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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

INDICATIONAL PROPERTIES OF BIOPHYSICAL PARAMETERS OF CELLS UNDER RADIOACTIVE CONTAMINATION

Specialty:	2406.01 - Biophysics
Field of science:	Biology
Applicant:	**Ismail Yusif Fridunbayov**

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The work was performed at the Department of Biophysics and biochemistry of Baku State University.

Scientific supervisors: Doctor of Physical and Mathematical Sciences, Professor
Rovshan Ibrahimkhalil Khalilov
Doctor of Philosophy in Biological Sciences, Associate Professor
Aygun Namik Nasibova

Official opponents: Doctor of Biological Sciences, Professor, Corresponding Member of ANAS
Novruz Magamed Guliyev
Doctor of Biological Sciences, Professor
Irada Nuraddin Aliyeva
Doctor of Biological Sciences, Associate Professor
Huseyn Azizulla Abiyev

Dissertation Council FD 2.31 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Baku State University

Vice chairman of the Dissertation council:
Doctor of Biological Sciences, Professor
Ellada Mirali Akhundova

Scientific secretary of the Dissertation council:
Doctor of Philosophy in Biological Sciences, Associate Professor
Samira Jafar Salayeva

Chairman of the scientific seminar:
Doctor of Physical and Mathematical Sciences, Professor
Eldar Ali Masimov

INTRODUCTION

Actuality and degree of elaboration of the theme. The relevance of the research is determined by the high level of anthropogenic impact on the biosphere, which leads to the emergence of complex environmental problems. Among these, the threat of radioactive contamination of the environment, associated with the activities of industrial facilities and the extraction of minerals, is of particular significance. Even in small doses, ionizing gamma radiation can cause serious changes in living organisms, damaging DNA and disrupting metabolic processes in cells. This problem is particularly relevant for regions with long-term technogenic impact, which includes the Absheron Peninsula[1]. Centuries of intensive oil production have shaped specific technogenic landscapes here, characterized by radioactive contamination[2].

In this regard, the Absheron Peninsula is a unique natural laboratory for studying the consequences of chronic radiation exposure in low doses on the components of the ecosystem[3]. In real conditions, radioactive contamination is often combined with other abiotic factors, such as ultraviolet (UV) radiation, heavy metal pollution, and soil salinization. The photosynthetic apparatus of plants, being sensitive to stress, is a primary target for ionizing gamma (γ) and ultraviolet radiation. Disruption of its functions directly affects plant growth, resilience, and their bioproductivity. Therefore, studying the functional state of photosynthetic organisms using modern, highly sensitive methods is of paramount importance for radioecological forecasting of ecosystem conditions.

Despite existing research dedicated to the impact of radiation on

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[1] Aliyev, Ch.S. Radioecological situation on Absheron peninsula oil fields / Ch.S.Aliyev, T.M.Huseynov, V.J.Hajiyev // Environmental Science and Pollution Research. – 2012. – Vol. 19, № 3. – s. 789-796.

[2] Garibov, A.A. Radioecological researches of soils of northwest part of Absheron / A.A.Garibov, R.I.Khalilov [et al.] // Proceedings of ANAS, Biological sciences. - Bakı: - 2008. - № 1-2. - s. 152-159.

[3] Абдуллаев, А.А. Современные проблемы радиоэкологии Апшеронского полуострова и пути их решения / А.А.Абдуллаев, В.М.Керимов, Н.Д.Мамедов // Экологический вестник Азербайджана. - Баку: - 2019. - № 2. - с. 45-52.

living systems, many questions remain open, particularly the insufficient study of the effects of chronic gamma radiation in combination with other stressors.

The formation of reactive oxygen species (ROS) as a result of radioactive exposure poses a serious danger to all living systems[4,5]. Therefore, when studying the influence of stress factors, including radioactive contamination, it is extremely important to investigate changes in biophysical parameters and their indicative properties.

Changes in biophysical parameters under the influence of radioactive contamination on living cells have been little studied to date, which makes this area of research relevant.

Thus, the study of the indicative properties of the biophysical parameters of cells of living systems under radioactive contamination in combination with other stressors is an actual task for biophysics.

Object and subject of research. The objects of our research were plants collected from different regions of Azerbaijan, in particular, from the territories of the Absheron Peninsula with an elevated radioactive background, as well as objects studied under laboratory conditions.

The field research objects include plants such as bean caper (*Zygophyllum fabago* L.), rush (*Juncus* L.), some species of medicinal plants such as rosemary (*Salvia rosmarinus* Spenn.), dog rose (*Rosa majalis* Herrm.), hawthorn (*Crataegus* Tourn. ex L.), and eucalyptus (*Eucalyptus* L. Her.).

The laboratory research objects include: the ornamental plant - Chinese rose (*Hibiscus rosa sinensis* L.), cereal crops; common barley (*Hordeum vulgare* L.), dent corn (*Zea mays var. indentata*), durum wheat (*Tríticum dúrum* Desf.), aquatic plants; (*Elodea canadensis* Michx.), (*Vallisneria* C. Micheli ex L.), the unicellular microalga - *Dunaliella salina* (*Dunaliella salina*, strain *IPPAS* D-

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[4] Ali-zade, V.M. Plant Functional Genomics for Crop Improvement under Abiotic Stress Conditions / V.M.Ali-zade, T.S.Shirvani, D.R.Alieva // Journal of Biosciences. - 2015. - Vol. 40, № 4. - s. 785-800.

[5] Халилов, Р.И. ЭПР-спектроскопия свободных радикалов в растениях при воздействии абиотических стрессовых факторов / Р.И.Халилов, А.Ч.Мамедов, А.Н.Насибова [и др.] // Биофизика. – 2018. – Т. 63, № 5. – с. 951–959.

294). The animal object was a laboratory white rat (*Wistar Albino*).

The subject of the research is to establish the patterns of changes in the biophysical characteristics of millisecond delayed light emission (ms-DLE), electron paramagnetic resonance (EPR) of intracellular structures of plants, microalgae, and laboratory rats under the influence of γ-radiation in comparison with other abiotic stress factors (UV-B radiation, heavy metal salt ions, salinity, temperature) in order to determine their bioindicative significance.

The purpose and tasks of the research. The aim of our dissertation is to study the indicator properties of the biophysical parameters of cells in living organisms under radioactive contamination, taking into account some abiotic stress factors. To achieve this goal, the following tasks were set:

- To study the bioindicator properties of the dominant plant species of the Absheron Peninsula using the ms-DLE method based on radioecological and dosimetric studies.
- To investigate the effects of UV-B radiation and other abiotic stress factors on the biophysical parameters of ms-DLE, EPR spectra, and photosynthetic activity of various research objects (ornamental, aquatic, medicinal plants, and microalgae).
- To assess the adaptive responses of the microalga *Dunaliella salina IPPAS* D-294 to the effects of abiotic stressors (increased salinity, heavy metal salt ions, gamma radiation, temperature, light) through the analysis of growth characteristics and ms-DLE parameters.
- To study the radioprotective properties of licorice root extracts (*Glycyrrhiza glabra* L.) on gamma-irradiated laboratory white rats (*Wistar Albino*) and to assess changes in internal organs using EPR spectroscopy.
- To investigate the dose-dependent effect of ionizing gamma radiation on seed germination and cytogenetic parameters in C_3 and C_4 type cereal plants (*Triticum durum* Desf., *Hordeum vulgare* L., *Zea mays var. indentata*) and to assess the role of photorespiration in their radiosensitivity.
- To substantiate the possibility of using the studied biophysical parameters of ms-DLE, EPR spectra as bioindicators for assessing

the state of the environment under the influence of stress factors on living organisms.

