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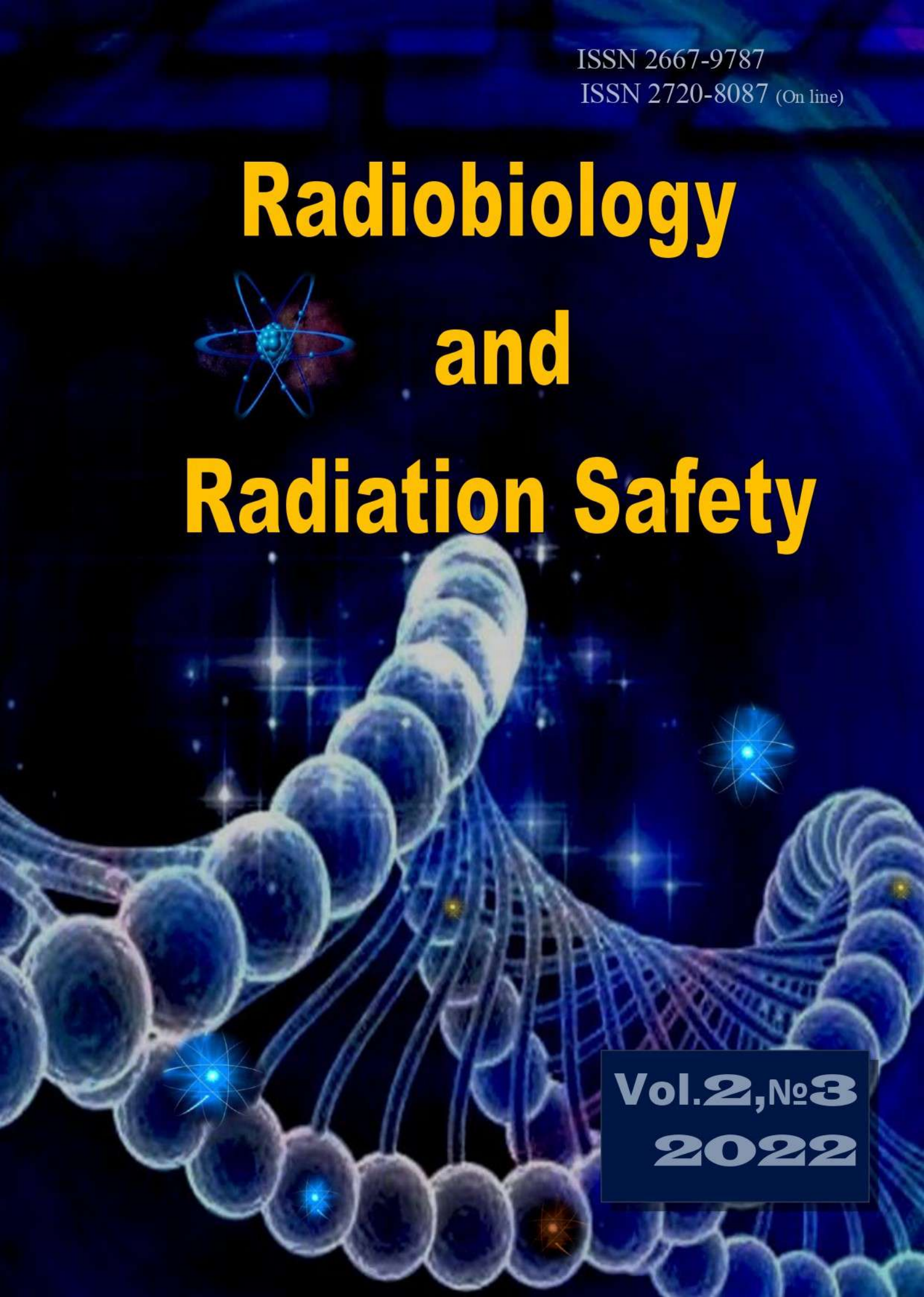

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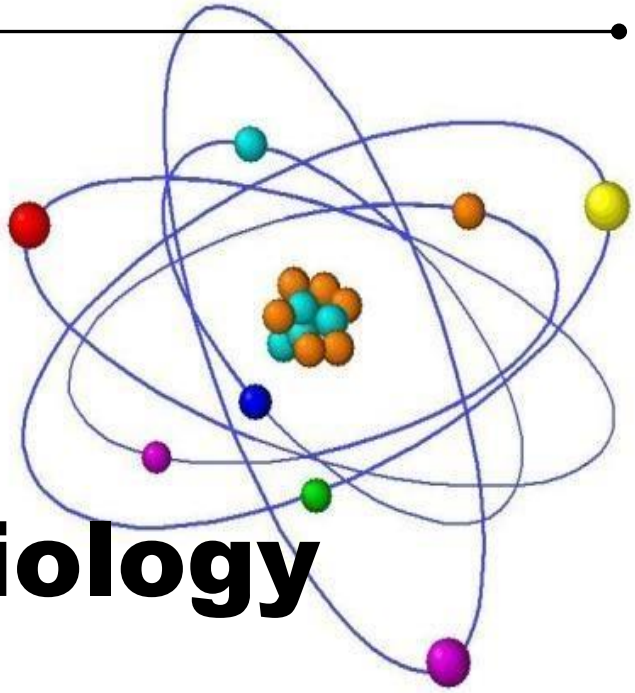

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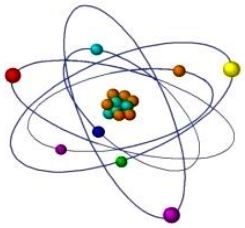
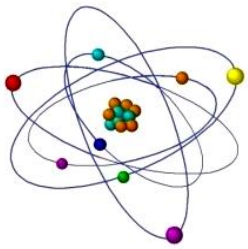


COMPARATIVE STATISTICAL ANALYSIS OF ANNUAL VARIATION OF THE INTENSITY OF GALACTIC COSMIC RAYS (IN TBILISI, ALMATY, APATITY, MOSCOW, NOVOSIBIRSK AND ROME)



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ABSTRACT: *Results of comparative statistical analysis of annual variation of the intensity of neutron component of galactic cosmic rays (CR) in Tbilisi, Almaty, Apatity, Moscow, Novosibirsk and Rome in 1995-2014 are presented. In the proposed work the analysis of data is carried out with the use of the standard statistical analysis methods of random events and methods of mathematical statistics for the non-accidental time-series of observations. In particular, the following results are obtained. In Tbilisi, twenty-year averages of CR intensity are lower than in Almaty, Moscow and Novosibirsk, and higher than in Apatity and Rome. The linear correlation coefficient for real values of CR intensity between Tbilisi and other measurement points varies from 0.73 (Tbilisi-Apatity pair) to 0.81 (Tbilisi-Rome pair). Almaty and Rome are the most optimal measurement points for recovering missing data on the intensity of cosmic rays in Tbilisi. The time variability regression equations of galactic cosmic rays intensity for Almaty has the form of the third order polynomial, for all other measurement points - the fifth order polynomial. The linear correlation coefficient for trend + background components of values of CR intensity between Tbilisi and other measurement points varies from 0.78 (Tbilisi-Apatity pair) to 0.87 (Tbilisi- Almaty pair). The linear correlation coefficient for random components of values of CR intensity between Tbilisi and other measurement points varies from 0.27 (Tbilisi- Almaty pair) to 0.65 (Tbilisi – Moscow and Tbilisi - Rome pairs). Connection of linear correlation coefficient between different components of galactic cosmic rays intensity in Tbilisi and in other measurement locations (real data, trend + background and random components) with distance from Tbilisi have the form of the second power polynomial. Real data on the CR intensity for Tbilisi are very highly representative at a distance of up to 500 km from the measurement point and highly representative at a distance of at least 3200 km from this city.*

Key words: galactic cosmic rays, neutron monitors, trend

INTRODUCTION

The study of cosmic rays, including galactic cosmic rays, is the most important experimental problem since they largely determine the most diverse processes occurring in the earth's atmosphere [1,2]. Thus, in many countries of the world, including Georgia, the intensity of the neutron component of galactic cosmic rays has been monitored for several decades [3-5].

In addition to traditional studies of various aspects of cosmic ray variations [6–8], a significant number of works are devoted to studying the relationship between cosmic radiation and the formation of aerosols in the atmosphere [9–13], general climatic effects of cosmic rays [14–16], and the influence of cosmic ray variations on such climate elements, such as cloudiness and air temperature [17-23], etc. Much attention is paid to the environmental aspects of cosmic radiation, including the study of their impact on human mortality [12, 24-26].

A number of the above mentioned studies were carried out at the M. Nodia Institute of Geophysics, TSU. In particular, in [9] has been proposed the scheme of the interaction of atmospheric aerosols and convective clouds, and also generation in the atmosphere and clouds of condensation, crystallization nuclei and ice crystals with allowance to ionization (including cosmic) and electrization processes occurring in the atmosphere.

In continuation of [9], the papers [10-12] present the results of studying the influence of cosmic radiation on the formation of secondary aerosols in the atmosphere associated with the formation of clouds.

The study of the relationship between annual variations in the intensity of galactic cosmic rays and the variability of cloudiness and air temperature in Tbilisi according to the data of 1963-1990 is presented in [18]. The paper [19] considers the results of the study of the connection between annual variations of intensity of galactic cosmic rays and the changeability of the total cloudiness, atmospheric precipitation and air temperature in 1966-2015 in Tbilisi. The statistical characteristics of the indicated parameters (trends, random component, linear correlations between real and random components, etc.) are studied. In particular, it was found that, within the variation range, the contribution of the studied parameters to atmospheric precipitation variability is as follows: total cloudiness - 17.1%, real values and random components of cosmic ray intensity - 37.8% and 28.0%, respectively.

Results of the study on influence of variations of the annual intensity of neutron component of galactic cosmic rays on the mortality of the population of Georgia in 1995-2014 in [26] are presented. In particular, the previously obtained results on a direct correlation between the intensity of cosmic rays and total mortality of the population have been confirmed. However, as it turned out, an increase in the intensity of cosmic rays mainly increases the mortality rate of the male part of the population of Georgia. The mortality rate of women is very weakly dependent on the galactic cosmic ray's influence.

It should be noted that at various cosmic ray monitoring stations, including Tbilisi, gaps in the series of observations are possible for various reasons. Therefore, it is very important to conduct a correlation and regression analysis of the connection between the series of measurement data at different stations. Such an analysis makes it possible to choose the most optimal station for recovering missing data, or for directly using the data of this station for a period of time with no measurements in the area under study. Note that a similar technique is widely used in meteorology [27].

Results of comparative statistical analysis of annual variation of the intensity of neutron component of galactic cosmic rays in Tbilisi, Almaty, Apatity, Moscow, Novosibirsk and Rome in 1995-2014 are presented below.

STUDY AREA, MATERIALS AND METHODS

Study area – Tbilisi (Georgia), Almaty (Kazakhstan), Apatity, Moscow, Novosibirsk (Russia) and Rome (Italy)–fig. 1. Distance from Tbilisi: Almaty – 2640 km, Apatity – 2970 km, Moscow - 1650 km, Novosibirsk – 3170 km, Rome – 2680 km.

Data about annual values of intensity of neutron component of galactic cosmic rays (CR) for Tbilisi is obtained at the Cosmic Rays Observatory of M. Nodia institute of geophysics. Data about CR for Almaty, Apatity, Moscow, Novosibirsk and Rome taken from - http://cr0.izmiran.ru/common/links.htm. All data are corrected for atmospheric pressure. The period of observation is 1995 - 2014. The unit of measurement is imp/min, omitted from the text and tables below.

In the proposed work the analysis of data is carried out with the use of the standard statistical analysis methods of random events and methods of mathematical statistics for the non-accidental time-series of observations [28-30].

The following designations will be used below: Min – minimal values, Max - maximal values, Range - variation scope, St Dev - standard deviation, Cv, % – coefficient of variation (Cv = 100·St Dev/Average), R - coefficient of linear correlation, Ra – coefficient of autocorrelation with lag = 1 year, Rk - Kendall rank correlation coefficient, Rs- Spearman's rank correlation coefficient, R^2 – coefficient of determination, K_{DW} – Durbin-Watson Statistic, Rand – random component of time-series of

observations, α- the level of significance, Real - measured data. The curve of trend is equation of the regression of the connection of the investigated parameter with the time at the significant value of the coefficient of determination and such values of K_{DW}, with which the residual values are accidental.

A background component usually enters into the curve of trend. The value of background component is most frequently unknown. From the physical aspect, random component can be represented in the form: Rand = Res + absolute value of the min value of Res. In this case random components have positive values with the minimum value = 0 (if the value of background component is known, the min Rand will be = Back). Accordingly, Trend+Back (sum of the trend and background components of time series) will be a curve of equation of the regression of the connection of the investigated parameter and the time minus absolute value of the min value of Res. So, Real = (Trend+Back) + Rand.

Fig. 1. Location of galactic cosmic rays measurement locations.

The degree of linear correlation was determined in accordance with [28]: very high correlation ($0.9 \leq R \leq 1.0$); high correlation ($0.7 \leq R < 0.9$); moderate correlation ($0.5 \leq R < 0.7$); low correlation ($0.3 \leq R < 0.5$); negligible correlation ($0 \leq R < 0.3$).

A comparison of mean values of CR in Tbilisi and another measurement locations were produced with the use of Student's criterion.

RESULTS

The results in table and fig. 2-6 and tables 1-5 are presented.

In fig. 3 and table 1 the time series of real data of annual variation of the intensity of neutron component of galactic cosmic rays in Tbilisi, Almaty, Apatity, Moscow, Novosibirsk and Rome in 1995-2014 and statistical characteristics of these data are presented.

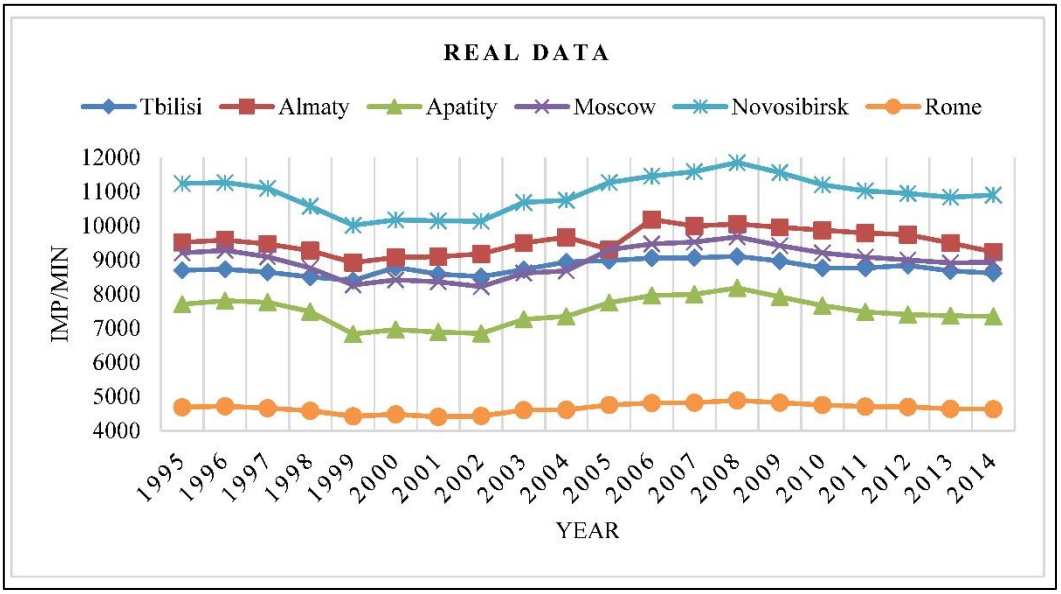

Fig. 2. Time series of real data of galactic cosmic rays intensity at measurement locations.

Table 1. Statistical characteristics of real data of galactic cosmic rays intensity at measurement locations.

Variable	Tbilisi	Almaty	Apatity	Moscow	Novosibirsk	Rome
Max	9100	10182	8177	9670	11845	4883
Min	8396	8919	6829	8214	10012	4404
Range	704	1263	1348	1456	1833	479
Average	8763	9541	7494	8967	10928	4652
St Dev	201	359	401	435	525	137
Cv, %	2.3	3.8	5.4	4.9	4.8	2.9
	Correlation Matrix					
Tbilisi	1	0.80	0.73	0.76	0.78	0.81
Almaty	0.80	1	0.81	0.83	0.85	0.88
Apatity	0.73	0.81	1	0.98	0.98	0.96
Moscow	0.76	0.83	0.98	1	0.99	0.98
Novosibirsk	0.78	0.85	0.98	0.99	1	0.98
Rome	0.81	0.88	0.96	0.98	0.98	1

As follows from Table 1, the average, maximum, and minimum real values of the CR intensity at the measurement points are, respectively, the following: Tbilisi – 8763, 9100 and 8396; Almaty – 9541, 10182 and 8919; Apatity - 7494, 8177 and 6829; Moscow -8967, 9670 and 8214; Novosibirsk – 10928, 11845 and 10012; Rome – 4652, 4883 and 4404. In general, for all measurement points, the variations in the CR intensity values in the time series of observations are small.

The greatest variations in the real values of the CR intensity are observed in Apatity (Cv = 5.4 %), the smallest - in Tbilisi (Cv = 2.3 %). In Tbilisi, twenty-year averages of CR intensity are lower than in Almaty, Moscow and Novosibirsk, and higher than in Apatity and Rome. The lowest values of CR intensity in Rome in comparison with other observation points are due to the sensitivity of the equipment and the measurement technique.

The linear correlation coefficient for real values of CR intensity between Tbilisi and other measurement points varies from 0.73 (Tbilisi-Apatity pair) to 0.81 (Tbilisi-Rome pair). In all cases - high correlation.

Note that Almaty and Rome are the most optimal measurement points for recovering missing data on the intensity of cosmic rays in Tbilisi. Although, if necessary, the data of all other stations may well be acceptable. The same is acceptable for any other station (table 1).

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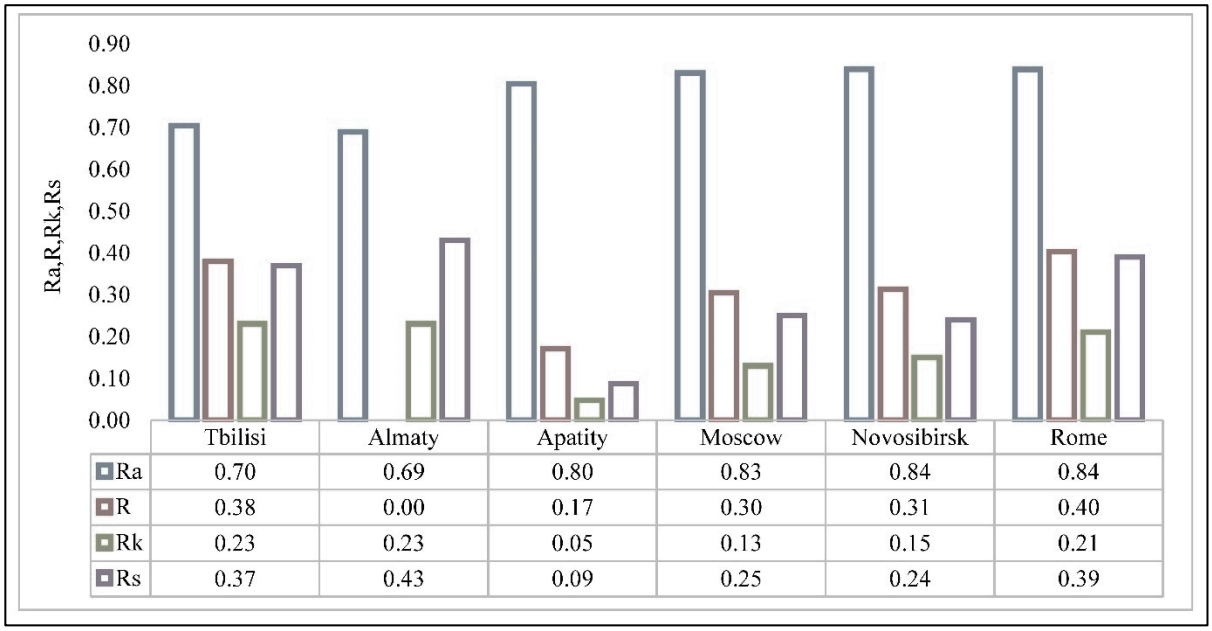

	Tbilisi	Almaty	Apatity	Moscow	Novosibirsk	Rome
Ra	0.70	0.69	0.80	0.83	0.84	0.84
R	0.38	0.00	0.17	0.30	0.31	0.40
Rk	0.23	0.23	0.05	0.13	0.15	0.21
Rs	0.37	0.43	0.09	0.25	0.24	0.39

Fig. 3. Indicators of stability over time of time series of galactic cosmic rays intensity at measurement locations.

In fig. 3 data about indicators of stability over time of time series of galactic cosmic rays intensity in measurement locations are presented. Thus, the Ra values for all measurement points are significant only with a lag = 1 year and vary from 0.69 (Almaty) to 0.84 (Novosibirsk and Rome). Values of R between CR intensity and time are more or less significant for all measurement points except Almaty. Values of Rk and Rs between CR intensity and time are more or less significant for all measurement points except Apatity. Thus, all series of observations are non-random to some extent and depend on time. Accordingly, to construct regression equations for the dependence of CR intensity values on time (trends) methods of mathematical statistics for the non-accidental time-series of observations are used.

Table 2. Types of time variability regression equations of galactic cosmic rays intensity at measurement locations.

Location/Variable	Regression Equation	R^2	K_{DW}
Tbilisi	Fifth order polynomial	0.783	2.13
Almaty	Third order polynomial	0.783	2.27
Apatity	Fifth order polynomial	0.922	2.35
Moscow	Fifth order polynomial	0.933	2.28
Novosibirsk	Fifth order polynomial	0.946	2.33
Rome	Fifth order polynomial	0.932	2.44

In table 2 data about types of time variability regression equations of galactic cosmic rays intensity in measurement locations are presented. As follows from table 2, this dependence for Almaty has the form of the third order polynomial, and for all other measurement points - the fifth order polynomial.

In fig. 4 and table 3 the time series of trend + background components of annual variation of the intensity of neutron component of galactic cosmic rays in Tbilisi, Almaty, Apatity, Moscow, Novosibirsk and Rome in 1995-2014 and statistical characteristics of these data are presented.

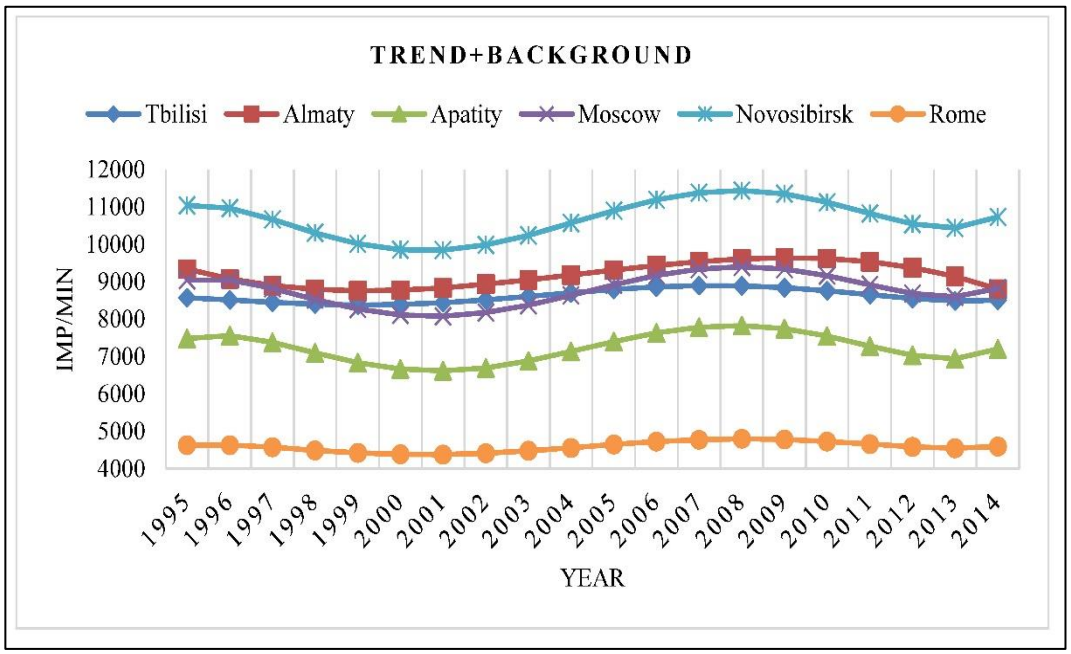

Fig. 4. Time series of trend + background components of galactic cosmic rays intensity at measurement locations.

Table 3. Statistical characteristics of trend + background components of galactic cosmic rays intensity at measurement locations.

