The effect of extremely-weak microwave radiation in the millimeter range on the bioluminescence of *Photobacterium phosphoreum* IMV B-7071

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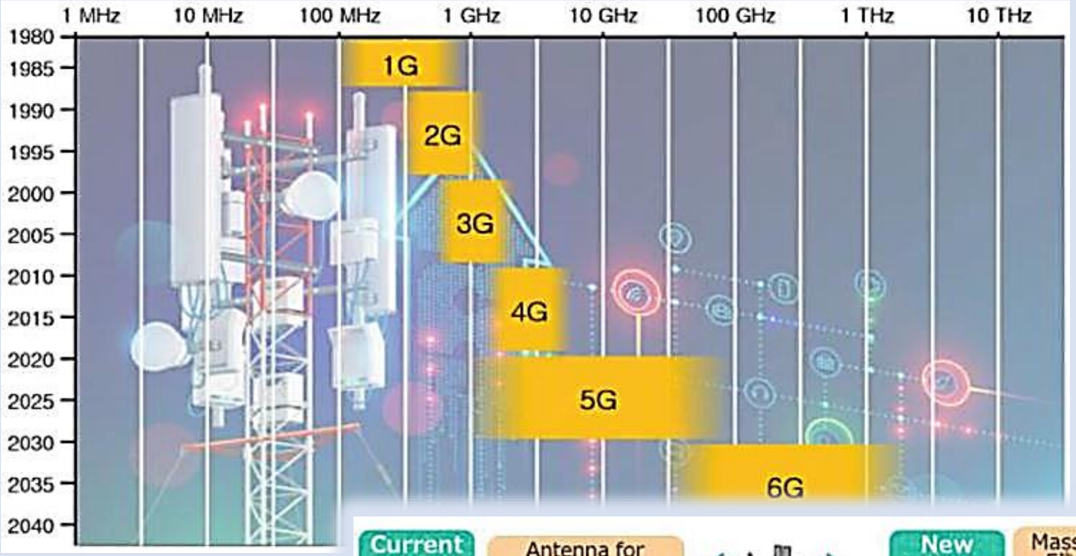
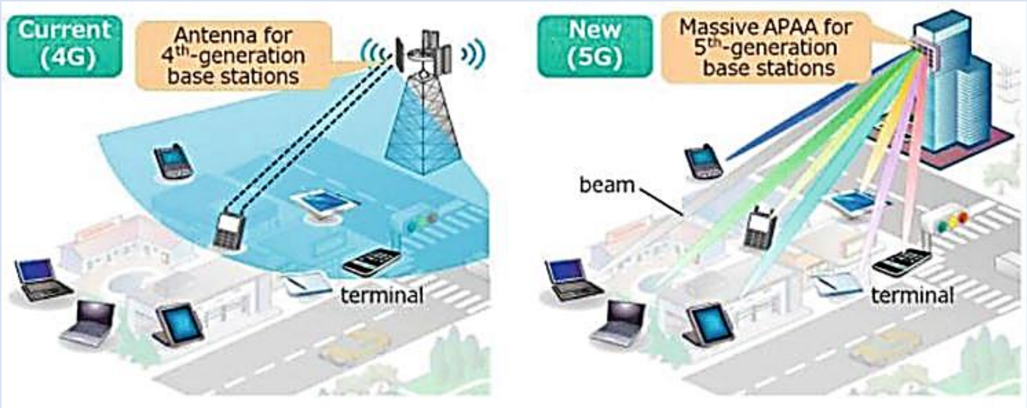
The development of telecommunication systems and the problem of electromagnetic pollution in the microwave range





Syeda Iffat Naqvi et el
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Presently, there has been a rapid development of modern information technologies and telecommunication systems, particularly wireless communication systems. The number of devices that use electromagnetic waves in the microwave spectrum in the extremely-high frequency range, from 1 to 30 GHz and above, is growing significantly. This radio frequency spectrum is used by mobile phones, Wi-Fi, radio relay systems, satellite communications, and other wireless technologies.

In the coming years, fifth-generation (5G) communication systems will become the main wireless communications standard in the European Community, the United States, China, and other countries. It is anticipated that in the near future, the entire territory of economically developed countries will be covered by a network of transmitting antennas operating in the frequency range of 5-100 GHz.

The development of telecommunication systems and the problem of electromagnetic pollution in the microwave range

But the next sixth-generation (6G) technologies are already on the horizon, operating in the frequency range of 50 to 1000 GHz. Various countries are developing the latest 6G systems that use the millimeter and submillimeter wave radio frequency bands.

SUMMARY OF 6G ANTENNA PREVIOUS WORK

Ref. No	Antenna	Frequency	Material	Fabrication	Results		
					Bandwidth	Gain / Directivity	Others
[7]	CP horn antenna	300 GHz	brass block	wire-cutting EDM	60 GHz	18.3 dBic	AR bandwidth : 7 GHz
[8]	WHEMS	60 GHz – 75 GHz	Rogers 3003	surface metal etching and plated vias	11 GHz	8 dBi – 10 dBi	Radiation efficiency : 95% 3-dB beamwidth of E-plane and H-plane :50° and 45°
[9]	RCA	300 GHz Then downscaled to 30 GHz	metal	3D printing	2 GHz (20 GHz if not downscaled)	13 dBi – 16 dBi	Radiation efficiency : 88% 3-dB beamwidth : 10 %
[10]	Transmit array	120 GHz	all dielectric	laser-drilling	9 GHz	32 – 34 dB	-

The Review and Analysis of Antenna for Sixth Generation (6G) Applications. 2020. DOI:10.1109/RFM50841.2020.9344731

The development of telecommunication systems and the problem of electromagnetic pollution in the microwave range

As the density of such devices increases, problems of electromagnetic pollution emerge, which can have a significant impact on the environment and human health. Even if the technological challenges of deploying such telecommunication networks are successfully addressed, the question of the environmental and biomedical consequences of a significant increase in the electromagnetic background in the millimeter range remains open. A particular concern is the significant, three or more orders of magnitude, excess of the natural electromagnetic background in the centimeter, millimeter, and submillimeter ranges.

There is an opinion that living organisms are not adapted to this range, and its influence can be extremely negative. In this regard, the public in countries where 5G and 6G communication systems are already being tested and deployed are asking questions about the possible negative impact of millimeter wave radiation on human health and the environment. Unfortunately, there are very few clear and scientifically based answers, which gives rise to unscientific speculations, groundless fears, and sometimes outright rejection of new technologies among the population of many countries. Even in economically developed countries with a high level of education, the lack of reliable information, misunderstanding of the problem, and negative emotional perception of the situation have led to individual acts of vandalism, during which people destroy antennas and base stations. Thus, this problem has already become a national security issue for countries implementing such new technologies.

