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THE USE OF EPR SIGNALS OF SNAILS AS BIOINDICATIVE PARAMETERS IN THE STUDY OF ENVIRONMENTAL POLLUTION

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Abstract. The effects of stress factors (ionizing gamma radiation and radioactive contamination) on grape snails were studied by Electron Paramagnetic Resonance (EPR) spectroscopy. Grape snails were collected from different regions of the Absheron peninsula (territory of ANAS, Govsan settlement and Guneshli settlement). EPR spectra of the pelvis and body parts of snails have been investigated. Depending on the radiation dose in the pelvis part of grape snails, a linear increase in the intensity of the EPR free radical signals is observed (g = 2.0023; H = 10 Gs). Generation of new paramagnetic centers in grape snails was also investigated as a result of radioactive contamination. Experiments using EPR spectroscopy have shown that this method is a very promising method for detecting new paramagnetic centers in living systems and can provide new information in environmental assessment and biomonitoring.

Keywords: *EPR signals, environmental pollution, snail, stress factors, ionizing radiation, radioactivity, bioindicative parameter.*

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1. Introduction

A number of scientific studies have been carried out in the field of biological systems using the EPR method. The first studies in this area were carried out in the 50-s years of the last century by L. Blumenfeld and his colleagues (Blumenfeld, 1973; Samoilova & Blumenfeld, 1961; Blumenfeld, 1976; Blumenfeld *et al*., 1961). L.A. Blumenfeld with his colleagues in his research for the first time was observed in yeast, a broad EPR signal.

Biogenic magnetic nanoparticles in magnetotactic bacteria have also studied by EPR (Mann *et al*., 1990; Fischer *et al*., 2011). The magnetic resonance characteristics of the received signals were studied. It has been established that the signal source of magnetotactic bacteria is ordered chains consisting of magnetic nanoparticles in size 20-150 nm, and their images were obtained by electron microscopes. Magnetite nanoparticles are very common in nature and are found in many biological objects. Even before synthesizing the first magnetic nanoparticles, they were discovered in natural biological complexes. It has been established that magnetic nanoparticles play a role in the metabolism and activity of living organisms.

For the first time, we discovered the presence of magnetic nanoparticles in plants by EPR method. Thus, we observed a broad EPR signal (g = 2.38 and ΔH = 320Gs) in plants using the EPR method and showed that this signal characterized the iron oxide magnetic nanoparticles (Khalilov *et al*., 2011; Nasibova *et al*., 2016; Khalilov *et al*., 2010). At the same time, our studies in the field of research and assessment of environmental pollution using plants were studied using the EPR method (Khalilov *et al.,* 2015; Nasibova *et al*., 2016; Nasibova *et al.,* 2019).

The aim of our work was to obtain detailed information about the behavior of the EPR signals of snails under the impact of stress factors. Obtaining new information is of great importance in environmental biomonitoring.

2. Materials and methods

In recent years, in our EPR studies, we study grape snails (Helix pomatia), which we collect in various areas of the Absheron Peninsula (ANAS territory, in the village of Govsan and the village of Guneshli). It should be noted that snails are distinguished by their high viability. They are resistant to biological, physical, chemical and radioactive stresses. Their vascular system is open. In the blood plasma solution they carry hemocyanin - a protein containing copper molecules This protein is the oxygen acceptor. Characteristic features - have a pelvis, a well-developed head with eyes and a flattened foot. Dimensions range from 3 mm to 25-60 sm. (Agamaliyev *et al*., 2012).

In addition, it is of great interest to the study of the pelvis caused by biomineralization in snails. Biomineralization is the process by which living organisms produce minerals and often results in the consolidation of tissues and mineralized materials (Gadd, 2008; Skinner *et al.*, 2007). In order to study the paramagnetic centers in snails during stress response, they were first irradiated at different doses (50, 100, 200, 350, 400, 500, 600, 700, 800 Gr) (Fig. 1). Artificial gamma irradiation of the samples was carried out on the K-25 equipment (power 32.31 rad/sec). The sources of irradiation were the isotopes ^{60}Co and ^{57}Co.

