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MONITORING OF GEOMAGNETIC FIELD FLUCTUATIONS WITH LUMINESCENT BACTERIA *PHOTOBACTERIUM PHOSPHOREUM*

In recent years, one of the important goals is to develop information systems for continuously monitoring the biological impact of the variable geomagnetic field. This problem is of importance from the viewpoint of forecasting such global processes as climate changes as well. ***Objective.*** *This study is focused on monitoring the intensity of the luminescence of Photobacterium phosphoreum depending on the geomagnetic field fluctuations in an automated registration mode. Pilot studies were performed using the proposed automated system for recording changes in the bioluminescence of P. phosphoreum.* ***Methods.*** *P. phosphoreum UCM B-7071 bacteria were cultured in a liquid nutrient medium. The study of the influence of the geomagnetic field requires simultaneous automated long-term calculations in real time. For this, a complex for continuous cultivation of P. phosphoreum B-7071 bacteria was created. A program for information processing of bacterial luminescence datasets was developed. The data on geomagnetic field fluctuations from the Subbotin Institute of Geophysics NAS of Ukraine (the Subbotin IGPH) were synchronized with the period of registration of bioluminescence changes. Then the twodatasets of the variables selected for chosen time intervals were processed. To evaluate the dependence of changes in the bacteria's luminescence intensity on geomagnetic field fluctuations, the correlation coefficients of the obtained arrays of experimental data were calculated.* ***Results.*** *The results obtained show the sensitivity of the Photobacterium phosphoreum luminescence to fluctuations in the geomagnetic field. The used data sampling method made it possible to average*

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the luminescence intensity values not only with 1-min resolution, but also with 1-hour, 3-hour, and other chosen resolutions. ***Conclusions.*** *The analysis of changes in the luminescence intensity of P. phosphoreum bacteria due to geomagnetic field fluctuations, with input data being synchronized in time, revealed a positive correlation. Additional analysis is needed to obtain more essential evidence for the bioresponse to the impact of illustrative indicators of geomagnetic field stormy fluctuations. Actually, further analysis of luminescence intensity changes during large natural geomagnetic disturbances or via modeling of their action in the laboratory conditions is needed.*

Keywords: *bioluminescence, geomagnetic field fluctuations, Photobacterium phosphoreum.*

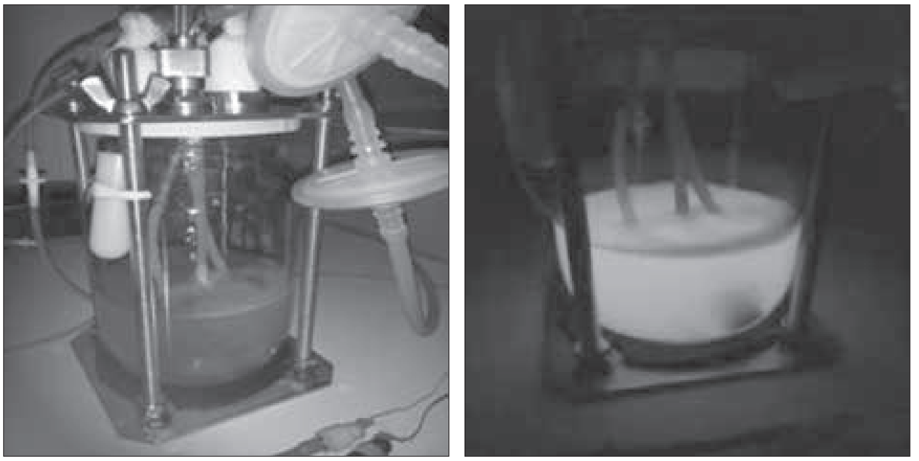
Bioluminescence as the capability of biological organisms to produce light of different intensity due to changing environmental conditions is known for thousands of years. This phenomenon occurs widely. Being bright enough to be detected, bioluminescence has attracted the attention of the scientific community. The researchers have sought to identify and explain the functions of bioluminescence, to follow changes in bioluminescent intensity and the sources of its fluctuations. Generally, there is a great amount of evidence that bioluminescence as such is a biomarker of various geophysical factors including disturbances of the Earth´s magnetic field (so-called geomagnetic field fluctuations) and other environmental conditions, e. g. pollution, etc. Especially, the luminescent bacteria *P. phosphoreum* appeared to be a biomarker of the variable geomagnetic field, i.e., to be of prognostic use [1, 2]. As a bioindicator of geomagnetic activity characterizing the disturbed geomagnetic field due to the variable solar forcing on the Earth and its environment, luminescent bacteria *P. phosphoreum* deserve a deeper study in order to use them as an effective tool for magnetic storm monitoring [3] and to understand the mechanisms of this effect. The luminescence of microorganisms is an intracellular process that is closely related to the main metabolic transformations in the cell. This process is unique to live intact bacteria. Cell restructuring leads to the violation of the connection between the luminescent reaction and other intracellular transformations [4, 5].