Research methods. Gamma irradiation of samples was carried out using the Rxund-20000 and MRX-γ25 installations, and UV-B irradiation was performed using a DRT-230 mercury-quartz lamp, which has a line emission spectrum. The radiation background of the studied territories was determined using a MKS-AT 1125 dosimeter-radiometer. Changes in the kinetics of ms-DLE were investigated using a phosphoroscope. EPR studies were performed on a spectrometer manufactured by Bruker (Germany). A scanning electron microscope (SEM) was used as a microscopy method. Cytogenetic analysis was performed using a ZEISS Axio Imager A2 light microscope. The number of microalgae cells was counted using a Goryaev counting chamber and a light microscope. The Student's t-test was used to determine statistically significant differences between the experimental results. All calculations were performed in the IBM SPSS Statistics 26.0 program based on data provided in five replicates (n=5). Differences are considered statistically significant at a significance level of $p \leq 0.05$ and below.

The main provisions submitted for defense. Biophysical parameters of ms-DLE and EPR spectra are highly sensitive bioindicators of the impact of abiotic stressors. Their dose-dependent changes in plants and microalgae under the influence of radiation, UV-B, and chemical factors lead to varying degrees of damage to the photosynthetic apparatus.

- Ms-DLE is a highly effective method for assessing the impact of abiotic stressors (gamma radiation, nanoparticles, heavy metal salts) on the photosynthetic apparatus of *Dunaliella salina IPPAS* D-294. This allows for the determination of their comparative toxicity and the identification of specific characteristics of the inhibitory effect.

- A direct relationship has been established between the level of abiotic stress factors and the EPR signal in various medicinal plants, which allows this parameter to be used as a bioindicator for assessing the ecological state of the territory.

- The extract of licorice root (*Glycyrrhiza glabra* L.) exhibits dose-dependent radioprotective activity in laboratory white rats

(*Wistar Albino*) subjected to gamma irradiation, which is confirmed by changes in the EPR spectra of brain and liver tissues. In particular, a decrease in the signal intensity of magnetic iron oxide nanoparticles (g≈2.32), which is an indicator of stress, is observed, as well as other observed changes, indicating a reduction in the negative consequences of radiation exposure.

- Radiosensitivity has been identified in C_3 and C_4 type plants. Gamma irradiation of cereal seeds at low doses (100–200 Gy) stimulates seed germination and mitotic activity in C_3 type plants (wheat, barley), but inhibits them in C_4 plants (corn). The role of photorespiration as a protective mechanism has been experimentally confirmed.

Scientific novelty of the work. For the first time in the dissertation:

- Based on radioecological and dosimetric studies conducted on the Absheron Peninsula, dose-dependent changes in ms-DLE parameters were determined in dominant plant species (*Zygophyllum fabago* L., *Juncus* L.) in local areas with high radiation backgrounds. This allows for the use of this parameter as a bioindicator of chronic radiation exposure in field conditions.
- Paramagnetic centers in some medicinal plants of Azerbaijan were investigated by the EPR spectroscopy method, the correlation of signal amplitudes with the degree of environmental pollution was established, and their indicator properties were identified.
- The effect of γ-radiation on laboratory rats (*Wistar Albino*) was studied using EPR spectroscopy. Based on the analysis of EPR spectra of the brain and liver of irradiated rats that received licorice root extract with their food, it was established that this extract has a radioprotective effect.
- New data were obtained on the dose-dependent effect of ionizing γ-radiation on the cytogenetic parameters and germination of seeds of some cereal crops (*Tríticum dúrum* Desf., *Hordeum vulgare* L., *Zea mays var. indentata*), which allowed for a comparative assessment of their radiosensitivity and the determination of their indicator properties.
- A comparative study of the effect of γ-radiation on seed germination and mitotic activity in plants with C3 (wheat, barley)

and C4 (corn) type photosynthesis was conducted. It was established that in C_3 type plants, irradiation at doses (100 Gy, 200 Gy) causes a stimulating effect, while inhibition is observed in C_4 type plants. The role of photorespiration as a protective mechanism has been experimentally confirmed.

Practical and theoretical importance of the work. The mechanisms of the influence of ionizing γ-radiation, UV-B radiation, and other abiotic stressors on the photosynthetic and genetic apparatus of plants have been investigated. The role of free radicals and paramagnetic centers in oxidative stress and plant adaptation has been established. Knowledge about the radiosensitivity and radioresistance of plants has been deepened, including a comparative analysis of C3 and C4 plants and the role of photorespiration. The mechanisms of adaptation of *Dunaliella salina* to extreme environmental factors have been studied. Data on the effects of heavy metal salts on Dunaliella salina can be used to develop biotests and optimize cultivation. Radioprotective effects of plant extracts of licorice root have been proposed and substantiated, opening up prospects for the development of radioprotective drugs. The parameters of ms-DLE and EPR spectra are proposed as express bioindicators for environmental monitoring (radioactive and chemical pollution, UV-B exposure). The results on the radiosensitivity of cereal crops can be applied to assess the resistance of varieties to radiation and develop agricultural technologies in areas with an elevated radiation background.

Approbation of the work. The results of the dissertation have been published in the following scientific publications: “Biological Issues” at the Republican Scientific Conference (Baku, 2008); “Achievements of Biological Science” at the Republican Scientific Conference (Baku, 2009); "Ecology. Man. Society" at the XIII International Scientific and Practical Conference (Kyiv, 2010); “XXI “Actual Problems of Biology” at the Republican Scientific Conference (Baku, 2010); I Republican Scientific Conference “Issues of Applied Ecology” (Baku, 2011); XVIII International Youth Scientific Conference "Students, PhD students and Young Scientists" of Lomonosov Moscow State University (Moscow, 2011);

“Theoretical and Applied Issues of Modern Biology” at the International Scientific Conference (Baku, 2011); “Prospects for the Peaceful Use of Atomic Energy” at the V International Conference (Baku, 2012); “Ecology, Nature and Problems of Society” at the II International Scientific Conference (Baku, 2012); “Radiation Research and Their Practical Aspects” at the VIII Conference (Baku, 2013); “Innovative Issues of Modern Biology” at the IV International Scientific Conference (Baku, 2014); “Modern Biology and Actual Problems” at the Republican Scientific Conference (Ganja, 2014); “Experimental Biology in Perspectives” at the Republican Scientific Conference (Baku, 2014); XXII International Youth Scientific Conference "Students, PhD students and Young Scientists" (Moscow, 2015); “Innovative Issues of Modern Biology” at the VII International Scientific Conference (Baku, 2017); “Coordination of Actions: Analysis of Actual Problems” at the International Scientific Conference (Baku, 2017); “Biological, Ecological Sciences and Applications” at the 1st International Conference (BESA) (Luxor, 2017); International Conference “Modern Trends in Physics” (Baku, 2017); “Actual Problems of Modern Natural and Economic Sciences” at the International Scientific Conference (Ganja, 2018); “Ecology and Soil Science in the XXI Century” at the IV Republican Scientific Conference (Baku, 2023); “Conservation of Biodiversity of Eurasia: Modern Problems, Solutions and Perspectives” at the 1st International Conference (Andijan, 2023); “Collection of Scientific Papers” at the VII Congress of Biophysicists of Russia (Krasnodar, 2023); "Biophysics of Complex Systems. Computational and Systems Biology. Molecular Modeling" Symposium with international participation (Moscow, 2023); “Modern Scientific Trends and Youth Development” at the XXIX International Scientific and Practical Conference (Warsaw, 2023); “Science of the XXI Century: Searches, Problems, Development Prospects” at the XXV International Scientific and Practical Conference (Paris, 2024); IV International Scientific and Practical Conference "Science, Technology, Innovation: Global Trends and Regional Aspect" (Tallinn, 2024); “Actual Problems of Science Development: Trends and Innovations” at the XIX International Scientific and Practical

Conference (Oslo, 2025); "A New Stage of Land Reform and Solving Environmental Problems" at the Republican Scientific Conference (Baku, 2025), "Application of digital and GWAS-based technologies for the wheat improvement" International Conference & Field Workshop (Baku, 2025).

