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# ABSTRACT

of the dissertation for the degree of Doctor of Science

# MECHANISMS OF THE INFLUENCE OF STRESS FACTORS ON THE FORMATION OF MAGNETIC PROPERTIES IN SOME LIVING SYSTEMS CHARACTERISTIC FOR THE ABSHERON PENINSULA

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# INTRODUCTION

**Actuality and degree of elaboration of the theme**. At the modern stage of scientific and technical progress, the development of interdisciplinary studies is characterized by searches carried out with the help of new high-tech approaches. In such a period, in connection with the protection and development of natural systems characteristic of the Apsheron Peninsula, conducting modern and promising scientific works aimed at their research, their biophysical and ecological research is one of the issues arising from historical necessity.

The Absheron Peninsula is rich in natural resources, consisting of living and non-living systems. Unfortunately, radioactive pollution, heavy metals, various toxic industrial and chemical wastes, as well as the intensification of other anthropogenic factors have a negative impact on various components of nature. Under the influence of these factors, the stratosphere is polluted, which leads to a decrease in the ozone layer and an increase in the intensity of ultraviolet (UV) rays falling on the Earth's surface, especially of its B-band (280-320 nm)[1].

At the same time due to the damaging effect of stress factors on natural systems, the changes taking place in them are intensively studied. Due to the susceptibility of living systems, characteristic of the Apsheron peninsula, to various stress factors (gamma radiation, radioactive pollution, UV radiation, etc.), the study of the mechanisms of influence of these factors is important and relevant. The impact of such stress factors on living systems leads to the formation of various toxic substances, reactive oxygen species (ROS), including free radicals. The study of structural-functional changes and new physico-chemical properties of living systems of the Apsheron peninsula under the influence of various stress factors is one of the urgent tasks.

It is clear that progress in this area, scientific achievement largely depends on the completeness of the research. To date, some

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[1] Nasibova, A.N., Achmedov, I.S., Khalilov, R.I. The effect of UV-B radiation on the thylakoid membranes of chloroplasts of higher plants // – Bulgaria. Proceedings of the University of Rousse, – 2009. – p. 171-177.

work has been carried out to study the influence of various stress factors on the living world. However, the phenomena of paramagnetism that arise under the influence of stress factors in living systems characteristic of the Absheron Peninsula have not been studied. Changes and new physicochemical properties of living systems as a result of exposure to stress factors have not been studied, and the mechanism of new phenomena has not been clarified. One of the main methods for studying these phenomena is the method of electron paramagnetic resonance (EPR) spectroscopy. This method is based on the interaction of matter with a magnetic field. The EPR method provides rich information about paramagnetic centers, which can be used to assess the degree of pollution of natural systems and biomonitoring of the environment, identifying structural and functional changes and new physicochemical properties caused by stress in living systems. At the same time, the EPR method is a very promising method for detecting the formation of magnetic nanoparticles in biological systems, providing new information for biomedical research.

Research in recent years shows that living matter, from bacteria to humans, is composed of naturally occurring magnetic iron oxide nanoparticles formed through biomineralization. It is known that the most common magnetic nanoparticles in living and inanimate nature are magnetite ($Fe_3O_4$) and maghemite ($\gamma$-$Fe_2O_3$). These biogenic nanophase magnetic structures have an important physiological role. Thus, they provide orientation in the Earth's magnetic field and can also be biomarkers of certain pathologies[2]. In this regard, it is very important to study the influence of stress factors on paramagnetic centers in living systems, to investigate the biogenic formation of magnetic iron oxide nanoparticles in them as a result of biomineralization, and also to study the mechanism of the formation of new magnetic nanoparticles.

Biomineralization of magnetic nanoparticles is a genetically controlled biochemical process that produces perfect ferromagnetic

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[2] Gubin, S.P. Magnetic nanoparticles: preparation, structure and properties / S.P. Gubin, Y.A. Koksharov, G.B. Khomutov [et al.] // Russian Chemical Reviews, – 2005. 74 (6), – p. 489-520.

crystals in living organisms[3]. Thus, the study of new physico-chemical properties, structural-functional changes that stress factors can create in living systems, and the clarification of their molecular mechanism are of great importance.

In modern times, synthesis of nanoparticles is carried out by various (physical, chemical, biological) methods. They are applied in almost all areas of human activity, especially in medicine, environmental protection, and many areas of industry. Iron oxide magnetic nanoparticles are the most widely used nanoparticles. These nanoparticles are used in various biological systems, including unicellular organisms, plants, fish, insects, birds, animals, etc. they can be formed biogenically in systems[3]. The study of the formation of biogenic iron oxide magnetic nanoparticles in living systems by the EPR spectroscopy method allows for a deep understanding of their formation and role. In modern times, magnetic nanoparticles, as well as functional nanosystems based on them, are popular objects of research in a number of fundamental and applied science fields due to their unique physico-chemical properties, "quantum size effects".

In this regard, the study of the phenomenon of paramagnetism in the natural systems characteristic of the Absheron Peninsula under the influence of stress factors is important from a fundamental point of view. With its help, it is possible to assess the degree of pollution of natural systems and biomonitoring of the environment, structural and functional changes caused by stress in living systems can be detected, and the results obtained can be applied in the field of modern biomedicine. The biogenicity of iron oxide magnetic nanoparticles and their low toxicity are of great interest for modern and promising biomedical research.

Taking this into account, the influence of stress factors on some living systems characteristic of the Absheron Peninsula was studied, and important as well as necessary results were obtained from the point of view of biophysical and biomedical application, and the

[3] Khomutov, G.B. Nanocomposite biomimetic vesicles based on interfacial complexes of polyelectrolytes and colloid magnetic nanoparticles / G.B. Khomutov, V.P. Kim, Y.A. Koksharov [et.al] // Colloids and Surfaces A: Physicochemical and Engineering Aspects, – 2017. V.532, – p. 26-35.

evaluation of the ecological state of the environment based on field studies and laboratory experiments. In this regard, as a result of the influence of stress factors, in various types of trees and shrubs widely distributed on the Absheron peninsula, in some plant samples germinated in laboratory conditions as a model system, as well as in some animal organisms, research of emerging paramagnetic centers, the study of morphological, structural-functional changes and emerging new magnetic properties necessitated the implementation of the dissertation work.

**The object and subject of the research:** During the dissertation work*, in vivo* and *in vitro* studies were carried out with plant samples. The main objects of research were many trees and shrubs that are widespread on the Absheron Peninsula. These include: leaves, stems, seeds and seed pods of camelthorn (*Alhagi pseudalhagi* L.), bean-caper (*Zygophyllum fabago* L.), fennel (*Foeniculum vulgare* M.), reed (*Scirpus lacustris* L.), rush (*Juncus Acutus* L.), oleaster (*Elaeagnus angustifolia* L.) which are dominant in the township of Ramana (territory of the currently inactive Iodine plant); olive (*Olea europea* L.), fig (*Ficus carica* L.), eldar pine (*Pinus eldarica* M.), and pomegranate (*Punica granatum*) leaves, which are valuable tree species widespread in the Absheron Peninsula; leaves of ornamental plants such as pyracantha bright red (*Pyracantha coccinea*), privet (*Ligustrum* L.), etc. Along with plants growing in nature, wheat (*Triticum L.*)*,* corn (*Zea mays L.*)*,* pea (*Cicer arietinum L.*) plant seeds were studied as a model system in laboratory conditions. Some aquatic plants have also been studied as model systems: aquatic limnophila (*Limnophila aquatica*) and elodea (*Elodea canadensis*). Experiments with some animal organisms were continued to demonstrate the generality and universality of the new results obtained in the course of the conducted research. At that time, the object of research was grape snails with shell (*Helix pomatia L.*) collected in various areas of the Absheron Peninsula (Academy Garden, Govsan gardens and Guneshli township), and laboratory rats (*Wistar Albino*) were selected as a model system. The subject of the study is the research of paramagnetic centers under the influence of stress factors in living systems, characteristic of the Apsheron

Peninsula. At the same time, showing the role of stress factors in the formation of new magnetic properties in living systems as a result of biomineralization was the main subject of research.

**Goals and objectives of the study:** The main objective of the research is to study in living systems characteristic of the Absheron Peninsula (various types of trees and shrubs, higher aquatic plants, chloroplasts, some animal organisms, etc.) to investigate paramagnetic centers, to study new magnetic properties arising in them under the influence of stress factors (ionizing gamma radiation, radioactive contamination, UV radiation) in a wide range of magnetic fields, and to clarify the molecular mechanism of these phenomena.

In this regard, the following tasks were set in the dissertation work:

- Investigation of the effect of radiation factors on various types of trees and shrubs (leaves, stems, seeds, seed pods) widespread in Absheron Peninsula;
- Investigation of the mechanisms of formation of magnetic properties in some types of plants affected by various stress factors in Absheron Peninsula;
- Study of the influence of stress factors on C3 and C4 plants (wheat (*Triticum* L.), corn (*Zea mays L.*)) as a model system in laboratory conditions and giving the mechanism of the results obtained;
- Investigation of iron oxide magnetic nanoparticles transport and paramagnetic centers in some higher aquatic plants (*Limnophila aquatica, Elodea Canadensis*) ;
- Investigation of the mechanism of formation of magnetic properties as a result of biomineralization event during stress *in vitro* studies;
- Investigation of paramagnetic centers and new magnetic properties generated by gamma radiation in some animal organisms;
- Giving the mechanism of formation of biogenic nanophase magnetic particles in living systems under the influence of stress factors in general.

**Research methods**. Irradiation of samples with gamma radiation was carried out on the installations “RXUND – 20000”, "URI" and "MRX-γ25", irradiation with UV-B radiation was carried out on a DRT–230 Mercury-Quartz lamp with a linear radiation spectrum. The radiation background in the study areas was determined with the help of the MKC-at 1125 dosimeter-radiometer. Isolation of chloroplasts was carried out based on standard methods[4]. EPR studies were performed on spectrometers "BRUKER - EMX" (Germany) and "VARIAN e-4" (USA) with a range of 3 cm. Among the microscopy methods, the research methods were a scanning electron microscope (SEM, ZEIS) and a transmission electron microscope (TEM, JEM-1400 (Japan)). Using a CANBERRA gamma spectrometer, the content of radionuclides in the studied samples and their specific activity were determined.

**The scientific novelty of the research.** For the first time in the presented dissertation work:

- When studying the impact of stress factors on living systems - various types of plant and animal organisms characteristic of the Absheron Peninsula, a wide EPR signal ($g \approx 2,2-2,5$; $\Delta H=320-500$ G) was observed using the EPR method in a wide range of magnetic fields.
- It has been established that radioactive contamination causes the generation of a characteristic broad EPR signal ($g=2.32$; $\Delta H=320$ G) in the leaves and seeds of plants, and the intensity of the generated signal in the studied leaf samples is more intense than in the seeds. It was found that with increasing radiation dose, an increase in the intensities of EPR signals occurs.
- It has been shown that a characteristic broad EPR signal ($g=2.32$; $\Delta H=320$ G), which appears in living systems under the influence of stress factors, characterizes magnetic iron oxide nanoparticles. It has been established that biogenic crystalline magnetic nanoparticles of iron oxide are formed in living systems as a result of the phenomenon of biomineralization when exposed to stress factors.
- *In vitro* studies have shown that under stress resulting from

[4] Гавриленко, В.Ф., Ладыгина, М.Е., Хандобина Л.М. Лабораторное руководство по физиологии растений // Высшая школа, – Москва: –1975.

damage to thylakoid membranes, Fe3+ ions at certain loci of the electron transport chain (ETC) are reduced and converted into magnetic nanoparticles of iron oxide. The results obtained were confirmed by microscopic studies (by TEM).

- When studying the temperature dependence of EPR signals obtained from fig (*Ficus carica* L.) leaves (260K-60K), it was determined that in the behavior of the parameters of the amplitude and the width of the paramagnetic resonance line of the detected EPR signals at temperature of 120-125 K occurs a characteristic Vervey phase transition characteristics to magnetic nanoparticles (magnetite-$Fe_3O_4$ and maghemite-$\gamma Fe_2O_3$).
- The study of the effect of stress factors on the morphological characteristics of plants belonging to the C3 and C4 types of photosynthesis (wheat and corn) showed that these factors have a stimulating effect on the growth of C3 plants and a retarding effect on C4 plants up to a certain dose. It was found that the discovered pattern is associated with the activation of the photorespiration process, which performs a protective function by preventing the formation of reactive oxygen species (ROS) in C3 plants.
- By the EPR method, it was found that pomegranate *(Punica granatum* L.) extract has restorative properties. It has been shown that in certain solids, $Fe^{3+}$ ions are capable of causing the generation of nanophase iron oxide magnetic particles in pomegranate extract.
- The effect of gamma radiation and UV radiation on grape snails with shell was studied by the EPR spectroscopy method. The parameters and behavior of EPR signals characterizing magnetic nanoparticles of free radicals and iron oxide from their shell and body parts depending on the radiation dose were studied. The basics of the mechanisms of acquired patterns are given.
- For the first time, it was established by the EPR method that in the liver organs of laboratory rats, under the influence of gamma radiation, biogeneration of crystalline magnetic nanoparticles based on iron occurs. The resulting magnetic nanoparticles were visualized using TEM.
- It was discovered for the first time by the EPR spectroscopy method that as a result of the influence of stress factors, biogenic

crystalline magnetic nanoparticles of iron oxide and magnetic properties are generated as a result of the phenomenon of biomineralization in living systems. The mechanism of this phenomenon is given. The received new event has been confirmed by various methods.

**The theoretical and practical significance of the study.** The results of EPR studies are informative in biophysical, biomedical and ecological research. The results obtained are important for use in medicine for diagnostic and therapeutic purposes. The results obtained in studying the effect of stressors on plants by the EPR method are important in assessing and biomonitoring the ecological state of the environment. The parameters of the EPR spectra recorded at room temperature of living organisms during research can be used as bioindication parameters in environmental studies. The results obtained in studying the effect of stress factors on grape snails with shell give reason to say that grape snails with shell can be used as bioindicators in assessing the ecological state of the environment. The application of the results obtained in experiments with laboratory rats in modern biomedical research is very important. The results obtained are also of practical importance in the development of scientific foundations of radiation safety. The results of the dissertation were used in the preparation and implementation of grant projects supported by the Azerbaijan Science Foundation and STCU.

**The main provisions submitted for defense.**

- Radioactive contamination causes the generation of an broad EPR signal ($g \approx 2.2$-$2.4$; $\Delta H \approx 320$-$450$ G) in various plant organs. An increase in the background radiation causes the formation of more new paramagnetic centers.
- The manifestation of a stimulating effect when stress factors are applied to C3 plants up to a certain dose may be due to the fact that the photorespiration process is triggered and performs a protective function, preventing the formation of ROS.
- In *in vitro* studies, broad EPR signals ($g=2.4$; $\Delta H=320$ G) characterizing the formation of iron oxide magnetic nanoparticles are detected in chloroplasts obtained from higher plant leaves.
- Based on the study of paramagnetic centers in various types

of tea leaves, it has been shown that they have high antioxidant activity. Determination of higher antioxidant activity in samples of fresh tea leaves than in samples of packaged tea, based on EPR spectra.

- The revealed pattern in the peculiarities of changing EPR signals from body parts and shell parts of grape snails with shell, depending on the radiation dose, allows these living organisms to be used as bioindicators in assessing the ecological state of the environment.
- Based on studies conducted on laboratory rats, identification of the formation of magnetic iron oxide nanoparticles in their liver organs under the influence of gamma radiation by EPR and confirmation of the results by TEM.
- Determination of the presence of magnetic nanoparticles in oil samples extracted from some oil fields of the Absheron Peninsula, and the advantages of this.
- Generation of biogenic crystalline iron oxide magnetic nanoparticles and emergence of new magnetic properties as a result of biomineralization event in living systems under the influence of stress factors.
- As a result of the phenomenon of biomineralization in living systems, biogenic crystalline magnetic iron oxide nanoparticles and new magnetic properties are formed when exposed to stress factors.