One of the important goals of the recent decades has been to develop information systems for continuously monitoring the biological impact of the variable geomagnetic field. Also, this problem is of importance from the viewpoint of forecasting such global processes as climate changes. Investigation of the dependence of significant sensitivity and the rate of changes in the bioluminescence intensity of microorganisms, in particular luminescent bacteria *P. phosphoreum*, in response to the geomagnetic field fluctuations is based on observational data.

This study is focused on monitoring the intensity of the luminescence of *P. phosphoreum* bacteria in an automated registration mode. On the other hand, we used the profiles of geomagnetic field fluctuations that were monitored by Ukrainian geomagnetic observatories supervised by the S. Subbotin IGPH and certified by the full participating members of INTERMAGNET (the International Real-time Magnetic Observatory Network). The initial information on these two sets of variables was transformed into datasets synchronized in time. Their processing has revealed some evidence of the correlation between these data arrays.

Materials and methods. The culture of *P. phosphoreum* UCM B-7071 was the object of this study. It is included in the collection of the Zabolotny Institute of Microbiology and Virology of NAS of Ukraine (the Zabolotny IMV). Cells were cultured at 21 °C in a liquid nutrient medium of the following composition (g/L): peptone — 5.0; yeast extract — 1.0; NaCl — 30.0; Na_2HPO_4 — 5.3; $KH_2PO_4 \times 2H_2O$ — 2.1; $(NH_4)_2HPO_4$ — 0.5; $MgSO_4 \times H_2O$ — 0.1; glycerine — 3.0 mL/L, distilled water — up to 1 L, pH 7.6 [6].

To obtain bioluminescence data, an automated continuous method for recording the bioluminescence of *P. phosphoreum* bacteria was used [7]. For this, a complex for continuous cultivation of *P. phosphoreum* IMV B-7071 was designed and



a *b*

Fig. 1. View of the cultivator in daylight (*a*) and in the dark (*b*)

used (Fig. 1). It includes an cultivator, an auxiliary system of continuous delivery, and a collection of culture fluid. The system is composed of several tubes, a pump, and a tank with a nutrient medium. The tube system pumps the nutrient medium into the cultivator with the help of a pump and also supplies the culture fluid from the cultivator to the UNI-T industrial digital multimeter.

An experimental luminometer setup based on a photomultiplier tube (FEU-115M, 1400 V) was used to register the bioluminescence intensity. The luminescence intensity of the sample is directly related to the electrical signal of the luminometer, so the luminescence intensity data are presented in conventional units [7].

The glow of bacteria was recorded with an average frequency of 4 values per second. It means that for each whole day, 346,000 values could be obtained, which is too large quantity for further processing. To decrease the data frequency, the averaging was performed using the Python programming language. A program allows sampling with a chosen resolution. This software processes a given sample of glow values from a text document according to the parameter of the time change and averages all values within 1 min. At the output, we get a text document (.txt format) with a sample of data in one value per minute.

For the luminescent bacteria' monitoring of geomagnetic field fluctuations, a necessity to assess the biological impact of the Earth's magnetic field variability on the luminescence intensity is obvious. Since biological (bacterial bioluminescence) and geophysical (geomagnetic field fluctuations) data used were measured at different frequencies, the data sampling method allows obtaining datasets of variables synchronized in time. Digitization was applied for geophysical data. As noted above, to follow the geomagnetic field fluctuations, the data from the Ukrainian geomagnetic observatory supervised by the Subbotin IGPH were used in this study, namely the changes in the horizontal X component (Cartesian coordinate system) of the magnetic field of the Earth (hereinafter referred to as X-component). Geomagnetic fields were measured in nanotesla (nT). Their values were taken from the official website of the Subbotin IGPH (http://www.igph.kiev.ua) [8]. However, on this site, the values of the geomagnetic field components are presented as continuous registrations of their changes (with so-called magnetograms) for individual days, i.e., in an analog format (Fig. 2). Such initial plots need transforming into a numerical format, i.e., digitizing to obtain the data frequency for one value per 1 minas done for the Earth's magnetic field earlier [9]. This task was resolved using the *GetDataGraphDigitizer*