Fig. 1. Control and irradiated snails in different doses

EPR spectra of objects were recorded at room temperature (297 K) on an ECS-106 EPR spectrometer from Bruker (Germany) X-band under the conditions indicated in the caption to the corresponding figures.

3. Result and discussion

In our research, we examined the morphological changes in snails affected by different doses of gamma rays. The mortality rate was not observed in the control sample for 10 days, very low mortality was observed in low-dose radiation samples, with the increase in dosage, the mortality rate was also higher.

After 10 days, the body and pelvis of the snails were separated, and after drying at room temperature, EPR spectra were obtained (Fig.2, 3).

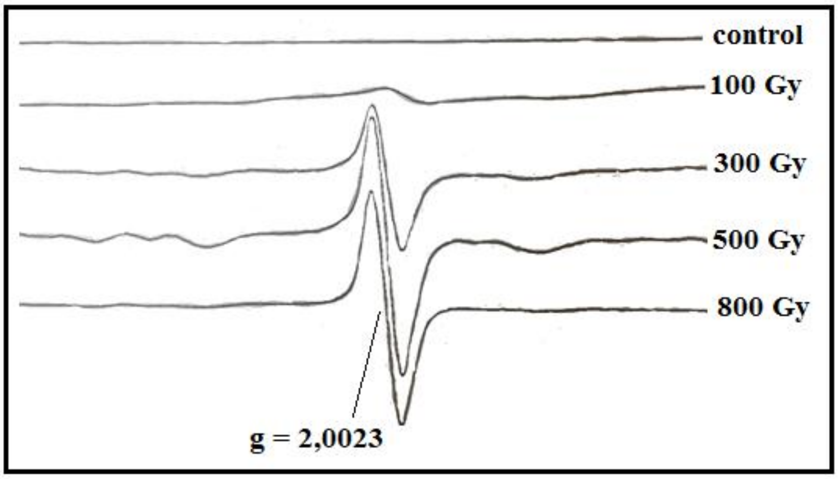


Fig. 2. EPR spectra of the snail'spelvis irradiated at different doses

Fig.2 shows the free radical EPR signals of the exposed to radiation at different doses snail's pelvis. As can be seen from the figure, the amplitude of EPR signals (g = 2,0023; ΔH = 10 Gs) increases linearly, depending on the dose. This important result can be used as a dosimetric tool in the future. Thus, the pelvis of the snail can be used as a bioindication parameter to measure the radiation background of the environment. Fig. 3 shows the EPR signals in a wide range of the magnetic field of a part of the snail's body exposed to various doses of gamma radiation. EPR signals have complicated nature and characterize metal complexes. These metal complexes include copper, iron and manganese. Here, EPR signals indicate that copper compounds have a higher concentration. Indeed, hemosionin is a major component of the blood system of snails.

It should be noted that the nature of EPR signals did not change much depending on the dose of radiation. This may be due to the fact that in the irradiated snails, the body was removed and dried out after 7 days. In the metabolism process, it takes longer time to form new paramagnetic complexes.

Therefore, in the following experiments, snails were kept for 4 months in soils (clean, mixed, and radioactive contaminated), which differed according to pollution levels (Fig. 4) and then their EPR spectra were recorded (Fig. 5, 6). The snails used in the experiments were collected from the ANAS territory and the Guneshli region. In studies under the influence of stress in snails, the effects of magnetization were revealed.