The development of telecommunication systems and the problem of electromagnetic pollution in the microwave range

There is a substantial array of data on the biological activity of electromagnetic fields across various frequency ranges, including millimeter waves. Attention is mainly focused on the activation of free radical oxidation processes under the influence of radio radiation, which can cause damage to biological objects. However, the vast majority of researchers believe that such relatively weak radiation does not pose a serious threat to the population.

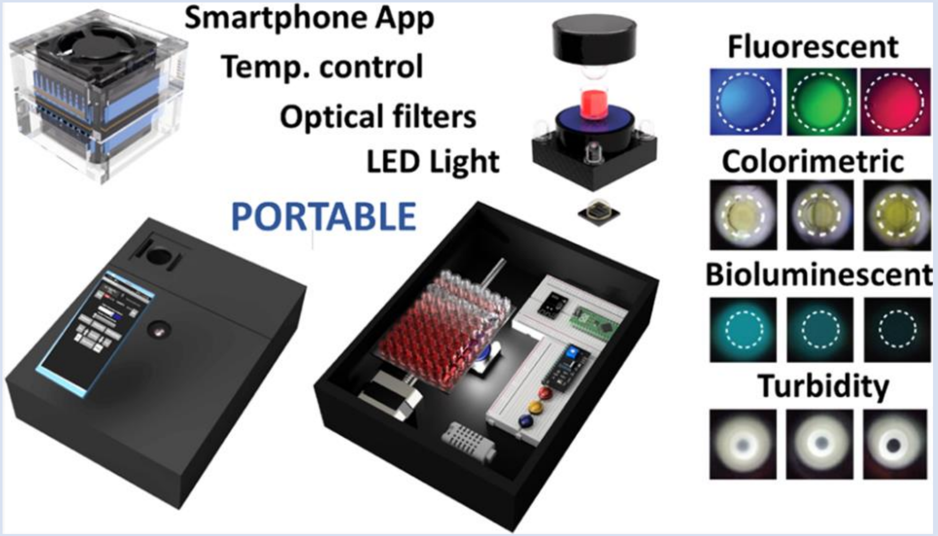
At the same time, scientists and doctors from 36 countries have appealed to the European Union, demanding the implementation of Council of Europe Resolution 1815 "On the hidden danger of electromagnetic fields and their impact on the environment" and proposing the creation of an independent working group to assess the consequences of the introduction of 5G communication on human health.

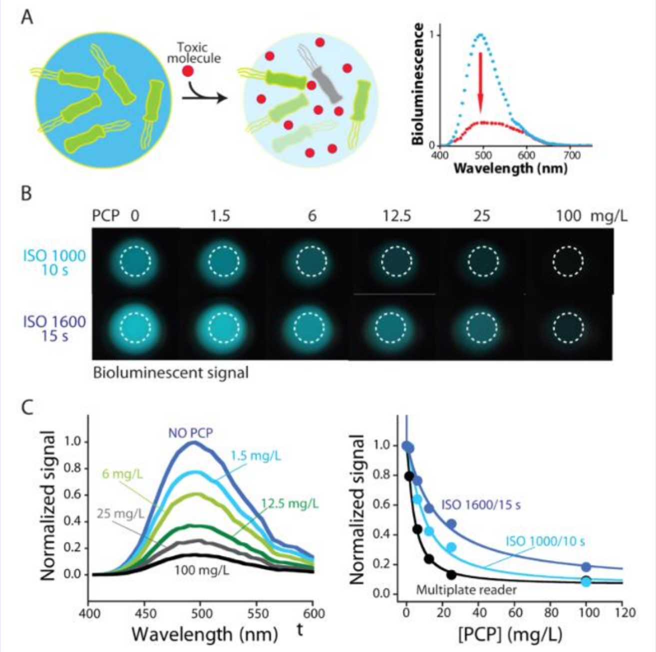
Therefore, interdisciplinary studies on the effect of ultra-high-frequency microwave radiation on biological objects and the search for the primary mechanisms of its action are an urgent scientific problem. To address this issue, it is important to have a specific list of biological objects, processes, and model systems that reliably demonstrate the biological effects of exposure to electromagnetic fields and are convenient for research into the physico-chemical and molecular-biological mechanisms of such exposure.

Search for test-systems for estimation of the biological activity of RF electromagnetic radiation.

Bacterial bioluminescence as a possible biological test system

The intensity of bacterial luminescence is widely used for biological testing of danger from various physical, chemical, and biological factors. Many researches are devoted to the effect of low doses of ionizing radiation, metal ions, and organic substances. There are a variety of commercial bioluminescent technologies for assessing water quality for human consumption. The use of bioluminescent analysis to assess the effect of non-ionizing radiation is reflected in some patents.



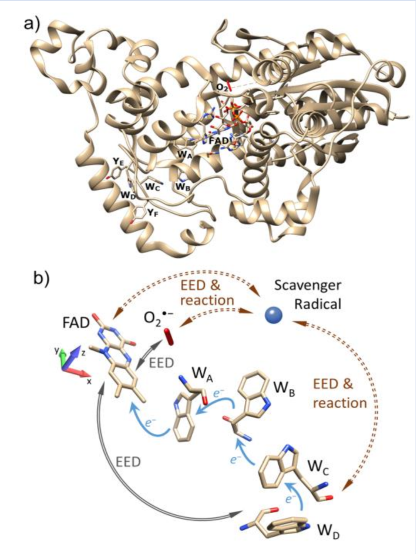


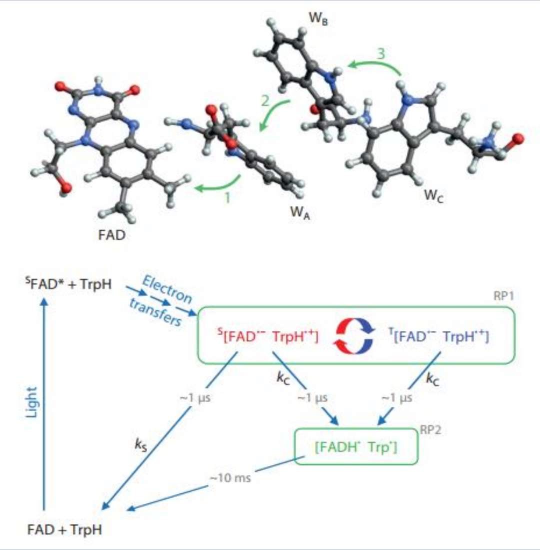
José Francisco Bergua Low-Cost, User-Friendly, All-Integrated Smartphone-Based Microplate Reader for Optical-Based Biological and Chemical Analyses. 2022. https://doi.org/10.1021/acs.analchem.1c04491

Search for test-systems for estimation of the biological activity of RF electromagnetic radiation.