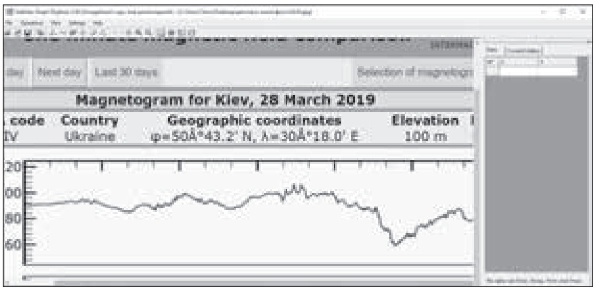


Fig. 2. Example of a magnetogram, for which the *GetGraphDigitizer* program was applied

program. This software allows one to digitize analog X-component magnetograms (Fig. 2.) into the dataset of time resolution needed. Hence, the biological and geophysical datasets are now synchronized in time and ready for further processing. Further investigations intend to use the resampling method to obtain synchronized datasets of different resolutions, namely on the 1- and/or 3-hour basis.

To investigate the influence of the geomagnetic field fluctuations on the bacterial luminescence intensity, simultaneous automated long-term calculations in real time are required. Methods of information analysis of correlations were applied to the generated datasets of both the X-component and *P. phosphoreum* bacteria's bioluminescence intensity values. The possible relationship between those variables was analyzed within a number of chosen one-day long-time intervals. The correlation analysis allows one to investigate the relationship between two or several random variables. The Pearson correlation coefficients were determined for the arrays mentioned within chosen one-day intervals from years 2018, 2019, and 2020. Calculations were carried out in Microsoft Excel 2013 using the correl function. The expression for calculations was as follows:

$$r = \frac{\sum (x - \overline{x})(y - \overline{y})}{\sqrt{\sum (x - \overline{x})^2 \sum (y - \overline{y})^2}}$$

where x is a component of dataset values (x and its mean $\overline{x}$) used on the same day as bioluminescence intensity dataset values (y and its mean $\overline{y}$) were measured on a given registration day, i.e. calculations for the zero time shift between the variables analyzed (denoted below as (0)) were performed.

In addition, the non-zero shift for dataset arrays was considered to quantitatively estimate correlation relationship between variables analyzed not only during the glow registration day, but also one and two days before and/or one and two days after that day (according to the method of overlapping epochs [5]), which is denoted as (—1) and (—2) and/or (+1) and (+2), respectively. Such pilot calculations are to some extent informative from the viewpoint of the explanation of possible mechanisms of bioactive impact of geomagnetic field fluctuations as far as variable luminescence intensity of *P. phosphoreum* bacteria is concerned.

Moreover, the graphs of bioluminescence intensity changes and X-component variability over chosen days were constructed for visualization of possible relationship.

Results and discussion. Since 2018, the Zabolotny IMV of Ukraine has been engaged into the research of the effect of the Earth's magnetic field variations on the bioluminescence characteristics of bacteria. Calculations of correlation coefficients were performed between monitored values of bioluminescence intensity performed with a frequency of 2—4 Hz and horizontal X component (Cartesian coordinate system) of the magnetic field of the Earth (the Subbotin IGPH of Ukraine, website http://www.igph.kiev.ua/), based on the transformed datasets (synchronized in time) derived from the initial information on input variables.