Based on the results of the dissertation, 43 scientific works have been published, including 10 articles and 33 abstracts of reports and materials from scientific conferences. Four articles are in international and six articles were published in republican publications. Four articles have an index in databases (Pub Med, Web of Science, Springer, Q1 Scopus).

Name of the organization where the dissertation work was performed. The dissertation work was completed at the department of Biophysics and biochemistry of Baku State University.

Structural sections and total volume of the dissertation.

The dissertation consists of an introduction (18 317 characters), 5 chapters: Chapter I (52 816 characters), Chapter II (44 621 characters), Chapter III (58 271 characters), Chapter IV (22 722characters), Chapter V (20 294 characters), a conclusion (7 681 characters), conclusions (1 852 characters), practical recommendations (709 characters), a bibliography of 241 literary references, and the list of abbreviations (1 492); it is presented on 212 computer pages, with a total volume of 228 775 characters. The dissertation includes 74 figures and 10 tables.

MAIN CONTENT OF THE DISSERTATION

In the INTRODUCTION section of the dissertation, the relevance of the topic, the degree of development, object and subject of research, the purpose and tasks of the research, research methods, the main provisions submitted for defense, scientific novelty of the work, practical and theoretical importance of the work, approbation of the work, name of the organization where the dissertation work was performed.

CHAPTER I. LITERATURE REVIEW

The radioecological state of the Absheron Peninsula is examined in detail, characterizing the main sources and levels of radioactive and chemical pollution. The mechanisms of the influence of ionizing γ-radiation and UV-B radiation on living organisms, on the damage to the genetic apparatus and the photosynthetic system, are analyzed. The principles and diagnostic capabilities of the chlorophyll ms-DLE and EPR spectroscopy methods in biophysical research are described. Information on the flora of medicinal plants of Azerbaijan, their potential use, and possible accumulation of pollutants is provided. Special attention was paid to the adaptive features of the halophilic microalga *Dunaliella salina* to extreme environmental factors, including salinity, temperature, and illumination, as well as its response to the toxic effects of heavy metal salt ions.

CHAPTER II. MATERIALS AND METHODS OF RESEARCH

As part of the research, the influence of various stress factors, such as radioactive contamination, UV-B radiation, gamma irradiation, as well as the effects of heavy metal salts and nanoparticles, on biological objects was studied. The main research objects were field plants: bean caper (*Zygophyllum fabago* L.), rush (*Juncus* L.), as well as some species of medicinal plants: rosemary (*Salvia rosmarinus* Spenn.), dog rose (*Rosa majalis* Herrm.), hawthorn (*Crataegus* Tourn. ex L.), and eucalyptus (*Eucalyptus* L. Her.).

Laboratory objects included: the ornamental plant Chinese rose (*Hibiscus rosa sinensis* L.), cereal crops (Hordeum vulgare L., durum wheat *Triticum durum* Desf., dent corn *Zea mays var. indentata*), aquatic plants (*Elodea canadensis* Michx., *Vallisneria* L.), the unicellular microalga *Dunaliella salina* (strain *IPPAS* D-294), and a laboratory white rat (*Wistar Albino*) as an animal object.

Radioecological studies were conducted on the Absheron Peninsula to identify local areas with an elevated gamma radiation background. Measurements of the exposure dose rate of gamma radiation (μR/h) were carried out at a height of 5 cm above the ground surface using a MKS-AT 1125 dosimeter-radiometer (Atomtex) with a NaI (TI) 025x40 mm detector. The influence of UV-B radiation on the photosynthetic activity of plant samples was studied. The initial activity was determined under visible light conditions (150/300 W lamps with focusing lenses and a water heat filter) using Carl Zeiss Jena interference light filters. UV-B irradiation (PRK-2 mercury lamp) was carried out at a distance of 25 cm using a UFS-2 light filter, which transmits UV and absorbs visible light. To minimize thermal effects, the samples were thermostated with ice. Dose-dependent effects of gamma radiation on biophysical and cytogenetic parameters were studied. The radionuclides cobalt-60 (^{60}Co) and cobalt-57 (^{57}Co) were used as sources, and irradiation was carried out on RXUND-20000 and MRX-γ25 gamma installations in the range of absorbed doses from 6 to 300 Gy. The effect of radioactive contamination on the photosynthetic apparatus was studied using the method of ms-DLE registration (time-dependent chlorophyll fluorescence) to assess its condition, analyze changes in parameters, and resistance to stress factors. The ratio of the initial and final amplitudes of ms-DLE and induction curves were analyzed. Laboratory cultivation of the microalga *Dunaliella salina IPPAS* D-294 was carried out to study its reaction to abiotic factors and toxic effects. Cultivation was carried out in a medium with varying concentrations of NaCl (1.5 M moderate, 3.0 M high salinity), at a temperature of 15-40 °C (optimum 27-30 °C) and light intensity up to 30 W/m^2 (optimum 25-30 W/m^2). The effect of heavy metal salts (Cu, Fe, Hg, Pb, Co, Cd, Ni) at concentrations of 10^{-8}-10^{-3} M, as well as iron (Fe) and copper (Cu) nanoparticles, were studied. Assessment of the functional state of the photosynthetic apparatus was performed by the ms-DLE method. Quantitative assessment of culture growth was performed by counting cells using a Goryaev counting chamber. EPR spectra were used to study paramagnetic centers in the photosynthetic apparatus and the generation of ROS under stress effects. Spectrum registration was performed on an EPR spectrometer (Bruker) at room temperature (modulation amplitude 0.4 mT, microwave power ~20 mW, central field 3000 Gs, width 5500 Gs). Cytogenetic studies were aimed at studying the effect of gamma radiation (100-300 Gy) on the germination and cytogenetic parameters of seeds of cereal plants (*Triticum durum* Desf., *Hordeum vulgare* L., *Zea mays var. indentata*). The objects were germinated at 24.5 °C for 3-5 days. Cytogenetic analysis of rootlets included treatment with colchicine, fixation, staining with acetocarmine, and preparation of crushed preparations. Microscopic analysis were performed at mitosis stages using a ZEISS Axio Imager A2 microscope. Visualization of the microstructure of the surface of biological objects was performed by the method of scanning electron microscopy (SEM). Statistical data processing was performed in IBM SPSS Statistics 26.0 software. The Student's t-test was used to determine statistically significant differences. All measurements were performed in five replicates (n=5). The results are presented as the arithmetic mean ± standard deviation (M ± SD) or mean ± standard error (M ± SEM). The critical level of significance: $p \leq 0.05$.

CHAPTER III. THE EFFECT OF ABIOTIC STRESS FACTORS ON THE PARAMETERS OF MILLISECOND DELAYED LIGHT EMISSION OF CHLOROPHYLL

3.1. Ms-DLE of chlorophyll from the leaves of bean caper (*Zygophyllum fabago* L.) and rush (*Juncus* L.) collected from radioactively contaminated territories

The field objects under investigation, collected from the territory of the defunct Iodine Plant in the settlement of Ramana, where the source of the radioactive background is activated carbon, include the plants bean caper (*Zygophyllum fabago* L.) and rush (*Juncus* L.). In addition to the field objects we studied, some trees and shrubs also grow there in the habitats of these plants, and the exposure dose rate (EDR) was measured (Table 1).

Table 1.
Results of exposure dose rate measurements

№	Plant Name	EDR (μR/h)
1	Rush (*Juncus* L.)	4-123
2	Bean Caper (*Zygophyllum fabago* L.)	3-135
3	Fennel (*Foeniculum Vulgare* Mill.)	2-110
4	Camel Thorn (*Alhagi* Gagnebin.)	2-119
5	Olive (*Elaeagnus angustifolia* L.)	3-108
6	Bulrush (*Scirpus Lacustris* L.)	2-116

Next, using the ms-DLE method, we investigated the changes in the induction curves in the plants (*Zygophyllum fabago* L., *Juncus* L.) collected from the radioactively contaminated territories of the Baku Iodine Plant in the Ramana settlement.