Variable	Tbilisi	Almaty	Apatity	Moscow	Novosibirsk	Rome
Max	8893	9638	7823	9397	11433	4792
Min	8372	8754	6613	8073	9842	4376
Range	521	884	1210	1324	1591	416
Average	8607	9180	7232	8767	10665	4586
St Dev	178	318	385	420	511	132
Cv, %	2.1	3.5	5.3	4.8	4.8	2.9
	Correlation Matrix					
Tbilisi	1	0.87	0.78	0.79	0.83	0.86
Almaty	0.87	1	0.76	0.81	0.83	0.87
Apatity	0.78	0.76	1	0.99	0.98	0.96
Moscow	0.79	0.81	0.99	1	1.00	0.99
Novosibirsk	0.83	0.83	0.98	1.00	1	0.99
Rome	0.86	0.87	0.96	0.99	0.99	1

The average, maximum, and minimum values of the trend + background components of CR intensity at the measurement points are, respectively, the following: Tbilisi – 8607, 8893 and 8372; Almaty – 9180, 9638 and 8754; Apatity - 7232, 7823 and 6613; Moscow - 8767, 9397 and 8073; Novosibirsk – 10665, 11433 and 9842; Rome – 4586, 4792 and 4376 (table 3).

As in the previous case (table 2) the greatest variations in the trend + background components values of the CR intensity are observed in Apatity (Cv = 5.3 %), the smallest - in Tbilisi (Cv = 2.1 %). In Tbilisi, twenty-year averages of trend + background components of CR intensity are lower than in Almaty, Moscow and Novosibirsk, and higher than in Apatity and Rome.

The linear correlation coefficient for trend + background components of values of CR intensity between Tbilisi and other measurement points varies from 0.78 (Tbilisi-Apatity pair) to 0.87 (Tbilisi-Almaty pair). In all cases - high correlation.

Finally, in fig. 5 and table 4 the time series of random components of annual variation of the CR intensity in measurement points in 1995-2014 and statistical characteristics of these data are presented.

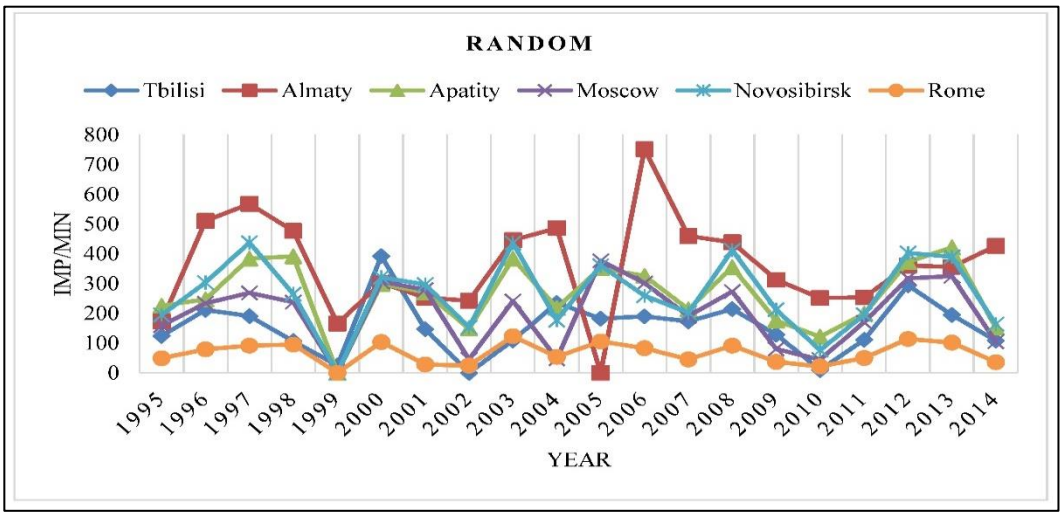

Fig. 5. Time variability of random components of galactic cosmic rays intensity at measurement locations.

Table 4. Statistical characteristics of random components of galactic cosmic rays intensity at measurement locations.

Variable	Tbilisi	Almaty	Apatity	Moscow	Novosibirsk	Rome
Max	392	750	421	376	437	122
Min	0	0	0	0	0	0
Range	392	750	421	376	437	122
Average	157	361	262	200	262	66
St Dev	94	167	112	112	122	36
Cv, %	59.7	46.3	42.6	56.3	46.5	54.3
	Correlation Matrix					
Tbilisi	1	0.27	0.56	0.65	0.61	0.65
Almaty	0.27	1	0.35	0.16	0.28	0.30
Apatity	0.56	0.35	1	0.87	0.92	0.91
Moscow	0.65	0.16	0.87	1	0.85	0.82
Novosibirsk	0.61	0.28	0.92	0.85	1	0.88
Rome	0.65	0.30	0.91	0.82	0.88	1

The average and maximum values of the random components of CR intensity at the measurement points are, respectively, the following: Tbilisi – 157 and 392; Almaty – 361 and 750; Apatity - 262 and 421; Moscow – 200 and 376; Novosibirsk – 262 and 437; Rome – 66 and 122. Minimum value of the random components of CR intensity at all measurement points is 0 (table 4).

The greatest variations in the random components values of the CR intensity is observed in Tbilisi (Cv = 59.7 %), the smallest - in Apatity (Cv = 42.6%). In Tbilisi, twenty-year averages of random components of CR intensity are lower than in all measurement points, except Rome.

The linear correlation coefficient for random components of values of CR intensity between Tbilisi and other measurement points varies from 0.27 (Tbilisi- Almaty pair, negligible correlation) to 0.65 (Tbilisi – Moscow and Tbilisi - Rome pairs, moderate correlation).

In table 5 data about the relationship between mean values of different components of galactic cosmic rays intensity in measurement locations are presented.

Table 5. Relationship between mean values of different components of galactic cosmic rays intensity in measurement locations.

Variable	Tbilisi	Almaty	Apatity	Moscow	Novosibirsk	Rome
Rand/Real, %	1.79	3.79	3.50	2.23	2.40	1.42
Rand/Trend+Back, %	1.82	3.94	3.63	2.28	2.46	1.44
Trend+Back/Real, %	98.2	96.2	96.5	97.8	97.6	98.6

As follows from table 5 the range of these ratios is as follows: Rand/Real – from 1.42 % (Rome) to 3.79 % (Almaty); Rand/Trend+Back - from 1.44 % (Rome) to 3.94 % (Almaty); Trend+Back/Real - from 96.2 % (Almaty) to 98.6 % (Rome). For Tbilisi, these ratios are respectively equal to 1.79 %, 1.82 % and 98.2 %.

In fig. 6 data about the connection of linear correlation coefficient between different components of galactic cosmic rays intensity in Tbilisi and in other measurement locations with distance from Tbilisi are presented.

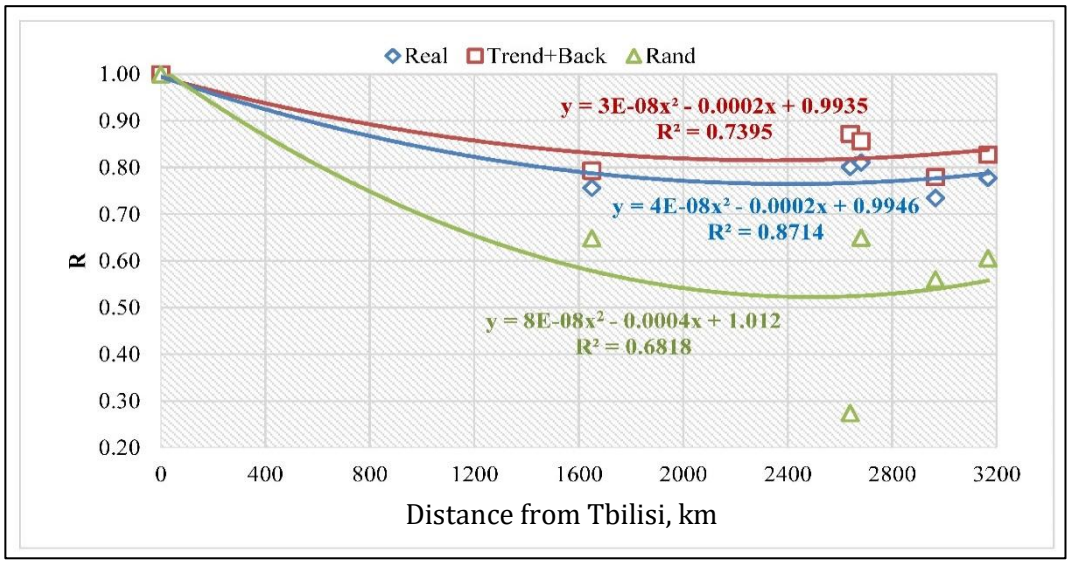

Fig.6. Connection of linear correlation coefficient between different components of galactic cosmic rays intensity in Tbilisi and in other measurement locations with distance from Tbilisi.

As follows from fig. 6 all these dependences have the form of the second power polynomial. In particular, it should be noted that the real data on the CR intensity for Tbilisi are very highly representative at a distance of up to 500 km from the measurement point ($R \geq 0.9$) and highly representative at a distance of at least 3200 km from this city ($0.7 \leq R < 0.9$).

CONCLUSION

In the future, we plan to conduct a similar study for time series of monthly values of cosmic ray intensity.

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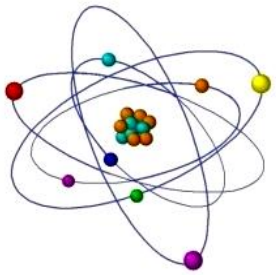
RADIOBIOLOGICAL ASPECTS OF PLANT EPIGENETIC POLYMORPHISM



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ABSTRACT: *The relationship of epigenetic variability with different individual radiosensitivity and adaptive capabilities was studied. Using a simple and convenient experimental model — maize seedlings with different germination terms and epigenetic patterns — the hypothesis was tested that genetically homogeneous but epigenetically different organisms have different radiosensitivity and radio adaptive capacity. Differences in the DNA methylation profiles of individual subpopulations of seedlings were used as a marker of epigenetic differences, and the yield of chromosomal aberration was used as an indicator of DNA vulnerability and its changes under different UV-C irradiation modes. In two series of experiments involving a UV-C acute single and exposure according to the scheme «adaptive - challenging», the investigation of the possible biological importance of epigenetic polymorphism has been performed. The study used a cytogenetic analysis of the yield of chromosomal aberrations and restriction analysis followed by ITS-ISSR- PCR. Significant differences have been established in chromosome aberration yield and DNA methylation profile in control and under UV-C exposure for seedlings of subpopulations differing in time of germination. The differences in the DNA methylation profiles and the yield of chromosomal aberrations in the control subpopulations of seedlings of different germination terms indicate the influence of the DNA methylation profile on DNA damage by regular metabolic factors, such as thermal vibrations or reactive oxygen species (ROS). This phenomenon can be explained by different chromatin conformation determining structural or "passive" resistance, which provides different DNA availability to damage. Methylation switching into de novo under different modes of radiation exposure could become a marker of gene expression changes due to induced repair and protection. The obtained data indicate the importance of epigenetic factors in determining the radioresistance and adaptive capacity of organisms. It points out that the epigenetic mechanisms that determine the choice of the metabolic pattern also contribute to the individual radiosensitivity and adaptive capacity of the organisms. This contribution is determined in two ways. First, the DNA methylation profile affects the initial damage processes, and second, the type of methylation switching into de novo is associated with the further development of protection and repair processes.*

Key words: radiation exposure, radiosensitivity, epigenetic polymorphism, DNA methylation profiles

INTRODUCTION

The issue of variability in species and population radiosensitivity and the identification of factors determining individual radiosensitivity is one of the key problems in radiobiology. Historically, the activity of metabolism, especially proliferative processes activity is the first of indicated and currently well-studied factors determining animal and plant radiosensitivity. The phenomenon is stated in the classic law of Bergonié J and Tribondeau L (1906). The connection between different radiation exposures with various DNA damage is intensively studied since the middle of the last century. Based on the study such representative markers of individual radiosensitivity as micronucleus test and chromosome aberration yield were suggested [1-3]. These markers are widely used from occupation medicine to investigation of plant radiation effects [1, 2, 4]. It is known that chromosome aberration yield is an integral index reflecting not

only DNA primary damage but also the effectiveness of protective and repair mechanisms associated with the functioning of the enzyme complexes. Individual radiosensitivity is now indicated to be connected with the polymorphism of more than 40 genes. They protect cells from mutagens and participate in their homeostasis. There are genes of the xenobiotic detoxification system, antioxidant protection, and DNA repair which polymorphism indicates different efficiency of the systems [5- 7].

Thus, genetic polymorphism is one of the reliable factors of population variability and individual radiosensitivity. The realization of genetic information (in other words gene expression) is related to a complex system of epigenetic regulation. Its mechanisms have been studied in recent decades. DNA methylation, covalent protein modification, and RNA interferences play reliable roles in epigenetic pattern choosing. Currently, the study of the mechanisms of gene expression is developed in different directions: from studying the organization of chromatin to identifying reactions to the effects of climatic factors and various stresses. DNA methylation is the most studied chromatin modification [8-12] and gene expression control factor [13-18].

This process is an integral component of a complex system of epigenetic regulation. Changes in DNA profiles associated with the switching of DNA methylation from maintenance to *de novo* mode are used as a marker of changes in gene expression upon environmental exposure [14-18]. In up-to-date biology along with the concept of genetic polymorphism, there is the concept of epigenetic polymorphism, which implies the existence of a variety of phenotypes while maintaining the unity of the genotype. The existence of epigenetic polymorphism in various biological communities from normal and cancerous human tissues to plant populations was shown in studies [15,17-21] However the biological role of this phenomenon, the connection between phenotypic and epigenetic heterogeneity with variability of sensitivity to environmental exposure and adaptation are still not studied.

A random sample of seeds of the same species, variety, and harvest is a simple and convenient experimental model to investigate the issue. It has some advantages. Firstly, there is data about germination time dependence on ecological factor effects [22]. This points to "gene – environmental" cooperation, i.e., the process is controlled not only genetically, but also epigenetically. Secondly, such a sample germinates asynchronously, individual seeds differ in germination term; this allows us to isolate different subpopulations of seeds and to study the relationship between the variability of germination time and epigenetic differences.

Our verification of the assumption showed that seedling subpopulations from seeds with various germination terms have different methylation patterns. In other words, they are epigenetically different. The following studies showed a change in germination time and methylation pattern in subpopulations with different germination times under chronic gamma-radiation exposure [23]. Different germination terms could be connected with both various metabolic pathways of seed before germination or different ripening degree. According to Woddingtone' conception (1944) germination time difference could be connected with different "epigenetic trajectories" or various positions on the same "epigenetic trajectory". The great majority of researchers explain the phenomenon with different ripening degrees i.e., differences in "physiological age" In other words, these are different positions on the same "epigenetic trajectory" [24-26]. Testing the hypothesis showed that differences in DNA methylation patterns are observed both within the subpopulation of seeds with the same ripening and germination terms and between subpopulations with different germination terms. Thus, it was shown that different seed germination times reflect both different "epigenetic trajectories" (metabolic pathways) along which maturation occurred and different positions on them [27].

The paper is devoted to the first stage of studying the relationship between epigenetic variability and both different individual radiosensitivity and adaptive capacity. The question of the relationship between DNA methylation polymorphism with different radiosensitivity and the formation of adaptive reactions under radiation exposure will be analyzed. The experimental model described above was used.

The chromosomal aberrations yield was used as a marker of DNA sensitivity to genotoxic factors, radioresistance, and its changes under radiation exposure.

MATERIAL AND METHOD

The subjects of the study were 3 – 7-day-old corn seedlings (the Polesska variety). Seeds were couched on plates, the bottoms of which were covered with filter paper and incubated in a thermostat at +22-+23^{0}C. The experiment was performed 7 times and had two experimental series. The goal of the first series of experiments was to study the relationship between germination term variability, DNA methylation pattern, and seedlings' radiosensitivity by the index of chromosome aberrations yield.

On the 2nd day germinated seeds were separated into 3 groups:

a) «fast-germinated» subpopulation (FG – the prime root length more than >1 cm);

b) «middle-germinated» subpopulation (MG – the prime root length more than > 0,1cm);

c) «slowly-germinated» subpopulation (SG – which didn't germinate on the 2nd day or just have hatched, the prime root length 1 mm).

3-day-old seedlings were exposed to 7,2 kJ/m^{2}UV-C irradiation (λ= 253 nm, the dose rate was 6,2 W/m^2). An OBN-150M bactericidal irradiator (Ukraine) equipped with Philips Special TUV-30W lamps was used.

In the second series of experiments, the goal was to study the adaptive response of seedlings from different epigenetic groups.

Two time intervals between adaptive and challenging doses were selected - 4 and 24 hours. The choice was based on our own results and literature data. First, it was shown that DNA methylation switched to de novo mode with an interval of more than 1 hour between exposures [23]. Second, we tried to take into account the duration of single- and double-stranded break repair. In the first case it takes from several minutes to several tens of minutes; in the second one – taking time is comparable to the duration of the cell cycle (from several hours to a day) [28,29]. The different intensity of the adaptive response under different intervals between adaptive and challenger dose effects indirectly gives information about the role of reparative processes in the formation of the chromosomal aberrations yield.

All repeated experiments indicated significant dependence on the season chromosome aberration yield of seedlings from seeds with moderate germination terms; this fact could be attributed to the heterogeneity index for the group. With these observations, we decided to use only two groups of seedlings from fast- and slowly-germinated seeds to analyze the connection between epigenetic polymorphism and the specifics of adaptive reaction development.

The following variants of exposure were used:

1). Non-UV-C irradiated seedlings;

2). Adaptive exposure (1 kJ/m^2);

3). Adaptive exposure, in 4 hours – challenging one (6,2 kJ/m^2);

4). Whole dose exposure (7,2 kJ/m^2); exposure simultaneously with the challenging irradiation of variant 3;

5). Adaptive exposure, in 1 day – challenging one (6,2 kJ/m^2);

6). Whole dose exposure (7,2 kJ/m^2); exposure simultaneously with the challenging irradiation of variant 5.

These groups showed stable results in both cytogenetic and molecular parameters.

Apical root meristem was used for the cytogenetic assay. Samples for cytogenetic assay were collected on the 4th day after irradiation. After separation from the roots, the apexes were placed in a Brodsky fixation solution (0,3 acetic acid: 1 ethanol: 3 formaldehyde mixture) for 2 hours and then washed with ethanol 3 – 4 times. Maceration was performed by alkaline hydrolysis with 20% NaOH for 2 hours. After that, the preparations were washed with distilled water for 15 minutes. Staining was performed with a mixture of acetoorcein and hydrochloric acid (1 acetoorcein: acetoorcein: 1 1MHCl) for 16 – 18 hours. The stained material was washed with 45% CH_3COOH, and squash preparations were prepared. To perform an analysis, ten parallel samples were prepared and 5000 – 10000 cells were analyzed. An analysis of chromosome aberrations was performed by the anaphase–telophase method, taking into account the specificity of plant tissues. The anaphase cell samples were at least 300 – 500 cells per preparation.

Isolation of DNA was performed from the 6-day-old corn seedlings with the set of reagents Diatom™DNAPrep100 based on NucleoS-sorbent. The standard protocol for DNA extraction provided by the manufacturer was used [30,31].

The concentration of DNA in the obtained solution was measured spectrophotometrically by a standard methodology described in the publications [30,31] using a Bio Photometer Plus Eppendorf v.1.35 spectrophotometer.

PCR analysis was performed in a four-channel Tertsik DNA amplifier (DNA-technology, Russia) with primers designed to minisatellite sequences ISSR (15-soro, 5'-AC-AC-AC-AC-AC-AC-AC-AC<C>-3'), transcribed sequences ITS1 (5'-TCC-GTA-GGT-GAA-CCT-GCG-G-3') and ITS4 (5'-TCC-TCC-GCT-TAT-TGA-TAT-GC-3'). Both types of primers were synthesized by “Metabion” (Germany). The set of reagents GenPak® PCR Core – the lyophilized dry mixes prepared for DNA amplification was used. The reaction mixture for ISSR-PCR (the total volume 20 µl) contained 1 unit of *Taq* polymerase inhibited for «quick start», 10 µl of PCR-diluent, 2,5 mM $MgCl_2$, 200 µM each dNTP, 0,1 µM primer (1,6 µl), 200 ng total genomic DNA (2 µl), 6,4 µl deionized water. The reaction mixture was covered with 20 µl of liquid petrolatum. The protocol for carrying out the reaction was provided by the manufacturer.

Amplification with ISSR primers included the following steps: 5 min initial denaturation at 94°C, 40 cycles; 45 s denaturation at 94°C, 45 s primer annealing at 52°C, 90 s elongation at 72°C; and 7 min final elongation at 72°C(Bartlett, 2003; Hernández, et.al.2013).

The reaction mixture for ITS-PCR (the total volume 20 µl) contained 1 unit of *Taq* polymerase inhibited for «quick start», 10 µl of PCR-diluent, 2,5 mM $MgCl_2$, 200 µM each dNTP, 0,1 µM each primer (per 0,8 µl), 200 ng total genomic DNA (2 µl), 6,4 µl deionized water. The reaction mixture was covered with 20 µl of liquid petrolatum. The protocol for carrying out the reaction was provided by the manufacturer.

Amplification with ITS primers included the following steps: 1,5 min initial denaturation at 94°C, 5 cycles; additional 40 cycles of denaturation at 94°C, 15 s; primer annealing at 55°C, 15 s and elongation at 72°C, 15 s; fixing, consisted of denaturation at 94°C for 10 s; primer annealing at 55°C for 10 s, and final elongation at 72°C for 5 min [32].

Experiments were performed in accordance with standard protocols for restriction analysis provided by the manufacturer.

Restriction analysis as well as amplification reactions were performed in a four-channel Tertsik

DNA amplifier (DNA-Technology, Russia). Three types of restrictases were used: MspI (C…C*GG; C…CGG), HpaII (C…CGG), and MboI (…GATC*) (Fermentas, Germany). The restriction endonucleases HpaII and MspI both cleave the nucleotide sequence CCGG, but the action of HpaII is inhibited if the internal cytosine is methylated. MspI is an isoschizomer of HpaII that cleaves both unmethylated and methylated HpaII sites.

The reaction mixture for restriction analysis (total volume 25 µl) contained 2 µl of 10xBuffer Tango, 500 ng of total genome DNA (5 µl), 17,1 µl (for reaction with MspI), or 17,7 µl (for reaction with MboI and HpaII) of deionized water, 0,6 units of the MspI enzyme (0,9 µl) or 0,2 units (0,3 µl) of MboI or HpaII. The mixture was covered with 20 µl of liquid petrolatum.

The conditions of the restriction reaction were as follows: incubation at 37°C for 16 hours, and reaction termination by incubation at 65°C (for HpaII and MboI) and 80°C (for MspI) for 20 min. The obtained PCR and restriction products were analyzed by electrophoresis in the 1,0% agarose gel supplemented with ethidium bromide in TBE buffer. The obtained gels were visualized by UV-transilluminator. To perform electrophoresis, the wells were filled with equal volumes of PCR and restriction products per 5 µl. A GeneRuler 50 bp DNA Ladder (Fermentas) containing fragments of 1000, 750, 500, 250 i 50 bp was used as molecular weight standards. Statistical analysis of experimental findings – the mean value and variance value were calculated by traditional methods.

RESULTS

The connection between germination terms variability, DNA methylation pattern, and radiosensitivity of seedlings. There are significant differences in chromosome aberration yield (Fig. 1) and DNA methylation pattern (Fig. 2) for control variants of FG- MG- and SG-subpopulations. The lowest chromosome aberration yield is indicated for FG-seedlings (1,6%) whilst MG- and SG 2,8%.

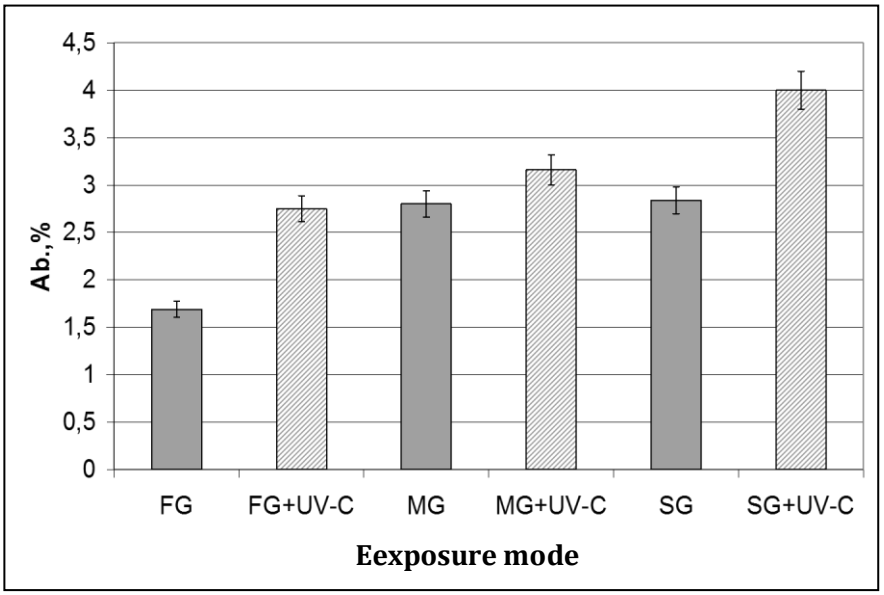

Fig. 1 Chromosome aberration yield (%) for FG – fast-germinated, MG – middle-germinated; SG – slow-germinated seedlings. Confidence interval, P = 0, 95.