Presently, the dominant hypothesis regarding the molecular mechanisms of biological magnetoreception and electromagnetic sensing is based on the idea of magnetosensitivity of free radical redox processes occurring in cryptochrome proteins that contain flavin cofactors. It is believed that such a mechanism is universal in the biological world and is characteristic of both the animal and plant kingdoms.

The essence of this hypothesis is that flavin, which is contained in cryptochrome proteins, goes into an excited state under the influence of light or a chemical process, as a result of which, with the participation of an oxygen molecule, a radical pair is formed that is sensitive to magnetic and radio-frequency electromagnetic fields. Thus, the rate of formation of the final products of this biochemical process can be controlled by magnetic and electromagnetic influences. It is believed that such a mechanism allows living organisms to sense the magnetic field and navigate in space relative to magnetic field lines.





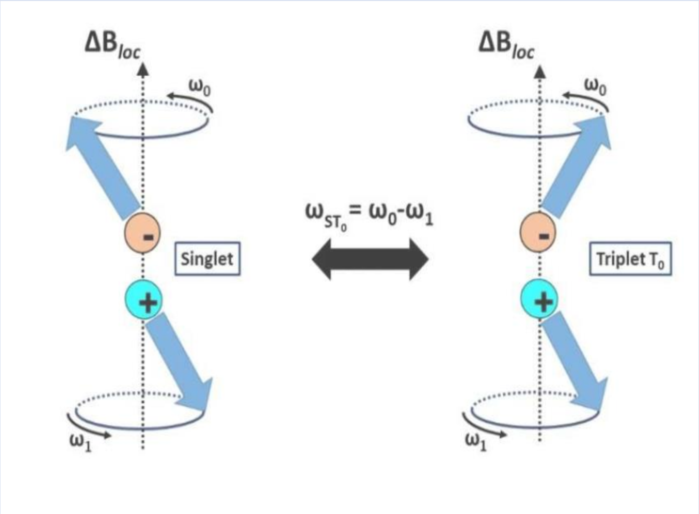


FIG. 1: Vector diagram representing singlet-to-tripletoscillations in a radical pair…

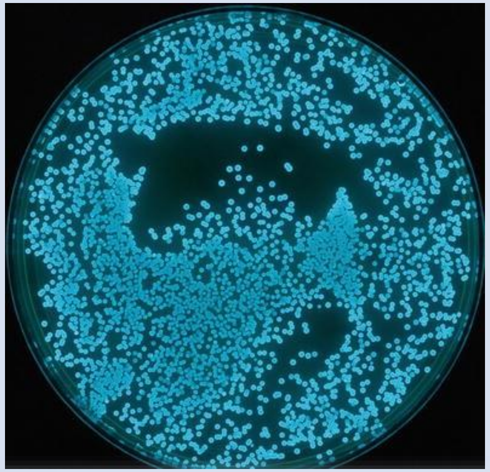
P.J. Hore, Henrik Mouritsen The Radical-Pair Mechanism of Magnetoreception. Annu. Rev. Biophys. 2016. 45:299–344.

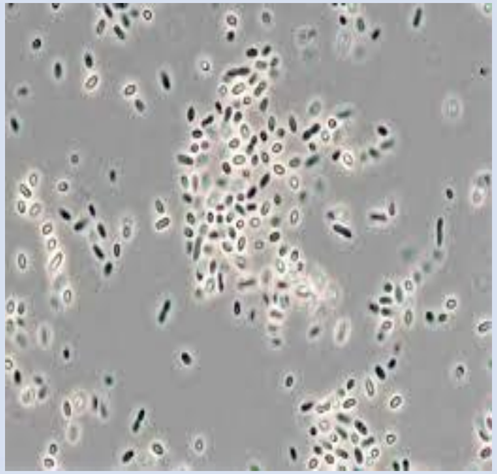
The Phenomenon of Bacterial Luminescence and Its Molecular Mechanism. Bacterial Luminescence as Possible Object for Studies of Magneto- and Electromagnetic Reception

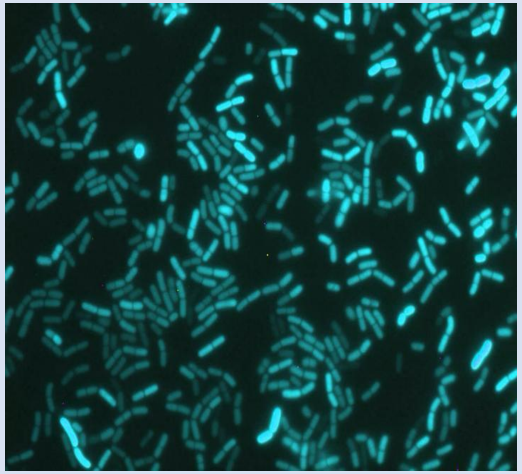
It is necessary to note that similar free-radical processes are carried out in bacterial luciferase, where the flavin cofactor is also involved. Bacterial luminescence is a bioluminescence phenomenon observed in certain species of bacteria. It arises as a result of redox reactions involving flavin and oxygen (fig. 1). The outcome of the reaction is the formation of oxidized flavin in an electron-excited state, which, upon returning to the ground state, emits energy in the form of light. This phenomenon is widespread among marine bacteria, particularly the *Vibrio sp.* and *Photobacterium sp*. Bacteria use this process for various purposes, including defense against predators, attraction of symbionts, intercellular communication, and protection against elevated concentrations of reactive oxygen species.

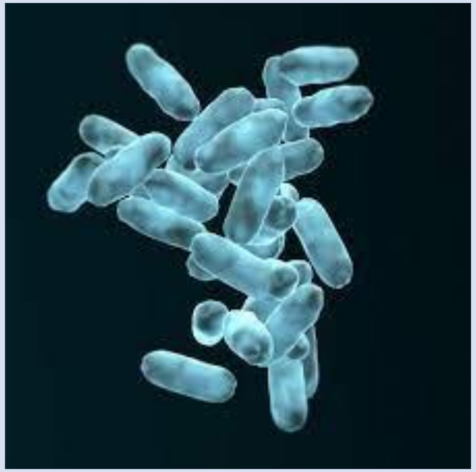
Thus, in our research, we assumed that the bioluminescent system of photobacteria can serve as a convenient biological model for studying the fundamental principles and mechanisms of magnetoreception and electromagnetic sensing in the biological world. On the other hand, the possible sensitivity of photobacteria to electromagnetic influences could allow us to use this phenomenon as a detector for estimating potential dangers to the human body. There is information that the photobacterium *Vibrio fisheri*, one of the strains used in luminescent analysis systems, is not sensitive to the magnetic component of high-frequency electromagnetic fields but reacts to the electric component (Catrin F. Williams et el. (2019), doi: 10.1117/1.JBO.24.5.051412). Therefore, the study of the effect of extremely high-frequency electromagnetic fields on bacterial luminescence and the subsequent development of test systems is highly relevant.