According to the results of these experiments, a rather dynamical correlation relationship was obtained in all pilot studies of the dependence of the luminescence intensity changes in the automated registration mode and the X-component values. This result appears to be due to the fact that the pilot studies were, unfortunately, performed during time intervals of subtle geomagnetic field variations, when no magnetic storms occured. Naturally, solar activity, inducing the geomagnetic field variability, including magnetic storms, was essentially low during the period of 2018-2020 and keeps insignificant in the current solar cycle 25, which is a reason to intend a new grand minimum in solar activity. Moreover, a complex characterization of the current state of the environment during one-day intervals considered is needed since the relationship analyzed is rather multidirectional. In addition, the results were affected by the shortcomings of the methodology of digitization and generation of datasets synchronized in time. These shortcomings were partially taken into account in the program modifications developed during pilot studies. Under taking into account these modifications, the calculated correlation coefficients can be followed below as summarized in Tables 1, 2, and 3.

Table 1. **Results of calculations of correlation coefficients for one-day intervals in 2018**

Registration date	Offset interval day				
	(–2)	(–1)	(–0)	(+1)	(+2)
Dec 12, 2018	0.28	0.15	–0.20	—	–0.23
Dec 25, 2018	–0.16	–0.37	0.45	0.63	–0.48
Dec 26, 2018	0.23	0.31	0.32	–0.30	0.28
Dec 27, 2018	0.01	0.01	–0.15	–0.38	0.40
Dec 28, 2018	0.14	–0.29	0.4	0.10	–0.03
Mean	0.10	–0.04	0.16	0.01	–0.01

Table 2. **Results of calculations of correlation coefficients for one-day intervals in 2019**

Registration date	Offset interval day				
	(–2)	(–1)	(–0)	(+1)	(+2)
March 4, 2019	0.04	0.41	0.7	0.08	0.52
March 5, 2019	0.42	0.14	–0.49	0.63	0.52
March 6, 2019	–0.52	0.32	0.20	–0.36	0.54
March 26, 2019	0.13	–0.12	0.79	0.52	0.36
March 27, 2019	0.44	0.46	0.69	0.32	0.21
March 28, 2019	0.66	–0.11	0.49	–0.56	–0.11
March 29, 2019	0.43	0.2	–0.51	–0.53	0.11
March 30, 2019	–0.19	0.24	0.73	–0.32	–0.01
March 31, 2019	–0.25	0.28	–0.13	–0.47	–0.41
Mean	0.13	0.20	0.27	–0.07	0.20

Table 3. **Results of calculations of correlation coefficients for one-day intervals in 2020**

Registration date	Offset interval day				
	(–2)	(–1)	(–0)	(+1)	(+2)
March 4, 2020	0.35	0.56	0.77	–0.72	0.54
March 5, 2020	–0.72	0.41	–0.57	–0.36	0.52
March 6, 2020	0.62	0.14	0.20	0.63	0.52
March 26, 2020	–0.63	0.32	0.79	0.25	0.54
Mean	–0.1	0.36	0.30	–0.05	0.53

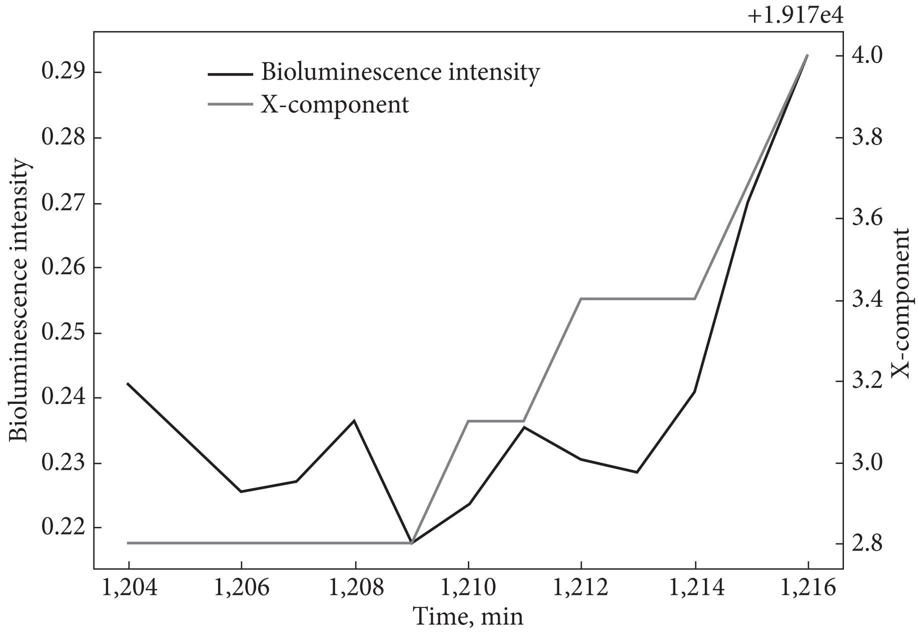


Fig. 3. Changes in the bioluminescence intensity of *P. phosphoreum* bacteria and X-component within time portion 1204 — 1216 on March 4, 2020.
Note: Here and in Figs. 4-6, bioluminescence intensity data are given in conventional units, and the geomagnetic field strength — in nanotesla (nT).