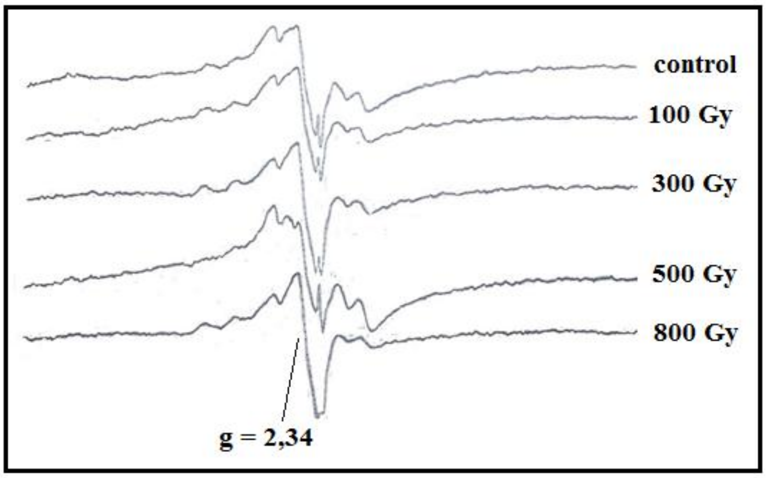
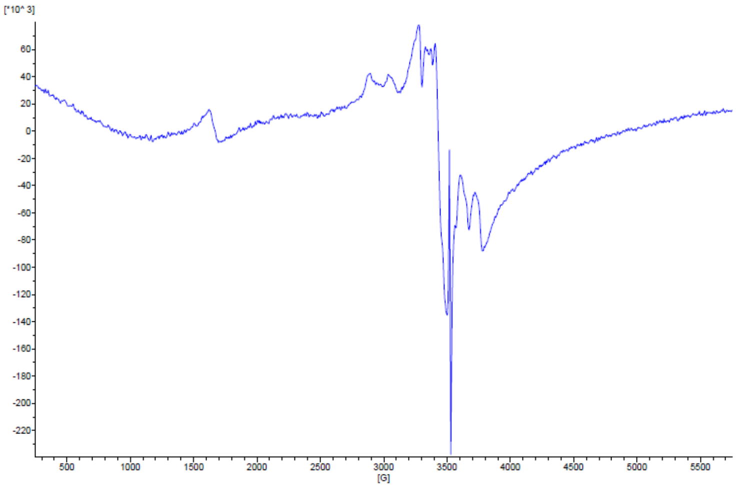


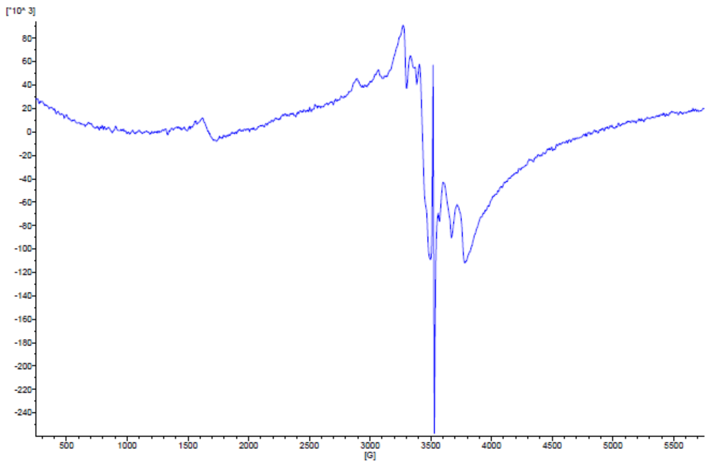
Fig. 3. EPR spectra from the body part of the snail irradiated at different doses

Fig. 4. Snails - in soils with varying degrees of pollution
(clean, mixed and radioactive contaminated)