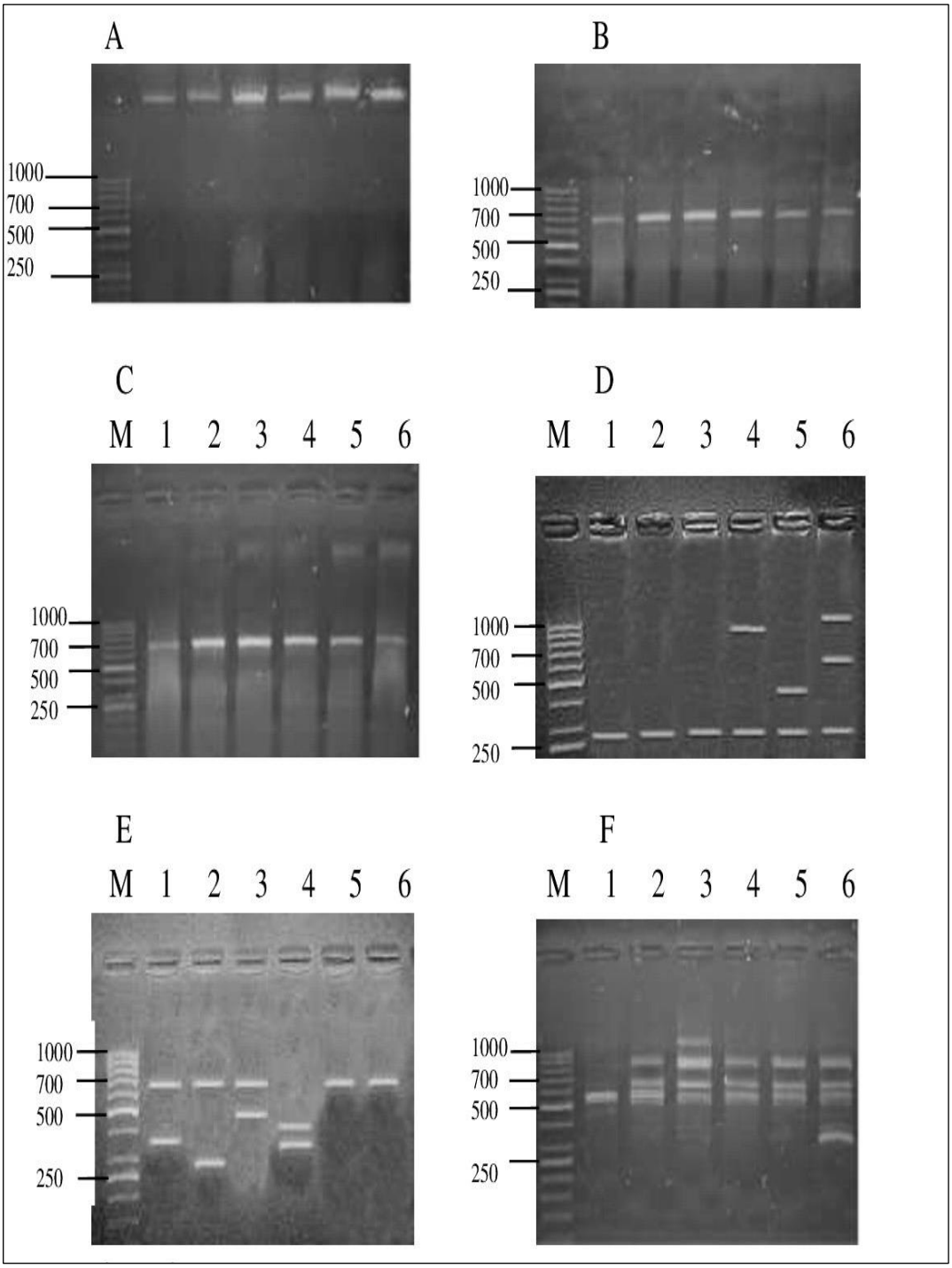

Fig.2. Connection between germination terms variability, DNA methylation pattern, and radiosensitivity of seedlings. DNA methylation data

A. The electrophoregram of isolated DNA quality control. **B**. The electrophoregram of native DNA ISSR- and. **C**. ITS – amplification; **D**. The electrophoregram of the MspI-restrict ITS-amplification. **E**. The electrophoregram of the MspI restricts ISSR –amplification; **F**. The electrophoregram of the MboI -restricts ISSR- amplification. **M** – high-molecular-weight marker; **1** – «FG» sample; **2** – «FG+UV-C» sample; **3** –«MG» sample; **4** – «MG+UV-C» sample; **5** – «SG» sample; **6** – «SG+UV-C» sample.

Differences in chromosome aberration yield are observed under radiation exposure to different subpopulations of seedlings. The lowest yield is up to 2,7% for FG- subpopulation, and the highest is for SG- ones (up to 4%).

For ITS-amplification of MspI-restricts within control variants from all subpopulations, there is one common amplicon 300 bp. It is the only band for FG- and MG- seedlings; for SG- there is another one 500 bp (Fig. 2D, positions 1, 3, 5). Radiation exposure causes the rearrangements of amplicon set for FG- and SG- seedlings by appearing longer amplicons 700 and 1000 bp.

ForISSR-amplification of MspI-restricts within control variants from all subpopulations there is only one common amplicon 700 bp. It is the only band for SG-seedlings; for FG- and MG- there are other bands 600 and 500 bp (Fig. 2E, positions 1, 3, 5). Changes for the SG- variant of amplicons are due to the appearance of a shorter amplicon of 300 bp; for MG- the disappearance of the 700 bp amplicon and appearing shorter bands of 600 and 450 bp.

The change in the set of amplicons for the variant FG - seedlings after radiation exposure is associated with the appearance of a "short" amplicon of 300 bp; for SG- seedlings, the amplicon 700 bp disappears, and shorter amplicons 600 and 450 bp. appear (Fig. 2E, positions2, 4, 6).

For all variants of the experiment, the amplicon 500 bp is common for ISSR-amplification of MboI - restricts. For control variants of SG - seedlings, amplicons 550, 600, and 1000 bp are still observed; for MG- seedlings -550 and 600 bp (Fig. 2 F, positions 1, 3, 5). The change in the set of amplicons for the variant of FG- seedlings after radiation exposure is associated with the appearance of longer amplicons of 550 and 750 bp; for SG seedlings the amplicon 1000 bp disappears; for the SG seedlings, a shorter amplicon 450 bp appears (Fig. 2 F, positions 2, 4, 6).

Thus, differences within chromosome aberration yield, in other words, the radiosensitivity of seedlings from the different subpopulations are related to various DNA methylation patterns through control and variety of their rearrangements under radiation exposure. Adaptive response of seedlings from different epigenetic groups. This experimental series also indicates differences in both chromosome aberration yield and DNA methylation pattern for control variants of seedlings from FG- and SG- subpopulations (Fig.3, 4)

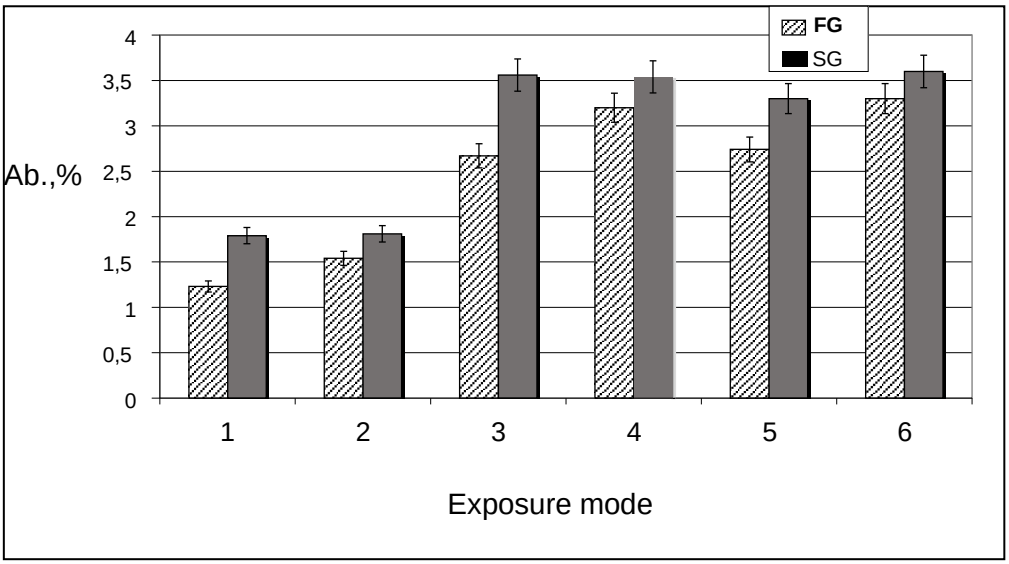

Fig.3. Chromosome aberration yield (Ab, %) for FG- and SG- seedlings.Confidence interval, P = 0,95.

1). Non UV-C irradiated seedlings;2). Adaptive exposure (1 kJ/m²);3). Adaptive exposure, in 4 hours – challenging one (6,2 kJ/m²);4). Whole dose exposure (7,2 kJ/m²); exposure simultaneously with the challenging irradiation of variant 3;5). Adaptive exposure, in 1 day – challenging one (6,2 kJ/m²);6). Whole dose exposure (7,2 kJ/m²); exposure simultaneously with the challenging irradiation of variant 5.

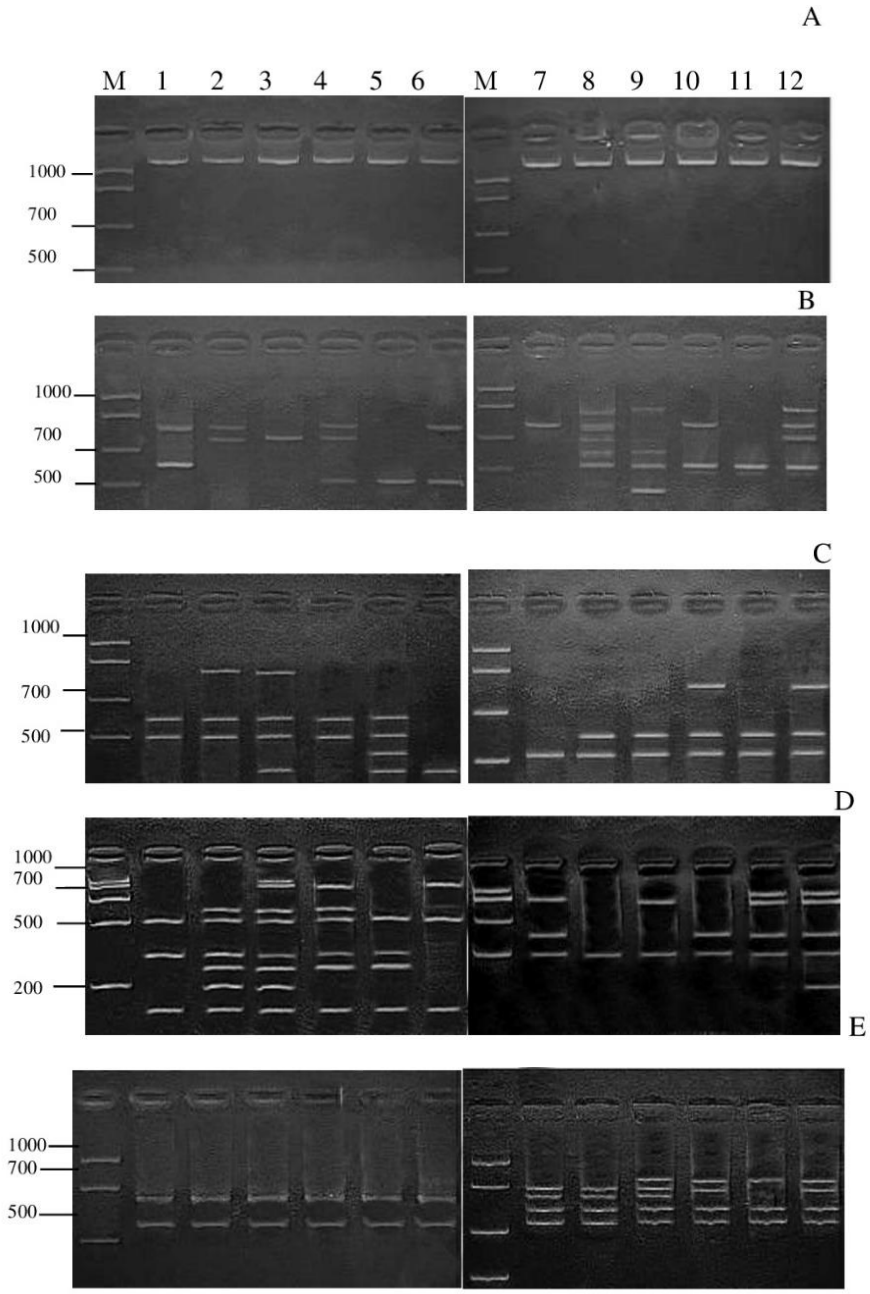

Fig. 4. Adaptive response of seedlings from different epigenetic groups.DNA methylation data

A) The electrophoregram of isolated DNA quality. B) The electrophoregrams of HpaII-restrict' ITS -amplification. C) The electrophoregrams of MspI- restricts' ITS -amplification. D) The electrophoregrams of MboI restrict ITS –amplification; E) The electrophoregrams of HpaII restrict ISSR-amplification.

For Figure 4 (A-E): M – high-molecular-weight marker containing fragments of 1000, 750, 500, 250 i 50 bp; 1. FG + non UV-C irradiation; 2. FG + adaptive exposure; 3. FG + adaptive exposure, in 4 hours - challenging one; 4. FG + whole dose exposure (7.2 kJ/m2); exposure simultaneously with the challenging irradiation of variant 3); 5. FG + adaptive exposure, in 1 day - challenging one; 6. FG + whole dose exposure; irradiation simultaneously with the challenging irradiation of variant 5; 7. SG + non UV-C irradiation; 8. SG + adaptive exposure; 9. SG + adaptive exposure, in 4 hours – challenging one; 10. SG + whole dose exposure; exposure simultaneously with the challenging irradiation of variants 9); 11. SG + adaptive exposure, in 1 day - challenging one; 12. SG + whole dose exposure; irradiation simultaneously with the challenging irradiation of variants 5 and 11.

Adaptive effect identification is by comparing results of exposure under "adaptive - challenging" and whole-dose mode. Comparison of variants 3 (adaptive dose 1 kJ/m2, challenging one 6,2 kJ/m2 after the 4-hour interval) and 4 (whole dose simultaneously with dose 6,2 kJ/m2 of the 3rd variant) shows different reactions of FG- and SG- seedlings. There is a lower yield of chromosomal aberrations for FG seedlings when exposed to an adaptive dose + stimulating dose (2.6%) compared with the full dose (3.2%), i.e., a clear adaptive response is observed. An adaptive response was not observed in SG seedlings. A comparison of variant 5 (adaptive dose of 1 kJ / m2, challenging exposure of 6.2 kJ / m2 after a 24-hour interval) and 6 (full dose at the same time as a dose of 6.2 kJ / m2 of variant 5) shows a significant adaptive response for FG- and negligible for SG seedlings.

For ITS amplification of HpaII-restricts, there is one common amplicon 800 bp within control variants of all subpopulations (Fig. 4B, positions 1,7). Adaptive radiation exposure causes rearrangements of DNA methylation of seedlings from different subpopulations: the disappearance of 600 bp band and appearance of 750 bp for FG-seedlings; appear 850, 775, 7,00, 600 and 500 bp for SG-group (Fig 4B, positions 2, 8).

Exposure under adaptive-challenging mode with a 4-hour interval also leads to rearrangements of methylation for FG- and SG-seedlings. 800 bp amplicon remains and appears new 500 bp band for FG-group; for SG-seedlings disappears 800 bp and in appears new 85,0, 600, 500 and 250 bp amplicons (Fig 4B, positions 3, 9). Exposure under adaptive-challenging mode with 24-hour intervals also leads to the same methylation changes for FG- and SG-groups. Common amplicon 500 bp appears there (Fig.4 B, positions 4, 10).

Two variants of the whole-dose exposure cause rearrangements of DNA methylation patterns of FG- and SG-seedlings. The 800 bp- amplicon maintains and 500 bp bands for both FG- and SG-groups (7,2 kJ/m2 simultaneously with challenging dose 4 hour-interval) appear (Fig, 4). Under whole-dose exposure (7,2 kJ/m2 simultaneously with challenging dose, 24-hour-interval) amplicons 800 and 500 bp were indicated for FG-group. These bands are common with the amplicons of SG-seedlings, where additional bands of 800 and 700 bp are observed (Fig 4B, positions 5 and 11, 6 and 12). For the ITS amplification of MspI - restricts there is one common amplicon 500 bp within controls of the subpopulations (Fig 4C, positions 1, 7). Under adaptive exposure the number of amplicons is different, only the amplicons 600 and 500 bp remain common (Fig 4C, position 2, 8). Underexposure mode 1+6,2 kJ/m2 with 4-hour intervals the same bands remain for FG- and SG-groups. Additional amplicons 850 and 250 bp appear for FG-seedlings. Underexposure mode 1+6,2 kJ/m2 with 24-hour intervals of 500 and 600 bp bands also remain for FG- and SG-groups. There are additional short amplicons of 350 and 250 bp for FG-seedlings (Fig 4C, positions 5).

Various rearrangements of DNA methylation patterns are observed for both whole-dose exposure modes. Methylation patterns of the FG-group have the same amplicons 500 and 600 bp with the control variant and a new one – 250 bp (Fig. 4C, positions 4, 6). Variant SG-seedlings have 500, 600 and 850 bp bands (Fig 4, positions 11 and 12).

For the ITS amplification of MboI - restricts there are no common amplicons within controls of the subpopulations (Fig 4D, positions 1, 7). FG-seedlings contain 500, 350 and 100 bp bands; SG-group - 700, 300 and 200 bp. Different rearrangements of DNA methylation patterns are observed under adaptive exposure: FG-seedlings have amplicons 600, 500, 350, 300, 200 and 100 bp, SG-group - 500, 300, 200 bp (Fig 4D, positions 2, 8). Thus, the common band is 300 bp only. Underexposure mode 1+6,2 kJ/m2 with 4-hour interval FG-seedlings have 8 amplicons and SG -variant has only 2 bands 700 and 200 bp that are the same for FG-group (Fig. 4D, positions 3, 9).

Underexposure mode 1+6,2 kJ/m2 with 24-hour interval FG- and SG-seedlings have 4 amplicons both, only two of which (300 and 200 bp) are common for these groups (Fig. 4D, positions 4, 10). For both whole-dose exposure modes patterns of ITS amplification of MboI - restricts are different for FG- and SG-seedlings. There are 5 amplicons observed for FG-group under exposure with 7,2 kJ/m2, simultaneously with variant 3; for SG-seedlings, it is only 2 bands 300 and 200 bp, which are common with FG-variant (Fig 4D, positions 5 and 6, 11 and 12).

For ISSR-amplification of HpaII- restricts control variants of FG- and SG-seedlings are different with a number of amplicons (2 and 4 respectively) and have common bands (Fig. 4E, positions 1, 7). Under radiation exposure the difference maintains. Exposure under adaptive-challenging mode with a 4-hour interval indicates an extra amplicon of 600 bp for FG-group and 750 bp for SG one (Fig. 4E, positions 2, 9).

Exposure under adaptive-challenging mode with 24-hour interval shows the same amplicons as in control for FG-seedlings and decreasing number of bands, with only one amplicon of 750 bp for the SG-group. Both variants of the whole-dose exposure led to different changes in methylation for FG- and SG-seedlings. Under whole-dose exposure simultaneously with challenging exposure of variants 3 and 5 there is the same methylation pattern for FG-seedlings as in the control variant (4E, positions 5, 6). Itappearancering of a new band of 700 bp for SG-group under whole-dose exposure simultaneously with challenging exposure variant 3 and 700, 750 bp under whole-dose exposure simultaneously with challenging exposure of variant 5 (4E, positions 11, 12).

Thus, in the second series of experiments, with different intervals between adaptive and challenging exposure FG- and SG- seedlings demonstrate various rearrangements of the methylation profile and the difference in adaptive response by the criterion "yield of chromosomal aberrations". All variants of both series of experiments show a strong relationship between differences in DNA methylation profiles and the yield of chromosome aberrations as a well-established marker of radiosensitivity.

DISCUSSION

The results of the two experimental series indicate a strong connection between chromosome aberration yield and DNA methylation patterns of seedlings from subpopulations with different germination terms.

The seedlings differ not only with methylation patterns of transcribed and satellite DNA in control variants but also with changes under radiation exposure.

The yield of chromosomal aberrations as an indicator of radiosensitivity also significantly differs for seedlings of different subpopulations in the control and various modes of radiation exposure.

In other words, the methylation pattern is associated with epigenetic and phenotypic diversity, which manifests in different germination terms, different radiosensitivity of seedlings, and adaptive capacity.

These results correspond with the other new data [33]. When studying 263 maize genotypes, it was shown that with the same general methylation, greater phenotypic diversity is observed with a greater variety of methylation sites. Considering both groups of facts, we can conclude that the combination of methylated sites is the key factor associated with the pattern of gene expression, which determines the

phenotype, including resistance to environmental factors. However, these results are only a statement of facts and are far from understanding the relationship of biophysical and information processes associated with DNA vulnerability and its conformational transformations. Under the radiation exposure of DNA, it is also the main target and structure, informational supporting the processes of protection and recovery. Thus, obtained data allow different interpretations.

The difference in the DNA methylation profiles and the chromosome aberration yield in the control variants of different subpopulations points to the impact of methylation on DNA damage by regular intracellular factors. It may be, for example, thermal vibrations or reactive oxygen species effects. This may be explained by various chromatin packaging which indicates structural or "passive" stability related to different DNA availability to damaging factors.

The methylation switching into de novo mode under radiation exposure could indicate changes in gene expression related to induction of repair and protective reactions. Different formations of adaptive response under various time intervals between adaptive and challenging exposure of FG- and SG-seedlings may indicate different effects of both single- and double-stranded DNA breaks repair. Comparing the obtained radiobiological data and the known effects of other stress factors, we could expand the interpretation of the results. DNA methylation is sensitive to environmental factors and defines the "epigenetic memory", the inheritance of the epigenetic pattern [34-37]. Methylation profiles might contain information about the conditions in which the organism was developed; it determines the variability of epigenetic programs, individual radiosensitivity; organism protection, and restoration pathway.

Thus, according to the state of the art for regulating gene expression, simultaneous study of chromosome aberration yield and DNA methylation patterns under various radiation exposure modes points to the significant role of epigenetic factors for individual radiosensitivity and adaptive reactions.

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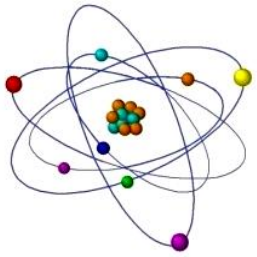
MICRONUCLACTED ERYTHROCYTES - AS A POTENTIAL NEW BIOMARKER OF LATE EFFECTS OF RADIATION IMPACT



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ABSTRACT*: The current task of modern biomedicine is to study the mechanisms of development of late effects of radiation-chemical impact, in order to develop simple and reliable criteria for predicting the severity of pathological processes and their consequences. The purpose of this paper was to determine the informativeness of current post-radiation changes in red blood system from the point of view of evaluating the severity of radiation pathology; In the groups of mice irradiated with sub- and semi-lethal doses, was studied the dependence of the probability of mice survival on the frequencies of micronuclear normochromic erythrocytes (MN-NCE) in the peripheral blood in the near and intermediate stages of the post-radiation period. The experiments were carried out on outbred mice (80 mice aged 8 week) and non-irradiated 10 mice. . Mice were randomly divided into groups irradiated at 3.5 Gy (group I, 30 mice), 5 Gy (group II, 30 mice), 6.5 Gy (group III, 20 mice), and sham-irradiated mice (group IV, 10 mice). Blood was collected from the tail vein of the mice. Blood smear was fixed into May-Grünwald' solution and dyed by Giemsa' s azur-eosin-methylene blue solution. Howell Jolly bodies in erythrocytes were counted for 1000 erythrocytes under a light microscope with inversion, lens magnification was 100x. In parallel with the blood examination, mice death caused by radiation were monitored daily and recorded to determine their survival. The data obtained by us shows that the increase in the frequencies of MN-NCE in the peripheral blood and the probability of mice death are time-correlated processes. The obtained results clearly indicate the high informative value of the frequencies of micronuclear erythrocytes in the peripheral blood in terms of assessing the depth of current pathological changes in erythropoiesis in the body's erythropoietic system (biological marker of the effect), however, when testing the genotoxic effects of various factors with the micronuclear test, the systemic factors of regulation of the frequencies of micronuclear erythrocytes should be taken into account.*

Key word: micronuclear normochromic erythrocyte, ionizing radiation, new marker.