**Approbation of the work.** The results of research on the topic of the dissertation were discussed at various national and international scientific conferences and symposiums: Prospects for the peaceful use of nuclear energy. III International conference (Baku, 2010); International conference. “Photosynthesis Research for Sustainability” (Baku, 2011); Prospects for the peaceful use of nuclear energy.. IV International conference (Baku 2011); International Conference of the Russian Academy of Sciences, Radiobiological Society, "Medical-biological problems of radiation action" (Moscow, 2012); International Scientific Conference. Soils of Azerbaijan: genesis, geography, melioration, rational use and ecology (Baku-Qabala, 2012); International Conference «Nuclear Science and its Application» (Samarkand, 2012); The V İnternational conference.

Perspectives of Peaceful Use of Nuclear Energy. ANAS, IRP (Baku, 2012); “Radiation studies and their practical aspects” VIII Conference, It is dedicated to the 65th anniversary of Academician M.K. Karimov. ANAS İnstitute of Radiation Problems (Baku, 2013); XII International Conference on Nanostructured Materials (Nano-2014) (Moscow, 2014); VII Congress on Radiation Research. (Moscow, 2014); The seventh Eurasian Conference Nuclear Science and its Application. (Baku-Azerbaijan, 2014); VII Congress of the Russian Photobiological Society. Pushchino-2014; The 22$^{th}$ International Conference. Mathematics. Computing. Education (Puschino-Russia, 2015); 3-rd International Conference on Integrative Biology (Valencia-Spain, 2015); XXIII International Conference “Mathematics. Computer. Education". Biophysics of complex systems. Symposium with international participation (Moscow, 2016); XXIV International Conference “Mathematics. Computer. Education" (Pushchino, 2017); International Conference Modern Trends in Physics. BSU (Baku, 2017); International Conference on Biological, Environmental Sciences Applications (Luxor-Egypt, 2017); XXVI İnternational Conference. “Mathematics. Computer. Education” (Puschino, 2019); First Eurasian Conference on Nanotechnology. Nanotech Eurasia. Khazar University (Baku, 2019); XXVII International Conference “Mathematics. Computing. Education” (Dubna, 2020); International Conference on the topic of the coronavirus pandemic: from scientific research to ensuring a healthy future (Baku, 2020); XIX International Scientific and Practical Conference- Applied and fundamental scientific research. (Brussels-Belgium, online, 2021); International Scientific and Practical “Problems of modern science and practice” (Boston-USA, online, 2021); International Conference. Modern Problems of Nuclear Energetics and Nuclear Technologies (Tashkent-Uzbekistan, online, 2021); VII İnternational Conference “Modern Trends in Physics” BSU (Baku, 2021); XXIX International Conference. "Mathematics. Computing. Education” (Moscow, Online, 2022); The XVIII International Scientific and Practical Conference “Advancing in Research, Practice and Education” (Florence-Italy, online, 2022); III International Scientific and Practical Conference “Theoretical

Aspects of Education Development" (Warsaw, Poland, 2023); İnternational Scientific and Practical Conference "Scientific Directions of Research in Educational Activity" (Osaka, Japan, 2023); VII Congress of Biophysicists of Russia (Krasnodar, 2023). The results of the dissertation were also discussed at the seminars of the Institute of Radiation Problems and Baku State University.

**Publications.** The main content of the dissertation work is reflected in 43 articles and 24 theses in the world's leading scientific journals, 16 articles and 18 theses published in local scientific journals, and 1 monograph. The main results of the dissertation were published in 102 scientific works, including 59 articles, 42 theses and 1 monograph.

**Structure and scope of the dissertation.** The dissertation consists of 365 pages, including introduction, 5 chapters, conclusion, main results, bibliography in 241 titles, appendices, list of abbreviations and symbols, 478957 characters (introduction – 20594 characters, chapter I - 134462 characters, chapter II - 32865 characters, chapter III - 154472 characters, chapter IV – 56619 characters, chapter V 11259 characters). The dissertation is also supplemented with 10 tables and 181 figures.

## THE MAIN CONTENT OF THE DISSERTATION

The INTRODUCTİON part of the dissertation work outlines the relevance of the topic, the object and subject of the study, goals and objectives, research methods, the main provisions submitted for defense, the obtained scientific innovations, the theoretical and practical significance of the research, and the approbation of the work. It also provides information about scientific papers published in accordance with the topic of the dissertation.

## CHAPTER I
## LITERATURE REVIEW

In the literature review, the results of scientific research conducted in various directions according to the topic of the dissertation are broadly interpreted. This chapter consists of nine paragraphs. Here is a broad overview of the results obtained from studying the effects of various stress factors (ionizing gamma radiation, radioactive pollution and ultraviolet rays) on living organisms[5], as well as the results of studying living systems using EPR spectroscopy[6,7]. In this chapter ,the essence of the phenomenon of biomineralization is described in detail, a brief description of magnetic nanoparticles in living systems, their significance, their use in targeted drug delivery and in other areas is given[8,9]. This chapter reflects the interpretation of scientific research on the effects of stress factors on certain animal organisms[10,11].

## CHAPTER II
## MATERIALS AND METHODS OF RESEARCH

The research works mainly studied leaves, stems, seeds and seed pods of plants (oleaster (*Elaeagnus angustifolia* L.), reed (*Scirpus lacustris* L.), rush (*Juncus Acutus* L.), camelthorn (*Alhagi pseudalhagi* L.), bean-caper (*Zygophyllum fabago* L.), fennel (*Foeniculum vulgare* M.)), which dominate the territory of the Absheron township of Ramana (the territory of the Iodine plant). In addition, the leaves of some tree and shrubby plants characteristic of

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[5] Gudkov, S.V. Effect of ionizing radiation on physiological and molecular processes in plants / M. Grinberg, V.S. Sukhov, V.A. Vodeneev // Journal of Environmental Radioactivity, – 2019. №202, – p. 8-24.

[6] Блюменфельд, Л.А., Тихонов, А.Н. Электронный Парамагнитный Резонанс // Соросовский Образовательный Журнал, – 1997. №9, – с.91-99.

[7] Тихонов, А.Н. Электронный Парамагнитный Резонанс в Биологии // Соросовский Образовательный Журнал, – 1997. №11, – с. 8-15.

[8] 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.

[9] Gubin, S.P. Magnetic nanoparticles: preparation, structure and properties / S.P. Gubin, Y.A. Koksharov, G.B. Khomutov [et al.] // Russian Chemical Reviews, – 2005. 74 (6), – p. 489-520.

[10] McDermott, M. Advancing Discovery of Snail Mucins Function and Application / M. McDermott, A.R. Cerullo, J.Parziale [et al.] // Frontiers in Bioengineering and Biotechnology, – 2021. № 9, – p. 1-12.

[11] Parkinson, C. Diagnostic necropsy and selected tissue and sample collection in rats and mice / C.M. Parkinson, A. O'Brien, T. M. Albers [et al.] // Journal of Visualized Experiment, – 2011. 54, – p. 2-7.

the Absheron Peninsula were studied: olive (*Olea Europea* L.), fig (*Ficus Carica* L.), Eldar pine (*Pinus eldarica* M.), firethorn (*Pyracantha Coccinea*), privet (*Ligustrum* L.), pomegranate (*Punica Granatum* L.), seedlings of wheat (*Triticum* L.) and corn (*Zea mays* L.) seeds, respectively, belonging to C3 and C4 types of photosynthesis under laboratory conditions and some higher aquatic plants (aquatic limnophila (*Limnophila Aquatica*), elodea (*Elodea Canadensis*)). To show the generality and universality of the new phenomenon that we discovered in living systems under the influence of stress—the phenomenon of the appearance of magnetic properties in them we also studied chloroplasts isolated from spinach leaves (*Spinacia* L.) and some animal organisms: grape snails with shell (*Helix pomatia*) and laboratory rats (*Wistar Albino*).

Gamma irradiation of the research objects was carried out on the installations "RXUND–20000" (D=2.722–0.249 Rad/sec), "URI" (D=9.965 – 6.781 Rad/sec) and "MRX-γ25" (D= 136.165 – 100.453 Rad/sec). The source of the radiation was the isotope $^{60}Co$. Lethal and semi-lethal doses of the samples were taken into account during irradiation. In addition, the studied samples were also irradiated with UV–B rays using mercury-quartz lamps DRT–230, which have a linear radiation spectrum. In the experiments, the UFS – 2 filter was used to produce environmental UV rays (290-360 nm).

The dosimeter-radiometer MKC – AT 1125 (Belarus), a highly sensitive device with extensive functionality, was used to determine the radiation background in the field.

Isolation of chloroplasts was carried out in several stages, and all stages were carried out at a temperature of 0-4°C, as a rule, in order to obtain a sufficiently functional active drug. According to the standard procedure, isolated chloroplasts were obtained and the following solution was used: 0.35 M NaCl in 0.05 M Tris-HCl buffer, pH = 8.00.

Using EPR spectroscopy, new paramagnetic centers formed in living systems under the influence of stress factors were studied. EPR spectra were recorded on 3 cm range spectrometers "VARIAN E-4" (USA) and "BRUKER-EMX" (Germany). During the studies, as a rule, EPR signals in intact leaves and isolated chloroplasts were recorded ultra high frequency (UHF)- power 10 mW, high-frequency modulation with an amplitude of 0.2-0.4 mT. The biological systems studied were also studied using SEM and TEM. The content of radionuclides in the samples was determined using a CANBERRA gamma spectrometer and their specific activity was determined.

# CHAPTER III
# INVESTIGATION OF PARAMAGNETIC CENTERS IN PLANTS UNDER THE INFLUENCE OF VARIOUS STRESSORS

The increased impact of various stressors on the environment negatively affects all its components, and as a result, the quality of living conditions of living systems is significantly reduced. Living and inanimate nature is exposed to various stressors, such as heavy metals, radioactive pollution, UV radiation, and various toxic industrial wastes. The impact of such stressors on living systems leads to the formation of ROS, free radicals, and various toxic substances[12]. Paramagnetic centers have been studied in them to study the mechanism of the occurrence of structural and functional changes, new physico-chemical properties that stressors can create in living systems.

**3.1. Comparative study of EPR signals of leaves, stems, seeds and seed peels of plants growing in areas with different radiation background.** In order to study paramagnetic centers during stress in plants growing in different areas of the Absheron peninsula, the primary research area was the Iodine plant area of Ramana settlement. There, during this period, the radiation background fluctuated between approximately 4-800 μR/h.

Dominant oleaster (*Elaeagnus angustifolia* L.), reed (*Scirpus lacustris* L.), rush (*Juncus Acutus* L.), camel-thorn (*Alhagi pseudalhagi* L.), bean caper (*Zygophyllum fabago* L.), fennel (*Foeniculum vulgare* M.) plants were collected from control (exposure dose strength (EDS) = 4-10 μR/hour) and radioactively contaminated (EDS=12-800 μR/hour) areas and their various organs were studied comparatively by EPR

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[12] Burlakova, E.B., Naidich, V.I. Radiation safety as a research problem // Herald of the Russian Academy of Sciences, – 2006. 76,l – p. 591–594

method.

Figure 1 shows the EPR spectrum in a wide range of magnetic fields (1000-5000 G) of the leaves of the scented plant, collected from a radioactively contaminated area (EDS=140 μR/hour), dried at room temperature. Figure 1 shows the EPR spectrum of *Elaeagnus angustifolia* L. leaves collected from a radioactively contaminated area (EDS=140 μR/h), dried at room temperature, in a wide magnetic field range (1000-5000 G). The signal of iron ions (g=4.6) and a narrow free radical signal (g=2.0023; ΔH=9G) were observed in this spectrum.

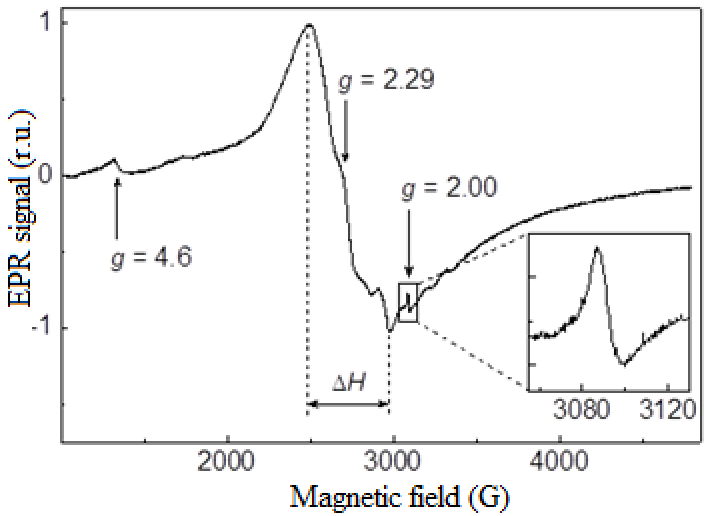



**Figure 1. EPR spectrum of the leaves of the fennel plant collected from a radioactively contaminated area (EDS ≈ 140 μR/hour) (EDS – exposure dose strength)**

In addition, the main place in the EPR spectrum is occupied by a broad signal (g=2.29, ΔH=450 G) detected by us for the first time in plant systems. Our ongoing research has shown that this signal belongs to iron oxide magnetic nanoparticles due to their parameters and behavior, and these nanoparticles, which are formed under the influence of stress factors, cause the emergence of magnetic properties in living systems.

When studying the effects of radioactive pollution on paramagnetic centers in leaves, stems, seeds and seed pods of plants, it was found that stress factors cause the generation of broad EPR signals (g≈2.32, ΔH≈350G) that characterize nanophase iron oxide particles. As the radiation background increases, the intensity of these signals also increases. It was found that the intensity of the signals recorded in the leaf and stem samples was higher than in the seeds (figure 2).

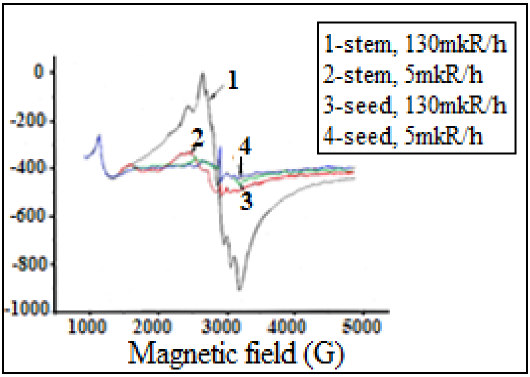



**Figure 2. EPR spectra of stems and seeds of rush (*Juncus acutus L.*) growing in control and radioactively contaminated areas**

Similar results were obtained in all studied plants. It was determined that the intensity of the signals characterizing the iron oxide magnetic nanoparticles recorded in the leaves and seeds of the plants growing in the radioactive contaminated area is higher than the intensity of the corresponding signals of the control samples. This suggests that more iron-based magnetic nanoparticles are generated in plant samples during radioactive contamination, and this phenomenon occurs more intensively in leaves (figure 3).

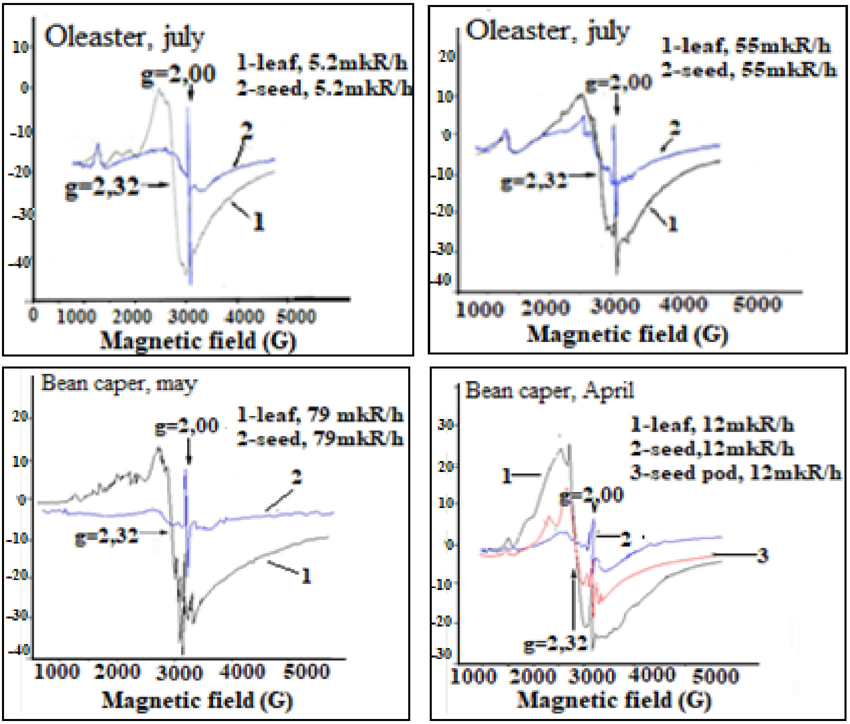



**Figure 3. EPR spectra of leaves and seeds of oleaster (*Elaeagnus angustifolia* L.) and bean caper (*Zygophyllum fabago* L.) plants grown in control and radioactively contaminated areas**

In order to determine the role of any extraneous factors (for example, temperature, drought, radiation, UV rays, etc.) in the obtained results, our studies were repeated in different seasons of the year (figure 4).