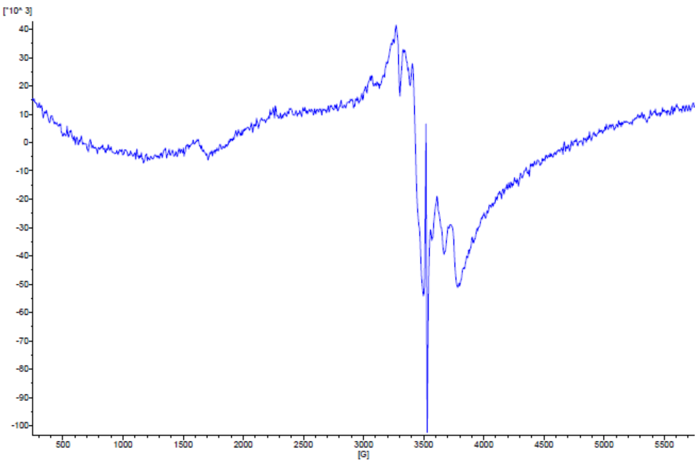
The EPR spectra of snails kept in all three soils are shown in the following figures (Fig. 5, 6).



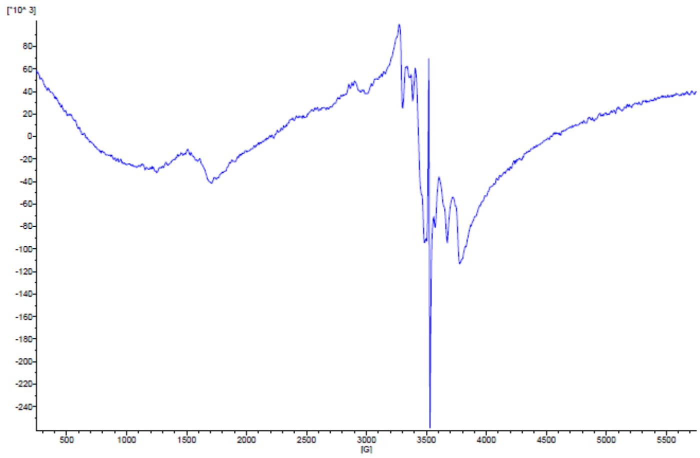
A – contaminated soil



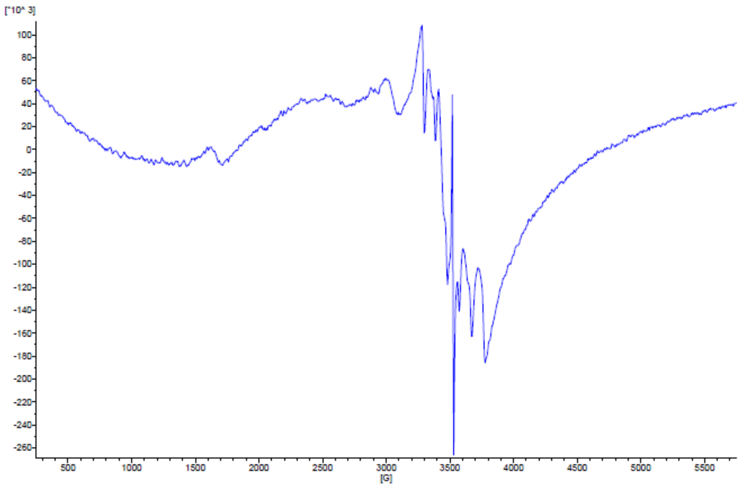
A – mixed soil



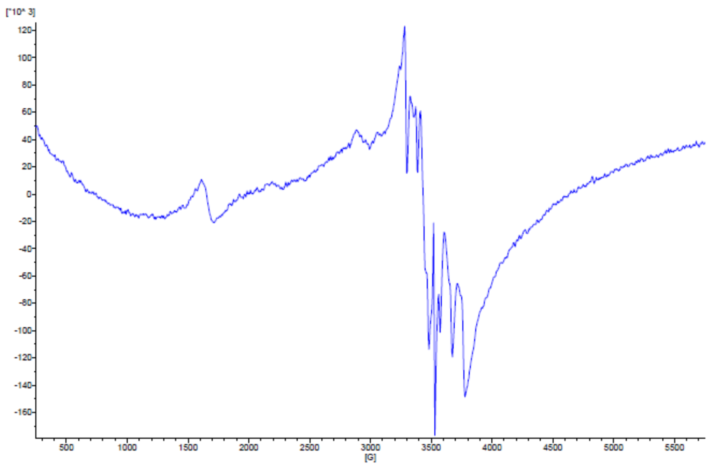