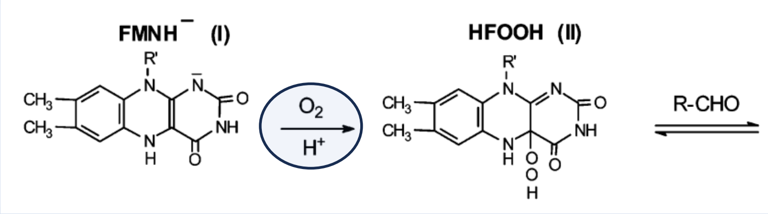
Bacterial Luminescence as Possible Object for Studies of Magneto- and Electromagnetic Reception











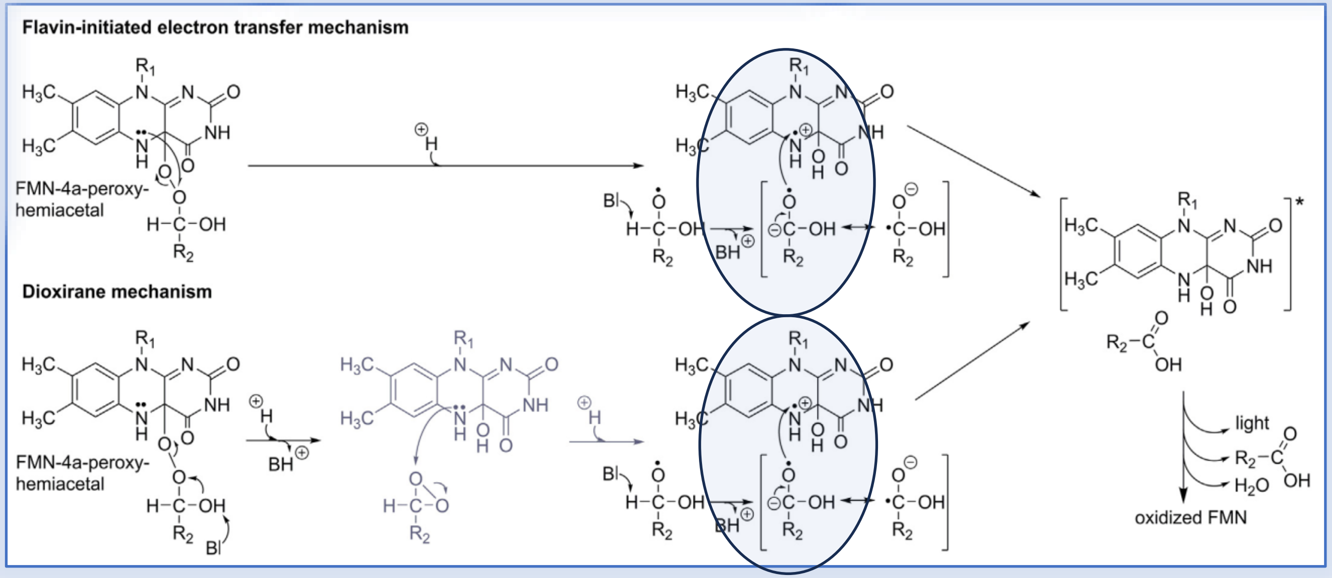


Fig. 1 Flavin-initiated electron transfer and the dioxirane mechanisms realized in bacterial luciferase

Eveline Brodl, Andreas Winkler, Peter Macheroux Molecular Mechanisms of Bacterial Bioluminescence Computational and Structural Biotechnology Journal 16 (2018) 551–564

The Purpose of the Study

The aim of this work was to study the peculiarities of bioluminescence dynamics of *Photobacterium phosphoreum* IMV B-7071 under the influence of a microwave electromagnetic field with a wavelength of 7.1 mm (42.2 GHz) and an intensity of 20 mW/cm^2. As is known, millimeter radio waves with similar frequency and amplitude characteristics are used in modern telecommunication technologies, such as 5G and 6G, as well as in some therapeutic procedures in medicine.

Methods

Bioluminescence studies were carried out in liquid media of standard composition in Petri dishes with a diameter of 5 cm. The height of the liquid bacterial suspension in the Petri dishes was 5 mm. The baseline bioluminescence was studied without stirring the bacterial suspension one or two days after inoculation in the culture medium, during which time the bacterial suspensions exhibited maximum luminescence. Bioluminescence was recorded by digital photo registration with subsequent image processing in ImageJ.

The temperature inside the suspension and at its bottom was measured with a thermocouple with an accuracy of 0.1°C. The temperature of the surface of the bacterial suspension was measured using a thermal imaging camera (Topdon Tc001) with an accuracy of 0.1°C.

The microwave electromagnetic field with a wavelength of 7.1 mm (42.2 GHz) and an intensity of 20 mW/cm^2 was generated using a G-141 generator. The electromagnetic influence was applied through a waveguide, directing millimeter-range electromagnetic waves into the center of the Petri dish from below through its 1 mm thick plastic bottom.