It was found that the increase in radiation background in all seasons of the year leads to the increase in the intensity of the broad EPR signal that characterizes magnetic nanoparticles in plants. Along with the signal of magnetic nanoparticles (g≈2.34), the signal of iron ions (g≈4.3) was recorded in plants.

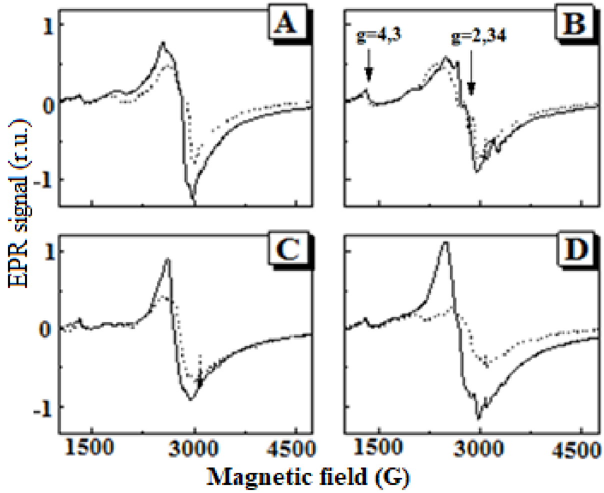
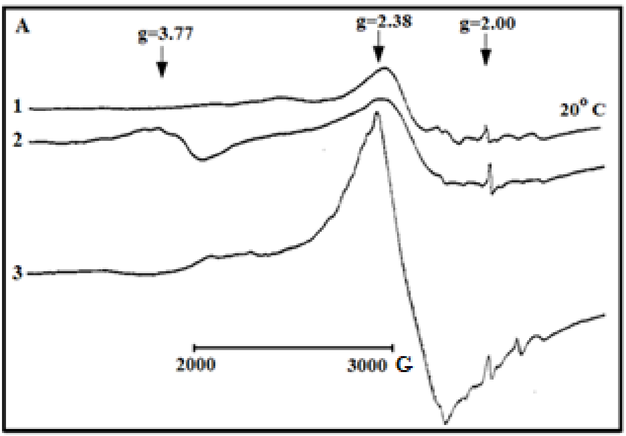



**Figure 4. EPR spectra of leaves of oleaster (*Elaeagnus angustifolia* L.) growing in areas with different radiation background in different seasons of the year. A) May, B) June, C) July, D) October. (The broken line is the control and the solid line is the spectra of the plants collected from radioactively contaminated areas.)**

The EPR spectra of the dominant plants in the area of the Iodine plant were also comparatively studied at room temperature (293-295K) and liquid nitrogen temperature (77-80K) (figure 5).



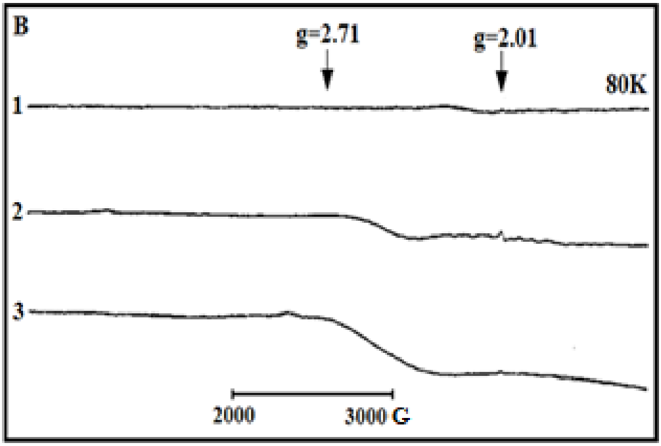



**Figure 5. EPR spectra of stems and leaves of *Alhagi pseudalhagi* plant (mixed) at different temperatures: 1- control plants (EDS ≈ 10 μR/h); 2, 3 - plants collected from areas with EDS ≈170 μR/h and EDS ≈220 μR/h, respectively. A–room temperature (293 K); B–liquid nitrogen temperature (80 K)**

Amplitudes of broad EPR signals recorded at room temperature of dried leaves and stems of plants sharply decreased with a decrease in signal recording temperature to 80 K. This can be caused by the expansion of signals at low temperatures.

As a continuation of the experiments, chemically synthesized magnetic nanoparticles were studied. EPR signals of synthesized magnetic nanoparticles on polyethylene matrix ($Fe_3O_4$ + PE) were recorded at different temperatures (figure 6).

In comparison with the signals recorded at room temperature, the expansion of the signals at low temperatures was observed with the decrease of their amplitudes. Thus, the broad EPR signals of the synthesized magnetic nanoparticles with a decrease in temperature were consistent with the similar signals recorded in plant leaves due to their change properties and behavior.

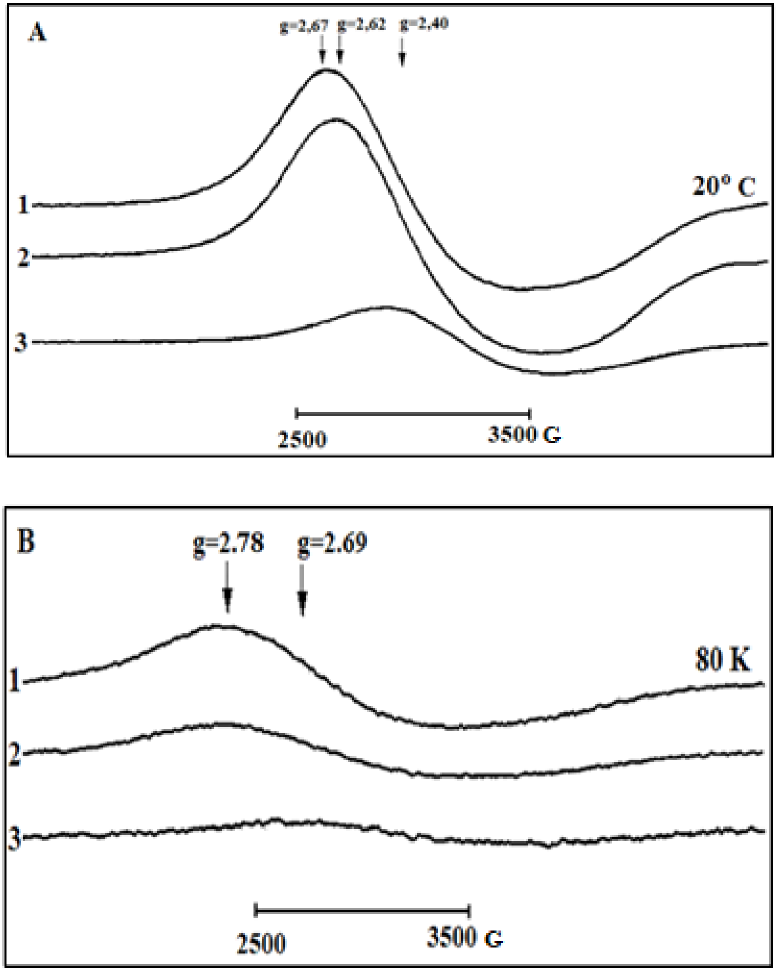



**Figure 6. EPR spectra of magnetite nanoparticles, temperature A-293K and B-80 K (1-Fe3O4+PE, 15 ml, 9.4 nm; 2- Fe3O4+PE, 10 ml, 9.4 nm; 3- Fe3O4+PE, 5 ml, 9.4 nm)**

Determination of the radionuclide content and specific activities of the plants growing in radioactively contaminated and control areas of the İodine plant showed that the increase in the radiation background caused an increase in the specific activities of $^{40}K$, $^{226}Ra$ and $^{228}Ra$ radionuclides in them (table 1).

**Table 1**

**Radionuclide content and specific activities of plants growing in radioactively contaminated and control areas of the İodine plant**

| The analyzed element | Specific activity of radionuclides (Bq/kg) | | | | |
|---|---|---|---|---|---|
| | Bean caper (*Zygophyllum fabago L.*) | | | Oleaster (*Elaeagnus angustifolia L.*) | |
| | Radiation background (μR/h) | | | | |
| | 5 | 170 | 200 | 5 | 140 |
| $^{40}K$ | 151 ± 18 | 305 ± 18 | 596 ± 17 | 205 ± 10 | 234 ± 26 |
| $^{226}Ra$ | 4.4 ± 1.4 | 14.2 ± 1.1 | 214.3± 1.4 | 2,2 ± 0.7 | 6.6 ± 2.1 |
| $^{228}Ra$ | 0.4 ± 0.8 | 0.5 ± 0.6 | 2,2 ± 0.8 | 1.3 ± 1.1 | 2.30 ± 0.04 |
| $^{235}U$ | 0.9 ± 0.2 | 0.8 ± 0.1 | 16.6 ± 1.0 | 0.20±0.06 | 0.20 ± 0.04 |
| $^{238}U$ | 19.5 ± 4.0 | 17.4 ± 1.9 | 360±21.72 | 4.3 ± 1.1 | 4.3 ± 0.8 |

Thus, while studying the effect of radioactive pollution on plants by the EPR method, we determine that biogenic iron oxide magnetic nanoparticles are generated as a result of biomineralization in plant systems during the stress factor.

The essence of this phenomenon is that as a result of stress, the bound iron in the living system changes to free iron and iron ions multiply in the body.

On the other hand, under the influence of a stress factor, the nativeness and integrity of plant chloroplasts are disturbed, and electrons are released into the environment as a result of breaks in ETC. Thus, thanks to the reducing environment, iron ions are transformed into iron oxide magnetic nanoparticles. It should be noted that the formation of iron oxide magnetic nanoparticles plays the role of self-defense of the body. Because otherwise, the increase of iron ions would lead to the formation of ROS and free radicals in the living system (Fenton reaction). However, the presence of a reducing environment leads to the formation of magnetic nanoparticles, which protect the living system from free radicals and ROS. In order to show the universality of the new phenomenon obtained, various tree species characteristic of the Absheron Peninsula were studied.

**3.2. Study of paramagnetic centers in extracts of the pomegranate plant (*Punica granatum*).** Of great interest is the study of the mechanisms of formation of magnetic nanoparticles of biogenic origin in living systems, the use of which is extremely necessary. To clarify this phenomenon, it is important to study paramagnetic centers in living systems. Therefore, in the pomegranate plant, which is widespread in our republic, including on the Absheron Peninsula, paramagnetic centers were studied, and the biogeneration of iron-based magnetic nanoparticles in them was studied.

The EPR spectra of pomegranate extracts were also taken under control with the addition of $FeCl_3$ and $FeCl_2$ separately to their composition (figure 7, A, B). Signals characterizing nanophase particles of iron oxide were recorded in samples to which $FeCl_3$ was added. However, this signal was not observed in the control sample (figure 7, A). When $FeCl_2$ was added to the pomegranate extract, a wide EPR signal was not generated (figure7, B). This shows that for the formation of characteristic EPR signals, the reaction medium (pomegranate extract) must have a reducing ability. The addition of $FeCl_3$ to pomegranate extract and some biological compounds present in the environment leads to the formation of magnetic iron oxide nanoparticles[13]. Thus, pomegranate extract acts as a reducing agent in experiments. The addition of FeCl2 solution to pomegranate water does not lead to the formation of a wide EPR signal. This indicates that Fe2+ ions are not being restored.

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[13] Nasibova, A.N., Khalilov R.I. Preliminary studies on generating metal nanoparticles in pomegranates (*Punica Granatum*) under stress // International Journal of Development Research, – 2016.6(3), –p. 7071-7078

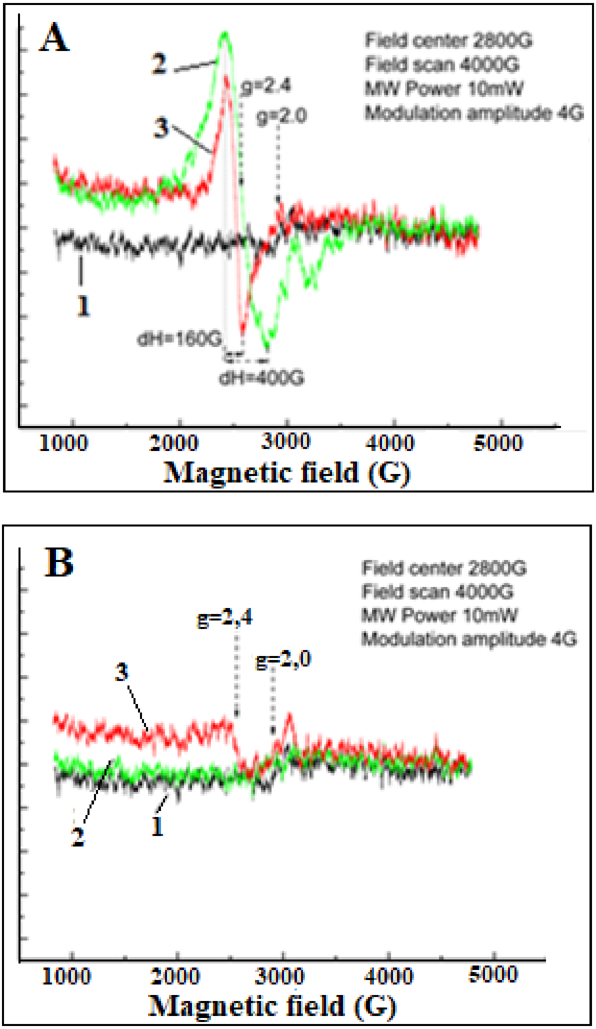


**Figure 7. EPR signals obtained from pomegranate (*Punica granatum*) extract. A) 1 - control; 2,3-added $FeCl_3$; B) 1 - control; 2,3-added $FeC_2$**

**3.3. Behavior of paramagnetic centers of fig (*Ficus carica* L.) leaves, characteristic of the Absheron Peninsula and dependence on some parameters.** Fig leaves collected from different regions of Absheron were studied using an EPR spectrometer. It was determined that two types of signals are registered in the spectra of leaves: broad EPR signal (g=2.32; ΔH=400 G) and free radical signal (g=2.0023; ΔH=10 G) (figure 8).

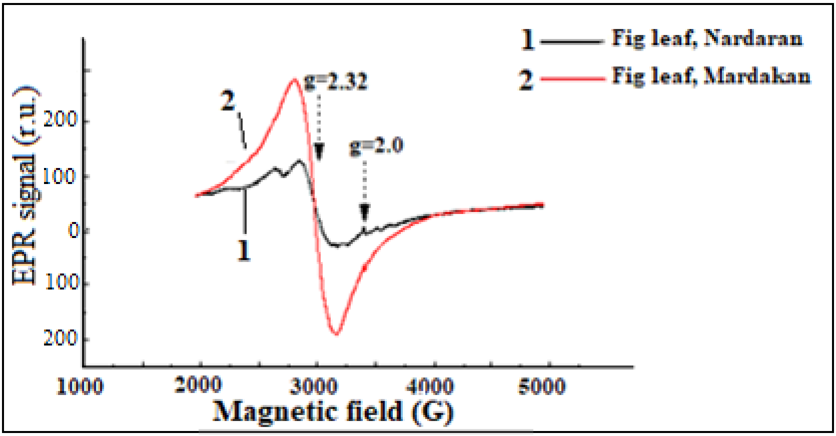


**Figure 8. EPR spectra of fig (Ficus carica L.) leaves collected from Nardaran and Mardakan**

The broad EPR signals we received (g=2.32; ΔH=400 Qs) (Figure 8) suggest for the first time that iron oxide magnetic nanoparticles in fig leaves (*Ficus carica* L.) growing on the Absheron peninsula (Nardaran and Mardakan areas) exists and these nanoparticles are generated as a result of biomineralization under the influence of various stress factors. In order to study the properties of the broad EPR signals that characterize the magnetic nanoparticles obtained in the leaves of *Ficus carica* L., we changed the parameters of the radio spectrometer and studied them at different angle variations and found that this signal has magnetic anisotropy (figure 9). Because, when rotating the quartz ampoule with dried leaf samples in the resonator of the spectrometer by 90°, 180°, 270°, 360° certain changes in the form of the EPR signals received from fig (*Ficus carica* L.) leaves were observed and at the same time, the signals shifted 100-150 G to the left in the resonance field. (figure 9). In this case, the behaviors and changes we detected in the EPR signals were found for magnetite

($Fe_3O_4$), maghemite ($\gamma$-$Fe_2O_3$) and superparamagnetic nanoparticles[14].