A – clean soil



B– contaminated soil

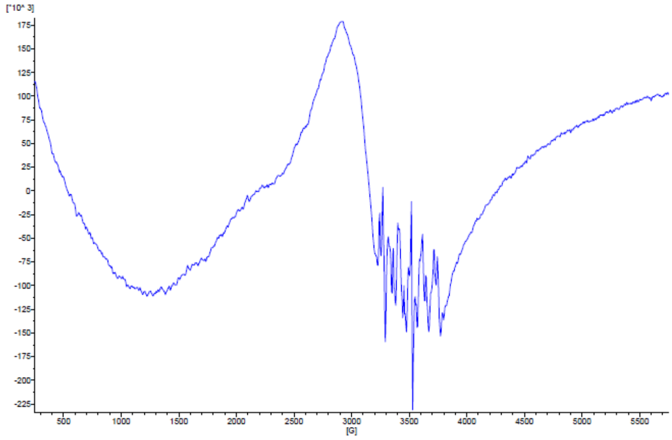


B– mixed soil

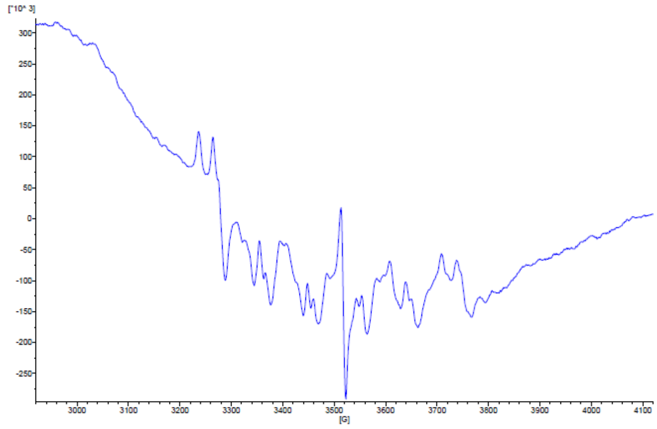


B – clean soil

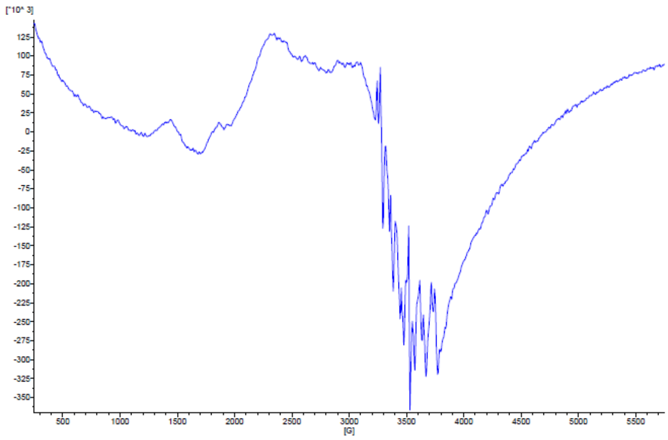
Fig. 5. EPR signals from body parts of snails stored in three types of soil
A – Territory of ANAS, B – Guneshli region