Bioluminescence of Photobacteria. Time Dynamics

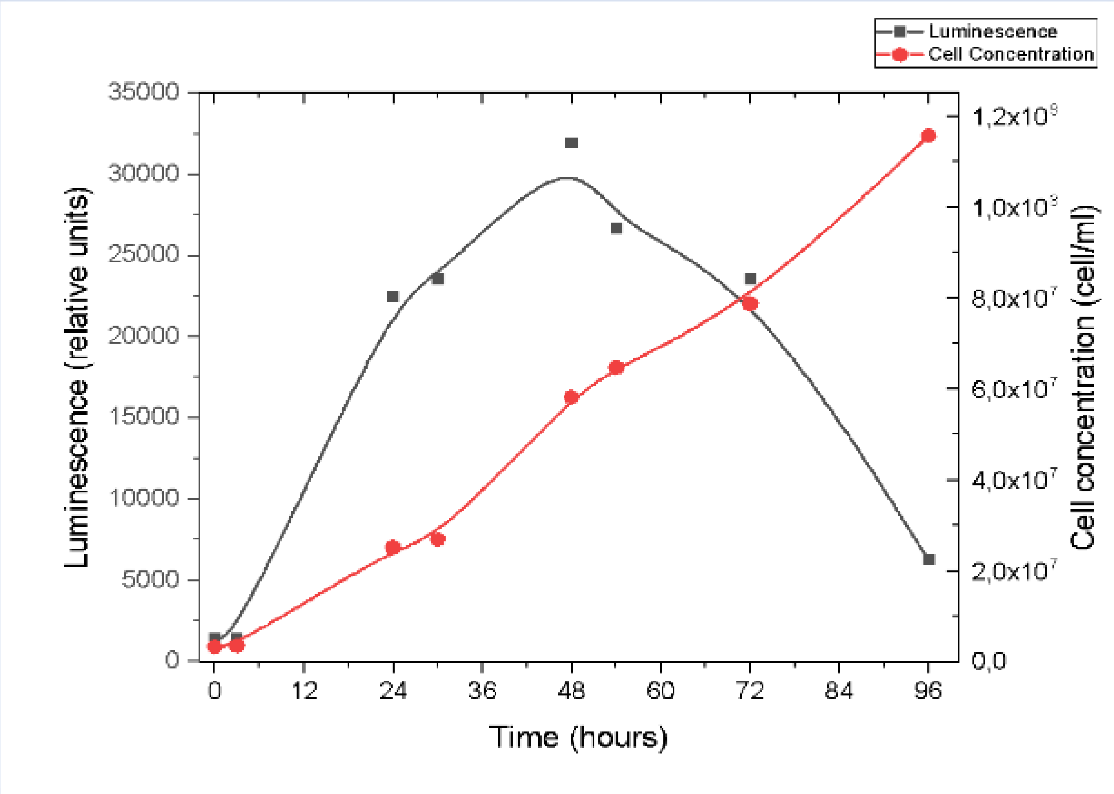


Fig. 2 Dynamics of background baseline bacterial luminescence (blackline) and cell concentration (redline) in the liquid medium during 96 hours (4 days).

The baseline bioluminescence was studied without stirring the bacterial suspension one or two days after inoculation in the culture medium, during which the bacterial suspensions showed maximum luminescence (fig.2 , black line).

During this period, the bacterial population actively grows and glows intensely. However, after two days, the luminescence begins to decrease against the backdrop of further increases in the density of the bacterial suspension (fig.8, red line).

According to modern theories, such luminescence dynamics are related to the bacterial quorum, where bioluminescent light acts as one of the communication channels during the initial period of active population density growth.

EMF influence. Thermal effects of exposure

In electromagnetic biology, there is an ongoing debate about the thermal and non-thermal effects of weak high-frequency electromagnetic fields. Given that in our study the intensity of the ultra-high frequency electromagnetic field at 20 mW/cm² was at the threshold between thermal and non-thermal effects, we conducted separate studies of temperature gradients and their dynamics in distilled water and in bacterial suspensions contained in Petri dishes. The results are presented on fig. 3. As can be seen from the figures, relatively weak temperature gradients (within 1-2, a maximum of 3°C in the region of the entrance of electromagnetic radiation) are formed in distilled water and in suspensions within a few minutes of exposure to ultra-high-frequency radiation. These gradients stabilize after about 1-2 minutes, indicating the formation of a stable slow convective structure in the bacterial suspension, sustained by the energy of electromagnetic radiation.

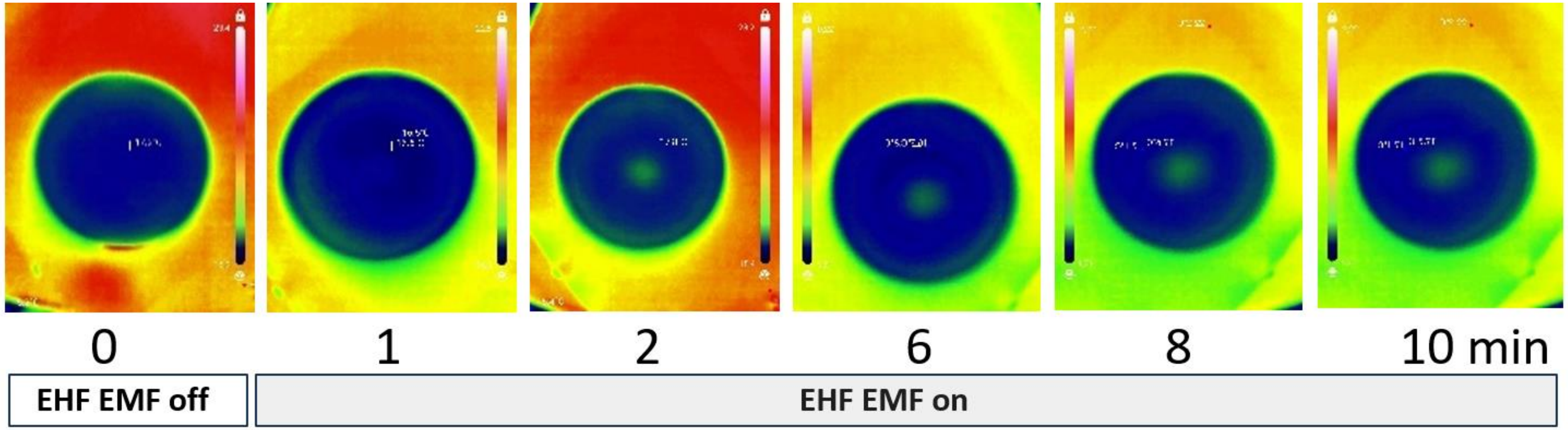
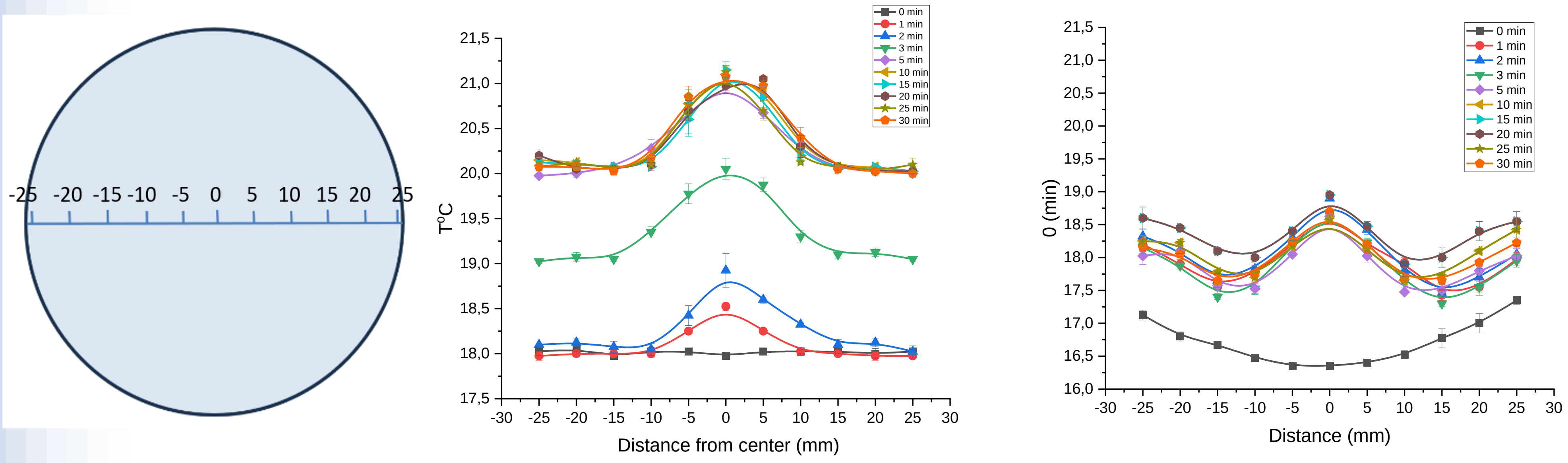