INTRODUCTION

The current task of modern biomedicine is to study the mechanisms of development of late effects of radiation-chemical impact, in order to develop simple and reliable criteria for predicting the severity of pathological processes and their consequences. This is primarily related to the problem of prevention and minimization of the risk of near and late stochastic effects of radiotherapy [1,2]. The classical approach to assessment and prognosis of the severity of radiation pathology is based on the study of the morphological and genetic characteristics of the white blood cells [3-8], however, it should be noted that there are interesting perspectives to study the red blood cells as well - in particular, a significant difference between strains of mice in the delayed effect of irradiation on the frequency of micronuclear

reticulocytes was noted. The results show that the delayed genomic effects of irradiation on the hematopoietic system of mice can persist in vivo for long periods of time and that there are differences between mouse strains in terms of sensitivity to radiation-induced genomic instability. [9]. This approach allows taking into account so-called non-targeted effects [10-12] of ionizing radiation and, accordingly, impacts of complex effects of ionizing radiation and other health risk factors [13,14], mechanisms of individual and population radiosensitivity [9,15,16,17]. In our early studies, was studied the informativeness of cyto and molecular-genetic markers in terms of prognosis of near post-radiation complications [17]. The purpose of this paper was to determine the informativeness of current post-radiation changes of erythropoiesis in the erythrocytic system from the point of view of evaluating the severity of radiation pathology;

MATERIALS AND METHODS

The experiments were carried out on outbred mice (80 mice aged 8 week). Mice were randomly divided into groups irradiated at 3.5 Gy (group I, 30 mice), 5 Gy (group II, 30 mice), 6.5 Gy (group III, 20 mice), and sham-irradiated mice (group IV, 10 mice). The animals were kept under standard conditions on a standard diet and consumed water ad libitum. The protocol for conducting experiments and keeping mice was approved by the Ethical Committee for conducting experiments on animals of the Tbilisi State Medical University.

Mice were exposed via Gamma irradiation on a GUPOS-3M with a source of Cesium-137 (137Cs) in a dry chamber at a temperature of 25±10 ^{0}C (dose rate of 1.1 Gy/min). Falsely irradiated mice (group IV, 10 mice) were placed in a radiation chamber without an irradiation source. Subsequently, the mice were divided into cages and observed.

Blood was collected from the tail vein of the mice, for sampling were chosen 3 mice from each group, after gamma rays expose sampling was done at days – 2, 7 ,12, 20, 35, 42, 60, 75, 85 and 100. Blood smears were prepared for counting *Howell* Jolly bodies. For smear preparation drop of blood was applied to a clean, dry glass slide, then was pulled toward the entire slide and dried in the air. After smears where dried, for fixation they were placed into May-Grünwald's solution during 3 minutes and then dried again. Blood smears were dyed by Giemsa's azur-eosin-methylene blue solution (diluted 1:5 with distilled water) for 20 minutes and washed with water flow to remove excesses of dye and dried in the air. Howell Jolly bodies in erythrocytes were counted for 1000 erythrocytes under a light microscope with inversion, lens magnification was 100x. In parallel with the blood examination, mice death caused by radiation were monitored daily and recorded to determine their survival.

The causal relationship between frequency *Howel* [l] Jolly bodies in erythrocytes and the life span of laboratory mice was analyzed on the basis of the Cox proportional hazard model. Basic calculations and visualization of the results were carried out using a mathematical package "STATISTIC 12".

RESULTS

In the groups of mice irradiated with sub- and semi-lethal doses, was studied the dependence of the probability of mice survival on the frequencies of micro nucleated normochromic erythrocytes (MN-NCE) in the peripheral blood in the near and intermediate stages of the post-radiation period.

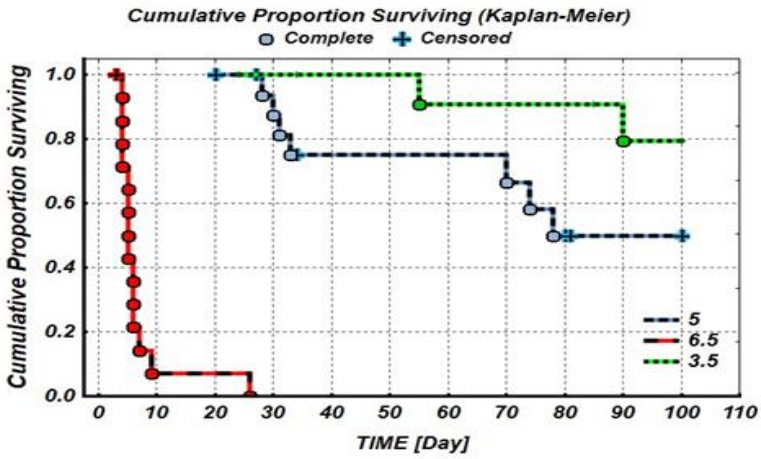

Fig.1. Survival curves of mice after total body irradiation (^{137}Cs γ-rays) with doses 6.5 Gy (circle), 5 (square) and 3.5Gy. (rhombuses). X-axis – Days after exposure, Y axis - part of animals at risk

Figure 1 shows dependence of the probability of survival of irradiated mice on the irradiation dose on post-radiation period in days. As expected, a sharp dose-dependence of the survival of animals is revealed, it is worth noting that the dynamics of the death of mice was not described by a smooth, but by a step function, this is especially notable at 5 Gy. dose-irradiated mice (range of 25-35 and 70-80 days), which should probably indicate a pronounced heterogeneity of mice in the study group in terms of radiosensitivity.

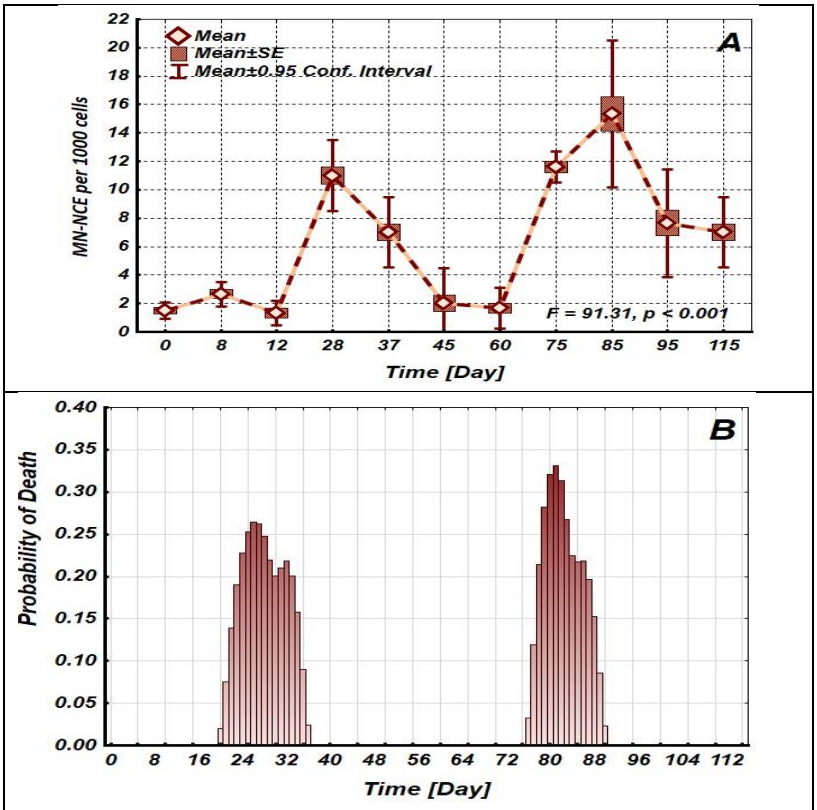

Figure 2. Mean number of micro nucleated normochromic erythrocytes (MN-NCE) per 1000 cells in group of animals irradiated with dose 5Gy (A) and probability density function (B) of the death of mice irradiated with a dose 5Gy.

In the dynamics of MN-NCE frequencies in the peripheral blood of 5 Gy. dose-irradiated mice, two clearly expressed peaks are recorded on days 20-30 and 75-85 of the post-radiation period (Fig. 2A). Visual analysis of the distribution functions shows that the increase in the frequencies of MN-NCE in the peripheral blood and the probability of mice death (Fig. 2B) are time-correlated processes. In order to quantitatively assess the credibility of this regularity, cross-correlation analysis of MN-NCE dynamics and animal death probability distribution functions were used (Fig. 3). Already by visual analysis it is clear that a kind of correlation exists between the intensity of animal death and the frequencies of micronuclear erythrocytes. which finds a quantitative reflection between the cross-correlations of these functions (Fig. 3)

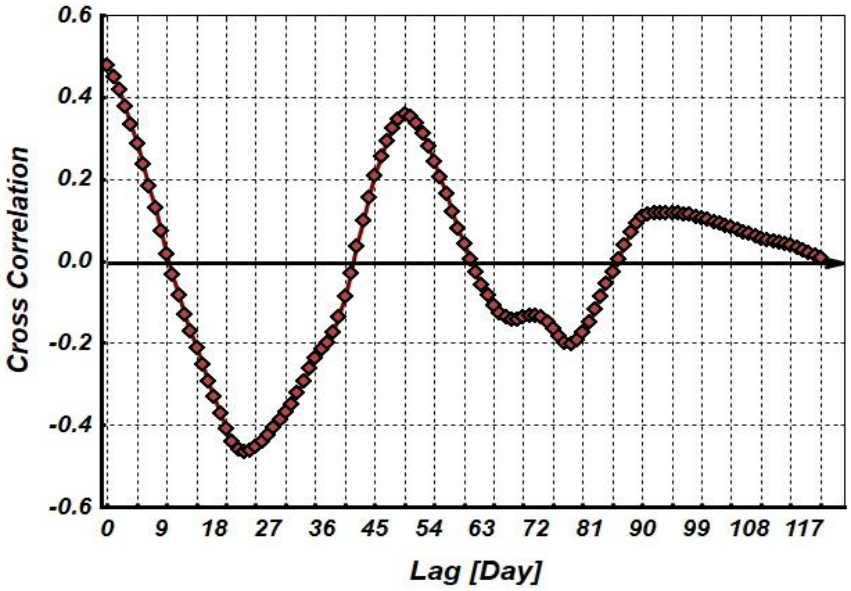

Fig.3. Cross-correlation function of the dynamics of MN-NCE frequencies in the peripheral blood of mice irradiated with a dose of 5 Gy. in the post-radiation period and the distribution of the probability of mice death

As can be seen from the graph, the maximum and minimum values of the function are approximately equal to 5, which indicates a high degree of correlation between the dynamics of MN-NCE levels and the distribution function of the probability of mice death.

DISCUSSION

As it is known, micro nucleus can be observed in cells of any proliferating tissue; however, they are most easily detected in cells without a nucleus in most mammalian species in erythrocytes (polychromatophilic - young and norm chromatophilic – mature). Micronuclei in erythrocytes are called Howell–Jolly body. A Howell–Jolly body is a cytopathological finding of basophilic nuclear remnants (clusters of DNA) in circulating erythrocytes. During maturation in the bone marrow, late erythroblasts normally expel their nuclei; but, in some cases, a small portion of DNA remains, which may be due to the genotoxic effects of various physical and chemical agents, or may be a manifestation of genomic instability associated with a number of internal and external factors [4,6].

The dynamics of Mn-NCE obtained from these positions in the initial stage of the post-radiation period should be associated with a high degree of reliability with the abortive rise of erythropoiesis in

irradiated mice (15-30 days after irradiation), which is followed by the gradual normalization of erythropoiesis in the later period (30-60 days). What concerns the second peak in the dynamics of Mn-NCE, it seemed less likely to us associate it only with the radio-induced instability of the genome (the biological mechanism of the nonlinear dynamics is unknown to us). It should be considered here that the production of Mn-NCE can be related not only to the damage of the genetic apparatus, but also to the intensification of erythropoiesis induced by the development of anemia; It is an experimentally proven fact that prior bleeding significantly increases the concentration of micronuclear reticulocytes in the peripheral blood of irradiated mice [11]. An increase in the concentration of Mn-NCE was detected under conditions of intensification of the proliferation-differentiation of erythroblasts induced by erythropoiesis [7], It should be noted here that even in early radiobiological studies, it was revealed that ionizing radiation causes not only damage to the genetic apparatus of the hematopoietic system, but also a reduction in the life span of circulating erythrocytes, which at a certain stage of the post-radiation period will become the reason for the intensification of erythropoiesis.

If we summarize the above, the superposition of the radio-induced instability of the genome and the intensification of erythropoiesis induced by radiation anemia can be considered as a hypothetical mechanism of the increase in the level of Mn-NCE on the 70-80th day of the post-radiation period, and from this point of view, the level of Mn-NCE in the peripheral blood is an integral part of the functional status of the erythrocyte system as a whole.

As for the close correlation of the probability of death of mice in the post-radiation period with the frequencies of Mn-NCE in the peripheral blood, the discussion of its specific biological mechanism is beyond the scope of this article, although the direct causal relationship of the radiation death of animals with the systemic stability of erythron to radiation exposure is clearly defined

CONCLUSION

The obtained results clearly indicate the high informative value of the frequencies of micronuclear erythrocytes in the peripheral blood in terms of assessing the depth of current pathological changes in erythropoiesis in the body's erythropoietic system (biological marker of the effect), however, when testing the genotoxic effects of various factors with the micronuclear test, the systemic factors of regulation of the level of micronuclear erythrocytes should be taken into account.

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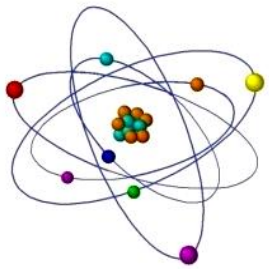
ROLE OF REGENERATION MECHANISM IN RADIOADAPTATION OF SEEDLINGS OF *PISUM SATIVUM L.*



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ABSTRACT: *The experimental dates are obtained, that at modified by decapitation of the main root of seedlings on the base of the increase of radioresistance (radioadaptations), fixed on the various parameters of growth activity of lateral roots, simultaneously a capacity is substantially reduced for the repair of sublethal damages, exposed by the method of fractionating of an acute dose of gamma-irradiation. The obtained facts allow concluding such mechanism of radioadaptive effect of decapitation, in which main part is acted by ephycell mechanisms. Additional comparative research of cyto- and histological parameters of cells of apex meristem of lateral roots of controls (intact) and experimental (decapitation) confirmed this conclusion.*

Keywords: radiobiology, regeneration, radioadaptation.

INTRODUCTION

A reaction of biological objects to the action of ionizing radiation is a comfortable model for the studies of general conformities to the law of transition in the state of stress. Special theoretical and practical interest among such states presents eustress [1], showing up in the increase of the initial level of resistance (adaptation state) a biological object under the influence of the proper doses of stressors. If ionizing radiation plays the role of stressors, appears the possibility of strictly measuring out an operating factor and expressly taking into account the consequences of his influence. Except for it, a radiation factor can be applied in the role of a testing factor.

In our and a row of other research the possible role of nonrepair mechanisms, in providing a radioadaptive answer was shown [2,3]. Obviously, the idea of the existence of the inducible/stimulation systems of recovery can be spread to a great number of other higher levels of structurally-functional integration of biological objects. Otherwise speaking, logically it was to assume that exists not only reparation and cell-repopulation mechanisms of radioadaptive answer (RA), but also organ, organism and other levels of this mechanism.

One of the necessary conditions of the study of RA is a search for such doses of radiomodifying influence, which cause passing the explored object in the post-factor period of a hyper compensate phase by values of the used parameters. From our data exactly doses, possessing such an ability can induce RA [4, 5, 6]. Exactly hypercompensate (phase of positive overshoot) processes in a biological object, showing up in the appearance of additional elements of the recovery (molecules of enzymes of repair, meristem cells and other) systems, provide the level of his radioresistance promoted in the end and in general case increase of the initial level of reliability [7].

At the level of separate organs of the plant the phenomenon of awakening of the plant buds can the example of hypercompensate processes serve under the influence of decapitation of stem apex or sped-up formation of lateral roots after the removal of the apex prevailing the apex of main root [8, 9].

Obviously, any affecting biological object, unbalancing him and inducing/stimulating thus transitional process with the phase of hypercompensation, can come forward at a certain dose and to the certain

parameters in a role of adapting.
If as an object of radiomodifying influence to choose the main root of seedlings that he can test on itself influencing of different degree – from the neutral and stimulants (hormesis) influencing to inhibitory and lethal. Obvious also, that practically any physical, chemical and/or biological factor can play this role. In this connection the measure (by a dose) of influence length of the chopped off area of the main root of seedlings was chosen. We assumed thus, that the certain dose interval of such influence will be rendered by stimulant (hypercompensate) influence on the formation of lateral counterfoils when mass (length and/or amount) of lateral roots will excel similar indexes at control seedlings. In subsequent, it was assumed to use the stimulating «doses» of decapitation as radioadapting.
Thus, the main task decided by the experiments described below – is to define the role of processes of regenerations as one of the possible mechanisms of radioadaptation.

MATERIALS AND METHODS

The pea seeds of the cultivar of Aronis (harvest 2006) were wet in plumbing water and grew in darkness at the stationary temperature of 22^0C. After 5 days of growth, the seedlings were parted into 7 groups, forming seven variants of experience. The seedlings of each group were subjected to the mechanical deletion of root apex proper length:1 – 0 mm (control); 2 - 3-4 mm; 3 – 10 mm; 4 – 20 mm; 5 - 30 mm; 6 – 40 mm; 7 – 50 mm.
Deletion of the apex was made via a blade of a safety razor, whereupon seedlings in an amount of 30 were placed in 0,5 l vessels with plumbing water and were put in the terms of permanent illumination. Further periodicity in days made measuring of the proper parameters (amounts of lateral counterfoils, their middle length, total length of lateral counterfoils, total and middle mass of lateral counterfoils).

For experiments on the research of radioadapting action of root decapitation, 4th days seedlings were parted into two groups, one of which was remote the apex of the main root length 3-4 mm. Controls and experimental seedlings (approximately for 40 things of every variant) are placed on 0,5-l vessels (for 20 things of seedlings on a vessel), filled with plumbing water and placed in a thermostat at 22^0C with permanent illumination. Thus, 4 groups of plants were formed for the realization of the next variants of the experiment: 1 – intact seedlings; 2 - decapitation plants («adapting dose»); 3–7 Gy on nondecapitation seedlings; 4–7 Gy on decapitation seedlings; («adapting dose» + 7 Gy on decapitation plants).
On 4 days after the decapitation, we looked after stimulation of root growth on the parameters of common amount of lateral counterfoils (180 % of control), middle length of lateral roots (117 % of control) and total length of lateral roots (140 % of control) and was accepted the decision to expose the proper variants of seedlings by test-dose. Irradiation was conducted on gamma-apparatus «RESEACHER» at a power of dose of 3,0 cGy/s.
For experiments with fractionating of acute dose of gamma-irradiation were used 3rd days seedlings which the followings variants were formed from: intact (control) roots: 1 – 0 Gy, 2 - 7 Gy, 3 - 3,5 Gy + 2 ÷ + 3,5 Gy, 4 - 3,5 Gy + 4 ÷ + 3,5 Gy; decapitation roots: 1 – 0 Gy, 2 - 7 Gy, 3 - 3,5 Gy + 2 ÷ + 3,5 Gy, 4 - 3,5 Gy + 4 ÷ + 3,5 Gy.

The irradiation of seedlings on the scheme that was presented higher was carried out at the age of 6 days (3 days after the decapitation). The dose of irradiation was 7 Gy. The time between equal factions of doses was 2 and 4h, which correspond to characteristic times of excision reparation.
The moment of irradiation was chosen coming from the results of looking at the sizes of growth parameters. Thus, the moment of irradiation was on stimulation of values of such parameters as the total length of lateral roots (157 % of control level on 3-day after decapitation and mass of dry matter of

lateral roots (about 200 % of control level).

The analysis of proliferate activity of cells of apex meristem of lateral roots was made on temporal preparations on the generally accepted method [10]. Determination of meristem was conducted coming from supposition, that she in some approaching is the truncated cone. Therefore, by micrometer, set on a binocular microscope, we determined length of meristem zone of root and its minimum and maximal radiuses of one. Further, was used a formula for the calculation of volume of the truncated cone:

$$V = h \cdot \pi(R^2 + Rr + r^2)/3$$

where: h - height of cone, R - large radius, r - small radius.

RESULTS AND DISCUSSION

If to estimate doses dependences by comparing absolute (total, integrate) indexes to which it is possible to deliver the common amount of lateral roots of seedling, a total length of lateral roots of one seedling, the total mass of dry matter of lateral roots of seedling, results of supervisions, presented on pictures 1 and 2. show that by the most effective dose of decapitation with point of view stimulation of number of lateral roots, their total length and total mass of dry matter (on seedling), there is a deletion of apex part of root length no more than 20 mm. The deletion of more extensive areas inhibits formation of lateral roots on the indicated parameters. If to estimate the reaction of decapitation seedlings on the specific parameters of formation of lateral roots (middle length of lateral counterfoil and mass of dry matter of one lateral root), there is diametrically an opposite picture (fig. 1-3). Stimulation on specific parameters is observed at the length of a remote area within the limits of 30 – 40 mm, that it is possible, obviously, to examine as a result of compensatory processes, i.e., diminishing of shortage of elements multiplying their single sizes and mass.

