REVIEW

Regarding the competition for the academic position "Associate Professor" in the professional field 4.3 Biological Sciences, scientific specialty "Biophysics", announced in State Newspaper No. 32/16.04.2021 for the needs of the Department "Biophysics and Radiobiology" at the Faculty of Biology of Sofia University "St. Kliment Ohridski"

Reviewer: Prof. Katya Marinova Georgieva, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences

Assistant Professor Margarita Angelova Kouzmanova, PhD is the only candidate in the competition announced for the needs of the Department "Biophysics and Radiobiology" at the Faculty of Biology. The materials presented have been prepared in accordance with the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for the conditions and procedures for acquiring academic degrees and occupying academic positions in Sofia University "St. Kliment Ohridski".

Brief biographical data

Margarita Kouzmanova graduated from the Faculty of Biology of Sofia University "St. Kliment Ohridski" with a master's degree in biology - teacher of biology and chemistry in 1981. In 1987 she was appointed as a specialist biologist in the Department "Biophysics and Radiobiology" at the Faculty of Biology. She successfully defended her Ph.D. thesis on "Study of the biological action and evaluation of the radioprotective efficiency of millimeter electromagnetic waves" and received the academic and educational degree "Doctor" in 1997. Margarita Kouzmanova continues to work in the Department of Biophysics and Radiobiology as an Assistant Professor since 1999. She speaks English and Russian languages.

General presentation of scientific papers

Dr. Margarita Kouzmanova has a total of 31 peer-reviewed scientific publications, as well as 20 publications (in full text) in proceedings of scientific forums, of which 12 are international. For participation in the competition for the academic position of "Associate Professor" she has presented 19 scientific publications, 7 of which have been published in journals with a rank of Q1, 2 in Q2, 2 in Q3, 1 in Q4, 2 publications with IF, but without quartile for the year of publication, as well as 5 publications in the category chapter of a book or collective monograph. It should be noted that a part of her publications is in leading specialized scientific journals with high IF, *Coordination Chemistry Reviews*, *Biochimica et Biophysica Acta, Plant Physiology and Biochemistry, Current Plant Biology, Photosynthetica* and others.

The presented publications are distributed as follows: In the habilitation work (indicator B4) are included 4 publications in Q1. The publications outside the habilitation work are 15: indicator Г7 - 10, of which 3 with rank Q1, 2 with Q2, 2 with Q3, 1 with Q4 and 2 publications with IF, but without quartile for the year of publication; according to indicator Г8 – 4 book chapters and 1 collective monograph. The total IF of the scientific papers submitted for participation in the competition is 40,141. A reference list of a total of 344 citations of the publications for participation in the competition is presented, as 286 of them are in scientific publications, referenced and indexed in Web of Science and Scopus (Indicator D). The scientific production, as well as the large number of their citations show the seriousness and relevance of her research activities. Dr. Kouzmanova has been a participant in 2 projects funded by the National Science Fund and 2 international projects. She has been a leader of 2 projects funded by the Research Fund of Sofia University, as well as the supervisor of 6 graduate students. During her entire career in Biological Faculty she has presented 56 posters at scientific conferences.

The enclosed documents show that Assistant Professor Margarita Kouzmanova meets the minimum national requirements for the academic position "Associate Professor" - indicator A (50 points) and indicator B (100 points) meet the minimum requirements, indicator G is 252 at a minimum value of 200, and indicator D is 630 at a minimum value of 50 and indicator E - 60 points.

Research activity

The scientific research of Assistant Professor Dr. Margarita Kouzmanova are in three main areas: Biological effects of magnetic and electromagnetic fields; Combined action of non-ionizing and ionizing radiation; Biophysics of photosynthesis.

In my review I will consider the contributions of the candidate, having in mind only the works submitted for participation in the competition with their numbering in the attached list.