Fig. 3 An example of the formation of temperature gradients over time on the surface of a bacterial suspension in a Petri dish upon microwave exposure is shown according to thermographic analysis data.

EMF influence. Thermal effects of exposure

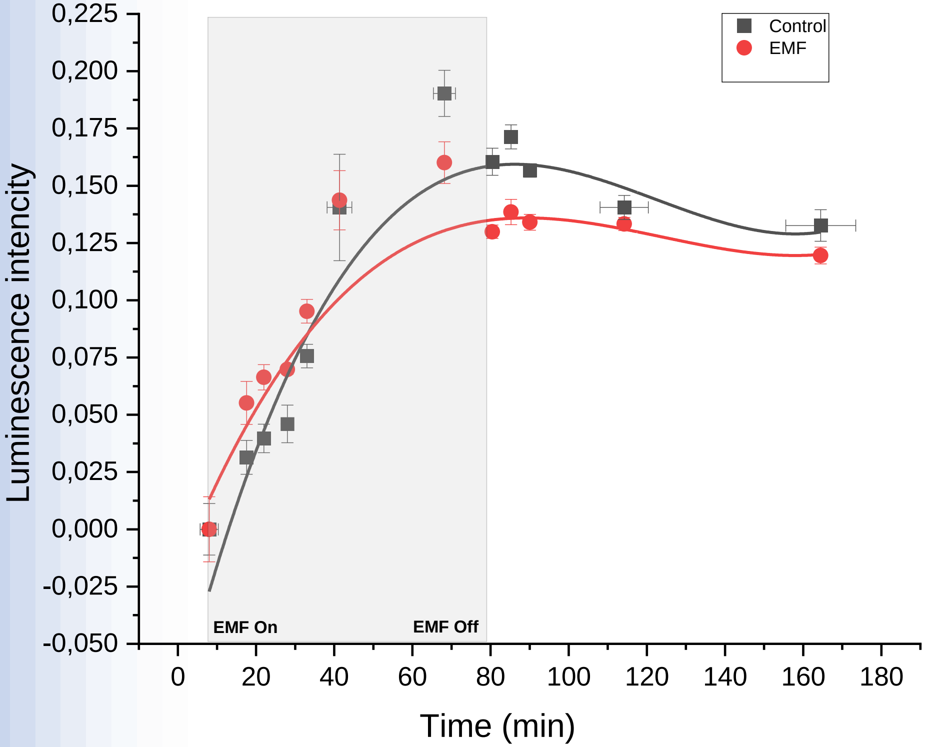


a b c

Fig. 4 A typical profile of temperature gradients in bacterial suspension when microwaves are turned on at an ambient air temperature of 18°C: **(a)** distances relative to the center of the Petri dish where the temperature was measured; **(b)** temperature at the bottom of the Petri dish; **(c)** temperature on the surface. Lines of different colors show the time from the moment of exposure to electromagnetic radiation.

The figure 4 shows that due to the evaporation process, the surface of the bacterial suspension has a lower temperature compared to the temperature in the middle of the volume. Moreover, the evaporation process is more active in the center of the dish, resulting in the lowest surface temperature there. It is important to note that according to our data and the findings of other researchers, small temperature variations in the range of 1-3˚C do not significantly affect the intensity of bioluminescence, which remains relatively constant within the range of 5-10˚C.

Effect of Extremely High Frequency Electromagnetic Fields on Background Baseline Bioluminescence Over Time



The analysis of the experimental results showed that the effects of extremely weak microwave electromagnetic radiation of the millimeter range on the intensity of bacterial luminescence take place, but they are very small and are on the border of statistical significance and accuracy of the bioluminescence registration method (fig. 5). In this regard, the use of bacterial luminescence as a test system for evaluating the biological activity of extremely weak electromagnetic fields in the millimeter range is inappropriate.

Fig. 5 Dynamics of bacterial luminescence intensity (M ± SEM) over time in control samples and under the influence of microwave electromagnetic radiation with a wavelength of 7.1 mm (42.2 GHz) and power density of 20 mW/cm^2.

Notes: Luminescence intensity was assessed over the entire area of the Petri dish. The size of the statistical sample at each time point is n=8. The figure shows the average values of luminance calculated for three RGB color channels. The increase in the intensity of bioluminescence over time is due to the fact that the experiment fell on the phase of slow growth of bioluminescence.

Effect of Extremely High Frequency Electromagnetic Fields on Background Baseline Bioluminescence Over Time

The figure 5 shows the dynamics of bacterial luminescence intensity in control samples and under the influence of microwave electromagnetic radiation with wavelength 7.1 mm (42.2 GHz) and 20 mW/sm2. In this study, the glow intensity was evaluated over the entire area of the Petri dish. The figure shows the average luminance values from three RGB color channels. The general increase in the intensity of bioluminescence over time is due to the fact that the experiment fell on the phase of slow growth of bioluminescence.