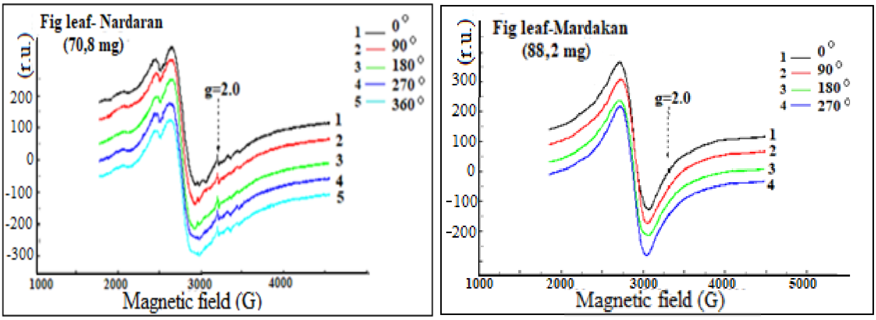


**Figure 9. EPR spectra of fig leaves (Ficus carica L.) at various angle changes.**

As a result of EPR study of fig (*Ficus carica* L.) leaves, important results were obtained. İn this regard, the temperature dependence of the EPR signals we received was studied in order to provide a wider identification and more complete characterization. As a result, it was found that the spectra obtained in fig leaves change depending on the temperature (figure 10). Thus, it was found that when the recording temperature decreases to approximately 120-125 K, the amplitude of the broad signal received from fig leaves gradually increases and reaches its maximum when $T \approx 120$-$125$ K. However, when the temperature drops below 125 K, the intensity of the signal is sharply reduced, and the signal itself has shifted to a small magnetic field (Figure 10). It is known that broadening of the EPR signal with decreasing temperature and shifting to a smaller magnetic field is characteristic of superparamagnetic nanoparticles[15].

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[14] Ацаркин, В.А. Влияние диамагнитного разбавления на спиновую динамику в манганитах / В.А. Ацаркин, В.В. Демидов, Д.Г. Готовцев [и др.] //Актуальные проблемы физики конденсированных сред, – Казань: – 2004. – с.13-27.

[15] Berger, R. Temperature dependence of superparamagnetic resonance of iron oxide nanoparticles / R. Berger, J.C., Bissey, J. Kliava [et al.] // Journal of Magnetism and Magnetic Materials, – 2001. V.234. I.3, – p. 535-544.

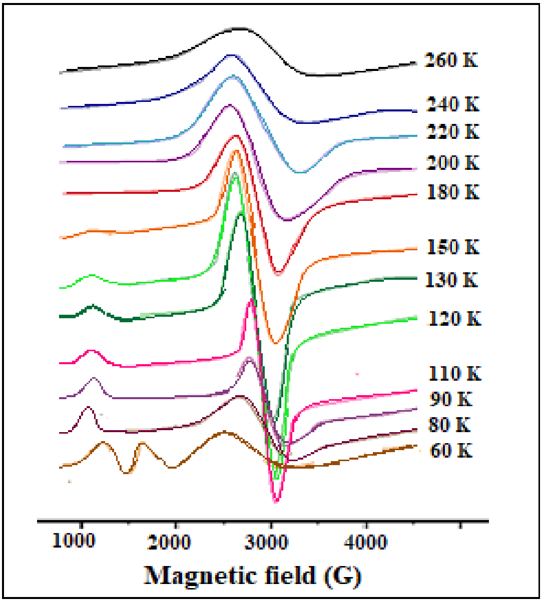



**Figure 10. Temperature dependence of EPR spectra of fig (*Ficus carica* L.) leaves**

Depending on the temperature dependence of the EPR signals of *Ficus carica* L. leaves, the intensity and width of the detected signals were recorded separately. At this time, a non-monotonic dependence was observed (figure 11). It was found that the characteristics of EPR signals characterizing magnetic nanoparticles (amplitude, g-factor, signal width) strongly depend on temperature and this signal has magnetic anisotropy.

When studying the temperature dependence of EPR signals, it was found that the characteristic Vervey phase transition occurs at a temperature of 120-125 K in the behavior of the amplitude of the magnetic resonance signal, the width of the magnetic resonance line, which is characteristic of superparamagnetic nanoparticles[16].

[16] Walz, F. The Verwey transition - a topical review //Journal of Physics: Condensed Matter, – 2002. V. 14, I. 12, – p. 1-8.

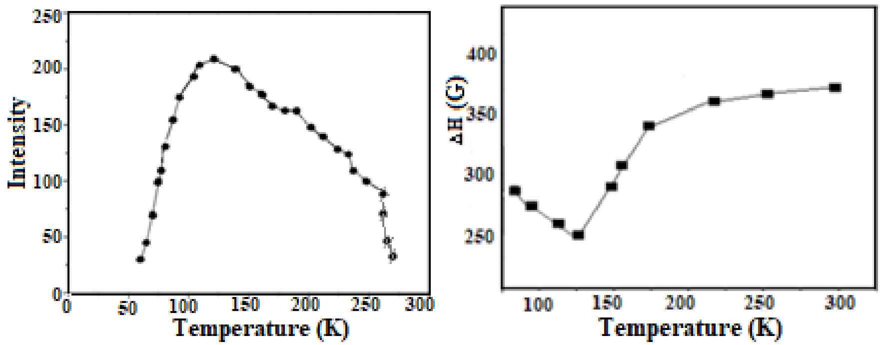



**Figure 11. Temperature dependence of the intensity and width of the EPR signal registered in fig (Ficus carica L.) leaves**

Thus, when studying the paramagnetic centers in some trees and shrubs characteristic of the Absheron peninsula, we find that under the influence of stress factors, biogenic iron oxide magnetic nanoparticles and magnetic properties are formed in them.

The formation of these nanoparticles is related to the transition of bound iron to free iron in the body as a result of the influence of stress factors, as well as the formation of a reducing system as a result of damage to the ETC of chloroplasts.

It should be noted that magnetic nanoparticles of iron oxide, formed in living systems as a result of stress, prevent the occurrence of the Fenton reaction - the formation of ROS, performing the function of protecting the body. Otherwise, the living system would be destroyed.

In order to show the universality of the obtained results, some plants were also studied in laboratory conditions. This is reported in the next paragraph.

**3.4. Effect of stress factors on C3 and C4 plants (wheat-*Triticum L.* and corn-*Zea mays L.*).** Among the stress factors, the effect of radioactive contamination and gamma radiation on wheat (*Triticum* L.) and corn (*Zea mays* L.) plants belonging to the C3 and C4 types of photosynthesis was studied. First of all, the morphological changes in wheat and corn seedlings caused by the above stress factors were studied, and the mechanism of the resulting patterns was established. At the same time, paramagnetic centers formed in these plants as a result of the action of stress factors were studied by EPR method.

During the experiments, wheat and corn seeds were germinated in control (clean) and radioactively contaminated soils under laboratory conditions (figure 12). It should be noted that the radioactively contaminated soil examined in paragraph 3.1 was collected from the site of the now defunct Iodine Plant, which was used in the production of iodine and was prepared in laboratory conditions using activated carbon sorbent, which is a source of radiation.

As can be seen from figure 12, the development, growth and germination density of wheat seedlings grown in radioactively contaminated soil were significantly higher than those of seedlings grown in the control soil. When studying the effect of radioactive contamination on the morphological characteristics of the corn plant, a different result was obtained. Thus, corn sprouts grown in radioactively contaminated soil were weaker than control ones in terms of growth and germination density.


**Figure 12. Control and sprouts of 10-day-old wheat and corn growing in radioactively contaminated soil (wheat on the left, corn on the right)**

The effect of gamma radiation on the morphological features of these plants was also studied. Figure 13 shows wheat seeds irradiated in different doses (50 Gy, 100 Gy, 200 Gy, 250 Gy, 300 Gy), and 10-day corn seed sprouts irradiated in different doses (50 Gy, 100 Gy, 150 Gy, 200 Gy). As the radiation dose increases, the differences in their appearance are clearly visible. It has been established that gamma radiation has a stimulating effect on the germination and development of wheat seeds. However, the growth of corn sprouts and the percentage of germination slowed down as the radiation dose increased. This result correlates with the result obtained when exposed to radioactive contamination on a corn plant.

Thus, we determine that gamma radiation has a stimulating effect on both height growth and germination percentage of wheat, a C3 plant, and an inhibitory effect on corn, a C4 plant.

Stimulating effect can be revealed during the impact of stress factors on C3 type plants due to activation of the photorespiration process in these plants during stress and playing a protective role by preventing the formation of reactive oxygen species (ROS).

The manifestation of a stimulating effect when exposed to stressors on C3 type plants may occur due to the fact that during stress, these plants start the process of photorespiration and they perform a protective role, preventing the formation of ROS.

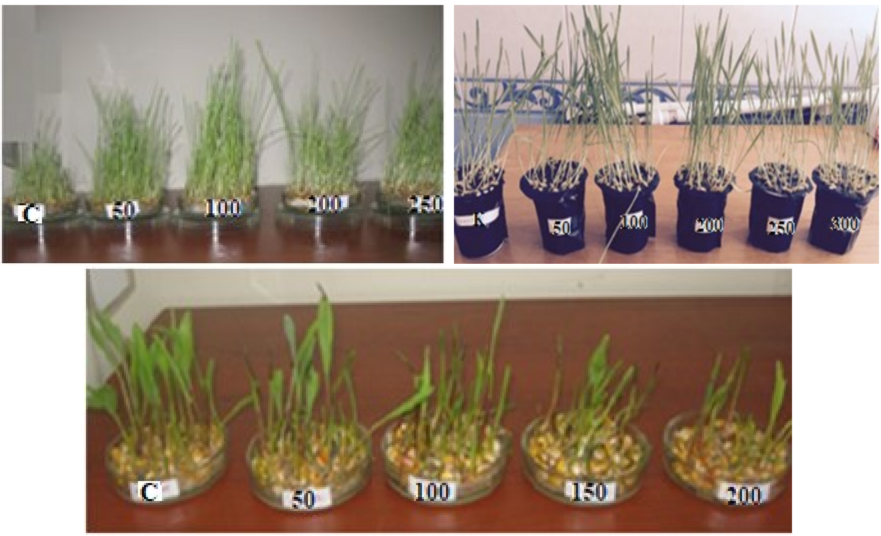
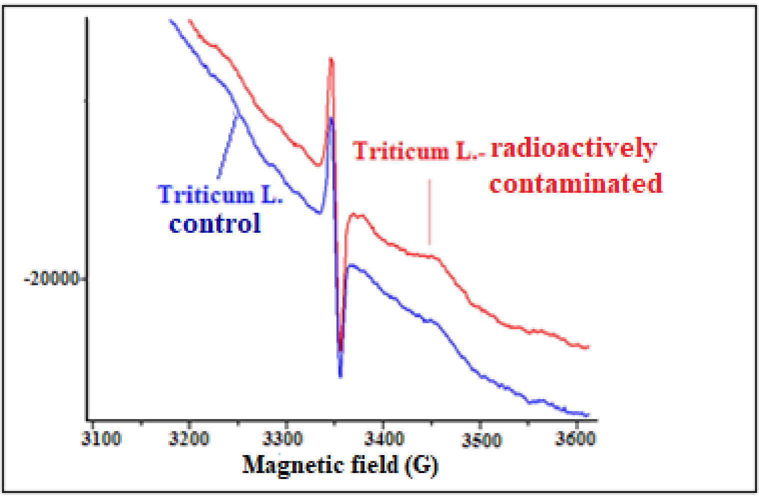



**Figure 13. Control and 10-day seedlings of wheat and corn seeds irradiated with gamma radiation in various doses.**

It is known from the literature that photorespiration accelerates when too many carbohydrate products of photosynthesis are formed. Such conditions lead to the oxidation of "excess" sugar, and its amino acids are formed. The resulting amino acids are consumed for the growth of plant leaves. Even a leaf that has completed its development is starting to develop again[17]. In our experiments, wheat sprouts belonging to the C3 type of photosynthesis grow sufficiently under the influence of stress factors. However, no clearly defined photorespiration was detected in C4 plants. The results show that photorespiration in C3 plants during stress plays a protective role, and suggest that photorespiration is a system that prevents the formation of ROS in C3 plants during stress.

The study of paramagnetic centers in the studied plant samples also allowed us to obtain important results. EPR spectra of both plant seedlings and their comparative analysis showed that radioactive contamination leads to an increase in the intensity of the narrowband signal of free radicals (g=2,0023; ΔH=10 G). In all cases, the amplitudes of free radical signals in seedlings growing in contaminated soil were higher than the control ones (figure.14).




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

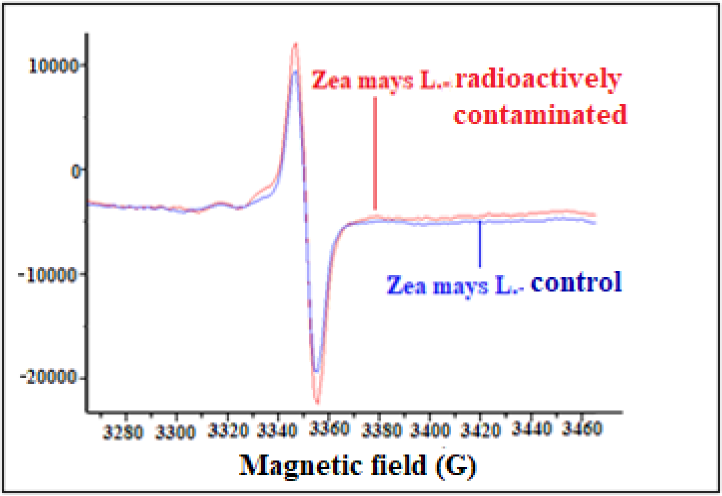



**Figure 14. The effect of radioactive contamination on paramagnetic centers in wheat and corn crops**

The research results have once again shown that radioactive contamination causes the formation of new paramagnetic centers in plant systems. This result can be applied to environmental biomonitoring.

In subsequent EPR experiments, the effect of various doses of gamma radiation on paramagnetic centers in a wheat plant was studied. Wheat seeds, subjected to control and irradiation with gamma radiation in various doses, were germinated with distilled water at room temperature. EPR spectra of ten-day seedlings were recorded (figure 15). As can be seen from the figure, in the control sample, only free radical signals were detected in a wide range of the magnetic field (500-5500 G). Here we do not observe the generation of a wide range of EPR signals that characterize magnetic iron oxide nanoparticles. Because since stress has no effect, iron remains bound in the living system, there is no recovery system, and magnetic nanoparticles are not formed either. As can be seen from the spectra, a wide low-intensity EPR signal (g=2.32; ΔH = 320 G) is generated in the irradiated sample at a dose of 100 Gy, characterizing magnetic iron oxide nanoparticles. Because irradiation at a dose of 100 Gy leads to the transition of bound iron into the free form of iron. Violation of the integrity of chloroplasts and ETC leads to the transformation of free iron ions of the reducing system into magnetic iron oxide nanoparticles caused by the dissociation of electrons into the medium.