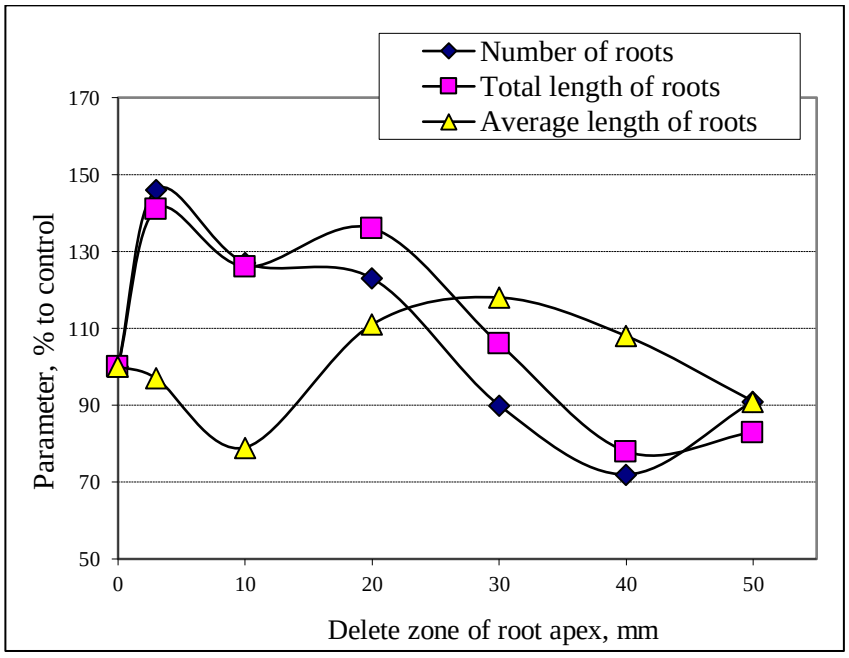

Fig. 1. Influence of length of delite zone of main root of pea seedlings on the growth parameters of lateral roots (7th day after decapitation)

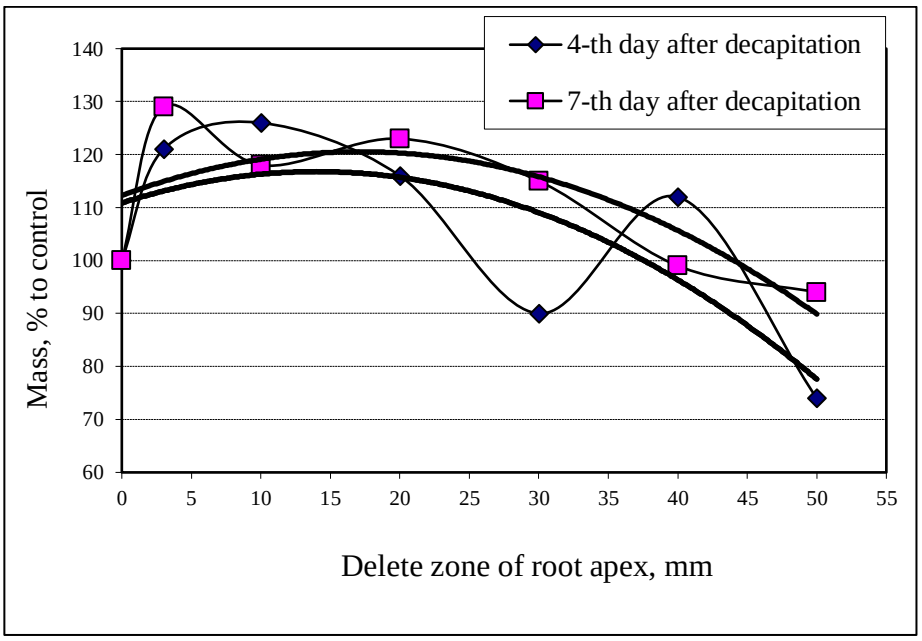

Fig. 2. Influence of length of the delited zone of main root of pea seedlingson mass of dry matter of lateral roots of one проростка (7th day after decapitation)

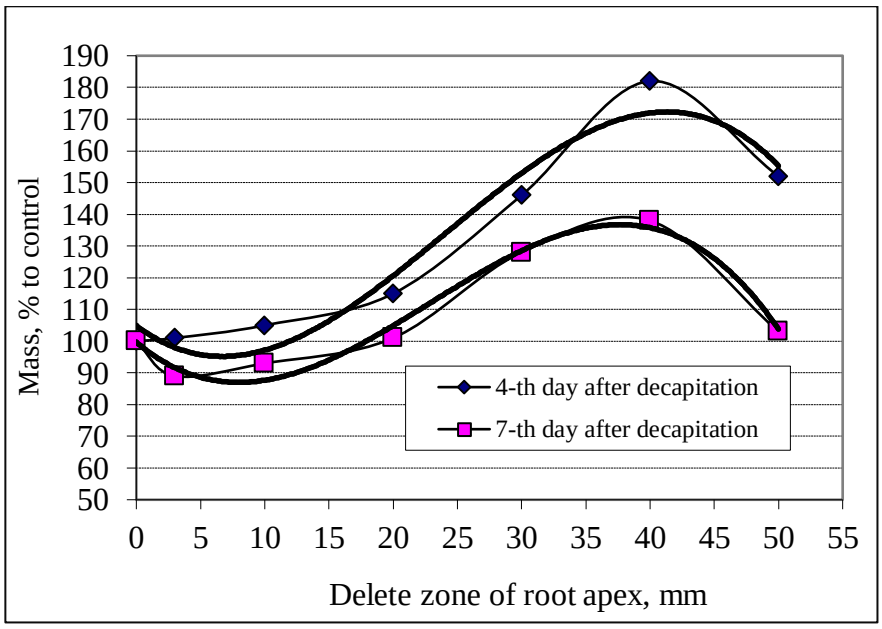

Fig. 3. Influence of length of the delited zone of main root of pea seedlings on mass of dry matter of one lateral root (7th day after decapitation)

Thus, the obtained results allow to draw conclusion about possibility of receipt of effects (and, as we supposed, radioadaptations) of stimulations in the wide range of levels (doses) of stress-factor as which used mechanical delete of root apex.It was further assumed to study connection between the stages of restoration process, fixed on the parameters of formation of lateral roots, and radioadaptive answer.The results of study of radioadaptation effect of decapitation are presented on a fig. 4-6. Evidently, that the irradiation of seedlings with decapitation roots (a test-dose 7 Gy) in the moment of

stimulation (4 day after decapitation of root apex) of formation of lateral roots (on total, middle length, mass of dry matter) exposed them more high level of radioresistance as compared to nondecapitation roots which were exposed to the rays in the same dose. Otherwise speaking, the decapitation of root at 4-day seedlings showed the radioprotective action which we interpret as radioadaptation.It is necessary especially to mark that a test-influencing is carried out in moment when the root system of experimental («adapted») variant has plenty of reactive elements and their large quantitative parameters. Thus, we once again were in a position to be satisfied of том, that one of terms of transition of the system in the state the promoted (by comparison to initial) stability there can be acquisition by her additional «reactive» elements.

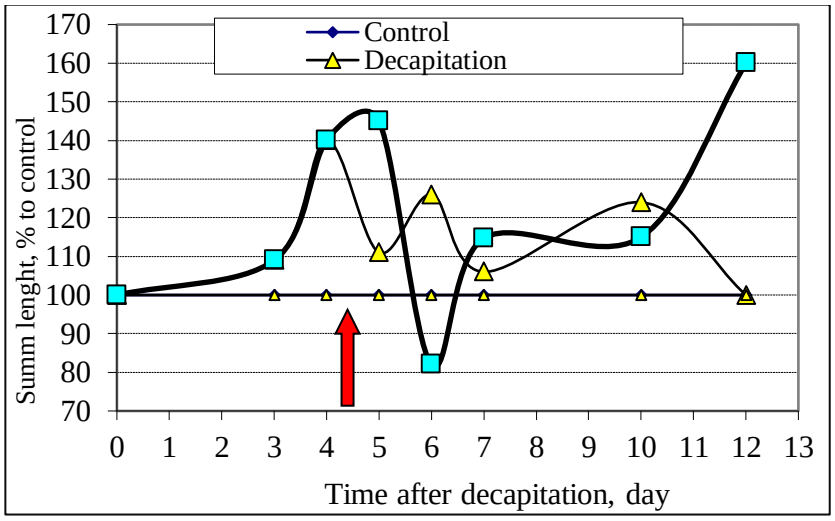

Fig. 4. Dynamics of the total length of lateral roots after the different variants of treatment

NB. A point shows the moment of irradiation in a dose 7 Gy.

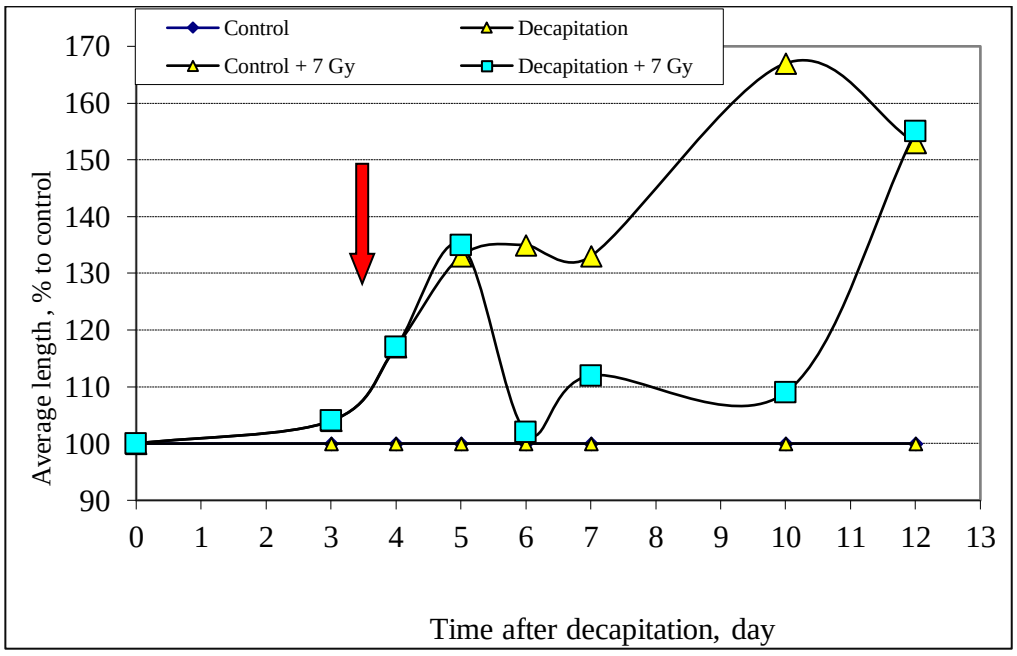

Fig. 5. Dynamics of average length of lateral roots after the different variants of treatment

NB. A point shows the moment of irradiation in a dose 7 Gy.

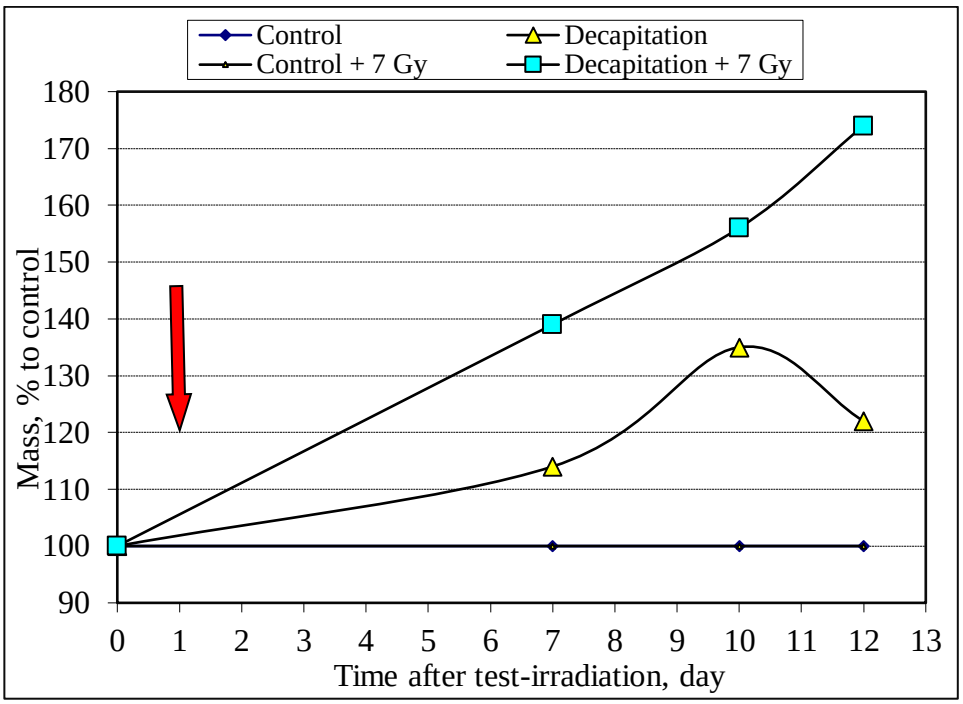

Fig. 6. The mass dynamics of dry matter of lateral roots of pea seedlings (irradiation at the age of 3rd day after decapitation) ***NB. A point is indicating the moment of irradiation in a dose 7 Gy. (3 days after decapitation).***

The large enough test-dose, applied in this experiment, allowed to study the degree of modified radiosensitivity yet on one parameter, namely on survivability of lateral roots after an irradiation. The lateral roots at which it was marked irreversible inhibition of growth activity had red apex, which testified to death of apex meristem. The count of stake (percent) of lateral roots with lost meristem in control and decapitation variants that were exposed to the rays allowed comparing their radioresistance. Appeared, that at the moment of completion of supervision the level of survivability of lateral roots of the intact variant was 45 %, and the decapitation variant – was 62 %, which also simply testifies to greater radioresistance of experimental (decapitated) variant.

The proiliferative activity of the cells of the apex meristem of the lateral roots were also studied in the moment of application of test dose and it is set that in the moment of application of testing irradiation a mitotic index (MI) of lateral roots meristem of the control variant was 1,5 %, and decapitation – 3,4 %, that almost in 2,3 time exceeded a control value. It ensues from findings that in modified by decapitation roots are created cyto- and histological conditions on the certain stage for the increase of the level of values of growth parameters. In the final analysis, in the moment of application of test dose the modified variants can have substantially more reactive elements (cells and organs as additional lateral roots), providing them promoted radioresistance. Comparison of the efficiency of fractionating dose of acute gamma-irradiation of lateral intact and decapitated embryonic roots of pea seedlings was used for the study of the efficiency of work of the repair in the apex meristem cells of lateral roots of the indicated variants. It was assumed that efficiency of repair recovery not will be substantially modified at decapitation variants, but can be even, vice versa, reduced because there was the increase of proiliferative activity, as we showed above. The last assertion has under itself a warrant, that the actively divided cells, i.e., cells, speed-up passing a cellular cycle, have less time on realization of excision reparation. [11].

On fig. 7-10 are presented the results of the study of the effects of fractionating of dose of acute gamma-irradiation. Evidently, the positive effect of fractionating of dose of acute gamma-irradiation on the change of parameter «total length of lateral roots» (fig. 9 and10.) has a positive sign only in intact seedlings, i.e., fractionating of the dose was instrumental in the less degree of inhibition, what acute of gamma-irradiation. In decapitated variants, a fractionating effect on this parameter is not exposed. The last circumstance can be explained by the acceleration of cellular divisions, minimizing the possible deposit of modified (stimulated) enzyme repair in providing of positive radioadapting effect of decapitation.

We will remind that the effect of fractionating of dose of acute gamma-irradiation was checked up at the moment of stimulation of growth parameters of lateral roots and exactly a test-irradiation gives the proof and reproduced the effect of radioadaptation at this decapitation.

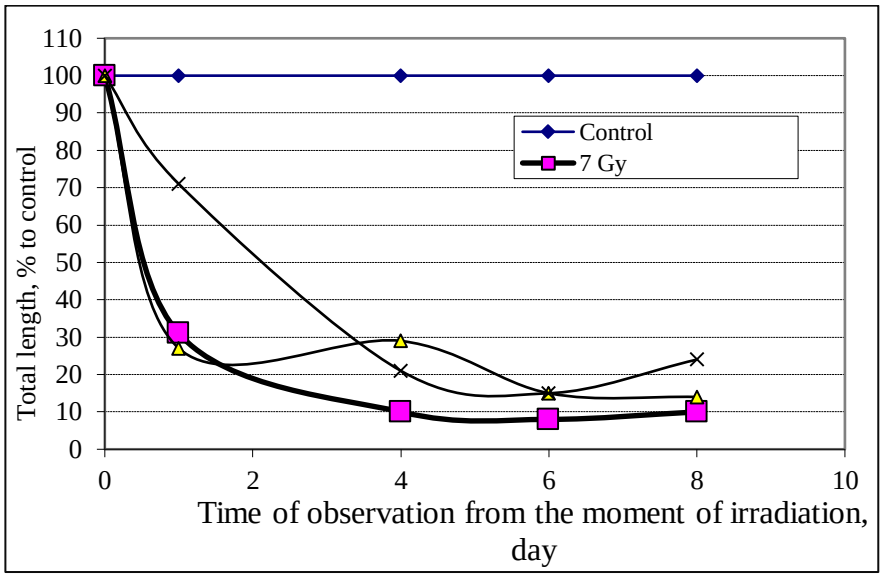

Fig. 7. Influencing acute and fractionating gamma-irradiation on total length of lateral roots of intact seedlings

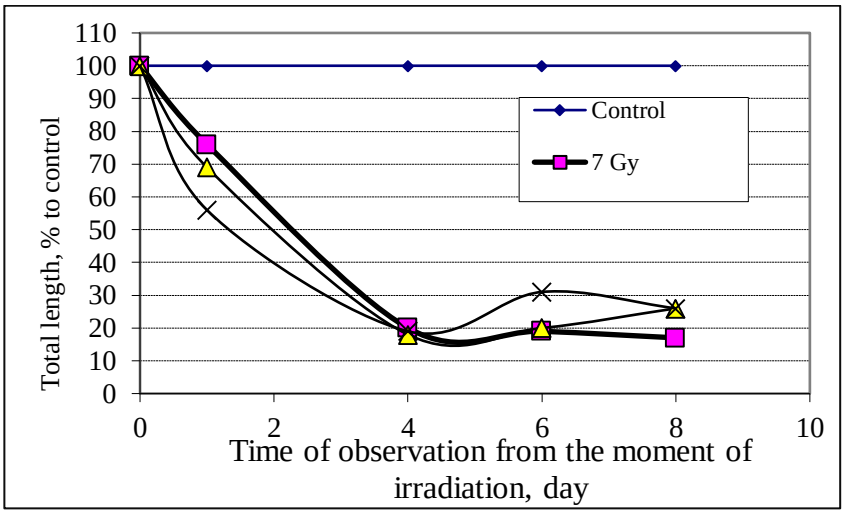

Fig. 8. Influencing acute and fractionating gamma-irradiation on total length of lateral roots of decapitated seedlings

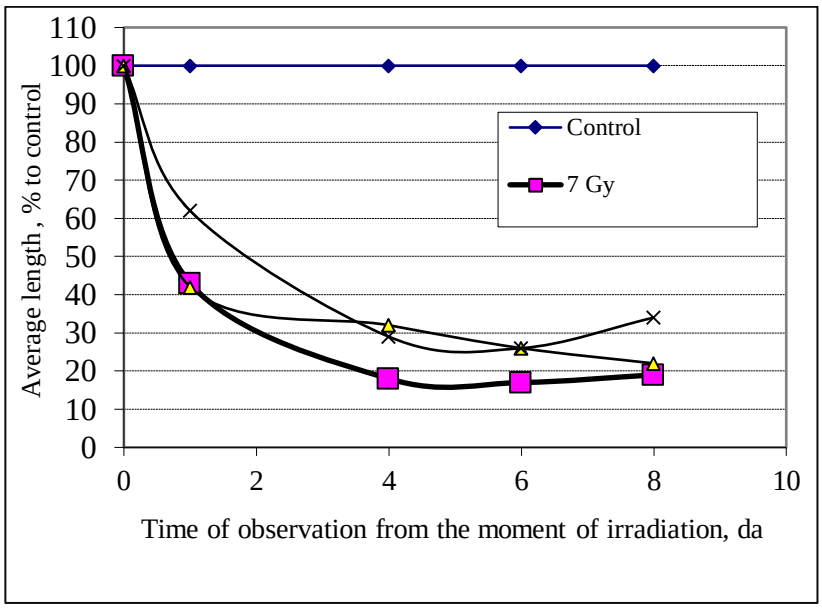

Fig. 9. Influencing acute and fractionating gamma-irradiation on average length of lateral roots of intact seedlings

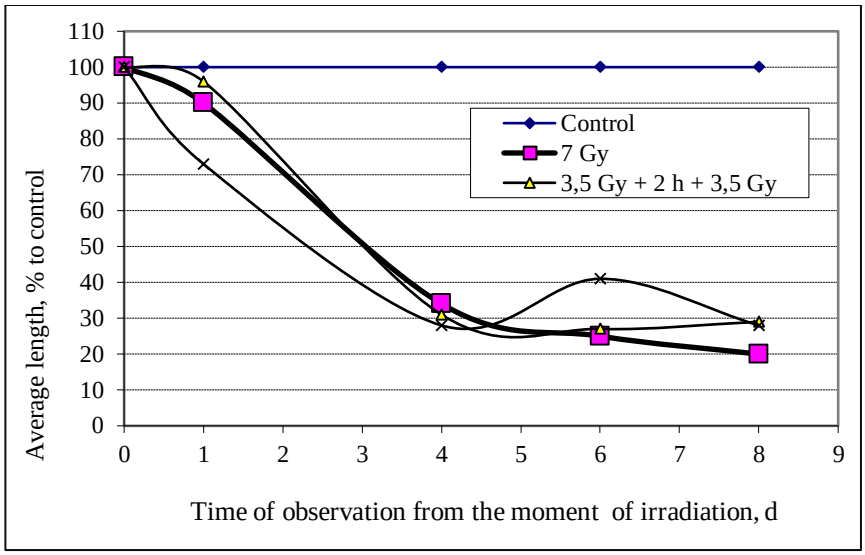

Fig. 10. Influencing acute and fractionating gamma-irradiation on average length of lateral roots of decapitated seedlings

The effect of fractionating of dose of acute gamma-irradiation on the change of parameter «average length of lateral roots» has a positive sign again only in intact seedlings. In decapitated variants a fractionating effect on this parameter even has some tendency to the negative values in an initial period of observation.

Thus, we got experimental confirmation, that at modified by decapitation of main root of seedlings on a background the increase of radioresistence, which fixed on the various parameters of growth activity of lateral roots, a capacity is substantially reduced for reparation of sublethal damages, exposed by the method of fractionating of dose of acute gamma-irradiation. The gotten facts allow to draw conclusions about such mechanism of radioadaptive effect of decapitation, in which determining part is acted by supercell mechanisms. Additional comparative research of cyto- and histological parameters

of apex meristem cells of lateral roots of controls (intact) and experimental (decapitated) confirmed this conclusion once again.

As set before, modified by decapitation roots created on the certain stage cytological and, probably, histological conditions for the increase of the level of growth parameters. On the basis of it, we assumed that, in the final analysis, at the moment of application of the test dose the modified variants could have substantially more reactive elements (cells and organs as additional lateral roots), providing them promoted radioresistance. With respect to lateral roots, as we specified before, their amount at decapitation variants substantially increases as compared to nondecapitation control.

In relation to multiplying a common amount in the apex meristem of lateral roots of the modified variants, we could talk only probably. In order for such suppositions, there were more reasons we carried out the proper experiment which mainly paid attention to the histological indexes of apex meristem of lateral roots of the modified (decapitation) and controls (intact) variants. Actually, there were only two variants in an experiment – intact and decapitation. The method of growing seeds and seedlings and decapitation are repeatedly described above. Studied histological parameters of apex meristems of lateral roots on 3th and 4th days after decapitation.

To that end, at first, determined the MI of apex meristem cells of lateral roots (AMCLR) experimental and seedlings of controls. The MI of the decapitation variant was 125 % to control on the 4th day after decapitation, which, in general, was repeated before the above-described results were obtained. All of it grounded us to count on, what the common amount of AMCLR of the experimental variant will excel the similar index of control variant. In this connection and, secondly, basic attention in this experiment was spared on determining the sizes of AMBK and their geometrical volume. The comparison of these two indexes, from our point of view, would help define what amount of AMCLR dispose control and experimental (decapitation) variants in the moment of application of test-dose. The comparison of volumes of apex meristem of experimental and control variants showed that on the 4th day after decapitation the first was 114 % to control, that, in an aggregate with the fact of equality of cells of the indicated variants a size, means, more cells in apex meristem of decapitation variant.

In theory, in accordance with the general theory of the systems of Urmantseva [12] a few methods of transformation of elements of the system are possible: change of the initial number of elements, change of their quality, and change of relations between them. Obviously, as mentioned above, different combinations of these three fundamental methods are possible. With respect to the quality of elements (in this case cells), the results of experiments on the study of effects of fractionating doses of acute gamma-irradiation specify one of the principle invariability of basic quality which interests us – the quality of radioresistance.

Undoubted and obvious is a change in the amount of the elements - cells, which, can be accompanied by the change of intercellular relations (co-operations). However, what causes the increase in radioresistance? From one side, it can be conditioned greater probability of saving a minimum of the necessary number of cells for renewal of functions of meristem [13], and, from another side, more cells can provide more intensive intercellular co-operations in the post-radiation stage of renewal.

Thus, the gotten results specify, that at modified by decapitation of the main root of pea seedlings on a background the increase of radioresistance (radioadaptations), fixed on the various parameters of growth activity of lateral roots, simultaneously a capacity has substantially reduced the level of sublethal reparation of intracell damages, exposed by the method of fractionating of dose of acute gamma-irradiation. Obviously, in the mechanism of radioadaptive effect of decapitation determining part is acted by supercell processes. Additional comparative research of cyto- and histological parameters of apex meristem cells of lateral roots of controls (intact) and experimental (decapitation) confirmed this conclusion.

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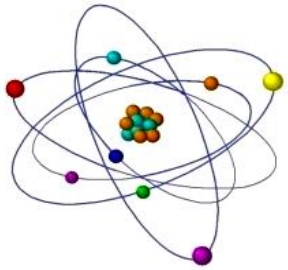
EFFECT HIGH DOSE OF Cs^{137} AND Co(II), Cd(II), Ag(I) IONS ON *Spirulina platensis*



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ABSTRACT: *Influence of metal ions Ag(I), Co(II), Cd(II) on Spirulina platensis and their constituent after high dose (400kGy) of Cs^{137} irradiation were studied using UV-VIS spectroscopy. It was shown that influence of metal ions Ag(I), Co(II), Cd(II) on Spirulina platensis and their constituents differs from each other its effectiveness. Effect high dose of Cs^{137} and Co(II), Cd(II), Ag(I) ions on Spirulina platensis study show that possible use of a high dose of gamma irradiation does not change the nature of the interaction of these metal ions for intact cells Spirulina patensis and its constituents.*

Key words: Spirulina platensis, metal ions, gamma-irradiation

INTRODUCTION

Spirulina was used as food by the Aztec civilization. It has been extensively studied for its nutraceutical value and is used as a food supplement. *Spirulina* contains various components that are beneficial for health, such as proteins, vitamins (such as pro-vitamin A, Bl, B2, B6, B12, E and D), essential amino acids, minerals, γ-linoleic acid, glycolipids, sulfolipids, and phycobilins (phycocyanin, allophycocyanin, and phycoerythrin). Khan et al. [1] reported the main bioactive molecules and explained in detail their effects on health and human nutrition. Furthermore, there are several studies reporting *Spirulina* therapeutic effects including the hypocholesterolemic effect [2]. It has some good medicinal properties against inflammation [3] and cancer [4], and cerebral ischemia [5], vascular reactivity [6], and anti-Parkinson [7]. Based on the findings, in work [8] were investigated whether *Spirulina platensis* supplementation can inhibit the development of liver fibrosis through its effect on inflammation and whole-body energy metabolism in a diet-induced mouse model of liver fibrosis. The expression and secretion of inflammatory genes in *splenocytes* were significantly reduced by *Spirulina platensis* supplementation, demonstrating the anti-inflammatory effects of *Spirulina platensis* in vivo. Although *Spirulina platensis* did not show appreciable effect on the prevention of liver fibrosis in this mouse model, it may be beneficial for other inflammatory conditions [8]. Numerous studies have suggested that zeaxanthin and lutein are crucial for visual health *Spirulina* can serve as a rich source of dietary zeaxanthin in humans [9].