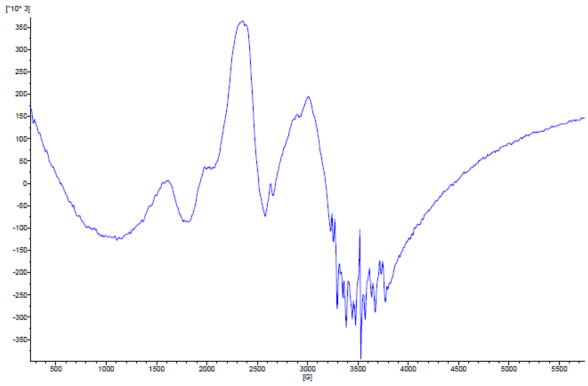
A – contaminated soil



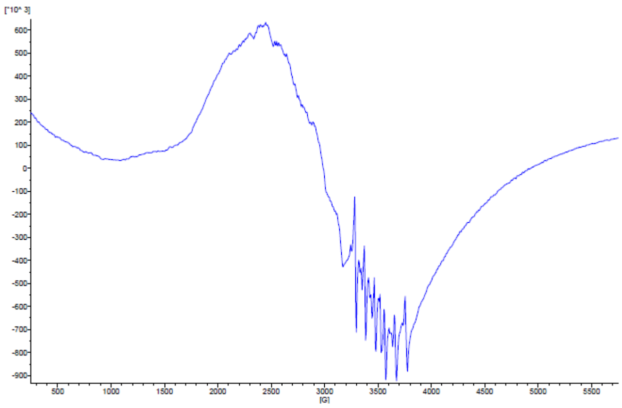
A – mixed soil



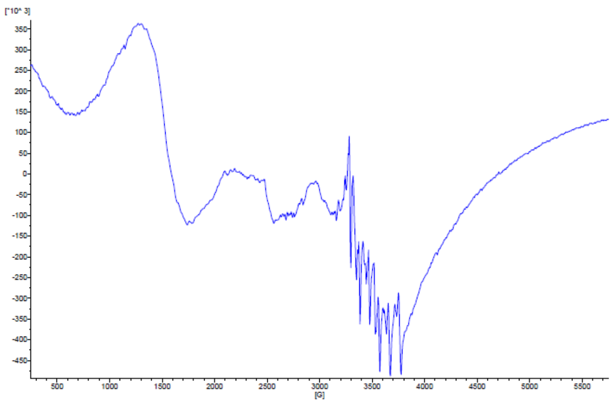
A – clean soil



B– contaminated soil



B– mixed soil



B – clean soil

Fig.6. EPR signals from pelvis parts of snails stored in three types of soil
A – Territory of ANAS, B – Guneshli region

In addition, the radionuclide composition and specific activity of snail's pelvis collected from the territory of ANAS and the Govsan settlement was also identified (table 1).

Table 1. The radionuclide composition and specific activity of snail's pelvis

Radionuclide composition and specific activity		
Bq/kg	İlbiz (Hövsan)	İlbiz (AMEA)
Be7	<5.67	<8.15
K40	32,22	30,57
Co60	<0.74	<1.09
Cs134	<0.83	<1.25
Cs137	<0.82	0,83
Tl208	<0.87	<1.20
Bi210	<0.96	<1.39
Pb210	<176.4	<256.52
Bi212	ND	ND
Pb212	1,91	1,55
Bi214	2,85	<2.72
Pb214	3,79	3,82
Ra226	ND	ND
Ac228	ND	ND
Pa234	ND	ND
Pa234M	45,86	ND
Th234	<21.47	<29.66
U235	<0.83	<1.21

In the pelvis of snails that have not been stressed were discovered a large number of radionuclides.

4. Conclusion

In studies were investigated the generation of copper, paramagnetic centers based on iron and the mechanism of action of stress factors on this phenomenon. The research results once again proved that stress factors, including radiation, play a stimulating role in the creation of paramagnetic centers in natural systems. This effect can be used as a bioindication parameter in environmental assessment of the environment.

Experiments using EPR spectroscopy have shown that this method is a very promising method for detecting paramagnetic centers in biological systems and can provide new information in environmental assessment and biomonitoring. The detection of the new paramagnetic centers in animal organisms can provide completely new biophysical information. The results will allow us to better understand the origin and role of biogenic paramagnetic centers in living systems.

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