Figure 1 presents the ms-DLE induction curves recorded from plants of bean caper (*Zygophyllum fabago* L.) where the radiation background varied within the range of 3-135 μR/h. The curves reflect a dose-dependent effect, expressed in a decrease in the amplitudes of all phases of the induction curve: the fast phase (FP), the slow phase (SP), and the stationary level (SL). This indicates a disruption of the functional activity of PSII, where the primary capture of light energy and electron transport occur. Ionizing radiation has an inhibitory effect on the ms-DLE parameters. The decrease in the kinetic indicators of ms-DLE indicates damage to the components of PSII and a disruption of the efficiency of electron transport within it, which reduces the probability of charge recombination ($P680^{+}Q_{a}^{-}$), which underlies the generation of ms-DLE, and indirectly reflects changes in the formation of a proton gradient on the thylakoid membranes.

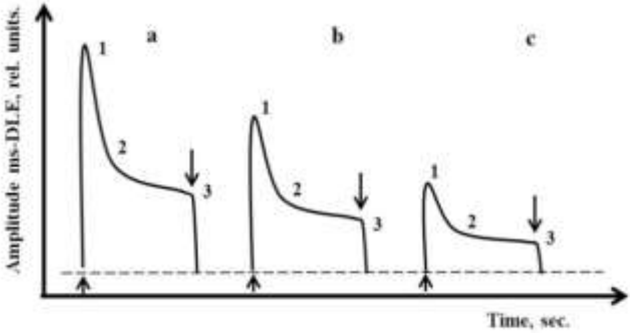


Figure 1. Induction curves of ms-DLE of chlorophyll from the leaves of bean caper (*Zygophyllum fabago* L.), where A - control, B - radiation dose 24 μR/h, C - radiation dose 45 μR/h. 1-FP, 2-SP, 3-SL

3.2. Ms-DLE of chlorophyll from the leaves of Chinese rose (*Hibiscus rosa-sinensis* L.) under the action of UV-B radiation

The study of the effect of UV-B radiation on the leaves of Hibiscus rosa-sinensis L. revealed its inhibitory effect on ms-DLE, which is an indicator of the state of PSII and ETC.

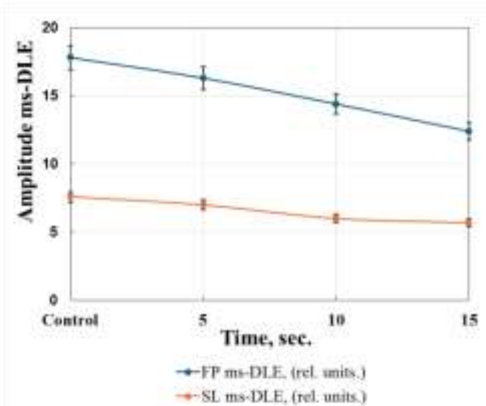


Figure 2. Dependence of the amplitude of FP and SL of ms-DLE induction in the leaves of *Hibiscus rosa-sinensis* L. on the time of UV-B radiation dose, ($p < 0.05$, n=5)

The study showed that exposure to UV-B radiation causes photoinhibitory stress. The main target of this stress is the damage to PSII. The fast component of ms-DLE shows greater sensitivity to stress changes compared to the slow component, which makes it an indicator in the work of the photosynthetic apparatus.

Thus, UV-B radiation causes dose-dependent suppression of ms-DLE, which indicates a disruption of the processes of primary charge separation in PSII and the subsequent transfer of electrons through the chloroplast ETC, leading to a decrease in the overall efficiency of photosynthesis. The ms-DLE parameters FP and SL are sensitive indicators of the stress effects of UV-B radiation on the photosynthetic apparatus of plants, reflecting the degree of its inhibition.

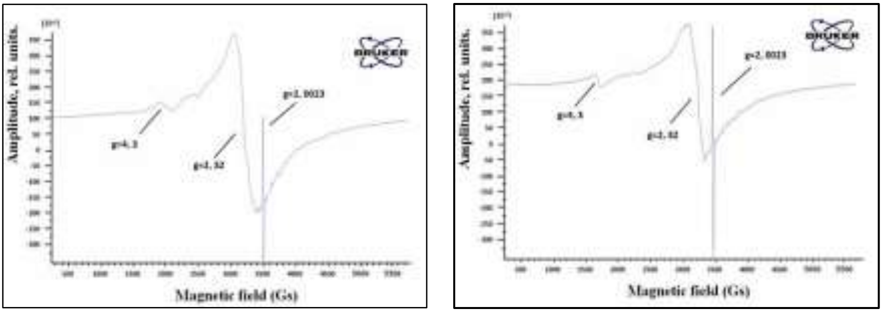
CHAPTER IV. STUDY OF PARAMAGNETIC CENTERS BY THE EPR METHOD UNDER THE INFLUENCE OF VARIOUS ABIOTIC STRESS FACTORS

4.1. Study of paramagnetic centers of medicinal plants by EPR spectroscopy

The objects of the research were the leaves of medicinal plants: rosemary (*Salvia rosmarinus* Spenn.), dog rose (*Rosa majalis* Herrm.), hawthorn (*Crataegus* Tourn. ex L.), and eucalyptus (*Eucalyptus* L. Her.). The leaves of the studied plants were collected from different regions of Azerbaijan. The rosemary plant was collected from Baku, Barda, Absheron, Mardakan settlement, dog rose and hawthorn plants were collected from Barda and Goychay regions, and eucalyptus from Absheron, Mardakan settlement, and Baku.

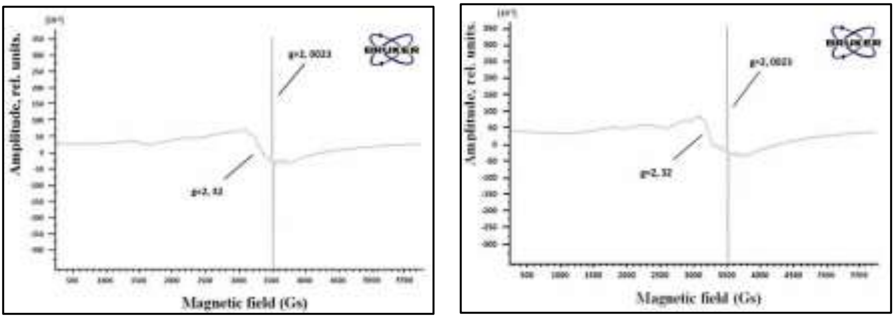
During the study of plant material, three characteristic signals were recorded in a wide range of the magnetic field: signals of free radicals (g=2.0023), signals of iron ions (g=4.3), and magnetic nanoparticles of iron oxide (g=2.32). A clear pattern was established: the higher the level of pollution of the area from which the leaves were collected, the greater the amplitude of the recorded signals. By studying the characteristics and changes of EPR signals for each plant species depending on the collection sites (Baku, Barda, Absheron, Goychay region), it is possible to identify correlations between the change in EPR signals and the level of environmental pollution, as well as to assess the potential for using plants as bioindicators.