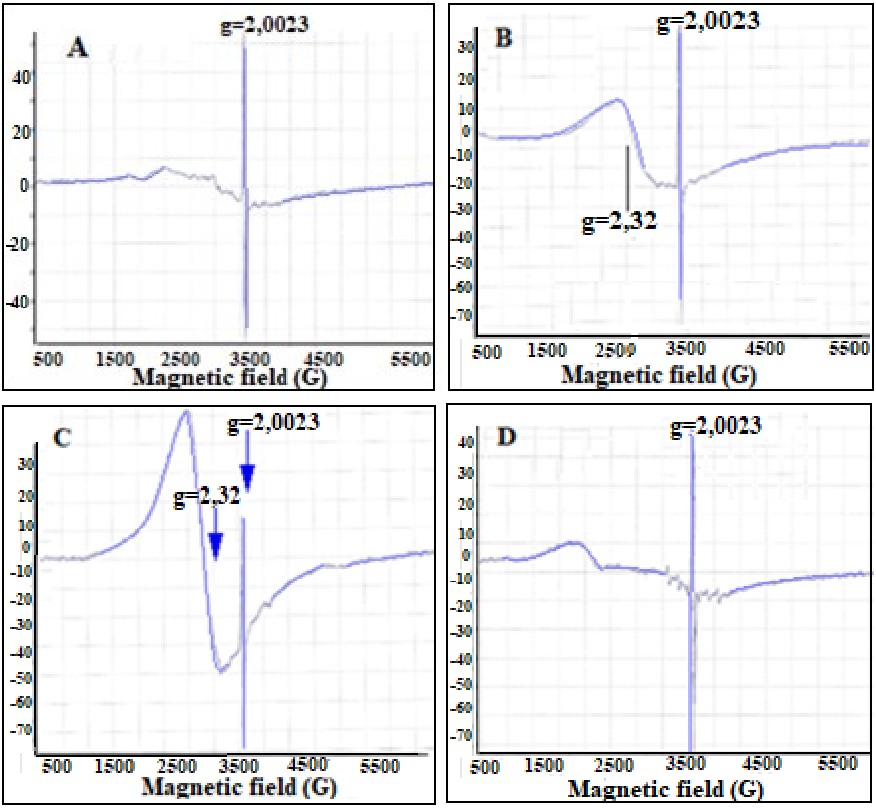



**Figure 15. EPR spectra of dried wheat seedlings (A-control, B-irradiation at a dose of 100 Gy, C-200 Gy, D-300 Gy)**

Reaching an irradiation dose of 200 Gy leads to an even greater increase in the intensity of this signal. This is due to the fact that an increase in the radiation dose leads to an increase in the number of iron ions in the living system. In addition, a greater number of breaks in the ETC leads to a greater release of electrons into the medium, which leads to the formation of more magnetic iron oxide nanoparticles in terms of quantity. When the radiation dose increases to 300 Gy, we observe how only a free radical signal is generated in a wide range of the magnetic field, as in the control sample. The amplitude of the wide EPR signal decreases sharply. This is explained by the fact that, although stress is present, the number of iron ions in the living system increases, but irradiation at a dose of 300 Gy already leads to the failure of the recovery system, and magnetic iron oxide nanoparticles are not formed, the living system is destroyed. Therefore, the signal characterizing magnetic nanoparticles is not recorded.

The results obtained in these experiments help us both clarify the mechanism of formation of magnetic nanoparticles in plants, and give reason to say that magnetic nanoparticles are formed in the plant itself. Our *in vivo* studies allow us to conclude that the effect of stressors leads to the generation of nanophase crystalline magnetic particles of iron oxide in living systems as a result of the phenomenon of biomineralization, to the appearance of new magnetic properties.

**3.5. Investigation of magnetic nanoparticle transport and paramagnetic centers in some aquatic plants.** To confirm the generality of the results of studies conducted with various species of trees and shrubs characteristic of the Absheron Peninsula, as well as with plant seeds grown in laboratory conditions as a model system, subsequent studies were carried out with higher aquatic plants (aquatic limnofila (*Limnophila aquatica*), elodea (*Elodea canadensis*)) using the EPR method.

A schematic representation of the experiments with aquatic limnophila is shown in figure 16. In the experiments, the Limnophila aquatica plant was placed inside a special container with a partition covered with cotton and water inside. The root part of the plant is placed on one side of the partition, and the upper part on the other side. Then, chemically synthesized magnetic nanoparticles were injected into the stem of the plant from the lower part.

The purpose of conducting these experiments was to investigate the possibility of transporting and collecting magnetic nanoparticles to other organs of the plant: the upper part of the stem, leaves.

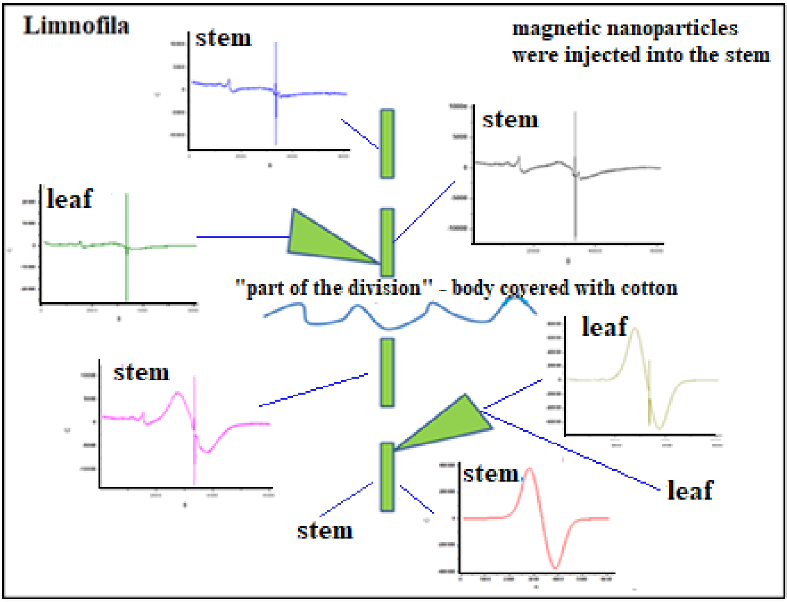



**Figure 16. Schematic representation of transport of iron oxide nanoparticles in Limnophila aquatica plant injected with magnetic nanoparticles into its stem**

After introducing magnetic nanoparticles into the lower part of the plant stem (near the root), the EPR spectra of the stems and leaves of plants located before and after the partition were recorded. It was determined that up to the partition, the signals characteristic of iron oxide magnetic nanoparticles appear in the stem and leaves (figure 17).

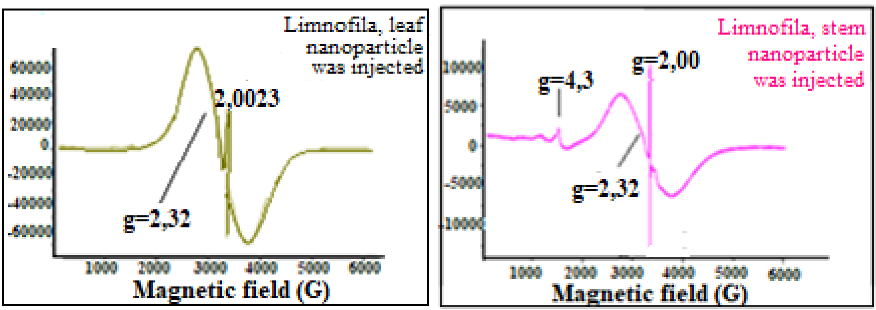



**Figure 17. EPR spectra of leaf and stem parts of *Limnophila aquatica* in section up to partition**

As can be seen from the figure 17, a narrow free radical signal and signals characterizing nanophase magnetic particles appear in the spectra. In addition to these signals, the signal of iron ions (g=4.3) was registered in the stem.

However, after the partition, the broad signal of magnetic nanoparticles is practically not observed (figure 18). This indicates that the transport of iron oxide nanoparticles in the stem and leaves of the plant is weak. The results obtained once again indicate that magnetic nanoparticles of crystalline iron oxide, detected in various plant organs by EPR method, are generated due to biomineralization in the living organism itself under the influence of stress factors.

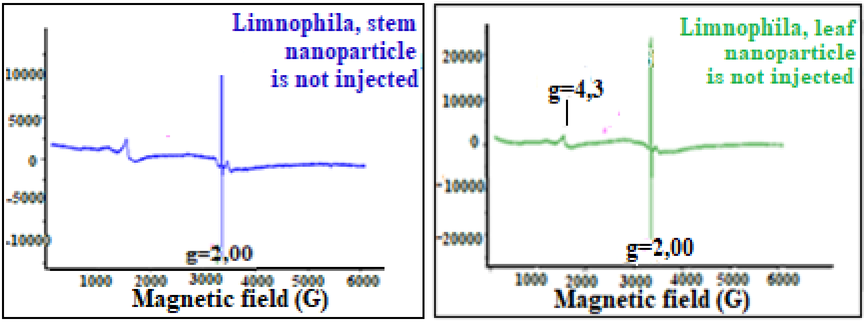



**Figure 18. EPR spectra of leaves and stems of *Limnophila aquatica* located after the partition**

**3.6. Generation of magnetic nanoparticles in chloroplasts.** While elucidating the mechanism of the new phenomenon that we found during in vivo studies - the occurrence of magnetic properties in plants during stress exposure, in vitro studies were conducted with chloroplasts isolated from higher plants (spinach – *Spinacia* L.) as a model system. To obtain the kinetic curves, the magnetic field was fixed at the maximum of the EPR 1 signal. The kinetics of oxidation-reduction transformations of P700, the reaction center of FS1, were examined based on the EPR 1 signal from oxidized $P700^{+}$ centers. The kinetics of photoinduced changes of the EPR 1 signal in control and 500 μM $FeCl_3$ salt-added chloroplasts were studied under the influence of far-red light that excites FS 1, near-red light that excites FS 2 and white light (figure 19).

It can be seen from figure 19 A that oxidation of P700 occurs under the influence of light with a wavelength of λ=707. After the EPR 1 signal reaches a stationary level, when this light is switched to λ=650 light, which excites FS 2 more effectively, the EPR1 signal decreases. This is due to the flow of electrons from FS 2 to $P700^+$. Re-introduction of far-red light leads to a slight increase in the EPR 1 signal. When the EPR 1 signal is at a stationary level, when the white light is turned on, which effectively excites both photosystems, the EPR 1 signal increases rapidly, and when the white light is turned off, it decreases sharply again. It was determined that when $FeCl_3$ with a concentration of 500 μM is added to the sample (figure 19 B), the oxidation-reduction rate of P700 reaction centers increases.

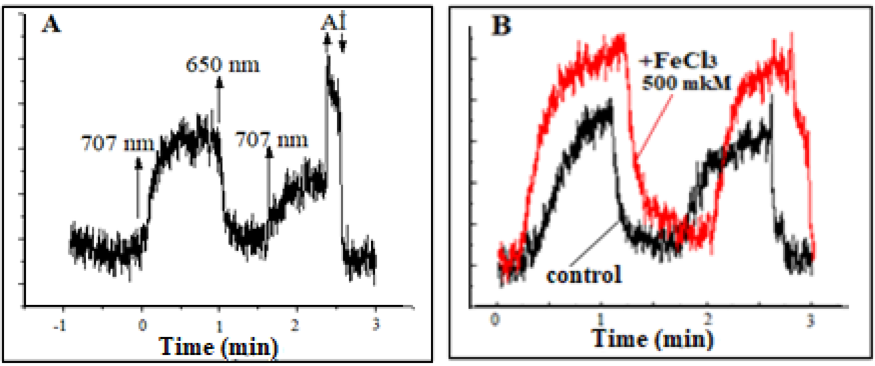



**Figure 19. Kinetics of photoinduced changes in EPR 1 signal rate in isolated chloroplasts of spinach (*Spinacia* L.). On and off times of continuous far red light ($7x10^{-4}$ J/sm$^2$ x sec), near red light ($10^{-4}$ J/ sm$^2$ x sec) and white light ($4x10^{-2}$J/ sm$^2$ x sec) with ↑ and ↓ respectively shown**

This can be explained by the presence of an excess electron accepting system ($FeCl_3$) in the chloroplasts. As a continuation of experiments with chloroplasts, chloroplast suspensions isolated from spinach leaves were separately adapted to white light and darkness, and $FeCl_3$ solution with a concentration of $10^{-4}$ M was added to them.

After the prepared solution was kept at room temperature for 5 hours, their EPR spectra were taken (figure 20). At the same time, control chloroplasts were kept at room temperature for 5 hours, and their EPR spectra were recorded. It was found that the high concentration of iron ions leads to aggregation of chloroplasts and inhibition of their photosynthetic activity. When chloroplasts were kept in the light and $FeCl_3$ solution was added to them, the effect of iron oxide magnetic nanoparticles formation was detected (g≈2.45).

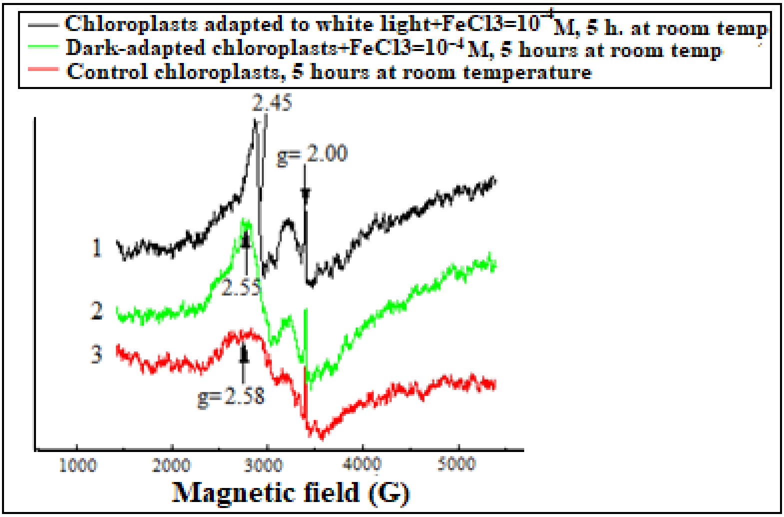



**Figure 20. EPR spectra of chloroplasts isolated from spinach (*Spinacia* L.) leaves. 1. Chloroplasts adapted to white light + [$FeCl_3$] =$10^{-4}$ M, 5 hours at room temperature. 2. Chloroplasts adapted to darkness + [$FeCl_3$] =$10^{-4}$M, 5 hours at room temperature. 3. Control chloroplasts, 5 hours at room temperature**

This means that the electron transport occurring in the thylakoid membranes of chloroplasts leads to the reduction of $Fe^{3+}$ ions, and at the same time, the presence of molecular oxygen ($O_2$) in the environment leads to the formation of nanophase iron oxide particles.

This is observed by the generation of characteristic broad EPR signals. *In vitro* experiments showed that photoinduced generation of magnetic nanoparticles occurs in chloroplasts isolated from spinach. It was determined that iron oxide magnetic nanoparticles ($Fe_3O_4$ - magnetite and $\gamma$-$Fe_2O_3$ - maghemite) can be formed in the presence of relatively small concentrations of iron ions in oxidation-reduction processes in living systems under certain conditions.

TEM method was used for visualization of magnetic nanoparticles. The obtained results were confirmed in experiments conducted with KEM. In these experiments, magnetic nanoparticles were observed in chloroplasts (figure 21).

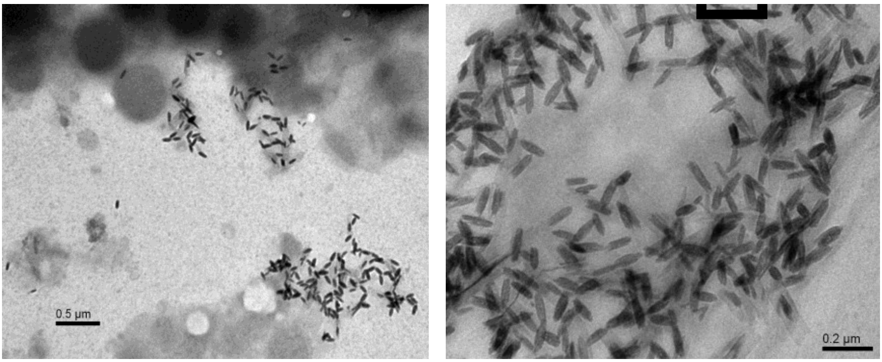



**Figure 21. TEM images of chloroplast solutions with added iron salt ($FeCl_3$)**

## CHAPTER IV
## FORMATION OF MAGNETIC PROPERTIES UNDER THE INFLUENCE OF RADIATION FACTORS IN SOME ANIMAL ORGANISMS

In modern times, biogenic nanoscale magnetite crystals are known to exist in living systems. In addition to plants, they are mainly involved in human and animal brain tissues[18]. The properties of these

[18] Kirschvink, J.L., Kobayashi-Kirschvink, A., Woodford, B.J. Magnetite biomineralization in the human brain // Proceedings of the National Academy of Sciences.USA: Biophysics, – 1992. V.89, – p. 7683-7687.

crystalline nanoparticles have been barely studied, and so far no scientific conclusions have been drawn about their source and destination. Various considerations and opinions are discussed about this[19,20].

