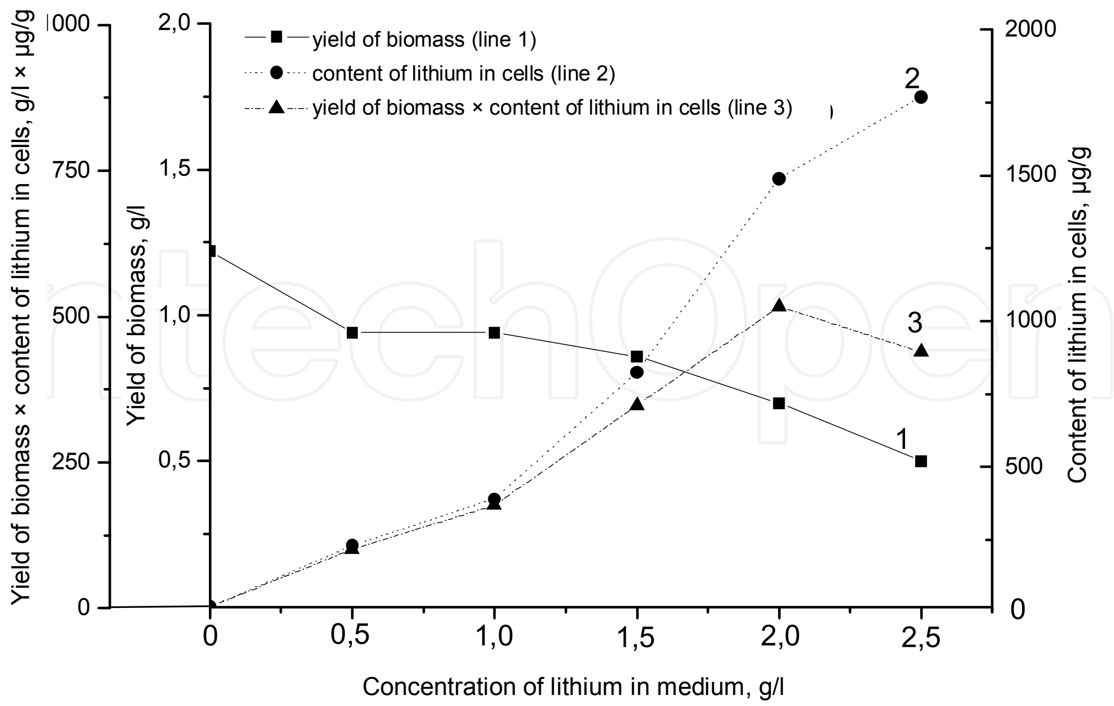


Fig. 6. Content of lithium in S. platensis cells and yield of biomass depending on lithium concentration in cultural medium and the value of optimal concentration