Biological effects of magnetic and electromagnetic fields

Publications № 1, 2, 3, 4, 5, 7 belong to this group

Changes in the properties of erythrocyte membranes under the action of a constant magnetic field have been studied using immunochemical and biophysical methods (publications 1-3). The effect of magnetic field depends on the physiological state of biomembranes, as well as on the presence of certain physical or chemical agents, including lectins. As chemical agents, lectins bind specifically to certain components of membranes, mainly glycoproteins. It has been found that the application of a static magnetic field of 5 mT significantly alters the binding of lectin to the erythrocyte membrane and increases the time required for its complete binding. The rate and extent of lectin binding may be an indicator of changes in the glycoprotein complex of the cell membrane. The obtained results show that the magnetic field causes significant changes in the content of ATP and electrophoretic mobility (EPM), which depend on the duration of exposure. The protective role of the magnetic field is more pronounced if the physical factor is applied before the chemical one, which is explained by the stabilization of membrane structure and the double electric layer when magnetic field is applied. It has also been found that the agglutination of erythrocytes strongly depends on the concentration of lectin, as well as on the presence or absence of a magnetic field. When a magnetic field is applied, a decrease in agglutination is observed. In addition, studies have shown that the effects of the magnetic field on erythrocyte membranes, as observed by changes in EPM and anion transport through the 3-band protein, are temperature dependent. This combined effect is related to lipid-protein interactions and the distribution of membrane surface charges (publication 3). These studies contribute to the elucidation of mechanisms of action of the magnetic field at the membrane level.

This thematic area also includes the research of Dr. Kouzmanova on elucidation of the biological action of high-frequency low-intensity electromagnetic fields, which are conducted mainly in two frequency ranges. To clarify the mechanisms of action of millimeter waves (MMW) with frequencies of 53.53 GHz (5.6 mm) and 42.19 GHz (7.1 mm) (used in medicine to treat a number of diseases), experiments were performed at the whole organism level (Wistar rats). MMWs have been found to modify immune reactivity by increasing histamine levels and lowering blood ceruloplasmin levels (publication 4).

Margarita Kouzmanova's research on the influence of electromagnetic fields used in communication systems is of great practical importance. High-frequency electromagnetic fields are widely used for the transmission of radio and television signals, as well as in wireless communications, so the study of the biological effects of radio frequency electromagnetic radiation could contribute to a better understanding of the possible dangers to human health. Changes in hemoglobin release as an indicator of haemolysis caused by increased membrane instability after *in vitro* irradiation of human erythrocytes with GSM900 electromagnetic field were studied (publication 5). It has been shown that the effects of low-intensity high-frequency electromagnetic fields on human erythrocytes depend on the field parameters, the exposure time and the time after irradiation, and the water content of the suspension as well. A decrease in the level of hemoglobin in the irradiated suspensions was found, which is explained by the stabilization of the erythrocyte membrane leading to a decrease in hemolysis. The treatment of the ornamental herb *Plectranthus sp*. with a 900 MHz electromagnetic field causes different changes in enzyme activity depending on the time after exposure and shows that plants perceive and respond to electromagnetic fields and are a good model for studying the effects of mobile phone radiation (publication 7).

Biophysics of photosynthesis

This group includes publications № 8, 9, 10, 11, 12, 14, 16, 17, 18

A significant part of the research of Dr. Kouzmanova in this field is devoted to the study of the highly informative biophysical methods, based on the analysis of the luminescent and optical characteristics of plant objects. Chlorophyll fluorescence is a widely used, fast and non-invasive method for assessing the functional state of photosynthetic apparatus, as well as for characterizing

its damage and acclimation ability under stress conditions. The main contributions of Dr. Kouzmanova in this field are:

Modifications have been made to the JIP test developed by Prof. Strasser, based on measurements of rapid chlorophyll fluorescence, which increase the information value of the method.

A linear correlation was found between the relative dark declines and the oxidized Q_A fraction, and it was suggested that experimentally measured fluorescence declines may be used for *in vivo* quantification of the redox reactions of Q_A and Q_B during the fluorescence increase from F_0 до F_M (publication 9).

Based on the simultaneous measurement of prompt fluorescence and delayed fluorescence, as well as the modulated reflection of light at 820 nm, the changes in the photosynthetic activity of bean leaves at different relative water content (RWC) during drying were analyzed. Using the obtained data, an artificial neural network capable of recognizing the RWC of "unknown" samples was constructed. After further development, this method could be used for quantifying of drought stress of crop plants *in situ* (publication 8).