Our studies with plant samples have shown that the EPR spectroscopy method provides excellent opportunities for detecting and characterizing iron oxide magnetic nanoparticles and their magnetic properties in living systems. Identification of spectra recorded in a wide range of the magnetic field plays a major role in obtaining important information.

In general, magnetic nanoparticles - magnetite ($Fe_3O_4$) and maghemite ($\gamma$-$Fe_2O_3$) play an important role in the functionalization of living systems. Our experiments with plants and chloroplasts have shown that these nanoparticles induce new magnetic properties in living systems and at the same time generate a broad EPR signal.

After studying the effects of stress factors on various plant systems, as a continuation of the research, the effects of stress factors on grape snails with shell (*Helix pomatia*) collected from different areas of Absheron (Academy garden, Hovsan gardens and Guneshli settlement areas) and laboratory rats (*Wistar albino*) were studied.

**4.1. The role of stress factors in the formation of magnetic properties in grape snails with shell.** Grape snails, chosen as model objects in research, are very resistant to the effects of stress factors. Oxygen transport in them is carried out by means of a copper-containing protein - hemocyanin. At the same time, the amount of iron in grape snails is low in tissues, iron ions are mainly included in enzymes. The formation of iron oxide crystalline particles in such a system testifies to their functional importance. In addition, the study of the shells formed as a result of biomineralization in grape snails is of great interest.

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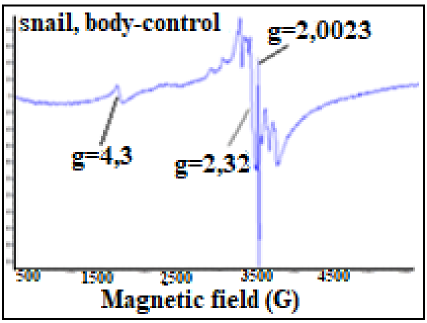
[19] Brem, F., Magnetic iron compounds in the human brain: a comparison of tumour and hippocampal tissue / F. Brem, A.M. Hirt, M. Winklhofer [et al.] // Journal of the Royal Society İnterface, – 2006. 3(11), p. 833-841.

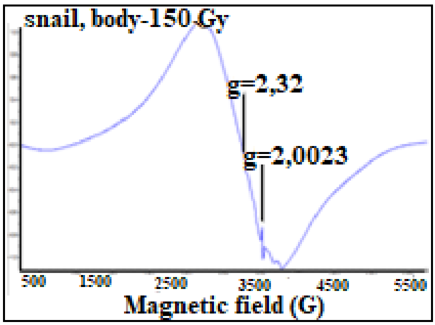
[20] Mourdikoudis, S., Pallares, R.M., Thanh, N. T. K. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties // Nanoscale, – 2018. V.10, – p. 12871-12934.

Due to these characteristics, grape snails with shell (*Helix pomatia*) were selected as one of the research objects when studying the formation of magnetic properties in animal organisms and the effect of gamma radiation on them was studied. It should be noted that before the irradiation of snails, they were placed in special containers with 15-20 individuals in each.

Control and packaged grape snails irradiated with gamma radiation in different doses (50 Gy, 100 Gy, 200 Gy, 350 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy) were collected from different areas of Absheron and studied by EPR method.

Life activities and morphological characteristics of control and irradiated grape snails were monitored for 30 days after irradiation. During this period, the percentage of death in control snails was not observed, it was determined that they were actively moving and actively feeding. In the samples irradiated with low doses (50 Gy - 350 Gy), a small number of dead individuals were found, and the percentage of deaths increased with the increase of the dose. Relatively low mobility and poor feeding were observed in irradiated snails. Thirty days after irradiation, the body and shell parts of the snails were separated from each other, dried under natural conditions at room temperature, and the paramagnetic centers formed in them were studied by the EPR method (figure 22, figure 24).



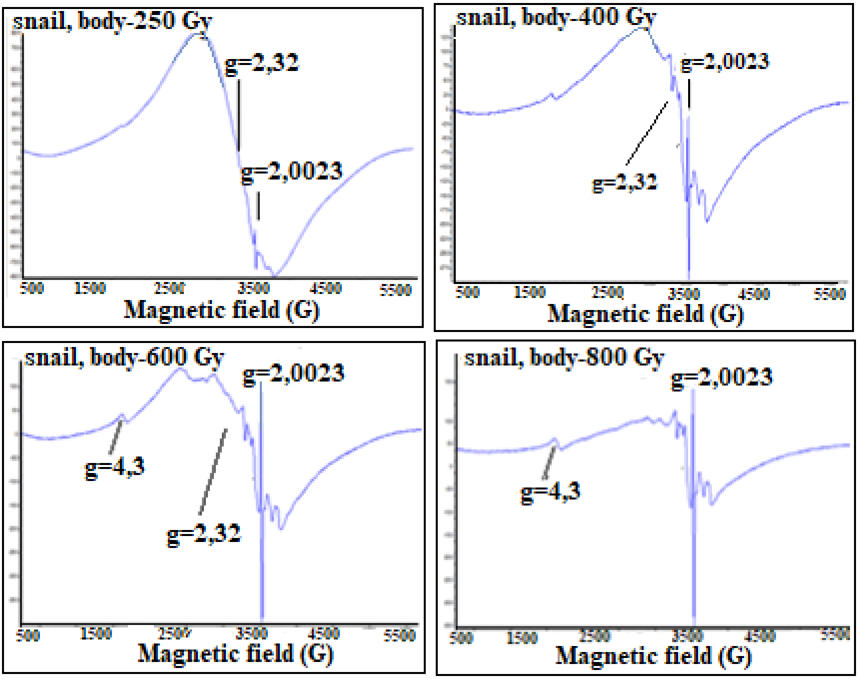



**Figure 22. EPR spectra of body parts of control and irradiated snails with different doses of gamma radiation**

It can be seen from figure 22 that free radical signals (g≈2.0023), broad EPR signals characterizing magnetic nanoparticles (g≈2.32) and signals of iron ions (g≈4.3) were recorded in the spectra taken from the body parts of snails. As can be seen from the spectra, a high-amplitude broad EPR signal (g=2.32) that characterizes iron oxide magnetic nanoparticles occurs during irradiation up to 250 Gy, compared to the control. Increasing the radiation dose from 400 to 800 Gy causes a gradual decrease in the intensity of this signal.

The main essence of such behavior of signals is that as a result of the effect of radiation in certain doses (200 Gy and 350 Gy), the increase of free iron ions in the body of snails and the presence of a reducing environment causes the formation of nanophase iron oxide particles in them. With the increase of the dose of radiation (600 Gy), despite the increase of free iron ions in the body of the snail, the gradual weakening of the reducing system reduces the formation of magnetic nanoparticles there.

Radiation at the highest dose of 800 Gy causes the complete failure of the reducing system, so the formation of nanoparticles almost does not occur.In addition, a linear increase of the intensity of free radical signals in the spectra was observed depending on the radiation dose. The dependence of the intensity of both signals on the radiation dose is given in figure 23.

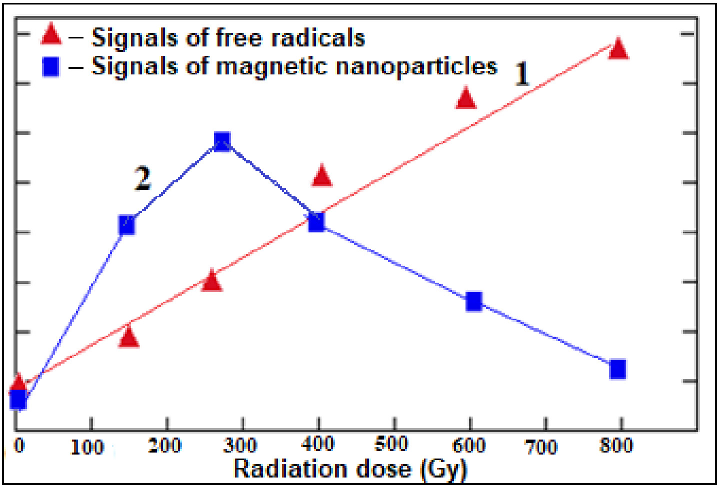
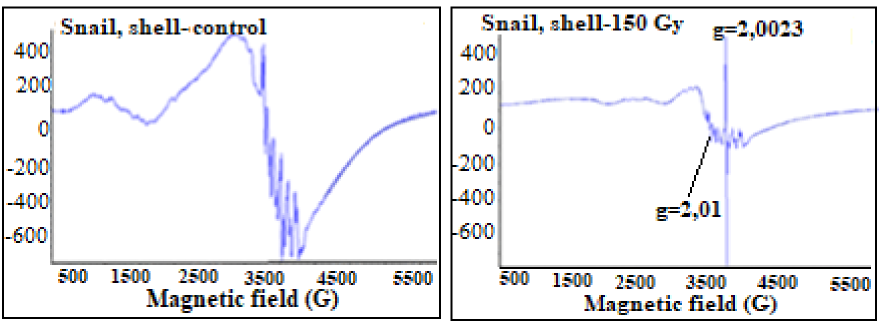



**Figure 23. Dependence of the intensities of free radical signals (1) and broad EPR signals characterizing magnetic iron oxide nanoparticles obtained from snail body parts (2) on the irradiation dose**



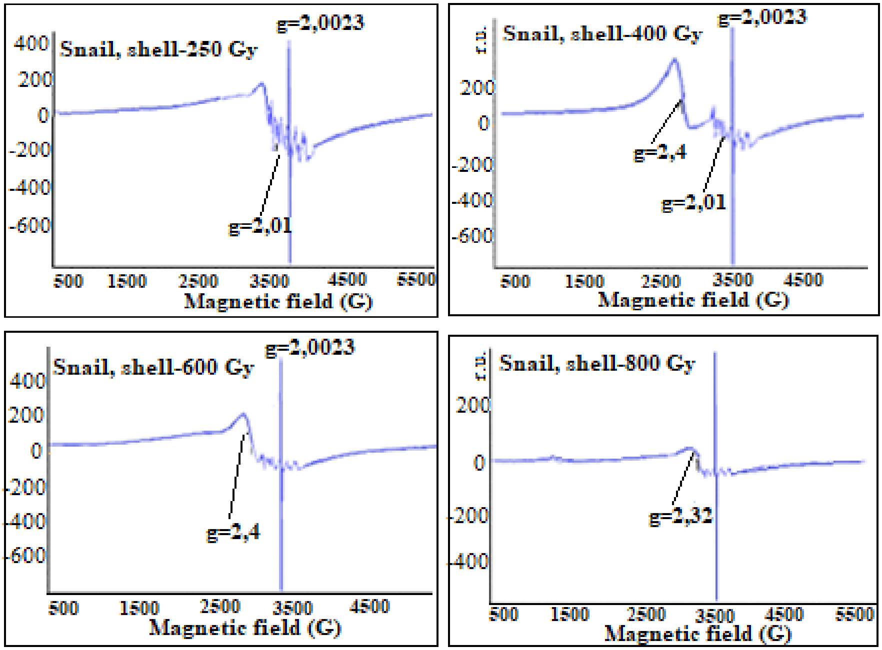



**Figure 24. EPR spectra of shell parts of control and gamma-irradiated snails at different doses**

EPR spectra of shell parts of snails were also registered (figure 24). In these spectra were detected free radicals signals (g=2.0023), signals of iron oxide magnetic nanoparticles (g=2.4) and signals of six-component manganese ions with an ultrafine structure (g=2.01).

It was determined that radiation at a dose of 150 Gy, 250 Gy causes the formation of signals characterizing iron oxide magnetic nanoparticles in shell samples, and the intensity of this signal gradually increases with the increase of the radiation dose. When the radiation dose reached 400 Gy, the intensity of the signal characterizing magnetic nanoparticles reached its maximum value. Irradiation at a dose of 600 Gy has caused a gradual decrease in the intensity of this signal. During irradiation with a dose of 800 Gy, it was observed that the intensity of the signal was reduced by two times.

Despite the absence of a reducing system in the snail's shell, the signals of iron oxide magnetic nanoparticles are recorded in them. The reason for this may be that the pelvis is directly connected to the body. The role of the body in the formation of the structure and composition of the shell is important and great[21]. In the spectra of the pelvic parts of snails, it was found that the intensity of free radical signals increased linearly depending on the radiation dose. The dependence of the intensity of both signals on the radiation dose is given in figure 25.

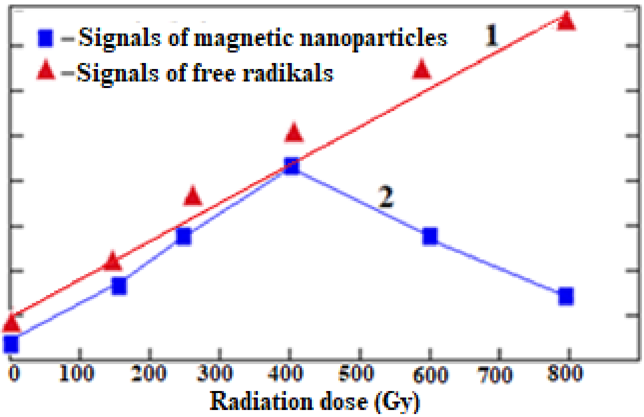



**Figure 25. Dependence of the intensities of narrow free radical signals (1) and signals of nanophase magnetic particles (2) which registered in the shell on the dose of gamma radiation**

Thus, while studying the effect of stress factors on living systems, it was determined that the stress factor causes the emergence of new magnetic properties in plant systems as well as in animal organisms (grape snails).

This result is very promising and actuality both in terms of biomedical application and ecological assessment.

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[21] Nasibova, A.N. The use of EPR signals of snails as bioindicative parameters in the study of environmental pollution // Advances in Biology & Earth Sciences, – 2019. V.4, №.3, – p.196-205.

The radionuclide composition of the body and shell of grape snails and their specific activities were also determined (table 2).

**Table 2**
**Radionuclide contents of the shell parts of snails collected from Hovsan settlement and Akademia garden and their specific activities**

| Radionuclides | Unit of measurement | Grape snail with shell, shell | Grape snails with shell, body |
|---|---|---|---|
| **$^{40}$K** | Bq/kg | 26.1 ±3.1 | 6.7 ±1.5 |
| **$^{232}$Th** | Bq/kg | 6.2 ± 0.3 | MDA=0.32 |
| **$^{226}$Ra** | Bq/kg | 3.1 ±0.5 | MDA=0.63 |
| **$^{228}$Ra** | Bq/kg | 4.3 ±0.4 | MDA=0.55 |
| **$^{137}$Cs** | Bq/kg | 1.39 ±0.12 | 0.91 ±0.14 |
| **$^{235}$U** | Bq/kg | 0.08 ±0.03 | MDA=0.03 |
| **$^{238}$U** | Bq/kg | 1.65 ±0.31 | MDA=0.65 |

It was found that more radionuclides ($^{40}$K, $^{232}$Th, $^{226}$Ra, $^{228}$Ra, $^{137}$Cs, $^{235}$U, $^{238}$U) are collected in the shells than in the body parts of snails. At the same time, higher specific activities of $^{40}$K, $^{228}$Ra, $^{238}$U, $^{238}$Th and $^{137}$Cs radionuclides were found in shells.

In the conducted studies, the generation of copper and iron-based paramagnetic centers and the mechanism of the effect of stress factors on this phenomenon were studied. The results obtained during the research proved once again that stress factors, including the radiation factor, play a stimulating role in the formation of paramagnetic centers in living systems. We determined by the EPR method that under the influence of gamma radiation iron oxide magnetic nanoparticles are generated in the body and pelvis parts of cupped vine snails. The resulting nanoparticles are formed quantitatively more in the body part than in the shell part.