A single dose of *Spirulina* can increase mean serum zeaxanthin concentration in humans from 0·06 to 0·15mmol/l,

Gamma irradiation has been used for microbial decontamination of food [10]. In [11] reported that gamma irradiation had a stimulatory effect on its growth and cellular constituents. In our works [12,13] influence of 7.2 kGy Cs^{137} gamma-irradiation have been studied with optical and differential scanning microcalorimetry (DSC) methods for cyanobacterium *Spirulina platensis* intact cells in the suspension, wet mass, and dry mass samples and also simultaneous effects of Cd(II), Pb(II) ions and γ-irradiation on stability of *Spirulina platensis* intact cells after 7.2 kGy Cs^{137} gamma irradiation and

without irradiation. In [14] combined effects of Cs^{137} gamma irradiation and heavy metal ions same concentrations on *Spirulina platensis* cells using UV-VIS spectrometry, when after one year the same *Spirulina platensis* (which was irradiated with 7.2 kGy one year ahead) again irradiated and recultivated were discussed.

In this work, we have studied simultaneous effects of a high dose (400kGy) Cs^{137}gamma irradiation and heavy metal ions Co(II), Cd(II), Ag(I) on *Spirulina platensis* intact cells after irradiation and recultivation and their constituents using UV-VIS spectrometry.

MATERIALS AND METHODS

Spirulina platensis IPPAS B–256 strain was cultivated in a standard Zarrouk [15] alkaline water–salt medium at 30ºC, illumination ~5000lux, at constant mixing in batch cultures [16]. The cultivation of the *Spirulina platensis* cells was conducted for 16 days. The growth was measured by optical density by monitoring of changes in absorption at wavelength 560nm using the UV–Visible spectrometer Cintra 10e. The intact cell suspension of *Spirulina platensis* at pH 10.1 in the nutrition medium was used for scanning the absorption spectra from 400 to 800nm. The concentration of *Spirulina platensis* was determined by the instrumental data [17, 18]. *Spirulina platensis* suspension (100 ml) has been irradiated with 400kGy gamma radiation from April to January, in the span of 10 months (Dose rate 0.018Gy/s) using ^{137}Cs as a gamma radiation source at the Applied Research Center, E. Andronikashvili Institute of Physics. Suspension after the irradiation (400 kGy) were filled up to 200ml with Zarrouk medium and they were recultivated for16 days. The optical density was measured every day with 24h intervals. The concentration of different compounds was estimated at the late exponential phase.

RESULTS AND DISCUSSIONS

Metal effect by irradiated and recultivated cells of blue-green microalgae *Spirulina platensis* was studied as a function of metal concentration (pH 10.1). Fig.1 shows the absorption characteristics after irradiation and recultivation of cells *Spirulina platensis*. The peak at 681 nm is due to the absorption of Chl a peak. At 621 nm is due to the absorption of phycocyanin (PC). At 500 nm is due to the absorption of carotenoids. A peak at 440 nm is due to soret band of Chla [19]. In fig.1 are shown effect of Cd(II), Co(II), Ag(I) ions on the absorption of the irradiation (400kGy) and cultivation cells after irradiation and cultivation of *Spirulina platensis*. It is seen from fig.1, that with increasing metal concentrations absorption intensity decreased for all metal ions. As can be seen from this figure, the absorption processes were relatively fast in the small concentrations for Cd(II), Co(II) and Ag(I) ions and then became slow. In [20] *Spirulina platensis* cells were exposed to different doses of gamma irradiation (Co^{60}). The optimum growth of *Spirulina platensis*was recorded at 2.0 kGy as compared to the control after the 14th day of incubation. The results of pigments analysis revealed that the chlorophyll *a* and carotenoid contents of *Spirulina platensis* reached their maximum rate at a dose of 2.0 kGy.

By us were also investigated the influence of the same metal ions on the same cellular components of *Spirulina platensis* the same irradiation dose. Effect of metal ions on the absorption intensity maximums for wavelengths 440nm, 500 nm, 621 nm and 681nm are shown in fig.2. At 440 nm with increasing Cd(II) and Ag(I) concentration the intensity of absorption increases, almost does not change for Co(II) ions. Increasing is very effectively for Cd(II) ions. Analogue results were received for carotenoids at 500 nm wavelengths in the case Cd(II), Ag(I), Co(II) ions. As for the change in absorption intensity at 621 nm, which is the peak of absorption of the major protein-phycocyanin of *Spirulina platensis*, as the concentration of silver ions increases, the absorption

intensity very efficiently increases, but almost does not change in the case for Cd(II) ions. An increase in the concentration of Co(II) causes the most noticeable decrease in the intensity of absorption intensity. It should be noted, that sequence of effectiveness first Ag(I), after that Cd(II) and after that Co(II) were observed for all constituents. Similar results were observed in absorption intensity for Ag(I) ions at 681 nm, which is the absorption peak of the Ch a of *Spirulina platensis*. In particular, as the concentration of silver ions increases the absorption intensity increases very efficiently. But unlike silver ions, an increase in the concentration of Co(II) and Cd(II) causes the most noticeable decrease in the intensity of absorption. If we consider that the maximum absorption of chlorophyll is 440 nm and 681 nm, then we can conclude that at both peaks of the chlorophyll absorption intensity of *Spirulina platensis* the above-mentioned metal ions act equally.

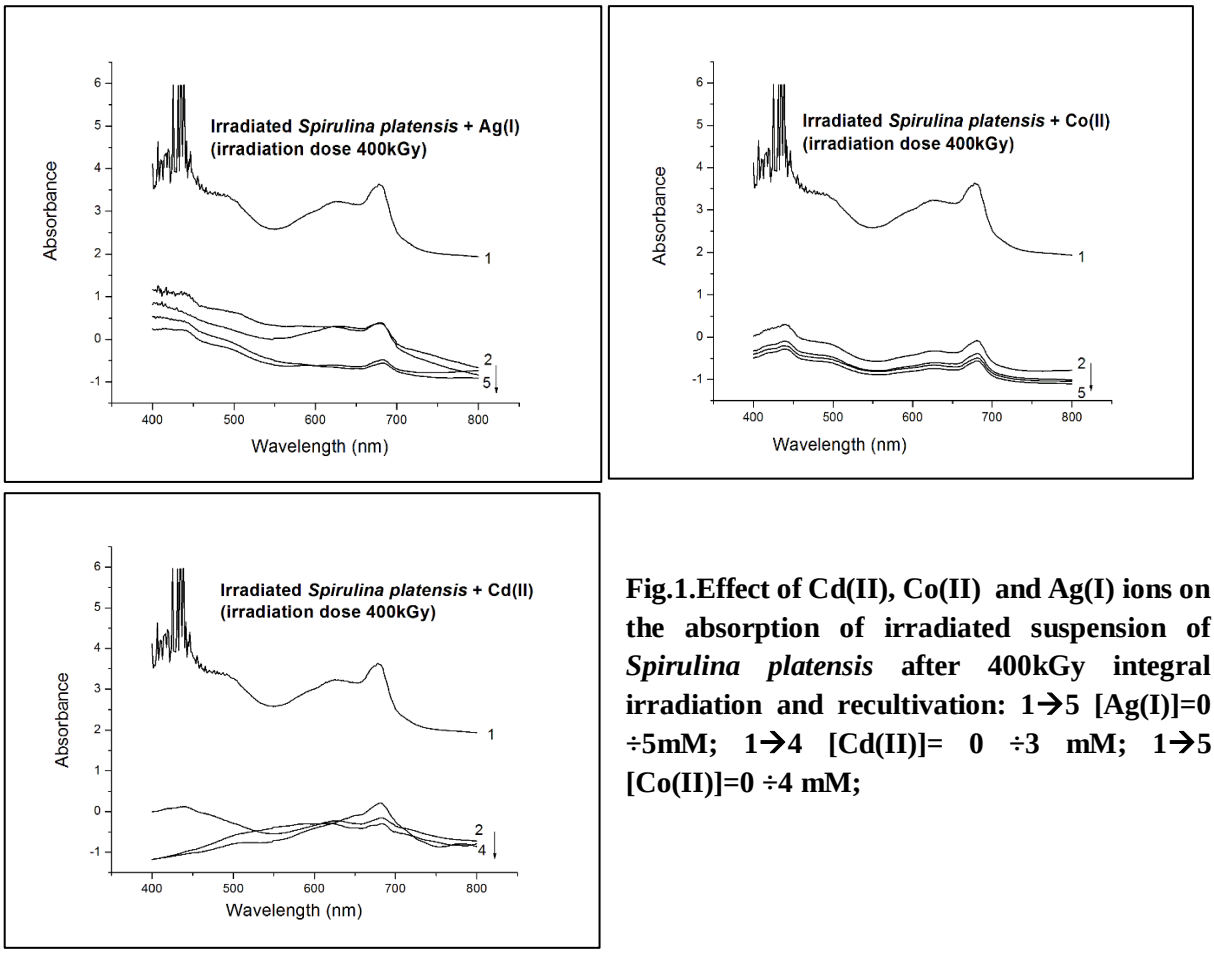

Fig.1.Effect of Cd(II), Co(II) and Ag(I) ions on the absorption of irradiated suspension of *Spirulina platensis* after 400kGy integral irradiation and recultivation: 1→5 [Ag(I)]=0 ÷5mM; 1→4 [Cd(II)]= 0 ÷3 mM; 1→5 [Co(II)]=0 ÷4 mM;

The role of *spirulina* orally administrated before or after whole body γ-irradiation on brain tissue is determined by FTIR-ATR and ESRThe role of *Spirulina* against brain tissue injury was discussed [21]. Thirty male albino whistler rats were divided. Deconvolution of secondary structure of amide I showed that 4Gy exerted significant increase in β-sheet. Electron spin resonance of lyophilized brain tissue indicated increase in the free radicals for 4Gy. *Spirulina* given before or after γ-irradiation ameliorate the changes in brain tissue.

If this results will be compared for the results which were received by us in works [13,22,23], for these metal ions in the case without irradiation, it is clear that the effectiveness of intact cells of cyanobacterium *Spirulina platensis* influence of metal ions (Ag(I), Co(II) is analogue for all the onstituents [22].

For silver ions, an increase in intensity is observed in both the irradiated after 3 times irradiation and recultivation) [23] and non-irradiated states [22].

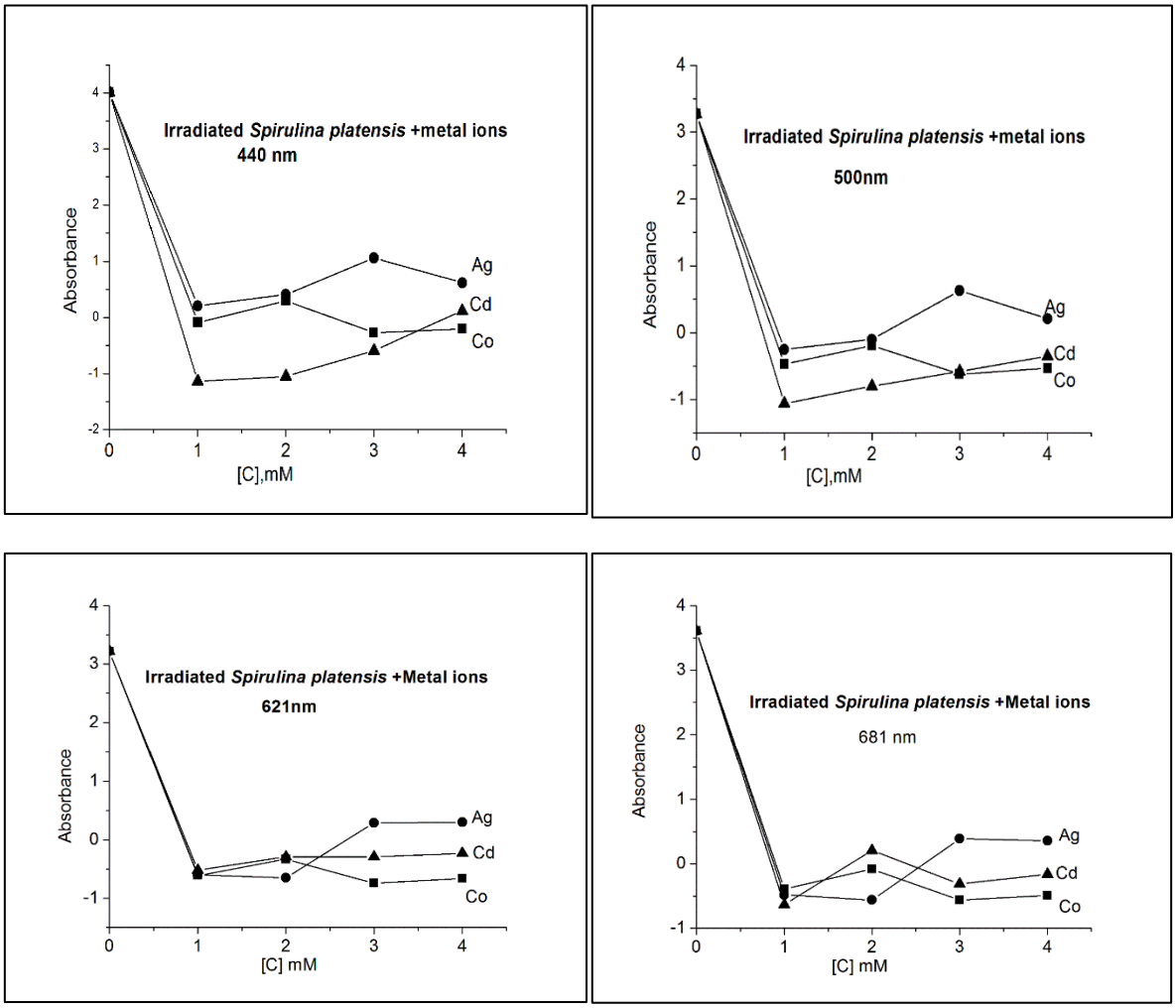

Fig.2. Changes in the absorption intensity of cellular constituents of irradiated *Spirulina platensis* at 621 nm (phycocyanin), at 440 nm (the soret band of Chl a), at 681 nm (the Chl a), at 500 nm (carotenoides) under the influence various metal ions after high dose (400kGy) integral irradiation and recultivation.

After 7.2 kGy 137Cs gamma irradiation and without irradiation it was shown that the addition of Cd(II) ions causes a decrease in optical absorption spectra band intensities. In the case of irradiation, the absorption band intensity decreases higher than without irradiation [13].

In work [24] was to investigate the tolerance and adsorption of five heavy metal ions by radiation-resistant microbes, a radiation-resistant strain NO.9 was identified according to 16S rDNA gene sequence analysis and biolog system, and the tolerance and adsorption to five heavy metal ions were analyzed. The results showed that it had a maximum tolerance of 2200mg/L to Pb2+, and it had adsorption ability to Pb2+、Cu2+、Hg2+、Zn2+ and Co2+under the situation as follows: 0.1g the amount of cells, at pH 6.0, 20°C and adsorbed for 40 min. it reached maximum adsorption. This 98.9% to Pb^{2+}, and the amount of adsorption was 39.56mg/g. It indicated that strain NO.9 had a strong tolerance and absorption to Pb^{2+}.

Thus, the effect high dose of Cs^{137} and Co(II), Cd(II), Ag(I) ions on *Spirulina platensis* study show that possible use ofa high dose of gamma irradiation together with Co(II),Ag(I) and Cd(II) ions do not change nature of the interaction of these metal ions for *Spirulina platensis*.

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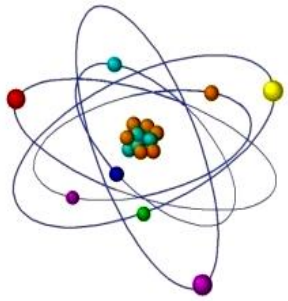
GAMMA-SPECTROSCOPY MEASUREMENTS OF RADIOACTIVITY AND ASSESSMENT OF RADIATION HAZARD INDICES IN SOILSAMPLES FROM SOME REGIONS OF THE BLACK SEA COAST



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ABSTRACT: *There are given results of radioactivity research in soil samples within the territory of some regions of the Black Sea coast. Ten samples have been collected from 10 locations. Up to 22 radionuclides were identified. Activity concentration of radionuclides of Th-232 family was in limits from 15.2 to 32.5Bq/kg (mean, 24.5Bq/kg), U-238 family – from 9.7 to 23.8 Bq/kg (mean, 17.2Bq/kg), U-235 family - from 0.62 to 1.31Bq/kg (mean, 0.93Bq/kg). Also individual radionuclides have been identified: K-40 – from 81.1 to 485Bq/kg (mean, 300Bq/kg), Cs-137 – from 8.8 to 191 Bq/kg (mean, 68.4Bq/kg); Be-7 – from 2.2 to 29.5 Bq/kg (mean, 12.3 Bq/kg). Radium equivalent activity varied from 44.9 to 89.4Bq/kg (mean, 73.2Bq/kg). Annual effective dose varied from 0.027 to 0.054mSv/y (mean, 0.043 mSv/y).*

Key words: radionuclides, soil, activity concentration, Black Sea coast

INTRODUCTION

Soil radioactivity is one of the main components of the Earth's radioactive background. Radioactivity in various areas of the globe differs considerably – from units up to hundreds of Bq/kg. Therefore, studying the terrestrial radioactivity in each territorial region is very important. Such studies are carried out in many countries. So, for example, in the work [1] there were studied soil samples selected in the territory of the Arnás River Catchment in the central part of the Spanish Pyrenees where six types of soil are widely distributed – CalcaricFluvisols, EutricGleysols, CalcaricRegosols, RendsicLeptosols, HaplicKastanozems, and HaplicPhaeozems; it was determined activity concentration of Th-232, U-238, Ra-226, Pb-210, Pb-210ex, K-40, Cs-137; the correlation between soil radioactivity and its mineralogical content was investigated. In another work [2] the activity of natural radionuclides in samples from 10 different regions in Qena governorate and Wadi EL-Lagita (Egypt) was investigated. Concentrations of radionuclides in soils ranged from 7.9±2.8 to 96.1±9.8 for Ra-226, 8±2.8 to 19 ±4.4 for Th-232 and 85.2±9.2 to 302.5±17.4 Bq/kg for K-40. The radiological health implication to the population that may result from these values is found to be low and almost insignificant, except in one case. No artificial radionuclide, however, was detected in any of the samples; hence, measurements have been taken as representing baseline values of these radionuclides in the soil in the studying areas. Similar research on naturally occurring radioactive materials (NORM) in the soil of Yalova, northwestern Turkey has been carried out in the work [3]. In addition, maps for the radionuclide activity concentrations of soil and the outdoor gamma dose rate distributions have been plotted for the region. The results of the study were discussed with the studies done in the close cities and the worldwide averages. In another work [4] soil samples (together with cement and fertilizers samples) collected in Sergipe State (Brazil) were studied; the radionuclide activities are below the Brazilian limit of the

exclusion and exemption criteria from the requirement of radiation protection. It also detected an unexpected Cs-137 in some samples; however, its activities do not represent a risk for the population. Surface and depth profile concentrations (down to 50 cm) of Th-232Th chain, Ra-226, and K-40 radionuclides were determined in undisturbed coastal and inland soils of La Plata city region, Argentina [5]. No dependence of the activity of the Th-232 natural chain on depth was found, whereas variations for Ra-226 and K-40 activities were observed. Positive correlations, determined by the Pearson correlation coefficients, were established between K-40, Ra-226 and Th-232 activity concentrations for the whole set of soil samples. Activity concentration of Th-232, Ra-226 and K-40 in soil samples from the Fen Complex area (Norwegian county of Telemark) was determined in the work [6]. In Georgia, regular research of natural (and also technogenic) radioactivity was not actually carried out. Rather detailed research of radioactivity in various environmental objects have been carried out in 1986, after the failure of the Chernobyl atomic power station and, basically, concerned technogenic radionuclides [7, 8, 9]. In these works it has been shown, that during this period in the territory of Western Georgia, basically in the strip adjoining the sea big concentrations of technogenic radionuclides were observed (in particular, Cs-137 concentration made from several hundred to some thousands of Bq/kg). It is possible to note also works [10, 11] in which results of research of radiation condition of the coast of the water area of the Black sea during a later period are given, in particular, presence of 7 natural (Ac-228, Ra-226, Bi-214, Pb-214, Pb-212, Pb-210, K-40) and 1 technogenic radionuclide (Cs-137) has been fixed in the soil in some areas of Adzharia (Batumi, Gonio, Sarpi, Chakvi, Kvariati). Some results of the last period are given in a study by Kekelidze et al [12]. Urushadze and Manakhov studied the content of technogenic radionuclides Cs-137 and Sr-90 in different types of soil in the territory of Georgia [13].

In the present work, there are given results of radioactivity research of soil samples within the territory of several regions of the Black Sea coast, Georgia.

MATERIALS AND METHODS

Study area

The study area of the Black Sea coast settles down between the settlement of Gonio and the city of Poti. In this area soil of two types – alluvial and red soils are most extended. Samples were collected near to following settlements and cities – Gonio, Batumi, Chakvi, Kobuleti, Shekvetili, Grigoleti, and Poti. In total 10 soil samples have been collected. The list of the investigated samples and their types is given in Table 1.

Table 1

List of control points (CP), types (ST) of investigated samples

#	CP	Lt(N); Ln(E)	ST
1	CP-1	41.554898; 41.578068	Rd
2	CP-2	41.605098; 41.578903	Al (Ac)
3	CP-3	41.616148; 41.587689	-“-
4	CP-4	41.636601; 41.655869	Al (St)
5	CP-5	41.669682; 41.691541	-“-
6	CP-6	41.733941; 41.738102	Rd
7	CP-7	41.804014; 41.777397	Al (St)
8	CP-8	41.958202; 41.762573	-“-
9	CP-9	42.018461; 41.762107	-“-
10	CP-10	42.184805; 41.648328	-“-

Note. Lt(N) – latitude (north); Ln(E) – longitude (east)

Sampling and analysis

Sample collection and preparation

Representative soil samples were collected from the soil surface (depth, 0.30 m) with the use of the special hand auger directly in plastic containers (volume up to 2.0 L). After drying in laboratory conditions samples were ground and sieved for their homogenization. Then samples were dried at the temperature of 105 - 110°C to constant weight and their bulk density and weight were determined. These values were used in the description of sample geometry. The samples were tightly sealed in Marinellibeaker (besides polyvinyl chloride adhesive tape was used also for hermetic sealing) and stored for 4 weeks before gamma-spectrometric analysis to avoid the escape of Rn-222 gas to attain a secular equilibrium between Ra-226, Th-232 and their respective progenies.

Gamma spectrometric analysis

The samples were analyzed for their radionuclides contents and activity concentrations using a Canberra GC2020 gamma spectrometer with a semi-conductor germanium detector with a relative efficiency of 24%. The gamma spectra acquisition time was 72 h. For the analysis, the software Genie-2000 S500 was used with additional modules, in particular, S506 – the Interactive Fit Program. The activity concentration of Th-232 was determined as averaged value for Ac-228, Ra-224, Pb-212, and Bi-212 (which the determination error varied from 1.4% to 6.4%), and that of U-238 was determined as averaged value for Th-234, Pb-214, Bi-214 (which the determination error varied from 7.1% to 16.2%). Since K-40 is directly a gamma-emitter, its activity concentration could be determined from its single photopeak at 1460 kev. Also identified was the technogenic radionuclide Cs-137. In samples "super-equilibrium" (allochthonous) Pb-210 (Pbal) was observed, the value of which was determined as the difference between measured activity values of Pb-210 and Ra-226 [1].