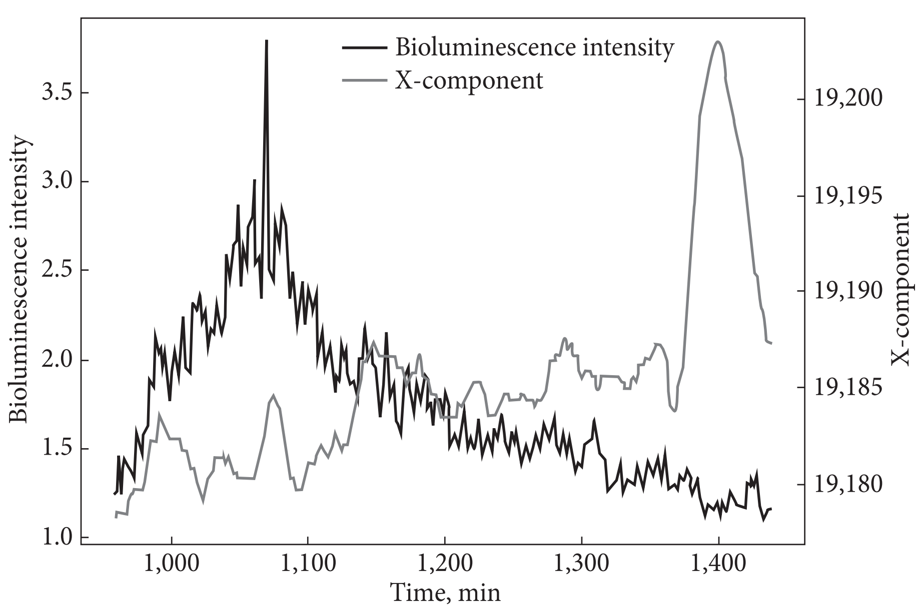


Fig. 4. Changes in the bioluminescence intensity of *P. phosphoreum* bacteria and X-component within the time portion 0930 — 1430 on March 5, 2020.
(See the note under Fig. 3)

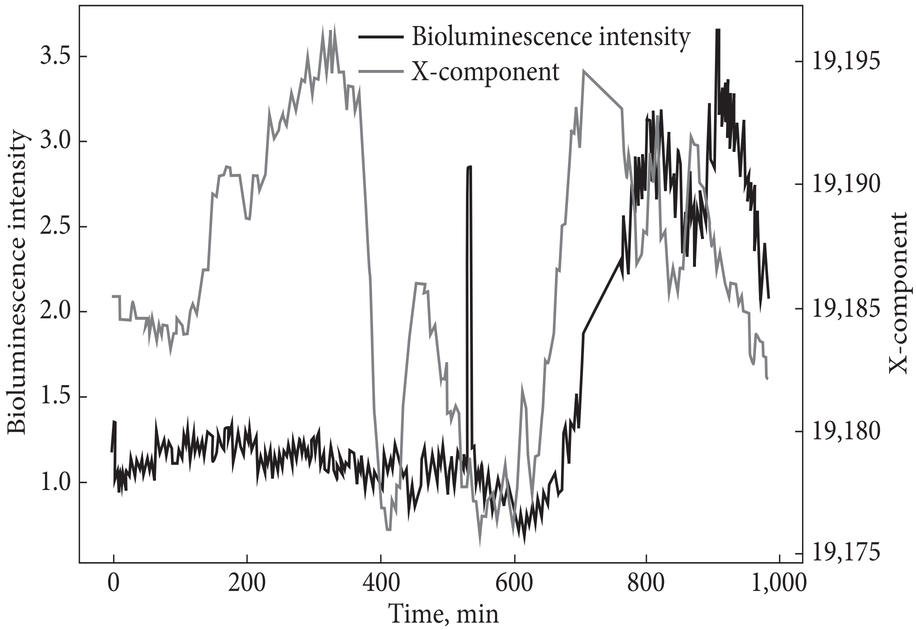


Fig.5. Changes in the bioluminescence intensity of *P. phosphoreum* bacteria and X-component from midnight to 10.00 on March 26, 2020.
(See the note under Fig. 3)