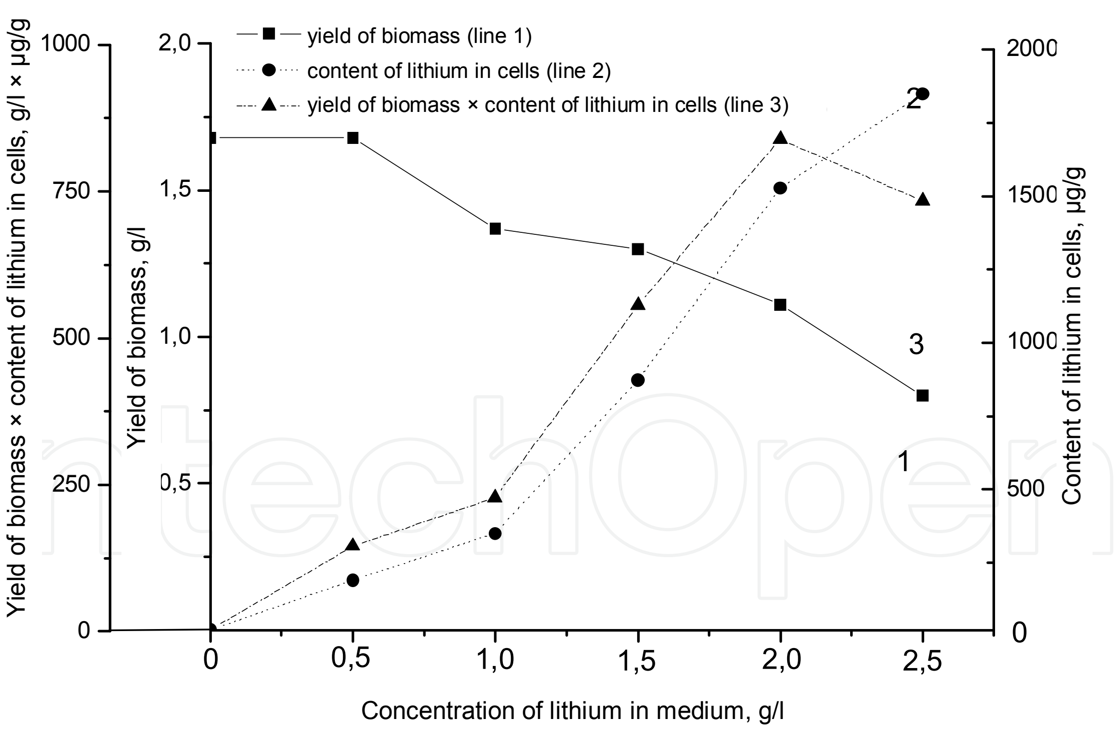


Fig. 7. Content of lithium in S. maxima cells and yield of biomass depending on lithium concentration in cultural medium and the value of optimal concentration

We have studied the effect of elevated lithium concentrations in the medium on mineral composition of cells in both cultures, as it was shown previously for other trace elements. Thus, in S. platensis the intracellular content of potassium had decreased by 30%

(comparing to control) when lithium concentration in the medium was 1.0 g/l and by 60% when it was 2.0 g/l. In cells of S. maxima at the same concentrations of lithium in the medium the potassium in cells decreased by 20 and 40%, respectively. In cells of S. platensis, starting with the lithium concentration in the medium 0.5 g/l, the sodium content, on the contrary, had increased by 50%. In cells of S. maxima this increase in sodium had not yet been observed, but at the lithium concentration in the medium equal to 2.0 g/l the sodium content in cells had grown 3 times in S.platensis, and 2.5 times in S.maxima as compared with the control.

At the same concentration of lithium in the medium (2.0 g/l), in the cells of both cultures manganese content grew 80% and iron content – 50%. The content of magnesium, calcium, zinc, copper, chromium did not change with the introduction of elevated lithium concentrations in the medium of both cultures.

This material demonstrates that electromagnetic radiation of millimeter range (EHF) at low non-thermal intensity, which can be characterized as extremely weak and possibly even superweak physical influence, can affect various aspects of metabolism of phototrophic cyanobacteria and microalgae, stimulate growth and yield of biomass of the cultures etc. It can also influence the accumulation of some essential trace elements in cells of cyanobacteria. It is shown that EHF radiation can reduce the toxicity of trace elements introduced into the medium. And, for the first time, it is established that the entering of elevated concentrations of certain trace elements into cultural medium causes shifts in general mineral profile of the cyanobacterial cells.

The development of bioelementology may lead to appearance of modified cells or technologies for creation of new cells which can be used for medical purposes. Without going into details, we only note that this tale may sooner become a reality with the correct formulation of tasks, based on the correct understanding of the hierarchy of “pre-living” processes and of the life itself, on the formation of new methodological approaches on its basis, on the proper division of essential substances in necessary and sufficient, primary and secondary, with a better understanding of the boundary between "pre-living" and "living", between the set of bioelements and life.

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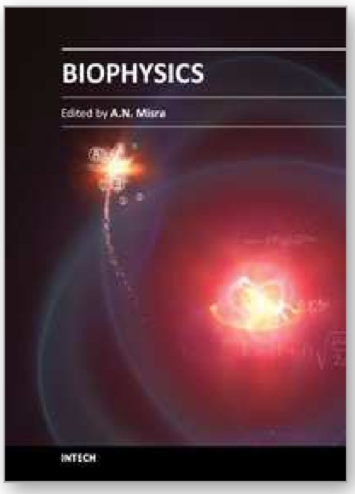

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Biophysics is a vast cross-disciplinary subject encompassing the fields of biology, physics and computational biology etc in microbes, plants, animals and human being. Wide array of subjects from molecular, physiological and structural are covered in this book. Most of these chapters are oriented toward new techniques or the application of techniques in the novel fields. The contributions from scientists and experts from different continents and countries focuss on major aspects of biophysics. The book covers a wide range of topics reflecting the complexity of the biological systems. Although the field of biophysics is ever emerging and innovative, the recent topics covered in this book are contemporary and application-oriented in the field of biology, agriculture, and medicine. This book contains mainly reviews of photobiology, molecular motors, medical biophysics such as micotools and hoemodynamic theory.