Experiments conducted with the EPR spectroscopy method and the results obtained during the study of grape snails showed that this method is of great importance in detecting the generation of paramagnetic centers in animal organisms for obtaining completely new biophysical and ecological information.

The obtained results allow a deep understanding of the formation and role of biogenic paramagnetic centers in living systems (plant organisms, chloroplasts, animal organisms).

**4.2. Effect of UV-irradiation on endogenous paramagnetic centers in grape snails with shell.** In addition to ionizing gamma radiation, the effect of UV-irradiation on grape snails with shell was studied by the EPR method.

Snails were packed in groups of 13-18 individuals and irradiated with a DRT-230 mercury-quartz lamp. Irradiation with UV-radiation was carried out during the 60 min., 120 min., 150 min., 180 min. Note that 10 min. radiation corresponds to 1.2 $J/sm^2$.

For 3 days, control and UV-irradiated grape snails with shell were kept under control in the laboratory, and their life activities and feeding were monitored. After 3 days, the shell and body parts of the snails were separated and dried at room temperature under natural conditions, and their EPR spectra were recorded in a wide interval of the magnetic field (500-5500 G). The spectra are given in figure 26 and figure 27. Figure 26 shows the EPR spectra of body parts of grape snails exposed to UV-irradiation at different doses and control. In the control sample, the free radical signal (g=2,0023) and the signals of six-component manganese ions (g=2,01) were detected. Along with these signals, EPR signals corresponding to the paramagnetic centers characterizing iron oxide magnetic nanoparticles were also recorded during UV- irradiation, and an increase in the integral intensities of these signals was observed with the increase of the radiation dose. UV- radiation also caused a linear increase in the amplitude of free radical signals.

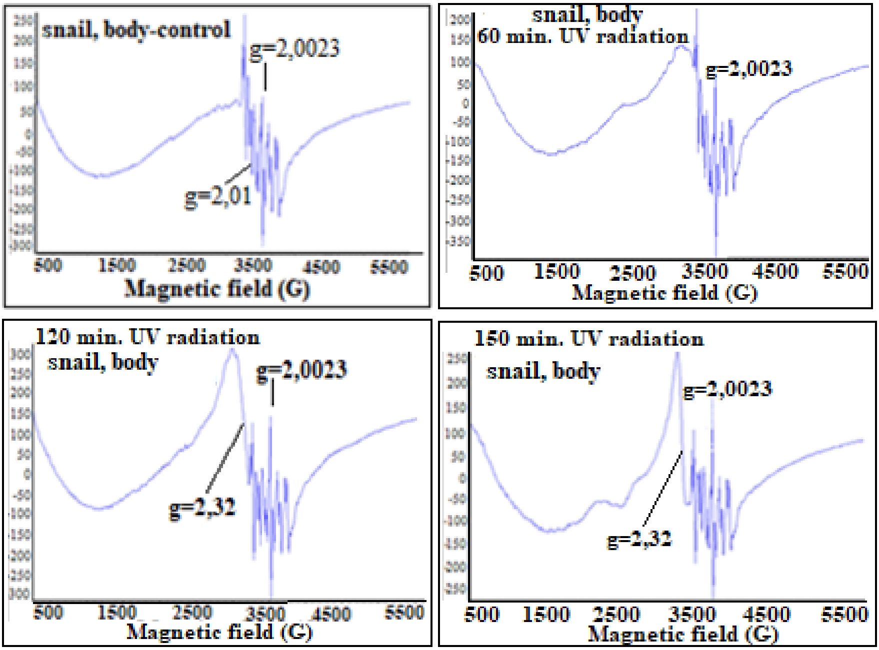
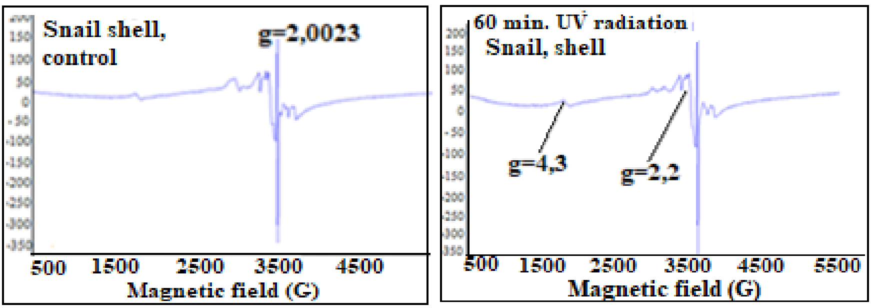



**Figure 26. EPR spectra of body parts of control and UV-irradiated grape snails with shell**

EPR spectra of shell parts of grape snails irradiated with UV-radiation are shown in figure 27. Here, a linear increase of free radical signals (g=2.0023) depending on UV-radiation was observed. In addition, signals of iron ions (g=4.3) were recorded in shell samples.



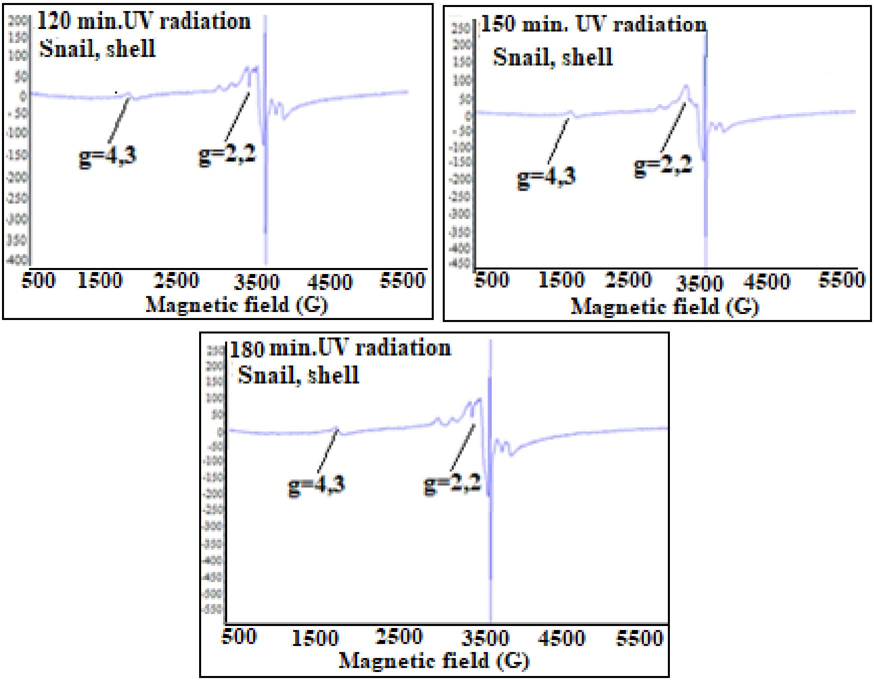



**Figure 27. EPR spectra of shell parts of control and UV-irradiated grape snails**

Signals of weak intensity (g=2.2) characterizing nanophase iron oxide magnetic particles were recorded in the spectra taken from the bowls. We observe that signals (g=2.2) characterizing nanophase iron oxide magnetic particles appeared in the spectra recorded in the shells under the influence of UV-radiation, as well as during the influence of gamma radiation. This is an indication that the shell is directly related to the body in grape snails with a shell, and that the body part has a great role in the creation and formation of the structure of the shell part of the snail. Thus, experiments have shown that stress factors (gamma radiation and UV- radiation) cause the emergence of magnetic properties in grape snail organisms.

**4.3. Study of magnetic properties in laboratory rats (*Wistar albino*) exposed to gamma radiation as a stress factor.** While studying the effects of stress factors on animal organisms, studies were also conducted with laboratory rats (*Wistar albino*). The effect of different doses of ionizing gamma radiation (3 Gy, 6 Gy, 8 Gy) on laboratory rats was studied. Paramagnetic centers were studied bu EPR method in control and laboratory rats exposed to different doses of gamma radiation.

In our experiments, laboratory rats were divided into four groups, each with six individuals: 1) control; 2) 3 Gy irradiated; 3) 6 Gy irradiated; 4) 8 Gy irradiated. Rats were irradiated in a RXUND 20000 device with a $^{60}Co$ source (P=0.286 rad/sec). Usually 37-55 days old rats were used in the experiments. Irradiated rats were kept under laboratory conditions (30 days) at room temperature. During this period, the life activities and behavior of the rats were kept under control and the morpho-physiological changes that occurred in them after the radiation were monitored. It was determined that their vitality and activity have decreased, and their nutrition has weakened.

Within a month after irradiation, no deaths were usually observed in the control and irradiated samples with a dose of 3 Gy. Laboratory rats irradiated with a dose of 6 Gy died in 2-6 months, and laboratory rats irradiated with a dose of 8 g usually died within 1 month. Then the laboratory rats were cut and their internal organs (heart, liver, lungs, spleen, kidneys) were removed (figure 28), and after drying at room temperature under natural conditions, their EPR spectra were registered (figure 29).

**Figure 28. Internal organs of control and irradiated rats (heart, spleen, kidneys, lung, liver)**

Signals of free radicals (g=2,0023) and iron ions (g=4,3) were observed in internal organs of control and irradiated laboratory rats. In addition to these signals, signals characterizing iron oxide magnetic nanoparticles (g=2,32) were detected in liver organs of laboratory rats.

Increasing the radiation dose led to an increase in the intensity of free radical signals in these samples. In addition, as a result of the effect of the radiation factor, the generation of the signal of iron oxide magnetic nanoparticles in the liver organs was found. When the radiation dose was 8 Gy, EPR signals with high integral intensity were observed (figure 29).

The obtained result is of great practical importance for application in biomedical research.

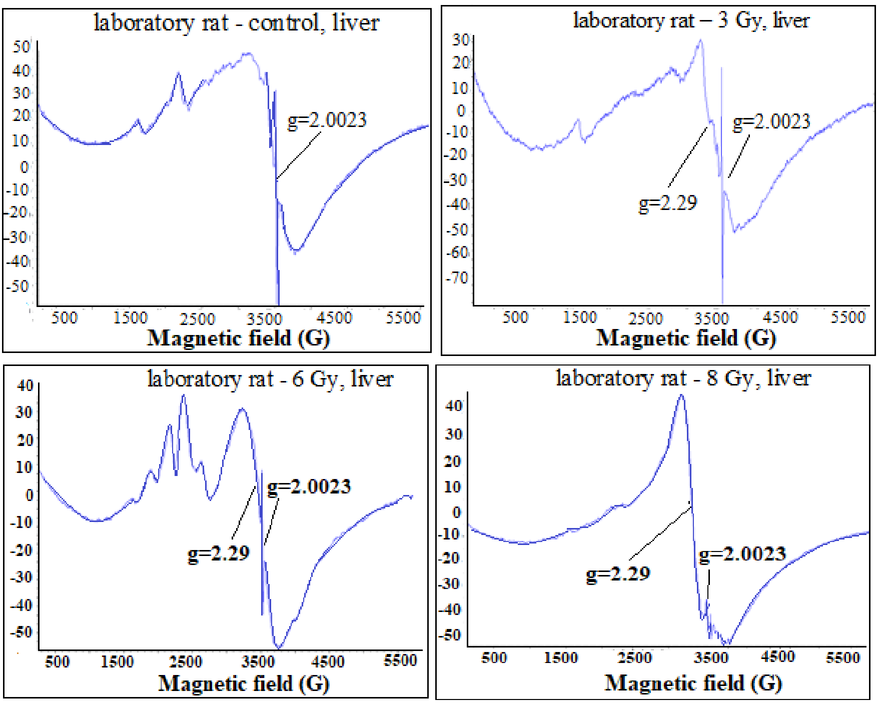
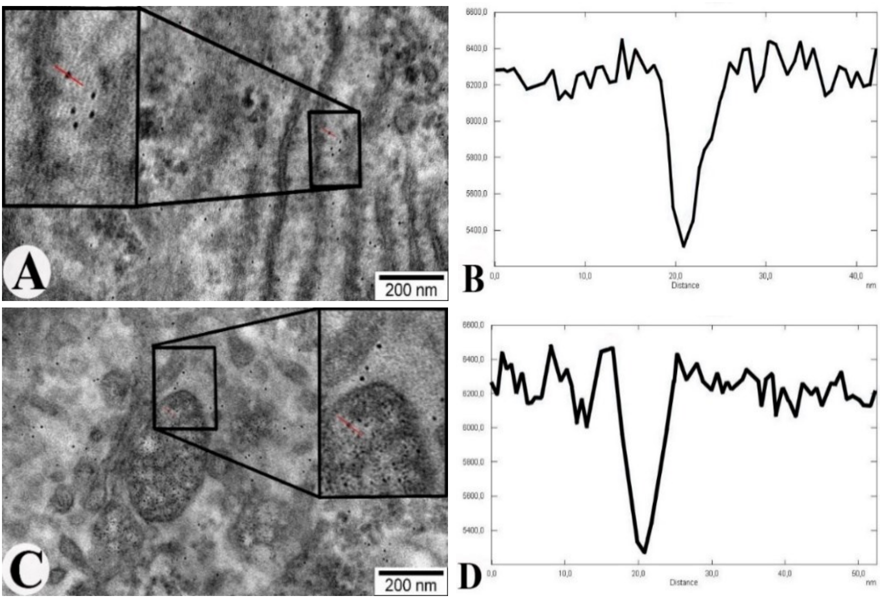



**Figure 29. EPR spectra of liver organs of control and irradiated rats at different doses**

After determining by EPR method that biogenic magnetic iron oxide nanoparticles were generated in the internal organs of laboratory rats, especially in the liver, under the influence of stress factors, these results were investigated and unequivocally confirmed by TEM (transmission electron microscope).

An ultrastructural study of the liver organs of laboratory rats irradiated with a dose of 6 Gy was performed. At this time, in the high magnifications of the electron microscope (100,000 times and more) in the cytoplasm of hepatocytes (figure 30 A), in cristae of mitochondria (figure 30 C), in glycogen (figure 30 E) and other structural elements bioaccumulative nanoparticles was observed (figure 30).

In addition to those structures being shown in the figures, their histograms are also given by means of "intensity profile" program (figure 30 B, figure 30 D, figure 30 F). It was determined that the degree of gray value of nanoparticles is 5300-5400, and their size is 10-12 nm. The mentioned gives reason to say that the nanoparticles visually visible in KEM are iron oxide magnetic nanoparticles (magnetite-Fe3O4).



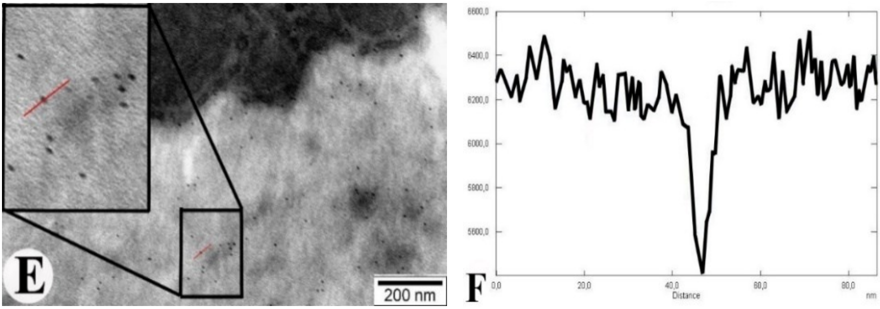
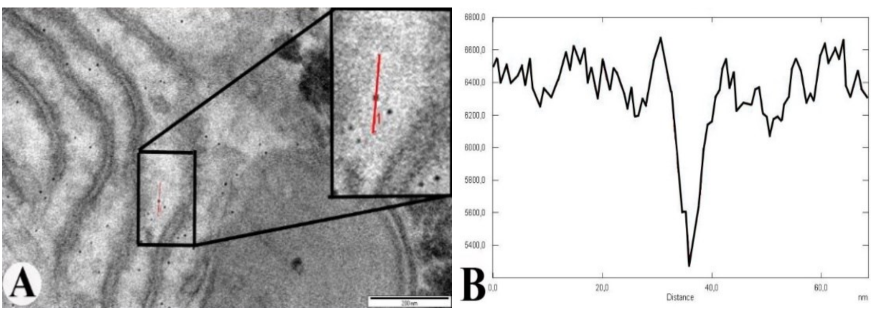



**Figure 30. Bioaccumulation of nanoparticles ($Fe_3O_4$) in liver hepatocytes in irradiated (6 Gy radiation) exposed rats. A, C, E – photo of an unstained ultrathin cross-section (50–70 nm) in an electron microscope, B, D, F – histograms of nanoparticles located in hepatocytes**

Thus, nano-sized magnetic particles were noted in the hepatocyte cytoplasm (figure 31A), lysosomes (figure 31C), mitochondria (figure 31 E) and also inside the nucleus (figure 31G). Histograms of those structures were prepared by means of the "intensity profile" program (figure 31 B, figure 31 D, figure 31 F, figure 31 H). The degree of gray value of detected magnetite ($Fe_3O_4$) nanoparticles was 5200-5400, and their size was 10-12 nm.