Assessment of radium equivalent activity Ra_{eq} (Bq/kg) and annual effective dose equivalent AEDE (mSv/y) depending on the soil type was carried out under formulas [14]:

$$Ra_{eq} = A_U + 1.43A_{Th} + 0.07A_K \qquad (1)$$

Where A_U, A_{Th}, and A_K are the activity concentrations (Bq/kg) of U-238, Th-232 and K-40, respectively;

$$AEDE = D \times N_h \times k_1 \times k_2 \qquad (2)$$

Where N_h is the number of hours in 1 y. (=8760 h), k_1 – the factor to convert effective dose rate into the absorbed dose rate in the air for adults, 0.7×10^3mSv/Gy, k_2 – outdoor occupancy factor (the fraction of time spent in the open air) which equals - 0.2, D – absorbed dose rate *D* (nGy/h):

$$D = k_U A_U + k_{Th} A_{Th} + k_K A_K \qquad (3)$$

Where k_U, k_{Th}, k_K – so-called dose coefficients which are equal to 0.462, 0.604 and 0.0417, respectively.

In the case of the presence of allochthonousPb, the contribution of its "allochthonous" parents Pb-214 and Bi-214 (and, accordingly, the activity *Raeq-al* of allochthonous Ra-226) to radioactivity was considered (under the assumption that its concentration is connected with excess radon in a soil layer), the contribution of which, according to Saito and Jacob [15], makes up a part equal to 98.5% of the gamma flow of the energy of U-238 radionuclides. In this case, the term equal to 0.985 • *ARa-al was* added to the calculation formula (1) where *ARa-al* is equal to *A*Pb-al = *A*Pb - *A*Ra [1]. A similar term (0.456•*ARa-al*) was added into equation (3) for the absorbed dose rate *Dal* and, accordingly, was considered for the calculation of AEDE*al*.

For samples characterization by the degree of radioactivity taking into account the accepted limiting

value *Raeq*- 370 Bk/kg (equivalent to γ-radiation dose of 1.5 mSv/y) [16] there were established several groups of samples by the value of radium equivalent activity, in particular: 1st group - not radioactive samples with activity no more than 30 Bq/kg; 2nd group – with low radioactivity in the range from 30 to 100 Bq/kg; 3rd group – with average radioactivity in the range from 100 to 300 Bq/kg; 4th group – samples with the raised radioactivity in the range from 300 to 1000 Bq/kg. The technique is described in more detail in works [17, 18].

RESULTS AND DISCUSSION

Up to 22 radionuclides were identified in samples: the Th-232 family (Ac-228, Th-228, Ra-224, Pb-212, Bi-212, and Tl-208), the U-238 family (Th-234, Pa-234, Th-230, Ra-226, Pb-214, Bi-214, and Pb-210), the U-235 family (U-235, Th-231, Th-227, Ra-223, Rn-219, and Pb-211), the individual radionuclides Be-7, K-40, and the techogenic radionuclide Cs-137.

The activity of identified radionuclides of the different families varied from 0.62 Bq/kg (for the U-235 family) to 65.5 Bq/kg (for the U-238 family). Among individual radionuclides, K-40 had the greatest activity (up to 485 Bq/kg). The activity of several radionuclides in some samples was below the minimum detectable activity (MDA). Activity concentrations of the main radionuclides in the studied samples, radium equivalent activity with no account taken of allochthonous Pb-210al (Raeq) and taking this into account (Raeq-al) and, accordingly, annual effective dose (AEDE and AEDEal), their average (*av*), minimal (*mn*) and maximal (*mx*) values are given in Table 2.

Table 2

The activity concentrations (A, Bq/kg) of the radionuclides, radium equivalent activity with no account taken of allochthonous Pb-210_{al} (Ra_{eq}) and taking this into account ($Ra_{eq\text{-}al}$) and, accordingly, annual effective dose (AEDE and $AEDE_{al}$), their average (*av*), minimal (*mn*) and maximal (*mx*) values

CP	A, Bq/kg											Ra_{eq}	$Ra_{eq\text{-}al}$	AEDE	$AEDE_{al}$
	Th-232	U-238	Ra-226	Pb-214	Bi-214	Pb-210	U-235	Be-7	K-40	Cs-137	Pb-210_{al}	Bq/kg	Bq/kg	mSv/y	mSv/y
CP-1	26.3	18.8	28.8	16.9	17.0	53.6	1.04	6.7	471	83.7	24.8	89.4	114	0.054	0.068
CP-2	15.2	9.7	14.0	8.0	7.7	43.6	0.62	4.9	192	21.1	29.6	44.9	74.1	0.027	0.043
CP-3	20.8	13.6	20.0	10.9	10.7	56.7	0.89	23.5	259	95.1	36.7	61.5	97.6	0.036	0.057
CP-4	32.5	23.8	34.8	21.6	21.5	55.2	1.31	29.5	195	47.6	20.4	83.9	104	0.048	0.059
CP-5	24.3	17.3	28.7	17.6	17.0	27.1	0.80	10.1	328	56.9	-1.6	75.0	73.5	0.045	0.044
CP-6	23.5	16.2	19.7	12.6	12.2	51.1	1.08	16.3	81	66.0	31.4	55.4	86.4	0.031	0.048
CP-7	18.7	16.3	16.3	14.4	13.7	65.5	0.92	5.4	485	191	49.1	77.0	125	0.048	0.076
CP-8	28.4	20.5	31.7	19.6	19.3	41.1	1.05	-	261	52.2	9.4	79.5	88.7	0.046	0.051
CP-9	32.4	17.9	22.3	18.5	18.4	17.4	0.80	2.2	292	8.8	-4.8	84.6	79.9	0.049	0.046
CP-10	22.6	17.6	22.1	18.5	17.1	33.6	0.81	-	436	61.4	11.4	80.5	91.8	0.049	0.056
av	24.5	17.2	23.8	15.9	15.5	44.5	0.93	12.3	300	68.4	20.6	73.2	93.5	0.043	0.055
mn	15.2	9.7	14.0	8.0	7.7	17.4	0.62	2.2	81.1	8.8	-4.8	44.9	73.5	0.027	0.043
mx	32.5	23.8	34.8	21.6	21.5	65.5	1.31	29.5	485	191	49.1	89.4	125	0.054	0.076

Within families of radionuclides, activity varied within sufficiently wide limits. In particular, the range (mean) was 5.5-35.0 (20.9) Bq/kg for Th-232, 7.7-65.5 (23.8) Bq/kg for U-238, and 0.62-1.31 (0.92) Bq/kg for U-235.

The activity of K-40 varied from 81.1 to 485 Bq/kg (mean, 300).

The mean activity concentration of Th-232 estimated in this study is 20.9 Bq/kg, which is lower than the world average of 33Bq/kg. The mean activity concentration of U-238 estimated in this study is 23.8

Bq/kg, and is thus lower than the world average of 45 Bq/kg. The main activity concentration of K-40 estimated in this study is 300 Bq/kg, which is lower than the world average of 400 Bq/kg.

Figure 1 shows the statistically significant correlation (R = 0.7272) between the Th-232 and U-238 activity concentrations.

Radionuclide Be-7 was observed in actually all samples with activity in the range 2.2-29.5 Bq/kg. Technogenic radionuclide Cs-137 was measured in all samples and was found to be in the range of 8.8 to 191 Bq/kg (mean, 68.4).

All samples by the level of equivalent activity belonged to the group with low radioactivity.

The highest values of equivalent activity were observed for soil type Al(St), with an average value of 80.1 Bq/kg, and rather less for soils Rd – 72.4 Bq/kg, and the least value was observed for Al(Ac) – 53.2 Bq/kg.

Determined minimal and maximal values of annual effective dose varied in the range of 0.027-0.054 mSv/y. These values (as well as equivalent activity) increase (annual effective dose - in the range 0.043-0.076 mSv/y) under the assumption, that allochthonous Pb-210 is caused by excess soil radon.

It is common knowledge that the concentration of radioactive elements in soils is determined by the radioactivity of initial rocks and the subsequent soil formation processes. The content and concentration of naturally occurring radionuclides, in general, correspond to those usually observed for different soils [19].

Due to specific processes of soil formation (the big role of migratory processes therefore hashing of various minerals occurs much more effectively than in rocks) range of activity changes of natural radionuclides in them is much less, than in rocks (where they are in the “sealed” condition). Owing to these reasons also, as it is apparent from the results, it is not observed the noticeable expressed dependence on soil type.

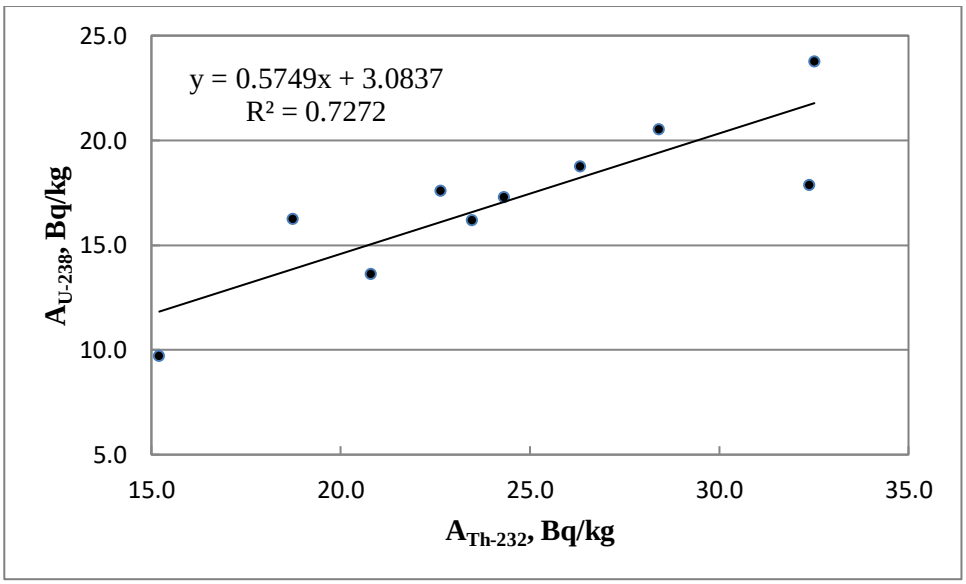

Figure 1. Correlation between Th-232 and U-238 activity concentration.

The presence of “super equilibrium” (allochthonous) Pb-210 connects with radon migration from the bottom layers of the earth to the top (and further in the atmosphere). Pb-210 is capable to accumulate on the walls of pores and faults at the passage of radon flow through the soil. At the passage of radon through a layer from underlying horizons, there is an accumulation in a layer of excess Pb-210, not supported by radium, formed by the expense of decay of radon arriving from below. Radioactive balance in the soil is thus broken towards an increase in activity of Pb-210. In a number of works presence of “super equilibrium” Pb-210 in the soil is connected with its deposition from atmospheric air.

Radionuclide Be-7 is formed in the upper atmosphere as a result of interaction with space radiation and then combines with deposits in the soil. This radionuclide was detected in several samples. Its absence from the other samples could be associated with the long period of sample storage, which could have led to a reduction in concentration to values below the minimum detectable level.

Technogenic radionuclide Cs-137 was observed in all samples in sufficiently appreciable amounts. Usually, its occurrence is connected with the failure of the Chernobyl atomic power station in 1986. By a number of data, in particular, according to systematic observations for the flat areas of East Georgia [20], values of Cs-137 activity are now, basically, in the range of 1 - 10 Bq/kg. With a certain degree of convention, it is possible to consider this level as background value for the whole territory of Georgia. The average value (68.4 Bq/kg) is greater than this quantity which can be due to non-uniform precipitations following the accident. The distribution of the naturally occurring radionuclide K-40 was similar to values observed by Kogan et al. [19].

The calculated values of the annual effective dose do not exceed the 1 mSv/y dose limit recommended for public radiation exposure control [21,22,23]. Table 3 compares the activity concentrations of radionuclides in investigated samples in the present study with those by other investigators in various countries of the world. The values in the current study were, on average, much lower than in other regions as well as compared to worldwide average values. In conclusion, it is necessary to note, that the received results represent doubtless scientific and applied interest for the investigated region that confirms the urgency of such research and the necessity of their regular character.

Table 3

Activity concentration (A, Bq/kg) of radionuclides and some other parameters in soil in different regions of the world

SR	A, Bq/kg					Ra_{eq}, Bq/kg	AEDE, mSv/y	Ref
	Th-232	U-238	Ra-226	K-40	Cs-137			
Sp	34.6	40.2	26.7	586.2	30.9			[1]
	23.7-49.4	19.9-60.0	20.8-34.9	446-799	4.4-64.7			
It	48	79		640	25.0			[24]
	20-70	24-231		242-1434	1.1-241			
Tk	51.8	24.5		344.9	26.3	125.0	0.070	[25]
	9.5-170.8	7.4-79.8		35.7-913.8	0.6-154.3	23.8-293.6	0.013-0.192	
Ch	101.0	79.3	75.1	535.8		260.8	0.147	[26]
	10.3-376.0	12.0-264.3	7.6-298.3	8.2-1747.1		28.3-850.3	0.017-0.468	
In	64.5		60.3	481.0		189.5	0.37	[27]
	36.1-136.1		15.0-205.6	65.8-795.9		110.0-436.2	0.06-0.24	
Ng	85.84		52.05	477.69	1.60	210.57	0.1170	[28]
	13.54-295.24		8.33-160.37	15.97-2723.22	0.89-3.53	28.47-701.53	0.0152-0.3891	
Ge	24.5	17.2	23.8	300	68.4	73.2	0.043	Present
	15.2-32.5	9.7-23.8	14.0-34.8	81-485	8.8-191	44.9-89.4	0.027-0.054	study
Ww	45	33	32	412				[29]
	0.05-360		0.5-1000	4-3200				

Note. Studied regions (SR): Sp – Spain; It – Italy; Tk – Turkey; Ch – China; In – India; Ng – Nigeria; Ge – Georgia; Ww - Worldwide average values.

CONCLUSION

Up to 22 radionuclides are identified in soil samples selected from the ten locations in some regions on the Black Sea coast. The mean activity concentrations of naturally occurring radionuclides Th-232 and U-238 families were lower than the world average values as well as the mean activity concentration of K-40. Technogenic radionuclide Cs-137 was measured in all samples and was found to be in the range of 8.8 to 191 Bq/kg (mean, 68.4). Comparison with reference data as well as analysis of obtained results was carried out.

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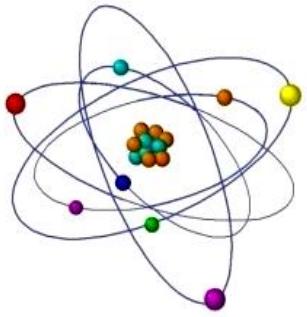
IONIZING RADIATION-INDUCED CHANGES IN THE ABSORPTION SPECTRUM OF ERYTHROCYTE MEMBRANE PROTEINS



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ABSTRACT*: Under influence of ionizing radiation different types of oxidative modifications of the protein occur, including carbonylation, direct amino-acid oxidation, oxidative cleavage of the protein backbone, or amino acid side chains. Aromatic amino acids are significantly more reactive with the dominant reaction pathway, including the connection of •OH to the aromatic ring. In the case of Tyr, the connection of •OH and subsequent hydrogen extraction lead to the formation of peroxyl radicals, which in the absence of reductants, form Tyr dimers that are implicated in the formation of intra- and inter-protein linkages [7, 8]. Erythrocyte membrane contains numerous integral membrane proteins, usually, they show absorption maximum between 275 and 280 nm, which are caused by the absorbance of the aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and, to a small extent, by the absorbance of cystine (i.e., of disulfide bonds). Our study aimed to determine Ionizing radiation-induced changes in the absorption spectrum of erythrocyte membrane proteins.*

Mice whole-body irradiation with ^{137}Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a "Gamma-capsule-2". The Erythrocyte membrane was separated according to Hasts Method and absorbance spectra were measured with a spectrophotometer.

Results show that absorption for proteins of erythrocytes' membrane at 280 nm wavelength time-dependent decreased after irradiation and after one month reaches 75% of the control level. This decrease may be related to Tyr-phosphorylation of B3p in radiation-induced oxidative stress conditions, which markedly reduces its affinity for ankyrin, leading to the release of band 3 from the spectrin/actin membrane skeleton, enhancement of the lateral mobility of band 3 protein in the bilayer, progressive vesiculation and loss from the plasma membrane of radiated cells, triggering a cascade of events inducing alteration of deformability, the resistance of erythrocytes membrane, its destabilization.

Key words: γ-radiation, erythrocyte membrane proteins, absorption spectra

INTRODUCTION

Post radiation damages cascade is divided into early, within 90 days after onset of the radiation exposure, which is characterized by massive cell death, tissue dysfunction, and late phase, which occurred some months or years after irradiation, where tissue damage is progressive and irreversible. The total effect of ionizing radiation (IR) exposure on the whole body and isolated cells is dependent on radiation dose, as well as individual radiosensitivity of tissues [1].

In living body tissues, IR exposure is carried out mainly by non-specific mechanisms that are also occurred at various damages (physical and chemical influence) [2, 3]. Cell damage can occur by direct bombarding the biological important macromolecules (proteins, lipids, and DNA) with high energetical photons or indirect action with highly reactive free oxygen radicals (ROS)produced in the fluid phases of tissues as a result of radiolysis of water [4,5]. Different types of oxidative protein modifications are known, including carbonylation, direct amino-acid oxidation, oxidative cleavage of the protein backbone, or amino acid side chains [6]. Aromatic amino acids are significantly more reactive with the dominant reaction pathway, including the connection of •OH to the aromatic ring. In the case of Tyr, the connection of •OH and subsequent hydrogen extraction lead to the formation of peroxyl radicals, which in the absence of reductants, form Tyr dimers that are implicated in the formation of intra- and inter-protein linkages [7, 8].

Erythrocyte membrane contains numerous integral membrane proteins including the glycophorins, the Rh proteins, and transport proteins such as band 3 (AE1, anion exchanger 1, SLC4A1), Na^+, K^+-ATPase, Ca^{2+}-ATPase, and Mg^{2+}-ATPase, etc.

Erythrocytes' proteins usually show absorption maximum between 275 and 280 nm, which are caused by the absorbance of the two aromatic amino acids tryptophan (Trp) and tyrosine (Tyr) and, to a small extent, by the absorbance of cystine (i.e., of disulfide bonds) [9, 10]. Our study aimed to determine Ionizing radiation-induced changes in the absorption spectrum of erythrocyte membrane proteins.

MATERIALS AND METHODS

The experimental protocol was in accordance with the guidelines for the care and use of laboratory animals as adopted by the Ethics Committee of the Tbilisi State Medical University (TSMU).

Animal care and maintenance

Three-month-old male mice (Mus musculus), were obtained from the Vivarium of Tbilisi State Medical University. They were housed in animal cages, with room temperature maintained at 200-220C, relative humidity of 50-70%, and an airflow rate of 15 exchange/h. Also, a time-controlled system provided 08:00-20:00 h light and 20:00-08:00 h dark cycles. All mice were given a standard rodent chow diet and water ad libitum from sanitized bottle fitted with stopper and sipper tubes.

After acclimatization to laboratory conditions for a week, the mice were divided into two different groups: I - control group (non-irradiated animals), II group - experimental group (gamma-irradiated animals). The blood samples were obtained from animals of the I group - non-irradiated mice, and II-d experimental group of irradiated mice after 48 hours (IIa subgroup) and one month (IIb subgroup) of irradiation.

Mice whole-body irradiation with 137Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a “Gamma-capsule-2”.

The Erythrocyte membrane was separated according to Hasts Method and absorbance spectra were measured with a spectrophotometer.

Erythrocyte membrane isolation by the Hast method

Blood samples, collected in tubes containing anticoagulants were centrifuged at 3000g for 15 min. The obtained erythrocyte sediment was washed 3 times with a 1: 4 volume of solution A, containing 130 μM KCl, and 20 μM Tris-HCl (pH-7.4). For hemolysis of the obtained erythrocyte sediment, the 1:10 volume of solution B, containing 5 μM Tris-HCl, and 1 mm EDTA, was added and the resulting mixture was left all night (for about 15 hours). The next day the suspension was centrifuged at 12,000 g for 20 min. The obtained precipitate was washed again with solution "B" 2-3 times before bleaching. The precipitate was washed again with a 1:10 volume of "A" solution. Absorbance spectra were

measured with a spectrophotometer (SPECTRO UV-UIS DUAL BEAM 8 AUTO CELL (UVS-2800) and (Lambda 38, PerkinElmer, Rodgau, Germani).

RESULTS AND DISCUSSION

Fig.1 shows the absorbance spectrum at the wavelength of 280 nm. The intensity of absorbance spectrum of erythrocytes' membrane proteins from a control group of mice was 2,392, in irradiated mice, 48 hours after radiation (subgroup IIa) the intensity of absorption slightly decreased (2,06) and after one month of irradiation (subgroup IIb) absorption spectrum decreased and was equal to 1,81 (Figure 1).

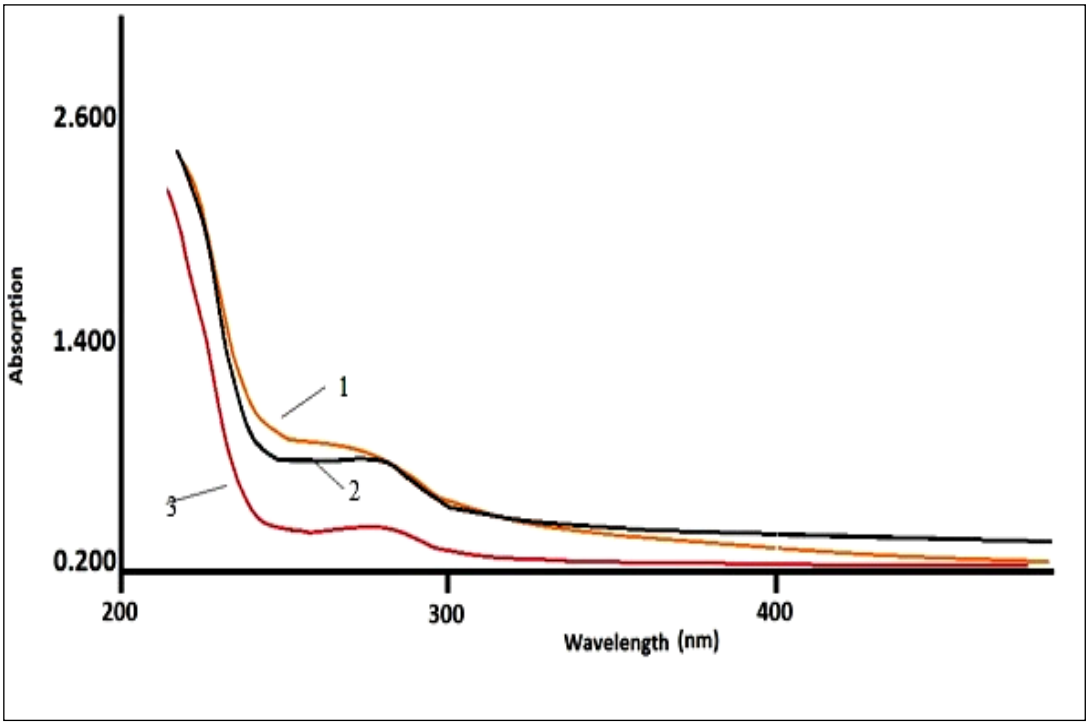

Fig.1 Absorption spectrum of erythrocyte membrane proteins
1 – control group, 2- after 48 hours from irradiation, 3- after one month from irradiation

Since band 3 protein is one of the most common proteins in the erythrocyte membrane and contains a large number of tyrosine residues (at least tyrosine 8, 21, 359, and 904) [11], we assumed that changes in the absorption intensity in the spectra of erythrocyte membrane proteins are to a certain extent associated with changes in tyrosine residues of Band 3 protein.

Band 3 protein is the major integral protein of the erythrocytes' membrane (composes approximately 25% of proteins), which has two primary functions, ion transport (mediates chloride–bicarbonate exchange) and maintenance of protein-protein interactions with cytoskeletal proteins (spectrin, actin, band 4.2) and glycolytic enzymes. The shape, osmotic resistance, and deformability of erythrocytes are critically related to the Band 3 protein (B3p) function [12]. Perhaps because of its many important functions, band 3 is also a prominent substrate of Ser/Thr kinases and is the major substrate of the cell's protein tyrosine kinases. In response to physiologic stimuli such as hypertonic conditions or oxidative stress, phosphorylation of band 3 on tyrosine residues, can increase by several orders of magnitude.

Literature data show, that erythrocytes membrane oxidative damage is leading to B3p dose-dependent clusterization in high-molecular-mass aggregates, through disulfide cross-linking dimerization [13, 14]. Since B3p is normally linked to the cytoskeleton by junctional complexes, the formation of large aggregates of B3p should be restrained by such interactions unless they are weakened by regulatory mechanisms. tyrosine phosphorylation via phosphotyrosine kinases (PTKs) or phosphotyrosine phosphatase (PTP) [15] which appears to be facilitating oxidatively modified B3p clusterization [13]. It was reported, that tyrosine phosphorylation of band 3 markedly reduces its affinity for ankyrin, leading to the release of band 3 from the spectrin/actin membrane skeleton, enhancement of the lateral mobility of band 3 in the bilayer, and progressive vesiculation and loss from the plasma membrane of stimulated cells [11]. It was suggested that B3p as a redox sensor, is regulated by phosphorylation; in oxidative stress conditions, rapid intense Tyr-phosphorylation of B3p affects its interactions with the cytoskeletal proteins triggering a cascade of events inducing alteration of deformability, the resistance of erythrocytes membrane, its destabilization [11] and finally leading to their hemolysis [13]. Irradiation of RBCs by gamma radiation could cause various degrees of damage to RBCs membranes. The damage to the cell membrane is dose-dependent [14].