The influence of microwave electromagnetic radiation led to an extremely weak increase in the intensity of bacterial luminescence by 2-3% relative to the control values during the first half of the 80-minute exposure and approximately the same magnitude of its decrease at the end of the exposure (see figure). It is worth noting that earlier studies, the results of which were published in 2019 (Hretskyi et el. (2019). doi: 10.15407/microbiolj81.06.058), used more powerful effects at the level of 15 Watts at the frequency of 40.7 MHz and 2.4 GHz, and there also observed similar dynamics, where a reliable increase in the intensity of the glow was observed at first photobacteria, and then its significant decrease was observed. But in this study, devoted to the influence of extremely weak millimeter electromagnetic radiation, changes in the intensity of bioluminescence were at the limit of statistical significance $p<0.05$, nevertheless they were observed in all three RGB color channels. Sometimes, in repeated experiments, a small decrease in signal intensity in the green and blue color channels was accompanied by a weak proportional increase in the signal in the red channel and vice versa.

Thus, we must state the fact that the effects of extremely weak microwave electromagnetic radiation of the millimeter range on the intensity of bacterial luminescence occur, but they are very small and are at the limit of statistical significance and accuracy of the bioluminescence registration method. In this regard, the use of bacterial luminescence as a test system for evaluating the biological activity of ultra-weak electromagnetic fields of the millimeter range is inappropriate.

Effect of Extremely High Frequency Electromagnetic Fields on Background Baseline Bioluminescence Over Time

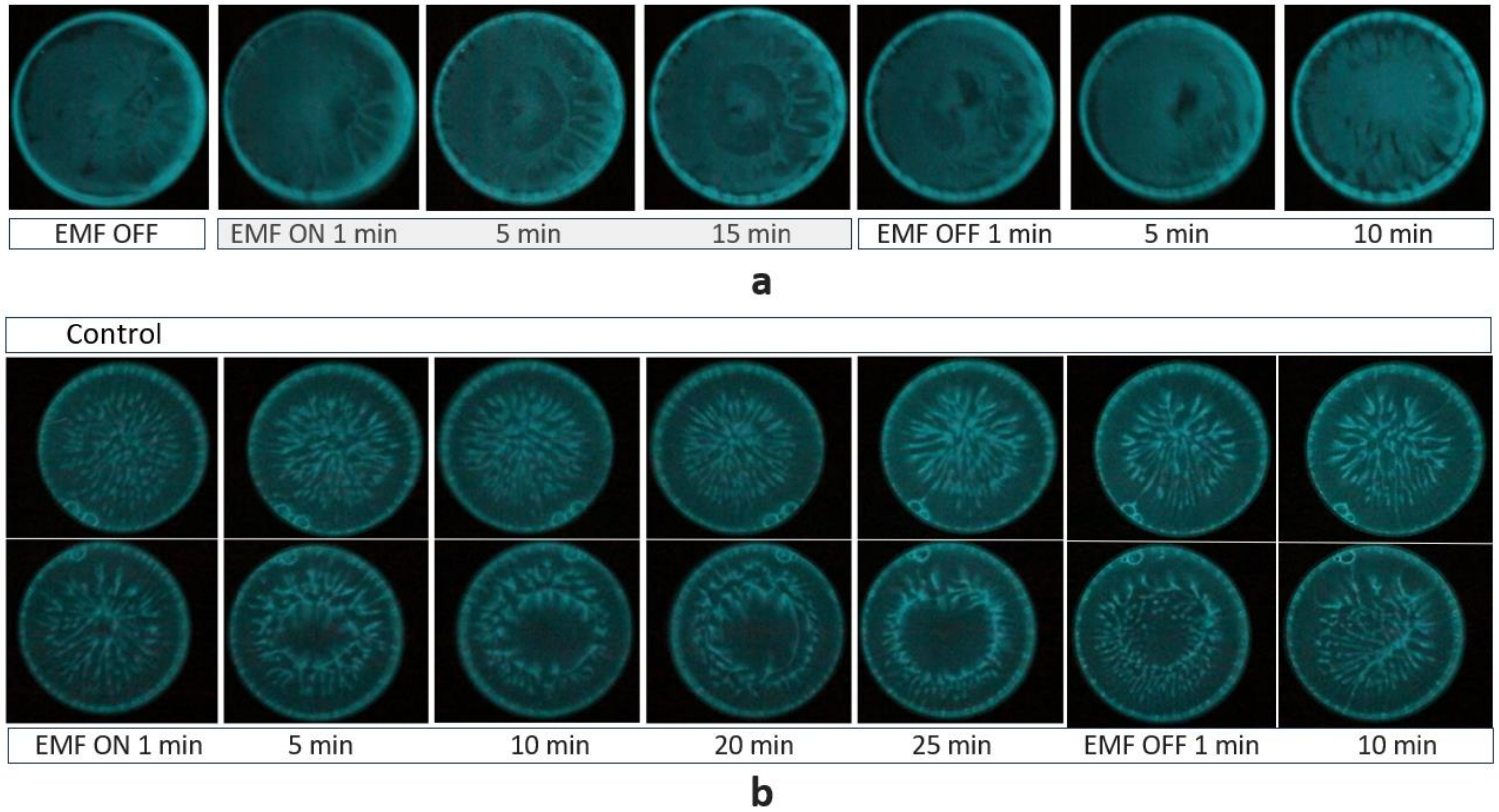


Fig. 6 Formation of dissipative dendritic structures in bacterial suspensions within Petri dishes under controlled conditions and subjected to extremely high frequency electromagnetic radiation is investigated

Notes: **(a)** Initially, the bacterial suspension is weakly structured and when the electromagnetic radiation is turned on the dissipative dendritic structures are formed, which disappear after the electromagnetic radiation is turned off;
(b) parallel photofixation of bacterial luminescence is conducted during experiments in both control samples and those subjected to extremely high frequency electromagnetic radiation.

Effect of Extremely High Frequency Electromagnetic Fields on Background Baseline Bioluminescence Over Time

However, during exposure to microwave electromagnetic radiation, we observed other effects of exposure to electromagnetic radiation, namely, the significant changes in the behavior of bacteria in suspension, which were accompanied by the formation of dynamic dendritic structures that changed over time (see figure 6 a). An important feature of the behavior of bacteria when electromagnetic radiation is applied is their migration towards the edges of the Petri dish. That is, regardless of small temperature gradients, bacteria in a certain way gradually avoid regions with an elevated temperature of the environment, which are heated due to the absorption of electromagnetic energy of electromagnetic waves by water molecules and their associates. After turning off the electromagnetic radiation, the bacteria return to the center of the Petri dish, but begin to form new dendritic structures, concentrating in the central part (see figure 6 a). Such dendritic structures continue to evolve in time for a long time.