The JIP test allows to determine the changes in chlorophyll fluorescence parameters before the visible manifestations of micro- and macroelements deficiency, which enables adequate fertilization of cultivated crops. It has been found that some of the fluorescent parameters can be used as phenotypic markers. The analysis of selected parameters of the JIP-test is a possible species-specific approach for identification/prediction of nutritional deficiency, which is important for agricultural practice (publications 10, 11, 14, 17).

Measurement of chlorophyll fluorescence and modulated reflection of light at 820 nm makes it possible to compare the tolerance of different plant ecotypes to abiotic stress. The comparison of stress reactions, tolerance and adaptability of two plane ecotypes (Bulgarian and Italian) under the influence of moderately high temperature shows the better tolerance of plants of the Italian ecotype and that the age of the leaves is essential for the stress response of plants (publication 18).

The JIP test was also used to characterize the parasite (*Cuscuta campestris*) – host (*Ipomea tricolor*) interaction. The effect of the parasite on the photosynthetic apparatus of the host has been found to depend on the physiological age of the host leaves (publication 16).

The accumulated theoretical knowledge and experience from the conducted investigations are summarized in a monograph, with special attention paid to the mechanism of generating delayed fluorescence in plants and to advantages from the simultaneous registration of both prompt and delayed fluorescence, together with the analysis of modulated reflection of light at 820 nm. Many examples of the application of chlorophyll fluorescence in the study of plant stress responses are given (publication 12).

The contributions from the research activity of Assistant Professor Dr. Kouzmanova have not only theoretical significance, but also a certain practical orientation.

Future research

Margarita Kouzmanova's future research is planned to continue in two main directions:

- Influence of electromagnetic fields emitted by antennas on the human body on isolated human erythrocytes
- Elucidation of the early cellular and molecular mechanisms responsible for the "bystander" effect and the radioadaptive response in peripheral blood lymphocytes by estimating the frequency of double-stranded DNA breaks

Teaching activities

As an Assistant Professor in the Department of Biophysics and Radiobiology Margarita Kouzmanova had a total of 2,088 teaching hours for the last 5 years (2015-2020). The presented documents show that her educational employment increases significantly after 2019. Dr. Kouzmanova has developed several lecture courses - Effect of physical factors on living systems (30 hours); Biophysics and radiobiology (10 hours for the specialty Biology and for the specialty Molecular Biology); Fundamentals of Radiobiology (45 hours for the bachelor's program in Nuclear Chemistry). She has developed a total of 42 hours of practical classes (for Biophysics; for the cycle Radiobiology and dosimetry of ionizing radiation; for the course on Action of physical factors). In addition to the 6 successful graduates, she has supervised 116 coursework of students majoring in Molecular Biology. The presented information shows the active teaching activity of Assistant Professor Margarita Kouzmanova.

In addition, she is actively involved in the life of Biological Faculty and Sofia University, performing a number of administrative functions. She has been a member of the organizing committees of several scientific conferences, as well as a member of a number of commissions such as the commission for organizing and conducting the National Competition in Natural Sciences and Ecology, participation in the Biological Faculty Open Doors and organizing visits of student groups. Margarita Kouzmanova also has a number of other administrative responsibilities.

CONCLUSION

The analysis of presented materials convincingly show that Assistant Professor Margarita Kouzmanova is an established scientist working in the current field of modern science, such as biophysics. The scientific works contain significant theoretical and practical contributions, which have received international recognition. The number of her research papers cited by foreign authors in prestigious scientific journals is significant. The evaluation of the scientific and teaching activity of Dr. Kouzmanova shows that she covers all requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, as well as the Regulations for the terms and conditions for acquiring scientific degrees and holding academic positions at Sofia University "St. Kliment Ohridski" for holding the academic position of "Associate Professor".

All this gives me arguments to evaluate positively her overall activity and to recommend to the respected members of the Scientific Jury to vote positively and to the members of the Faculty Council of the Biological Faculty of Sofia University "St. Kliment Ohridski " to elect Assistant Professor Dr. Margarita Angelova Kouzmanova for the academic position "Associate Professor".

Date: 19.07.2021 Reviewer:

Sofia /Prof. Katya Georgieva, Ph.D./