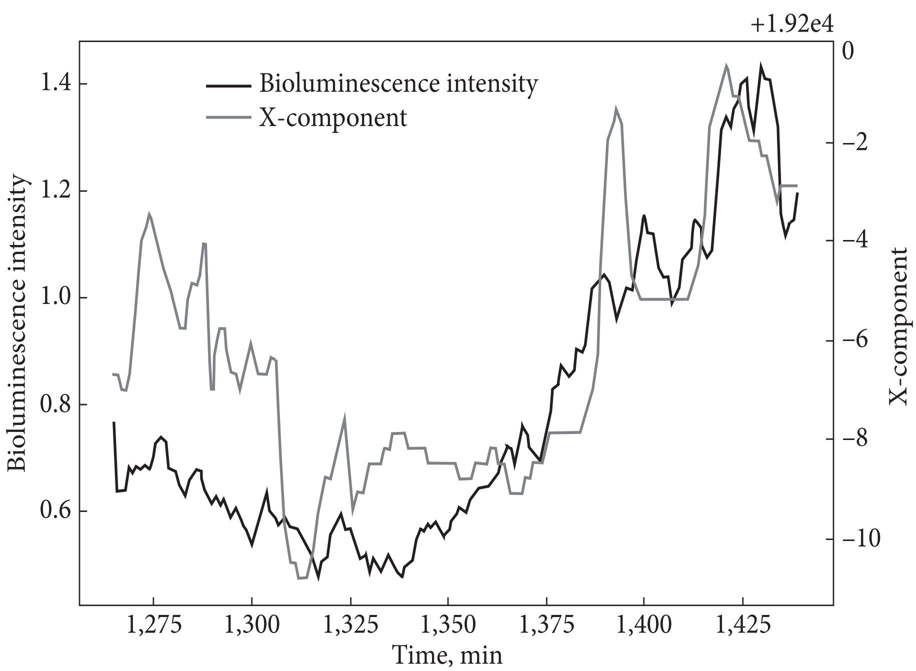


Fig. 6. Changes in the luminescence intensity of *P. phosphoreum* bacteria and X-component within the time portion 1204 — 1440 on March 6, 2020.
(See the note under Fig. 3)

The dynamical character of calculated r is most likely due to the fact that bioluminescence studies were performed against the background of different states of geomagnetic field fluctuations, but in the absence of magnetic storms as most significant disturbance events. The basic information on quantitative estimates is now being acquired. The qualitative aspects are discussed below. Further research will clarify the relationship under consideration in more detail.

To visualize the relationship of variables analyzed, the time-dependent profiles of both the registered bioluminescence and X-component about midday (1-minute data) on March 4, 2020, are illustrated in Fig. 3. Their relationship is evident: the correlation coefficient is 0.77.

In Fig. 4, the dependence of the registered bioluminescence on the X-component can be followed within the time portion 0930 — 1430 on March 5, 2020. The correlation coefficient is quite high but negative (–0.57).

The registered luminescence intensity and X-component profiles from midnight up to 10.00 on March 26, 2020 are shown in Fig. 5. The relationship between them is quite complex, which is also confirmed by their profiles in Fig. 6. The comparison of the relationships between profiles in Figs. 3—6 indicate a quite high sensitivity of luminescence intensity to the X-component variability. In addition, the results of the calculations are summarized for the chosen days in 2020 as seen in Table 3. The significant multidirectional correlation coefficients in those pilot studies of the dependence of two variables synchronized in time can be stressed for zero-shift and non-zero-shifts variants of the calculations.

The calculation results are also summarized in Table 3 for chosen days in 2020. Again, significant multidirectional correlation coefficients can be stressed for zero-shift and non-zero shifts variants of calculations.