Figures 3, 4, and 5 present the EPR spectra of paramagnetic centers of plant leaves collected from different regions of Azerbaijan.



a b

Figure 3. EPR spectra of paramagnetic centers of leaves of (a) rosemary (microwave frequency: 9.858 GHz, power: 2.149 mW), (b) eucalyptus (microwave frequency: 9.859 GHz, power: 2.151 mW) collected in the Mardakan settlement



a b

Figure 4. EPR spectra of paramagnetic centers of leaves of (a) dog rose (microwave frequency: 9.855 GHz, power: 2.164 mW), (b) hawthorn (microwave frequency: 9.855 GHz, power: 2.164 mW), collected in the Barda region

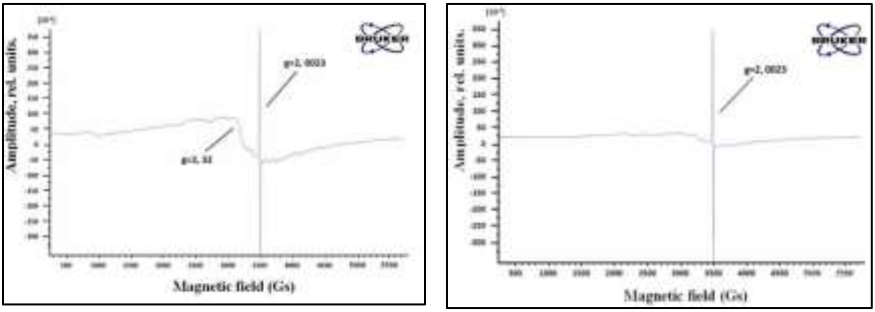


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Figure 5. EPR spectra of paramagnetic centers of leaves of (a) dog rose (microwave frequency: 9.857 GHz, power: 2.166 mW), (b) hawthorn (microwave frequency: 9.857 GHz, power: 2.171 mW), collected in the Goychay region

Iron ions (Fe^{3+}, g=4.3) are a cofactor of electron transport chain (ETC) proteins in photosynthesis, such as cytochrome b_6f, ferredoxin, and Photosystem I (PSI). Stress can disrupt these iron-containing proteins, which is reflected in the EPR signal.

Manganese ions (Mn^{2+}, g=2.01) are the main component of the water-oxidizing complex (WOC) of PSII. They are involved in the oxidation of water. The six-component Mn^{2+} signal can indicate the presence of free or weakly bound manganese, or changes in the manganese cluster of the WOC under stress, which affects the initial stages of photosynthesis. Magnetic nanoparticles of iron oxide (g=2.32) are an indicative stress parameter. Their formation is not a direct component of photosynthesis, but it is associated with the plant's response to stress, including heavy metal pollution and oxidative stress. The accumulation of excess iron and ETC damage, leading to the formation of ROS, can contribute to the oxidation and precipitation of iron in the form of oxide nanoparticles. EPR spectroscopy allows not only the identification of various paramagnetic centers but also the connection of these centers to disturbances in photosystems and ETC caused by abiotic stress. The accumulation of magnetic iron oxide nanoparticles in plant tissues serves as a sensitive indicator of severe stress. The formation of magnetic iron oxide nanoparticles in plant tissues is an indicator of severe stress, indicating the accumulation of excess iron and damage to cellular structures.

Thus, the level of accumulation of these nanoparticles can serve as a reliable indicator of pollution, which makes these plants promising for biomonitoring.

4.2. Study of the radioprotective properties of licorice root extract (*Glycyrrhiza glabra* L.) in gamma-irradiated laboratory white rats (*Wistar Albino*) by EPR spectroscopy

Within the framework of this work, a study of the protective properties of licorice (*Glycyrrhiza glabra* L.) on Wistar laboratory rats, which were subjected to radiation exposure, was conducted. For the experiment, γ-irradiation doses of 6 Gy and 8 Gy were chosen, which are significant but sublethal, as the lethal dose for rats is about 8-10 Gy. Three-month-old rats were divided into three groups: (1) a control group, (2) a group that underwent γ-irradiation (source ^{60}Co, D=0.286 rad/sec) at doses of 6 Gy and 8 Gy, (3) a group that, after similar irradiation, received licorice root extract for three months. At the end of the experiment, internal organs were extracted, which were dried at room temperature and prepared for further analysis by EPR spectroscopy on a (Bruker, Germany) installation. The main task of the work was to study paramagnetic centers in the brain and liver tissues of rats using EPR spectroscopy to assess the radioprotective properties of licorice root extract.

To compare with the irradiated groups that received licorice, control animals that were also administered licorice, but without irradiation, were used (figures 6, 8). Its protective properties are due to the presence of biologically active compounds, primarily glycyrrhizin acid and flavonoids (liquiritigenin, isoliquiritigenin). These substances have pronounced antioxidant and anti-inflammatory properties, neutralizing free radicals formed during the irradiation and promoting cell recovery. It was found that at a dose of 8 Gy, licorice effectively protected the rat's brain and liver due to its antioxidant components (figures 7, 9).

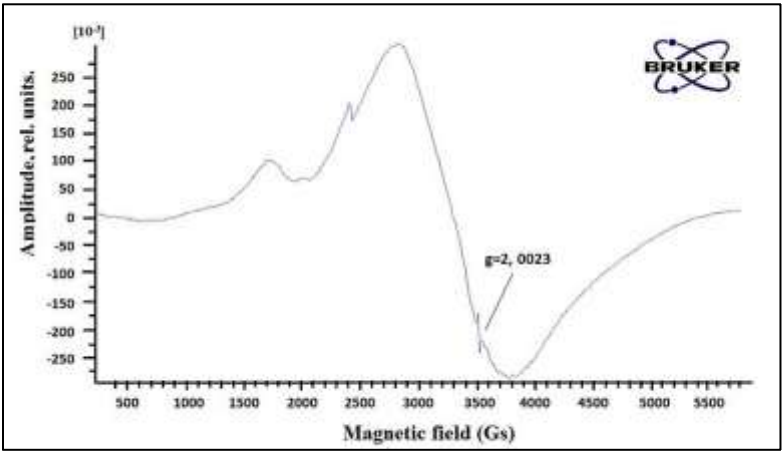

Figure 6. EPR spectrum of the brain of control laboratory rats fed with licorice root extract, (microwave frequency: 9.862 GHz, power: 2.154 mW)

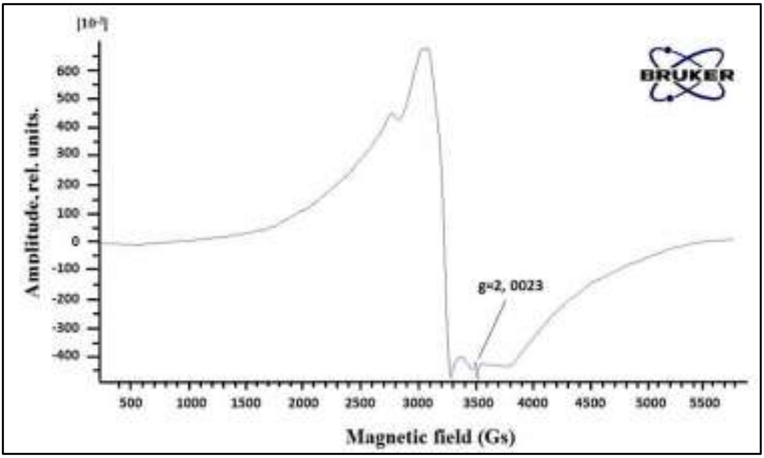

Figure 7. EPR spectrum of the brain of laboratory rats fed with licorice root extract, γ-irradiated 8 Gy, (microwave frequency: 9.869 GHz, power: 2.142 mW)

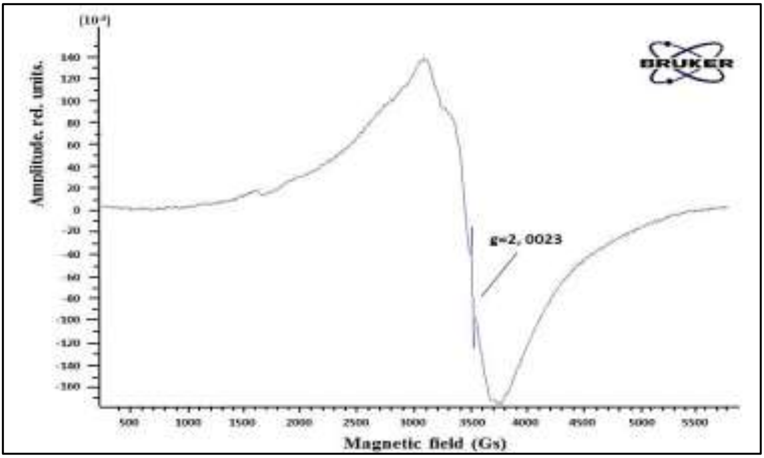

Figure 8. EPR spectrum of the liver of control laboratory rats fed with licorice root extract, (microwave frequency: 9.860 GHz, power: 2.161 mW)