In the experiments, during the study of various structural elements of the laboratory rat's liver by means of TEM, the degree of gray value was between 5200-5400 during the irradiation of the mentioned nanoparticles in both doses (6 Gy and 8 Gy), which indicates that these particles are magnetite - $Fe_3O_4$.



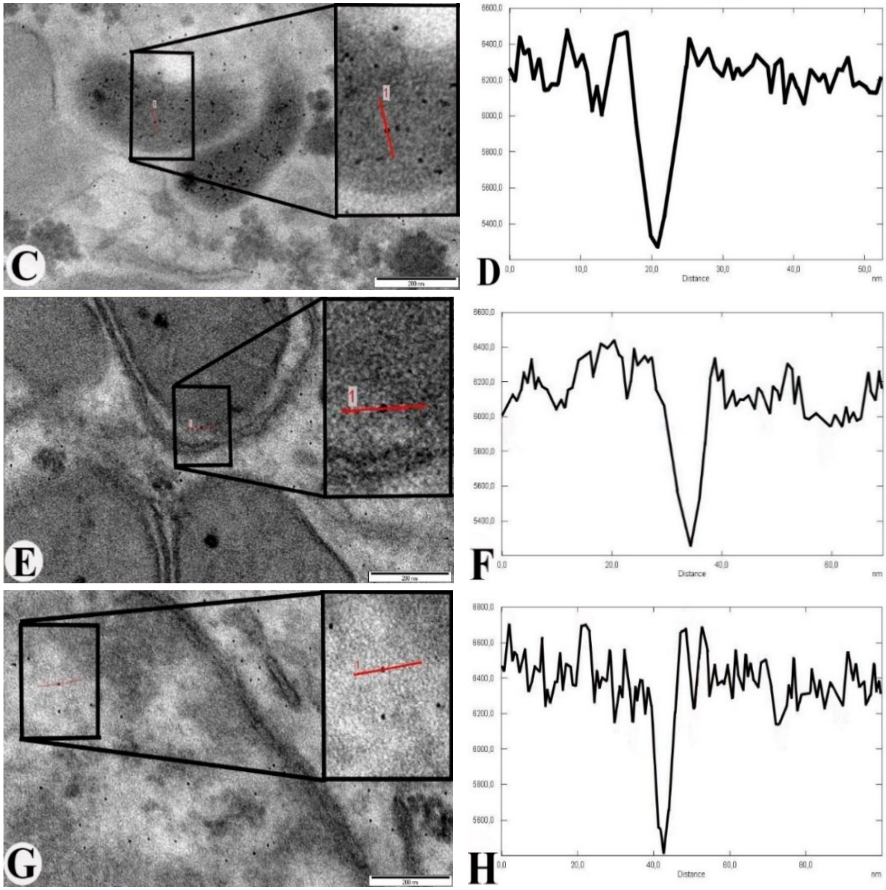


**Figure 31. Bioaccumulation of nanoparticles ($Fe_3O_4$) in liver hepatocytes in laboratory rats exposed to irradiation (8 Gy gamma radiation). A, C, E, G – photo of an unstained ultrathin cross-section in an electron microscope (50-70 nm), B, D, F, H – histograms of nanoparticles located in hepatocytes**

Thus, after experiments conducted on laboratory rats, it became known that, as in other living systems we have studied (various types of plants, chloroplasts, grape snails with shell), and in laboratory rats under the influence of stress factors (gamma irradiation) biogenic nanophase iron oxide particles are generated as a result of biomineralization. Thus, EPR signals characterizing iron oxide magnetic nanoparticles (g=2,3; ΔH=320 Qs) were recorded in their liver organs. The obtained results were also confirmed by the TEM method. It was possible to visualize 10-12 nm size magnetite ($Fe_3O_4$) nanoparticles in liver organs of laboratory rats after 6 Gy and 8 Gy doses of gamma radiation by TEM.

$Fe_3O_4$ nanoparticles were detected in cytoplasmic structural elements (mitochondria, lysosome, glycogen, nucleus and cytoplasm itself) during the study of the ultrastructure of liver hepatocytes.

# CHAPTER V
# FORMATION OF MAGNETIC PROPERTIES IN OIL SAMPLES OF BIOGENIC ORIGIN

**5.1. Study of paramagnetic centers in oil samples from different oil fields of Absheron.** It is known that oil is of biogenic origin, a product of the living world (plants, animals, microorganisms) and is also characteristic of the Absheron peninsula. In this regard, iron oxide magnetic nanoparticles that we found in plant and animal organisms were also investigated in oil samples of biogenic origin typical for the Absheron Peninsula. It is known that Azerbaijan is one of the leading countries in the field of hydrocarbon production. This determines the importance and relevance of the efficiency and environmental safety of oil production, transportation, and processing processes. Intensive study of underground resources of Azerbaijan was accompanied by radioactive soil contamination in several cases. This shows the importance of analyzing the results and monitoring of the effects of radiation on the environment.

Paramagnetic centers were studied in the oil samples taken from different oil fields of Absheron by us. Our studies have shown that iron oxide magnetic nanoparticles can also be present in the structural elements of oil.

As is known, a number of microorganisms can feed on oil and break down hydrocarbons, as well as produce iron oxide particles. The presence of magnetic nanoparticles in oil makes it possible to control technologically important properties of oil (viscosity, precipitation of paraffin, etc.) with the help of an external magnetic field. It should be noted that oil refers to a number of liquid colloids, including biological (blood, lymph, plant sap, etc.) entities, so-called non-Newtonian fluids. These fluids are primarily characterized by a strong dependence of viscosity on motion deformation (gradient of fluid motion velocity). This feature, and the high viscosity of heavy oil, causes technical problems that complicate the production and transportation of oil. Magnetic nanoparticles can be included in fractal aggregates of colloidal components of oil. It is this position that allows oil to influence the structure of aggregates using the influence of an external magnetic field, to change the characteristics of oil (reduce viscosity, reduce the deposition of paraffin)[22,23].

It should be noted that iron oxide magnetic nanoparticles are characterized by chemical stability as well as magnetic properties and are quite widespread in living and non-living nature. In some literature, the presence of iron compounds in oil was detected (by X-ray diffractometry), but no information was given on the size of the iron particles and their interaction with the colloidal particles of the oil [24].

The characteristics of eleven oil samples taken from oil wells in different areas of Absheron (Umbaki, Ateshgah, Gala field, Bibiheybat, Balakhani, etc.) were studied by the EPR method.

The analysis of EPR spectra recorded at room temperature in a wide range of magnetic field in the experiments showed that the presence of iron oxide magnetic nanoparticles is characteristic for some oil samples.

Since these nanoparticles have ferromagnetic properties, it may be possible to change the viscosity of oil samples through an external

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[22] Лесин, И.В., Кокшаров, Ю.А., Хомутов, Г.Б. Магнитные наночастицы в нефти // Нефтехимия, – 2010. Т.50, №2, – с.114-117.

[23] Лесин, В.И. Кокшаров, Ю.А. Хомутов, Г.Б. Магнитные наночастицы в составе агрегатов коллоидных частиц нефти // Нефтяное хозяйство, – 2009, с. 95-97.

[24] Лесин, В.И. Фрактальная теория вязкости неньютоновской нефти, основанная на учете взаимодействия коллоидных частиц: обзор и новые результаты // Актуальные проблемы нефти и газа, – 2021. №1, – с.26-46.

magnetic field. This is one of the results aimed at the development of new effective nanotechnologies for managing the main physico-chemical properties of oil.

Figure 32 shows a typical broad EPR signal from an oil sample.

Since these nanoparticles have ferromagnetic properties, it may be possible to change the viscosity of oil samples through an external magnetic field. This is one of the results aimed at the development of new effective nanotechnologies for managing the main physico-chemical properties of oil.

Figure 32 shows a typical broad EPR signal from an oil sample.

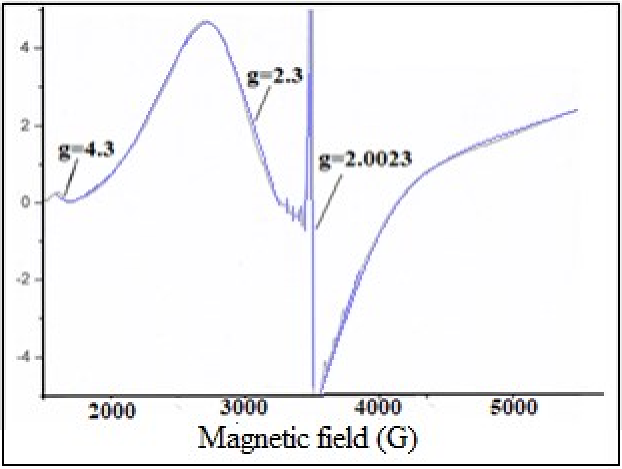


**Figure 32. EPR spectrum of oil sample**

As can be seen from the figure, in addition to the EPR signal characterizing iron ions (g=4.3), the broad EPR signal characteristic of iron oxide nanoparticles (g=2.3) and the narrow free radical EPR signal observed in all oil samples (g=2, 0023) was recorded. The similarity of EPR signals belonging to paramagnetic sources in various natural systems (plants, animal organisms, oil) shows that the phenomenon of biomineralization occurs in those natural systems, and as a result, paramagnetic centers are formed under certain conditions.

The presence of iron oxide magnetic nanoparticles in the oil samples extracted from some oil fields of the Absheron Peninsula allows influencing the oil extraction and oil transportation processes in those fields by means of the magnetic field[25]

The results obtained can help solve scientific and technical problems of increasing oil recovery from wells, ensuring continuous transportation of oil through pipelines, creating cost-effective and environmentally friendly oil refining technologies based on targeted changes in oil viscosity using physical and chemical methods. The problem of reducing the viscosity of oil and the problem of reducing the growth rate of paraffin deposits on the walls of oil wells and oil pipelines are important and relevant in the oil production industry, also, the economic and environmental efficiency of the oil industry as a whole depends on their solution.

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## MAIN RESULTS

1. For the first time, a broad EPR signal (g=2.32; ΔH=320 G) was observed in a wide interval of the magnetic field (0-500 G) the study of the effect of radioactive pollution on plant samples typical for the Absheron Peninsula by the EPR method. It was determined that the increase of the radiation background causes an increase in the intensity of this signal. The behavior of these EPR signals is very important in studying and monitoring the ecological status of the environment and can be used as bioindicator parameters.

2. As a result of *in vivo* and *in vitro* studies, it was found that a characteristic broad EPR signal (g≈2,2-2,4; ΔH≈320-450 Qs) generated in living systems during stress characterizes iron oxide magnetic nanoparticles. It was determined that lowering the recording temperature of the spectra of synthetic magnetic nanoparticles from room temperature to 80 K causes a sharp decrease in the amplitude of this signal and a significant expansion of its width. The characteristics of temperature-dependent EPR signals of synthetic magnetic nanoparticles were identical to the temperature-dependent changes of broad EPR signals of plant samples.

3. During *in vitro* studies, it was found that trivalent iron ions are reduced and turned into magnetic nanoparticles in certain loci of ETC as a result of damage to thylakoid membranes. This is observed by the formation of characteristic EPR signals (g=2.4; ΔH=320 G). The obtained results were confirmed by microscopic studies using the TEM method.

4. The study of the temperature dependence (260K - 60K) of the EPR spectra of fig (*Ficus carica* L.) leaves showed that the behavior of the parameters of the amplitude and width of the paramagnetic resonance signal at a temperature of 120-125 K produces a Vervey phase transition characteristic of superparamagnetic nanoparticles.

5. For the first time, the reducing properties of pomegranate (Punica granatum) extract were determined by the EPR method and it was shown that $Fe^{3+}$ ions at certain concentrations cause the generation of nanophase iron oxide particles in pomegranate extract.

6. The effect of stress factors on morphological parameters and paramagnetic centers of plants belonging to C3 and C4 types of photosynthesis was studied. It was found that stress factors (radioactive pollution, ionizing gamma radiation) up to a certain dose have a stimulating effect on the growth of C3 plants and the paramagnetic centers formed in them, and a retarding effect on C4 plants. It was determined that the regularity obtained can be related to the activation of the photorespiration process, which performs a protective function by preventing the formation of ROS in C3 plants.

7. The study of paramagnetic centers in different types of tea leaves showed that they have high antioxidant activity. Based on the EPR spectra of tea leaves, it was found that the antioxidant activity was lower in packaged tea samples compared to fresh tea leaf samples. Additionally, it was determined that the antioxidant activity was higher in the leaves growing in the lower parts of the plant stem, that is, in the older leaves.

8. During the study of the effect of gamma radiation and UV-irradiation on the grape snails with shell, it was determined that as the radiation dose increases, the intensity of free radical signals in their shell and body increases linearly. A non-monotonic change of the broad EPR signals characterizing iron oxide magnetic nanoparticles was found depending on the dose of gamma radiation, and a linear change depending on the dose of UV radiation.

9. EPR studies with laboratory rats have shown that ionizing gamma radiation induces a signal characteristic of iron oxide magnetic nanoparticles ($g=2,32$; $\Delta H=320$ G) in liver organs. The obtained results were proven in experiments with TEM, bioaccumulation of nanoparticles (magnetite – $Fe_3O_4$) in the structural elements (hepatocyte, sinusoid, etc.) of the liver organ of rats irradiated with gamma radiation in different doses (6 Gy, 8 Gy) was visually observed, it was determined that their degree of gray value is 5300-5400, and their size is 10-12 nm.

10. Iron oxide magnetic nanoparticles that we found in plant and animal organisms were also found in oil samples of biogenic origin typical for the Absheron Peninsula. It was determined that the effect of the magnetic field on the technologically important physico-

chemical properties of oil occurs with the presence of magnetic nanoparticles generated as a result of biomineralization. In order to ensure continuous transportation of oil in those fields and to increase production, it is possible to influence the oil extraction and oil transportation processes with a magnetic field.

11. Summarizing the results obtained during our research, we show for the first time that biogenic crystalline iron oxide magnetic nanoparticles and magnetic properties are formed as a result of biomineralization in living systems under the influence of stress factors. Crystalline magnetic nanoparticles are generated during stress exposure in the living system itself. The essence of the mechanism of occurrence of this event is related to the generation of iron ions generated in living systems during stress with the help of a reducing system into iron oxide magnetic nanoparticles. This process plays the role of self-defense of the organism.

## RECOMMENDATIONS

1. The occurrence of magnetic properties in living systems under the influence of stress factors can be applied in biophysical, ecological, nanobiotechnological studies, and in the field of biomedicine, and lead to fundamental results.

2. The regular change of the signals characterizing the iron oxide magnetic nanoparticles and the parameters of the free radical signals recorded in plants by the EPR method depending on the radiation dose can be applied to use them in the evaluation and biomonitoring of the ecological state of the environment.

3. The characteristics of changes in the parameters of the EPR spectra taken from the body and shell parts of the snails exposed to gamma radiation at different doses showed that the snails can be used as bioindicators.

4. Studies with laboratory rats have shown that biogenic iron oxide magnetic nanoparticles are generated in the liver organs of stressed rats. As magnetic nanoparticles are very popular objects for diagnosis and therapy in modern medicine, the obtained result is very important in terms of application in biomedicine.

5. The detection of magnetic nanoparticles in oil samples can help to solve the scientific and technical problems of increasing the oil production of wells, ensuring uninterrupted transportation of oil through pipelines, and also creating economically effective and environmentally safe technologies for oil processing using an external magnetic field.

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