The formation of the dendritic structure of the bacterial suspension can be initiated by its mechanical mixing, acoustic or vibration effects. After the cessation of the external disturbing influence on the aqueous nutrient medium, the dendritic structure of the bacterial suspension continues to change over time (see figure 6 b, Control). But under the action of electromagnetic radiation, as in the first case, shown in figure 6 a, a glow ring is formed around the center of the Petri dish, and there is no glow in the central part (see figure 6 B). After turning off the electromagnetic radiation, the dendritic structure returns to the center of the Petri dish and continues to evolve in time.

Dissipative Effects of Extremely High Frequency Electromagnetic Fields

An interesting question pertains to the mechanism underlying the formation of dendritic structures within bacterial suspensions. Such formations can be result of interference of physical-chemical and biological processes.

We assume that the Benard-Marangoni hydrodynamic effect as the primary physical-chemical basis, elucidating the convection's genesis due to temperature gradients and phase separation with different surface tensions within the aqueous medium. The variations of surface tension, contingent upon temperature and the presence of surface-active compounds, produced by bacterial cells to accelerate themselves and ease locomotion in aquatic environments, contributes to this explanation. Absorption of energy of extremely high frequency electromagnetic radiation induce the forming of weak temperature gradients within the aqueous media, thereby forming corresponding gradients in surface tension.

Consequently, bacteria gravitate towards regions with diminished hydrodynamic resistance within the medium. In fact as result, we observe the formation of dissipative structures within complex aqueous multi-component dynamic system. Such an explanation can be considered as a working hypothesis that to be tested.

Effect of Extremely High Frequency Electromagnetic Fields on Background Baseline Bioluminescence Over Time

The validity of this hypothesis can be supported by the formation of dissipative structures in Petri dishes under the influence of extremely weak millimeter radiation, when a 1% solution of sodium dichlorophenolindophenol dye is added to the center of the dish (see figure 7). Upon impact of the electromagnetic radiation the dissipative structures form, where regions of the aqueous medium containing the dissolved dye do not mix with regions devoid of the dye. This creates a characteristic dissipative pattern, which somewhat resembles the dendritic structures observed in bacterial suspensions. However, in the latter case, the behavior of photobacteria is more complex, leading to the formation of dynamic dendritic structures that observed in our experiments.

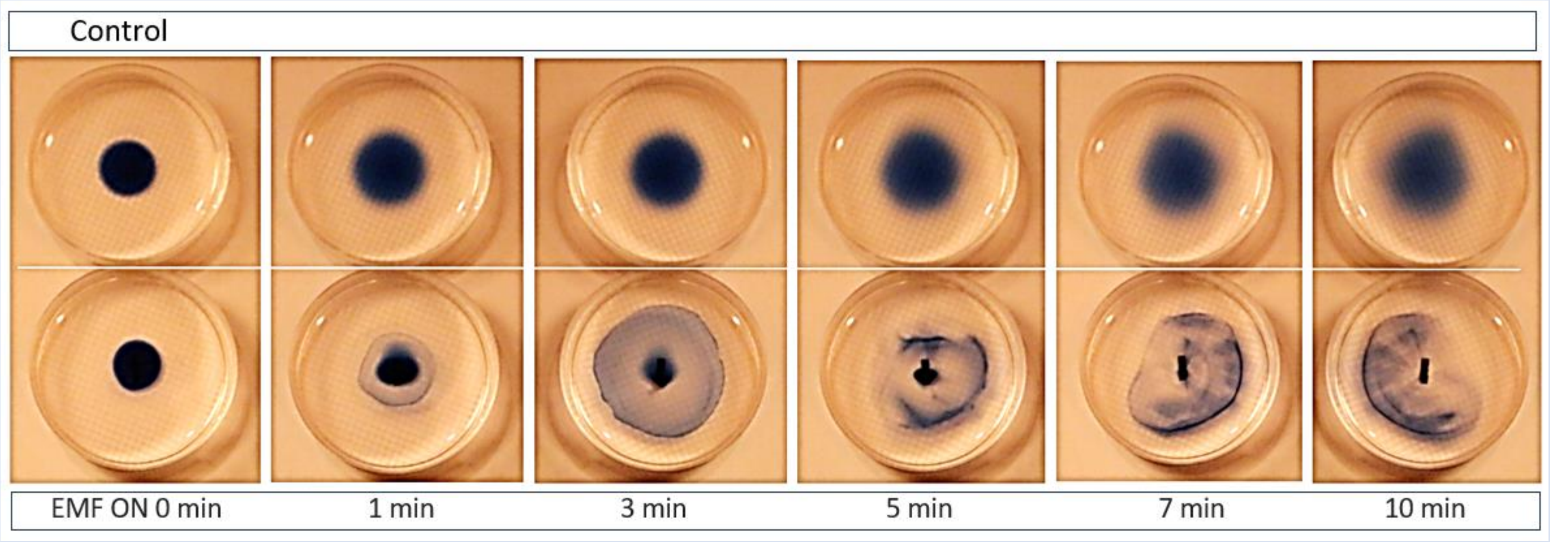


Fig. 7 Formation of dissipative structures under the influence of extremely high-frequency electromagnetic radiation in experiments with sodium dichlorophenolindophenol dye during time of experiment.

Notes: The upper part shows the usual diffusion of the dye in the water over time without the influence of electromagnetic radiation. The bottom part shows the formation of dissipative structures under the influence of electromagnetic radiation. The ambient air temperature in the laboratory is 19°C.

Conclusions:

Thus, bacterial luminescence is a time-dynamic phenomenon. Under controlled conditions, the maximum baseline glow is observed after 1-2 days from the moment of inoculation of bacteria into the nutrient medium.

The extremely weak microwave radiation of the millimeter range does not significantly affect the total baseline luminescence of bacteria, but small local temperature gradients in the bacterial suspension, which arise under the influence of electromagnetic waves, lead to the formation of appropriate dissipative structures in bulk of water media, in which bacteria are concentrated or, on the contrary, avoided.

What physical and chemical properties of the aquatic environment determine this strategy of photobacterial behavior? Are the hydrodynamic characteristics of the environment decisive for the photobacterial response? What are the molecular biological mechanisms that determine the sensitivity for such influence and for the corresponding behavior that is not associated with the activation of the bioluminescent system of photobacteria? This is a range of interesting questions, the answers to which will allow us to better understand the molecular mechanisms of the effect of extremely high-frequency electromagnetic fields on biological systems.

Thank you for attention

We would like to express our sincere gratitude to the organizers of the RAP 2024 conference for their support of scientists from Ukraine