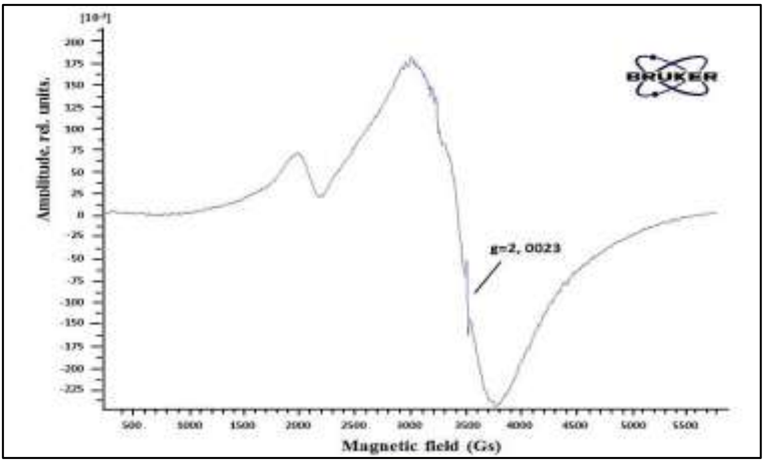

Figure 9. EPR spectrum of the liver of a laboratory rat fed with licorice root extract, γ-irradiated 8 Gy, (microwave frequency: 9.860 GHz, power: 2.147 mW)

The study confirmed that licorice extract has radioprotective properties, protecting the cells of the brain and liver from radiation exposure[6]. This effect is associated with the antioxidant activity of its components. The EPR spectroscopy method confirmed the mitigation of the negative consequences of irradiation, and also revealed that the action of licorice can affect biomineralization[7] in tissues, which opens up new directions for studying its mechanisms of action.

CHAPTER V. STUDYING THE EFFECT OF RADIOACTIVE CONTAMINATION ON THE GENETIC APPARATUS OF THE STUDIED PLANT SPECIES

5.1. Studying the effect of radiation on the dynamics of seed germination (*Hordeum vulgare* L.), (*Tríticum dúrum* Desf.), and (*Zea mays var. indentata*)

For laboratory work, the following seed samples were used: common barley (*Hordeum vulgare* L.), durum wheat (*Tríticum dúrum* Desf.), and dent corn (*Zea mays var. indentata*). Studies were conducted with control seed samples and samples irradiated with various doses of ionizing γ-radiation (100 Gy, 200 Gy, 300 Gy), with 500 seeds of each sample. The seeds were irradiated on the MRX - γ25, (Co^{60}) equipment. The number of fixed rootlets grown from the seeds in the size (1 – 1.5 mm) is shown in Table 2. The counting of the total number of germinated seeds (control and irradiated with various doses of γ-radiation (100 Gy, 200 Gy, 300 Gy) is presented in tables (3, 4, 5).

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[6] Атамова, Н.Г. Изучение радиопротекторных свойств экстрактов *Glycyrrhiza glabra* L. L. на облученных крысах линии Wistar / Н.Г.Атамова, А.М.Мусаев // Вестник НАНА, Серия биологические и медицинские науки. - Баку: - 2022. - Т. 77, № 1. - с. 50-57.

[7] Nudelman, F. In vitro models of collagen biomineralization / F. Nudelman, A.J.Lausch, N.A.Sommerdijk [et al.] // Journal of Structural Biology, – 2013. 183 (2), – p. 258-269.

Table 2.
Number of fixed rootlets in the size (1 – 1.5 mm)

№	Variants	Seed Samples		
		Hordeum vulgare L.	*Tríticum dúrum* Desf.	*Zea mays var. indentata*
1	Control	75 ±4,1	75±5,1	80±5,8
2	100 Gy	85 ns ±4,5	50*±4,5	50**±4,1
3	200 Gy	45*±3,1	60 ns ±5,0	40***±3,9
4	300 Gy	40***±2,8	30** ± 3,5	25***±2,5

Note: *, **, * denote statistical significance at a probability of p≤0.001, p≤0.01, and p≤0.05, respectively, and ns - no significance**

Table 3.
Counting the total number of germinated seeds of *Hordeum vulgare* L.

Variants	Total number of seeds	Number of germinated seeds			Germination, %
		Rootlet size (1-1.5 mm)	Rootlet size (0.1-0.8mm)	Total	
Control	500	75 ±4,1	50 ±3,2	125 ±5,5	25,0 ±1,9
100 Gy	500	85 ns ±4,5	80**±4,2	165**±6,1	33,0** ±2,5
200 Gy	500	45*±3,1	50ns±3,5	95*±4,8	19,0* ±1,5
300 Gy	500	40***±2,8	30**±2,2	70***±4,0	14,0***±1,2

Note: *, **, * denote statistical significance at a probability of p≤0.001, p≤0.01, and p≤0.05, respectively, and ns - no significance**

Table 4.
Counting the total number of germinated seeds of *Tríticum dúrum* Desf.

Variants	Total number of seeds	Number of germinated seeds			Germination, %
		Rootlet size (1-1.5 mm)	Rootlet size (0.1-0.8mm)	Total	
Control	500	75±5,1	110±6,8	185±9,3	37,0±1,8
100 Gy	500	50*±4,5	225***±11,2	275**±12,1	55,0**±2,4
200 Gy	500	60 ns ±5,0	250***±13,5	310***±14,4	62,0***±2,9
300 Gy	500	30** ± 3,5	95* ± 6,1	125*** ± 7,0	25,0*** ±1,4

Note: *, **, * denote statistical significance at a probability of p≤0.001, p≤0.01, and p≤0.05, respectively, and ns - no significance**

Table 5.
Counting the total number of germinated seeds of *Zea mays var. indentata*

Variants	Total number of seeds	Number of germinated seeds			Germination, %
		Rootlet size (1-1.5 mm)	Rootlet size (0.1-0.8mm)	Total	
Control	500	80±5,8	75±5,2	155±8,1	31,0±1,6
100 Gy	500	50**±4,1	10***±1,5	60***±4,8	12,0***±1,1
200 Gy	500	40***±3,9	5***±0,9	45***±4,2	9,0***±0,8
300 Gy	500	25***±2,5	0***±0,0	25***±2,5	5,0***±0,5

Note: *, **, * denote statistical significance at a probability of p≤0.001, p≤0.01, and p≤0.05, respectively, and ns - no significance**

Based on the results of tables (3, 4, 5), a diagram of the dynamics of the percentage germination of cereal plant seeds was presented (figure 10).

Figure 10 shows how the dynamics of the percentage germination of cereal crops changes: in C3-plants (barley), irradiation of seeds at a dose of 100 Gray caused an increase in the number of germinated seeds compared to the control. Increasing the dose of ionizing γ-radiation to 200 Gray and 300 Gray led to a significant decrease in the number of germinated seeds. In the second C3-type plant (wheat), irradiation of seeds at a dose of 100 Gray caused an increase in the number of germinated seeds compared to the control, and with an increase in the dose of ionizing γ-radiation to 200 Gray, an even greater increase in seed germination activity was observed, while at a dose of 300 Gray, seed germination significantly decreased. For the C4-plant (corn), doses of γ-radiation of 100 Gray, 200 Gray, and also 300 Gray, showed a consistent decrease in seed germination rates compared to the control.