Hence, it can be stated that the correlation is not linear. Since during time intervals of the pilot studies, there were no significant changes in the geomagnetic field like the occurrence of magnetic storms, additional analysis is needed to obtain more essential evidence of the bioresponse to the impact of illustrative indicators of geomagnetic field stormy fluctuations. Actually, it should be noted that this question can be answered more confidently via studying changes in bioluminescence during large natural geomagnetic disturbances or via modeling their action in the laboratory conditions.

Conclusions

1. The results obtained confirm the presence of a positive correlation between the luminescence of *P. phosphoreum* bacteria and geomagnetic field fluctuations. The data sampling method, which makes it possible to average the luminescence intensity values not only for 1-minute resolutionbut also with 1-hour, 3-hour, and other chosen resolutions, points a way to compare functional changes in biological species due to the influence of different physical factors of the environment, in particular geomagnetic field fluctuations.

2. The developed software code in Python provides automated retrieval of geomagnetic field discrete values from observational analog data (website links to choose time intervals of interest). The approach applied eliminates errors and increases the rate of digitization with a further calculation of correlation coefficients using datasets of luminescence intensity changes and geomagnetic field fluctuations synchronized in time. Some evidence of positive correlation between the arrays studied was revealed for a number of one-day intervals in the years 2018, 2019, and 2020.

3. The analysis of changes in the luminescence intensity of bacteria *P. phosphoreum* and geomagnetic field fluctuations, with input data being synchronized in time, revealed their relationship to be of high sensitivity. Additional analysis is needed to obtain essential evidence of the bioresponse to the impact of illustrative indicators of geomagnetic field stormy fluctuations. Actually, further analysis of luminescence intensity changes during large natural geomagnetic disturbances or via modeling of their action in the laboratory conditions is needed.

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МОНІТОРИНГ ФЛУКТУАЦІЙ ГЕОМАГНІТНОГО ПОЛЯ З ВИКОРИСТАННЯМ
ЛЮМІНЕСЦЕНТНИХ БАКТЕРІЙ *PHOTOBACTERIUM PHOSPHOREUM*

В останні роки набуває важливості завдання розробки інформаційних систем для постійного моніторингу біологічного впливу змінного геомагнітного поля. Ця проблема має значення з точки зору прогнозування таких глобальних процесів, як зміна клімату. **Мета.** Дослідження було присвячене моніторингу інтенсивності люмінесценції бактерій Photobacterium phosphoreum у режимі автоматизованої реєстрації. Для досягнення мети моніторинг світіння люмінесцентних бактерій під впливом коливань геомагнітного поля проводили, використовуючи запропоновану автоматизовану систему для запису змін біолюмінесценції *P. phosphoreum*. **Методи.** Культивування бактерій *P. phosphoreum* УКМ В-7071 проводили в рідкому поживному середовищі. Вплив геомагнітного поля вивчали шляхом автоматизованих довгострокових одночасних вимірювань інтенсивності біолюмінесценції в режимі реального часу за допомогою створеного комплексу для безперервного культивування бактерій *P. phosphoreum* УКМ В-7071. Дані коливань геомагнітного поля, отримані від Інституту геофізики ім. С.І. Субботіна НАН України, були синхронізовані з періодом реєстрації змін біолюмінесценції. За допомогою розробленої програми для обробки даних проведена оцінка залежності отриманих масивів експериментальних даних змін інтенсивності бактеріальної люмінесценції від коливань геомагнітного поля. **Результати.** Отримані результати показують чутливість люмінесценції *P. phosphoreum* УКМ В-7071 до коливань геомагнітного поля. Запропоновано метод дискретизації даних, що уможливлює усереднення значень інтенсивності люмінесценції не тільки з однохвилинною, але й з годинною, тригодинною та іншою роздільною здатністю. **Висновки.** Аналіз змін інтенсивності люмінесценції бактерії *P. phosphoreum* флуктуацій геомагнітного поля, за умови синхронізації вхідних даних у часі, виявив позитивний кореляційний зв'язок. Необхідно провести додатковий аналіз для отримання суттєвих доказів впливу на біологічну відповідь ілюстративних показників флуктуацій геомагнітного поля, а також аналіз змін інтенсивності люмінесценції впродовж великих природних геомагнітних збурень або шляхом моделювання їх дії в лабораторних умовах.

Ключові слова: *біолюмінесценція, флуктуації геомагнітного поля, Photobacterium phosphoreum.*