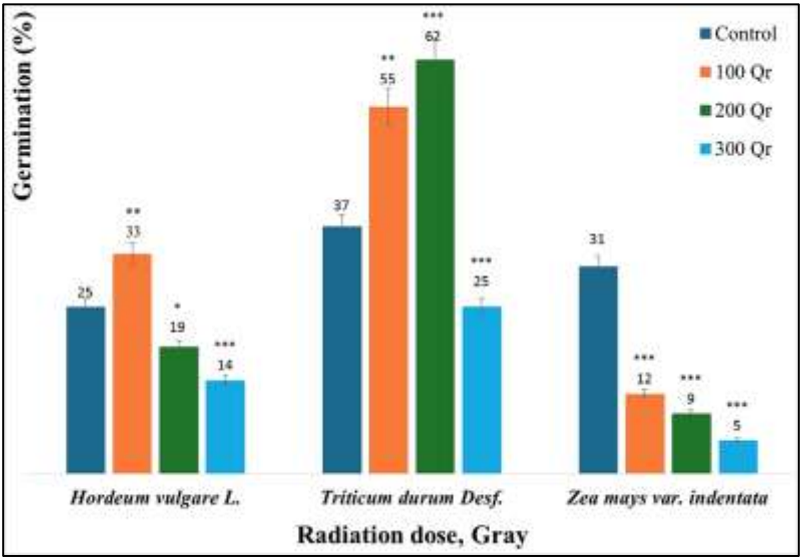

Figure 10. Dynamics of seed germination (*Hordeum vulgare* L., *Tríticum dúrum* Desf., *Zea mays var. indentata*), (ns - not significant, * - $p \leq 0.001$, ** - $p \leq 0.01$, and * - $p \leq 0.05$)**

Studies have shown that ionizing γ-radiation triggers the process of water radiolysis in cells, leading to the formation of highly reactive ROS, such as the superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2). These molecules cause oxidative stress, damaging key macromolecules: DNA, proteins, and membrane lipids, which inhibits metabolic processes, including germination.

5.2. Studying the effect of gamma radiation on the cytogenetic parameters of the studied plant species (*Hordeum vulgare* L., *Tríticum dúrum* Desf., *Zea mays var. indentata*)

A cytogenetic analysis was performed to calculate the mitotic index of these seeds. The cytogenetic analysis of the seeds (*Hordeum vulgare* L., *Triticum durum Desf.*) and (*Zea mays var. indentata*) at various doses of ionizing γ-radiation (100 Gy, 200 Gy, 300 Gy) is presented in tables (6). Referring to the obtained results of cytogenetic studies, diagrams of the dynamics of the mitotic cycle of control and irradiated cereal seeds were compiled (Figure 11).

As can be seen from Figure 11, at low doses of gamma radiation (100 Gy, 200 Gy), stimulation of active division in the mitotic cycle is observed in C3 plants, and at a higher dose (300 Gy), the stimulation process weakens. In C4-type plants, radiation has the opposite effect.

The process of photorespiration, which is one of the protective mechanisms of the plant cell, manifested itself to a greater or lesser extent in each of our subsequent experiments, depending on the radiation dose. This affected the change in morphological and genetic parameters in laboratory plants of the C3 and C4 types.

Table 6.
Calculation of the mitotic index

Variant	Total number of fixed seed rootlets (1-1.5 mm)	Germination, %	Number of dividing cells					Mitotic cycle (Mitoticl %)
			Cells analyzed (N)	Prophase (P)	Metaphase (M)	Anaphase (A)	Telophase (T)	
***Hordeum vulgare* L., chromosome set 2n=14**								
Control	75 ±4,1	25,0 ±1,9	322	32 ±2,1	16 ±1,4	11 ±0,9	6 ±0,5	20,18 ±1,12
100 Gy	85ns ±4,5	33,0** ±2,5	266	39* ±2,5	19 ns ±1,8	13 ns ±1,1	7 ns ±0,8	29,32** ±2,05
200 Gy	45* ±3,1	19,0* ±1,5	278	36 ns ±3,0	17 ns ±1,5	10 ns ±1,0	8 ns ±0,7	25,53* ±1,98
300 Gy	40 *** ±2,8	14,0 *** ±1,2	204	12 *** ±1,1	6 *** ±0,7	3 *** ±0,4	1 *** ±0,2	10,78 *** ±1,04
***Tríticum dúrum* Desf., chromosome set 2n=28**								
Control	75 ±5,1	37,0 ±1,8	283	28 ±2,5	19 ±1,9	16 ±1,6	9 ±0,9	25,44 ±1,55
100 Gy	50* ±4,5	55,0** ±2,4	262	37* ±3,1	16 ns ±1,8	14 ns ±1,5	8 ns ±0,8	28,62* ±1,82
200 Gy	60 ns ±5,0	62,0 *** ±2,9	234	33 ns ±2,9	20 ns ±2,1	13 ns ±1,4	11 ns ±1,1	32,90 *** ±2,13
300 Gy	30** ±3,5	25,0 *** ±1,4	218	11 *** ±1,2	4 *** ±0,5	2 *** ±0,3	3 *** ±0,4	9,17 *** ±0,95
***Zea mays var. indentata*, chromosome set 2n=20**								
Control	80 ±5,8	31,0 ±1,6	431	29 ±2,8	22 ±2,1	14 ±1,5	8 ±0,9	16,93 ±1,21
100 Gy	50** ±4,1	12,0*** ±1,1	393	11*** ±1,3	6*** ±0,8	7*** ±0,9	4*** ±0,5	7,12*** ±0,75
200 Gy	40*** ±3,9	9,0*** ±0,8	382	6*** ±0,8	5*** ±0,7	3*** ±0,4	5** ±0,6	4,97*** ±0,51
300 Gy	25*** ±2,5	5,0*** ±0,5	298	5*** ±0,6	3*** ±0,4	2*** ±0,3	2*** ±0,3	4,02*** ±0,44

Note: *, **, * denote reliability with a probability of $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively, and ns - no significance**

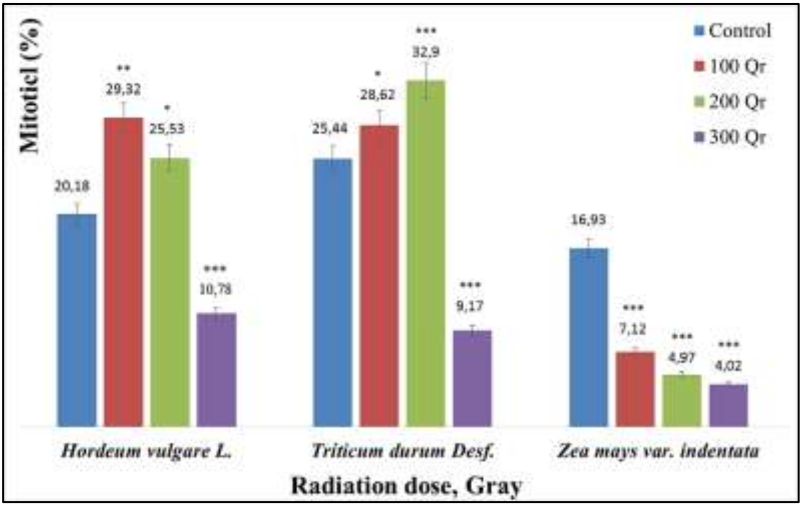

Figure 11. Dynamics of the mitotic cycle of seeds (*Hordeum vulgare* L., *Tríticum dúrum* Desf., *Zea mays var. indentata*), (ns - not significant, * - $p \leq 0.001$, ** - $p \leq 0.01$, and * - $p \leq 0.05$)**

5.3. Studying the role of photorespiration in the genetic of studied C3 and C4 plants under radioactive contamination

We conducted a series of genetic and morphological studies. As a result of the studies, the effect of radiation as a stress factor was revealed. For example, C3 cereal plants, wheat and barley, were taken for comparison with the control. As can be seen from the experiments, at low doses, a stimulating effect on the activity of rootlet growth is observed compared to the control. The growth slows down at a dose of 300 Gy of ionizing γ-radiation. In C4-type plants, for example, dent corn (*Zea mays var. indentata*), the activity of rootlet growth compared to the control decreases with an increase in the ionizing radiation dose. The intensity of active rootlet growth also affects the indicators of genetic research, having a direct impact on cell division in the mitotic cycle in both C3 and C4 type plants (Figure 12). There is a disruption of division in all its stages up to the formation of mutant traits such as (bridges, duplications). Our research has shown that photorespiration is the main mechanism of protection against oxidative damage to the vegetative organism[8,9].

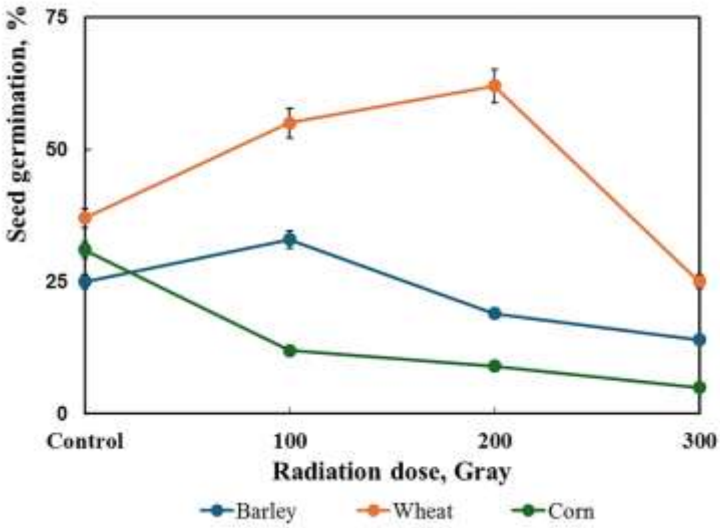

Figure 12. Dynamics of seed germination of C3 type cereals (*Hordeum vulgare* L., *Tríticum dúrum* Desf.) in comparison with C4 type plant seeds (*Zea mays var. indentata*), (ns - not significant, * - p ≤ 0.001, ** - p ≤ 0.01, and * - p ≤ 0.05)**

A comparative analysis of the studies (figures 12, 13) shows differences in the reaction of C3 and C4 plant types to radioactive contamination, largely due to their fundamental features of photosynthetic metabolism, at the center of which is photorespiration.

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[8] Ahmad, P. Reactive oxygen species, antioxidants and signaling in plants / P.Ahmad, M.Sarwat, S.Sharma // Journal of Plant Biology. – 2008. – Vol. 51, № 3. – p. 167-173.

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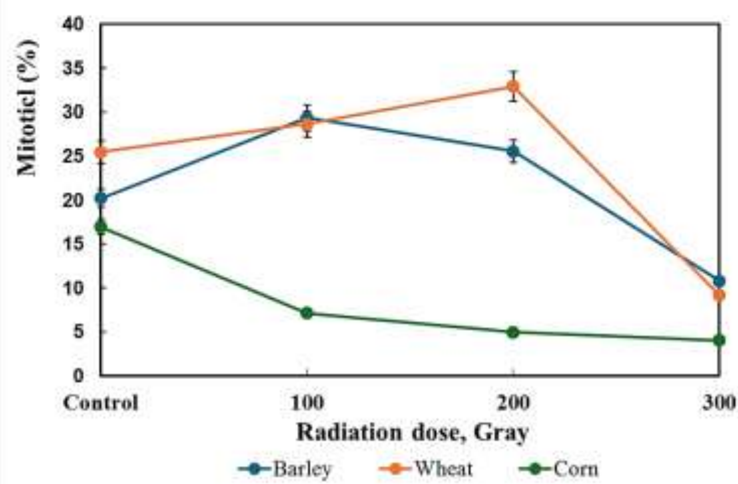

Figure 13. Cytogenetic analysis of cereal plants of the C3 type (*Hordeum vulgare* L., *Tríticum dúrum* Desf.) in comparison with the C4 plant type (*Zea mays var. indentata*), (ns - not significant, * - p ≤ 0.001, ** - p ≤ 0.01, and * - p ≤ 0.05)**

Based on the research, it can be concluded that photorespiration is not just a side process of photosynthesis, but the main metabolic pathway that determines the differential reaction of C3 and C4 plants to radioactive contamination. In C3 plants, an active photorespiratory cycle can provide a certain level of protection against moderate radiation stress, contributing to the dissipation of excess energy and the control of oxidative stress, which is confirmed by the temporary stimulation of growth and mitotic processes. In C4 plants, where photorespiration[10] is virtually absent, a more pronounced and dose-dependent inhibition of these processes is observed under the influence of ionizing γ-radiation.

[10] Чиков, В.И. Фотодыхание // Саратовский Образовательный Журнал, – 1996. № 11, – с. 2–8.

CONCLUSIONS

1. Based on radioecological and dosimetric studies of contaminated territories of the Absheron Peninsula, dose-dependent inhibition of ms-ZES parameters was observed in dominant plant species (*Zygophyllum fabago* L., *Juncus* L.), indicating damage to PSII and ETC, which allows for their use as bioindicators in environmental assessment.
2. UV-B radiation causes a dose-dependent decrease in the ms-DLE parameters in the studied plants, which is an indicator of the disruption of the processes of primary charge separation in PSII and electron transport in the ETC, and also reduces the overall efficiency of photosynthesis.
3. When studying some medicinal plants by the EPR spectroscopy method, signals associated with magnetic nanoparticles ($g \approx 2.32$) were found. The parameters of these signals can be used for biomonitoring purposes.
4. The microalga *Dunaliella salina IPPAS* D-294 shows characteristic mechanisms of adaptation to the effects of abiotic stressors (heavy metal salt ions, ionizing γ-radiation, temperature, light), which is manifested in a change in the ms-DLE indicators. The identified mechanisms indicate a significant resistance of the microalga *Dunaliella salina IPPAS* D-294 to stress factors.
5. Based on the data obtained using EPR spectroscopy in laboratory white rats (*Wistar Albino*), it was established that the licorice root extract (*Glycyrrhiza glabra* L.) has effective radioprotective activity under radiation exposure. The results obtained indicate the ability of the extract to minimize the negative effects of γ-radiation at the cellular level in the tissues of the brain and liver of laboratory white rats.
6. It has been established that ionizing γ-radiation has a dose-dependent effect on the cytogenetic parameters and germination of cereal seeds (*Tríticum dúrum* Desf., *Hordeum vulgare* L., *Zea mays var. indentata*). The stimulating effect of low doses (100 Gy, 200 Gy) on C3 plants, in contrast to the inhibition in C4 plants, emphasizes the role of photorespiration in the mechanisms of adaptation to radiation stress.

PRACTICAL RECOMMENDATIONS

1. The ms-DLE and EPR spectroscopy methods are recommended for use in radioecological studies to assess the state of plant objects and identify areas of radiation and chemical contamination.
2. The obtained data on the radiosensitivity of cereal crops can be used in agricultural selection to create varieties resistant to radiation exposure and to develop strategies for agricultural management in areas with an elevated radiation background.
3. The results on the effect of heavy metals on *Dunaliella salina* can be used to develop biotests to assess the toxicity of aquatic environments.
4. The identified radioprotective properties of licorice extract open up prospects for research on the development of radioprotective drugs.

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2. Nağıyev, C.Ə., Mahmudova, Ü.T., Fridunbəyov, İ.Y., Xəlilov, R.İ., Abşeron yarımadasında təbii və süni radionuklidlərin monitorin-qi: / – Azərbaycan xalqının böyük oğlu, ulu öndər H.Ə.Əliyevin anadan olmasının 85-ci ildönümünə həsr olunmuş "Biologiyanın müasir problemləri" mövzusunda Respublika Elmi Konfransının materialları, BDU, Bakı, Azərbaycan, 25-26 Aprel 2008. – s. 295-296.
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Address: Baku, academic Zahid Khalilov street, 33, AZ 1148

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