The results of the study indicate that absorption for proteins at 280 nm wavelength time-dependent decreased after irradiation. This decrease may be related to Tyr-phosphorylation of B3p in radiation-induced oxidative stress conditions [16], which markedly reduces its affinity for ankyrin, leading to the release of band 3 from the spectrin/actin membrane skeleton, enhancement of the lateral mobility of band 3 in the bilayer, progressive vesiculation and loss from the plasma membrane of radiated cells, triggering a cascade of events inducing alteration of deformability, the resistance of erythrocytes membrane, its destabilization.

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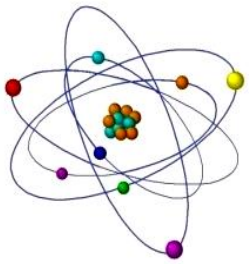
THE EVOLUTION OF RADIORESISTANCE



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ABSTRACT: *The relationship between the information capacity of the eukaryotic genome and the potential for epigenetic variability is shown. The progressive evolution of the genome is due to the need to increase the information capacity of the genome and improve genetic reliability systems to ensure a stable operation of the growing genetic apparatus of prokaryotic organisms.*

Key words: radioresistance, evolution, genetic reliability

The first direct experimental evidence of the genetic determination of radioresistance (GDR) was the production of mutations in *E. coli* that lead to a change in radioresistance. A mutant form B/r was isolated from a UV-irradiated suspension of cells which significantly exceeded the initial strain in terms of radioresistance (RR). For eukaryotes, several dozens of genes that affect RR are currently known, and many of them have been mapped. At the same time, it was shown that they are located randomly in the genome without forming clusters, i.e., they are characterized by chromosomal non-localization. In addition, the GDR system has a number of other properties: polygenicity, recessiveness (predominantly), and non-specificity. Undoubtedly, progressive evolution could not take place without the improvement of systems that ensure the stability of the genome in general and against radiation exposure in particular. In this regard we were primarily interested in the phylogenetic aspect of RR, therefore, it is extremely important to conduct research on the comparative RR of biological objects that are at different stages of phylogenetic development and differ in structural and functional organization. Such studies have a rather rich history, and their results have been expressed in a number of attempts to create the concept of Radiotaxon.

The range of RR variation determined, for example, by LD_{50}, is quite wide - from several *Gy* (mammals) to several thousands of *Gy* (bacteria, viruses, lichens), i.e. within four orders. In 1961 Terzi M.[1] for the first time tried to establish the relationship between RR and the structural organization of the genome of 32 species of organisms using the efficiency of genome inactivation as an indicator of RR:

$$E = 3,7 \times 10^{11} \frac{D}{N}$$

where: *E*–inactivation efficiency; *D*–radiation dose, *P*; *N*–molecular weight of the genome, daltons.

As a result of the analysis - 4 groups of organisms were identified that differ significantly in E: 1) (E_{sr} = 0.64) single-stranded RNA and DNA viruses; 2) (E_{sr} = 0.62 × 10^{-1}) double-stranded viruses; 3)

(E_{sr}= 1.23 10^{-2}) bacteria (with the exception of *Haemophilus influenzae* which fell into the second group) and haploid yeast; 4) (E_{sr} = 0.69 × 10^{-3}) mammalian cells, as well as di- and polyploid yeasts. According to TerziM.the difference in the effectiveness of the inactivation of the selected groups of organisms could be due to the differences in the structural organization of their genetic systems.

This direction was further developed in the work of Kaplan G.and Moses L. (who drew attention to a significant correlation between RR and the content of nucleic acids, and especially in the works of Sparrow A. and coauthors [2]. Studying the dependence of RR (determined by D_0) on the volume of the interphase nucleus, Sparrow A. divided a sample of 79 organisms into eight groups which he called radiotaxa, within which the correlation between D_0 and the volume of the interphase nucleus was 0.85-0.99. However, the organisms that differ fundamentally in the structural organization of the genome fell into the same radiotaxon. For example, some viruses, bacteria, and yeasts fell into radiotaxon 4, while other bacteria, yeasts, and mammalian cells fell into radiotaxon 5. On the contrary, the forms similar in the genetic organization often appeared in different radiotaxa. Thus, different strains of the bacterium E. coli got simultaneously into four radiotaxa - from the 4th to the 7th inclusively. Based on the obtained resultsSparrow A. was forced to conclude that radiation taxonomy has nothing to do with the biological classification of species and does not reflect their phylogenetic relationships.

Of course, with all the evidence of the connection of RR with the taxonomic position of the organism, it was difficult for the researchers to agree with such a categorical conclusion of SparrowA., and the research continued in the direction of searching for a more adequate assessment of the radioresistance of organisms.

ShalnovM.I. [3] identified six radiotaxa (according to the correlation of D_0 with the genome size), each of which had its own regression curve with the corresponding coefficient *Ki* that has the dimension of the radiation chemical yield. ShalnovM.I. also drew attention to the fact that along with the complication of the genome structure in the process of progressive phylogenetic development the radiation-chemical yield of reactions leading to reproductive cell death decreases, i.e., increasing the reliability of the genetic systems. An increase in RR upon the transition from taxon to taxon due to the improvement of DNA repair mechanisms could be characterized by a dimensionless factor *fi* and *Gi* at the radiation-chemical yields corresponding to each radiotaxon determined by the structural and functional organization of the genome. In accordance with this, the coefficients *Ki* of six regression lines, obtained as a result of the product of the factors *fi* and *Gi*form, according to ShalnovM.I. steps of adaptive variability of the genome in the direction of the increasing of radioresistance. Proposed by Shalnov M.I. approach allowed him to assess the contribution made to the overall resistance of the genome by changes in its structural and functional organization and improvement of enzymatic repair processes. So, according to ShalnovM.I., in the course of phylogenesis, the RR of the genome increased by 100 times due to changes in the structural and functional organization and as a result of the improvement of enzymatic repair systems, also by 100 times (and that - by 10^4 times).

Developing the ideas of Shalnov M.I. and KorogodinV.I. [4] introduced the concept of "genome reliability" and analyzed the distribution of organisms by radiotaxa from the point of view of genome reliability. As a measure of genome reliability, KorogodinV.I. proposed to use a value equal to the amount of the radiation energy the absorption of which in DNA is necessary and sufficient for the appearance of one elementary damage. As an estimate of the reliability of the genome the product D_0C was used, where D_0 is the radiation dose at which, on average, one lethal damage occurs in each cell, and C is the amount of DNA in the genome. If D_0 is expressed in *Gy* (Gray) and C is expressed in nucleotides, then the reliability of the genome (K) is:

$$K = 3,31\times10^{-6} D_0 C \text{ (eB)}$$

The introduction of this ratio allowed KorogodinV.I. to answer the question about the relationship between the reliability of the genome and its size. If *K* remained constant during the entire time of the phylogenetic processes, then for an increase in the size of the genome (and this is an inevitable process that ensures progressive evolution) which varies within 8 orders of magnitude (from 1.3×10^3 base pairs in the tobacco necrosis satellite virus to $2,3 \times 10^{11}$ bp in Tradescantia Virginiana) living organisms would have to "pay" with a proportional increase in the radiosensitivity (RS). However, the data of radiobiological experiments indicate that the differences in the RS of biological objects are much smaller than might be expected and are less than five orders of magnitude.

KorogodinV. I. singled out not six, but four radiotaxa combining the 4th and 5th into one and discarding the 6th due to the unrepresentative information. The distribution of biological objects according to radiotaxa corresponded well to their distribution according to the levels of structural organization of genetic systems. The set of organisms with the same level of structural organization of the genome KorogodinV.I. suggested calling it a karyotaxon. According to KorogodinV.I., the reliability of the genome of the organisms of the first three karyotaxons is mainly due to physicochemical factors - the transition from the single-stranded structure of nucleic acids (karyotaxon 1) to the double-stranded structure (karyotaxon 2) and then to the DNA-protein complex of the haploid genome (karyotaxon 3). A sharp increase in the reliability of the genome of organisms of the 4th karyotaxon is due to the appearance of the mechanism of "diploid-specific" repair. However, according to SarapultsevB.I. and GeraskinS.A. [5] data on the reliability of eukaryotic polychromosomal genomes do not require additional hypotheses about the existence in eukaryotes of any special ways to increase the reliability of the elementary genome for their interpretation. In particular, the number of repaired double breaks per chromosome of a eukaryotic cell does not exceed the number successfully repaired by prokaryotic genomes. The mentioned authors believe that the phylogenetic development of the elementary genome reliability systems is probably fully completed within the framework of the prokaryotic genome and the high reliability of the eukaryotic cell genome is mainly due to the transition to the polychromosomal organization of genetic information storage and the effect of polyploid protection.

The hierarchy of radiotaxa directly reflects the main stages of the structural reorganization of the genome in the course of a progressive phylogenetic process from "bare" single- and double-stranded virus-type nucleic acid molecules to pro- and eukaryotic genomic molecules organized into a nucleoid and a true nucleus. The latter circumstance unequivocally testifies to the general biological significance of radiotaxonomy and allows to raise the question of the biological meaning of the phenomenon of radiation resistance of organisms.

Thus, radiotaxonomic studies while remaining within the framework of radiobiological studies were quite successful and led to the establishment of a relationship between either the taxonomic position and radioresistance (RR) or between the physical size of the interphase nucleus and RR (karyotaxa). For some time it seemed that these investigations and the corresponding results were of significance only for radiobiology. However, a certain paradoxical nature of these results, namely the low RR of eukaryotic organisms compared to the RR of prokaryotic organisms, forced radiobiologists to search the solutions to this problem using molecular genetic and phylogenetic methods and approaches.

Radiobiologists are faced with the second most important radiobiological paradox the resolution of which can have not only general radiobiological but also general biological significance.

In connection with the need to resolve this paradox, it is difficult to overestimate the research of Shalnov M.I. who established that in parallel with the phylogenetically determined structural and functional complication of the genome, there was a decrease in the radiation-chemical yield of damage

to nucleic acid molecules leading to the inactivation of the irradiated object. Without going into the essence of the mechanisms that ensure a decrease in the yield of damage (recombination, enzymatic repair, a “coat” of histone proteins), it should be stated that the reliability of genetic systems increases in the process of progressive phylogenetic development of biological systems.

The idea of the adaptive significance of a high level of reliability of the genome of eukaryotic organisms in relation to the action of the ionizing radiation factor had to be abandoned because since the birth of life the radiation factor has varied within a maximum of three orders of magnitude and could not cause a difference in the RR of some representatives of prokaryotic and eukaryotic organisms by four orders of magnitude. Nor could RR be the result of developed nonspecific resistance, since there is also an inverse relationship between the phylogenetic "advancement" of species and their resistance to other extreme factors. This pattern is explained by the fact that the progressive direction of the development of life on Earth which has so far been the predominant direction has led mainly to the emergence of adaptations that help isolate biological objects from the action of extreme environmental factors (including biotic factors) or the development of means to avoid dangerous environmental factors. In other words, the development of the adaptations did not follow the path of acquiring “oak” resistance, but along the path of acquiring highly organized behavioral responses (in plants, in particular, the division of ontogenesis into actively functioning and passively experiencing unfavorable phases).

Thus, it remains to be assumed that the reliability of the genome which is generally calculated as the product of D_0 and the volume of the genome (see above) expressed as the number of nucleotides characterizes, first of all, its ability to function reliably under normal conditions, and not radioresistance. The factor of the spontaneous degradation of nucleic acid molecules (a consequence of the thermodynamic instability of the DNA molecule, the influence of reactive oxygen species, as well as a consequence of the erroneous processes of DNA repair and replication) in itself is significant enough to act as a phylogenetic adaptation factor. Indeed, the reliability of the genome of most eukaryotic organisms is far superior to that of prokaryotic organisms. Such superiority is ensured by a whole hierarchical system of means for ensuring the reliability of the genome.

How could a whole hierarchical system have arisen in the process of progressive phylogenesis that ensures the reliability of the genome (which in its turn just ensured the possibility of progressive development)? And in general, what is a progressive phylogenetic process? And why do organisms coexist in the biosphere at present which differs so much from each other in the complexity of their genetic apparatus and, accordingly, in the reliability of its functioning?

Here, there is a reversal (transformation) of the 2nd radiobiological paradox. So, if at first, it seemed incomprehensible the existence of eukaryotic organisms with their relatively low radioresistance, now the existence of prokaryotic organisms with their relatively low level of genome reliability and low information capacity becomes unclear.

In most cases, prokaryotic organisms have a high RR which to a much greater extent than in eukaryotic organisms correlates with the high resistance to other external extreme factors of a physical and (or) chemical nature. The minimum ability of prokaryotic organisms to maintain the constancy of the internal (intracellular) environment is due to the comparative primitiveness of their genetic apparatus which in its turn causes high resistance to the destructive action of environmental factors. A kind of “payment” for the high and nonspecific (universal) resistance of prokaryotic organisms is their inability to maintain their genetic individuality as evidenced by the high level of their genetic variability.

Prokaryotic organisms used one of two possible ways to ensure adaptability to the environment - the way to increase the stability of the genome by reducing its physical size and, consequently, the

level of organization (complexity). In contrast, eukaryotic organisms in the process of progressive phylogenetic development used another opportunity - autonomization (or avoidance) from environmental factors through the acquisition of a complexly organized genetic apparatus (with the participation of proteins that did not have high thermal stability) which provides complex behavior in a variety of environments. Since complex behavior requires a large amount of memory a necessary condition for the functioning of eukaryotic genomes is their high reliability which is achieved due to the duplication of genetic information and its mosaic location on nonhomologous chromosomes as well as due to the existence of recombination and repair systems. The last two of the listed mechanisms are "inherited" from prokaryotic organisms and constitute the repair "foundation" of the entire system that ensures the reliability of the functioning of the genome (storage, processing, and transmission of genetic information) and on this basis the phenome.

Finally, the high information capacity of the eukaryotic genome provides a wide opportunity for epigenetic variability (differentiation) of cells which by specializing, probably formed the basis for the emergence of multicellular organisms and subsequently multi-tissue organisms. The direction of the phylogenetic process of genome change is determined not only and not so much by adaptive variability and selection of forms with nonspecific RR but by a tendency to increase the information capacity of the genome and the reliability associated with this process of improving genetic systems to ensure stable (accurate) operation of the growing genetic apparatus.

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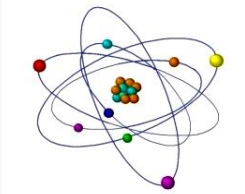
DEVELOPMENT OF BIODOSIMETRY METHODS IN CONNECTION WITH THE IMPROVEMENT OF MEDICAL RADIOLOGICAL EQUIPMENT



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ABSTRACT: *In the last decades the use of ionizing radiation for medical procedures, for treatment and diagnostic purposes, has greatly increased. It is known that in addition to the unconditional benefit, radiation rays can adversely affect healthy tissues which cause radioinducedcomplications. The total effect of the radiation exposure on the whole body and on individual cells depends on the dose of radiation as well as the individual radiosensitivity of the tissues The problem of determining the exact absorbed dose, studying the individual biological reactions of the body under radiation exposure, the search for the most characteristic biological changes for these effects occupies one of the key places in modern radiobiology. Ongoing search for relevant effective biomarkers continues Biological dosimetry is a set of tests that make it possible retrospectively to determine the dose of ionizing radiation absorbed by the body. The analysis of chromosomal aberrations by different techniques is the most developed method of quantifying dose to individuals exposed to ionizing radiations. During the last few decades progress has been made in the field of radiation biodosimetry and numerous biomarkers have been proposed at the level of genes, proteins and other macromolecules. In the field of radiation therapy, everything is changing very quickly. A method of cancer treatment using interactions between radiosensitive drugs and neutrons and proton therapy are introduced. Two major forms of radiation energy are employed in medicine: one is transmission radiation used in both radiology and radiation oncology treatment planning using the external beam, and the other is emission radiation used in nuclear medicine and brachytherapy planning. Some limitations of existing biomarkers in developing methods of radiotherapy are shown. The currently used radiation-dosimetric biomarkers can no longer be universal and a constant search for new effective biomarkers is required. According to the IAEA, extensive international multicenter studies are needed to improve the methodology for the clinical application of biodosimetry. The article provides an overview of the development of biodosimetry methods in connection with the improvement of medical radio equipment*

Key words: cancer, radiotherapy, biodosimetry, markers, individual radiosensitivity

INTRODUCTION

Advances in the treatment of oncological diseases are increasing at an enchanting pace. Despite the annual expansion of the implemented methods, irradiation continues to occupy one of the first places.

When radiation was first used to treat cancer in 1901, it marked a real revolution in medicine. However, this method was developed only with the advent of certain innovative technologies. Today, thanks to advances in physics, technology and computing, radiation therapy methods are becoming significantly more accurate, effective and safe.

X-rays are electromagnetic waves in the range between ultraviolet and gamma radiation. Accordingly, the X-ray machine is a source of ionizing radiation, a serious overdose of which leads to the destruction of the integrity of DNA and RNA chains.

The radiation destroys the DNA structure of cancer cells. Since these cells are defective, the DNA

structure is not restored, as a result of which the cells lose their ability to divide and grow and subsequently die. Healthy cells, which are also exposed to radiation during treatment, have a higher ability to repair because they are not infected: therefore, the likelihood that they will not be damaged during radiation therapy increases [1]. Meanwhile, in all cases, when using X-rays, there is a danger of damage to healthy tissues, which causes radioinduced complications.

There are three methods of exposure: contact (the source of radiation is in contact with human tissues), remote (the source is at some distance from the patient) and radionuclide therapy (the radiopharmaceutical is injected into the patient's blood). Contact radiation therapy is sometimes called brachytherapy.

In addition to radiotherapy, there is also an increasing need to use different doses of radiation for diagnostic purposes. Medical X-ray apparatus as a source of ionizing radiation and high voltage is potentially dangerous. Therefore, a distinctive feature of the operation of X-ray equipment is to ensure the safety of personnel and patients. This is possible with strict compliance with the requirements for the parameters of X-ray technology. The technical serviceability of the equipment and compliance with the norms of its operation is of great importance. More scientific data regarding radiation in medical use and more communication to the medical staff and the public are warranted to optimize the benefit of medical radiation in clinical services [21]. The use of ionizing radiation (IR) medical procedures, for treatment and diagnostic purposes, has recently very increased. Although the general radiobiologic principles underlying external beam and radionuclide therapy are the same, there are significant differences in the biophysical and radiobiological effects. This is raising the problem of management of the results of IR. Results obtained will allow physicians to have a real image of changes in patients' organism caused by irradiation and to make follow up of changes and medically manage them. For persons working with ionizing radiation the basis for developing safety measures is dosimetry. However, physical dosimetry provides only extrapolation information about the dose absorbed by the human organism and does not take into account the individual radiosensitivity of the organism [17]. Different types of radiation may produce different biological effects and the magnitude of the effect can vary according to the rate at which radiation is received (dose rate). The dose rate is a primary factor in determining the biological effects of a given absorbed dose.

The problem of determining the exact absorbed dose, studying the individual biological reactions of the body under radiation exposure, and the search for the most characteristic biological changes for these effects, occupies one of the key places in modern radiobiology. So, in the middle of the last century, a special direction in radiobiology arose –biodosimetry. Biological dosimetry is a set of tests that make it possible retrospectively to determine the dose of ionizing radiation absorbed by the body. The biological dosimetry methods applied in patients undergoing various medical irradiations to low doses.

Post-irradiation damage results are divided into early and late phases. Late irradiation effects appear even after some months or years. Late effects of tissue damage are progressive and irreversible. The total effect of the radiation exposure on the whole body and individual cells depends on the dose of radiation as well as the individual radiosensitivity of the tissues. Because it is a strong mutagen, ionizing radiation primarily causes changes in the genetic structure of living organisms. That's why cytogenetical indexes and parameters are the best markers to detect the biological effects of ionizing radiation [7,8]. Biological dosimetry methods, which are based on the chromosomal damages are very important, because unlike physical dosimetry, it provides the difference between individuals with different sensitivity to radiation. To choose the correct type and doses of radiation are the means not only for optimal results, but also to overcome the radioresistance [1,14].

The analysis of chromosomal aberrations by different techniques is the most developed method of quantifying dose in individuals exposed to ionizing radiations [5,11,13]

In the late 90-s after biodosimetry was started in Georgia and cases of overdose were detected [25], We conducted a survey of medical personnel who had contact with X-ray equipment. These were difficult years after the collapse of the Soviet Union and old, faulty equipment and working conditions in a number of institutions labor was often violated. As a result, the number of dicentric chromosomes in employees exceeded our background data and, in several employees, the received dose exceeded the total dose allowed for professionals (>0,4Gy) The same laboratory conducted a survey of medical staff in a new, well-equipped department, and did not reveal any violations [27]. Since the beginning of this century, both sources of medical exposure and biomarkers that determine the effectiveness and safety of radiotherapy have been constantly improved.

Radiation dosimetric biomarkers have found applications beyond the radiation protection area and now are actively introduced into clinical practice. Cytogenetic assays appeared to be a valuable tool for individualized quantifying radiation effects in patients, with a high capacity for assessing genotoxicity of various medical exposure modalities and providing meaningful radiation dose estimates for prognoses of radiation-related cancer risk [23]. The most common, tested and correct genetic markers of exposure are radiation-specific cytogenetic disorders - stable and unstable aberrations of the chromosomal type.

One of the first additional methods of biodosimetry was the method of determining the level of micronuclei in peripheral blood lymphocytes. [3,6,15] After confirming the informational value of micronuclei, their levels began to be studied in other tissues too [22]. Gradually, other methods of premature chromosome condensation, cytokinesis are being introduced. [10].

The response of different persons to the mutagenic impact varies and depends on individual sensitivity. The data on the proposed biomarkers can be used to predict potential responses to mutagenic factors in specific persons allowing to consider individual sensitivity [26].

The study by us biomarkers (dicentrics, micronuclei, DNA comets) in patients with a tumor of the same localization (laryngeal carcinoma) irradiated with a linear accelerator in 2 gray/fraction mode with a total dose of 70 gy and with “Electra Synergy Platform” apparat, revealed the individual reaction to radiation therapy. Despite one and the same tumor localization and identical received dose of radiation, changes in the studied parameters were not homogeneous. Biomarkers determine not only the absorbed dose but also register the genotoxicity of radiotherapy. It was also demonstrated that the level of micronuclei in buccal cells reliably registers the genotoxic effect of radiation and the individual sensitivity of the patients [12,26].

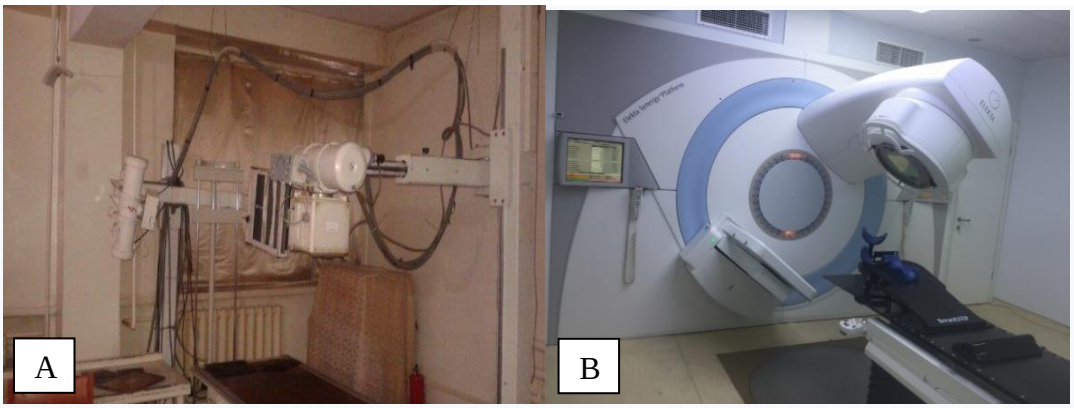


Fig.1 Old (A) and modern (B) X-ray machine

Taking into account that the radiosensitivity of tumors is different even of the same genesis, it is very important to determine the optimal curative regimen for individual patients [24].

During the last few decades, progress has been made in the field of radiation biodosimetry and numerous biomarkers have been proposed at the level of genes, proteins and other macromolecules [16]. Advancements made in radiation biodosimetry at the level of genomics, transcriptomics, metabolomics, proteomics, cytogenetics and electron paramagnetic resonance (EPR) to deal with radiological/nuclear mass casualty incidents have been reviewed [9,18,24].

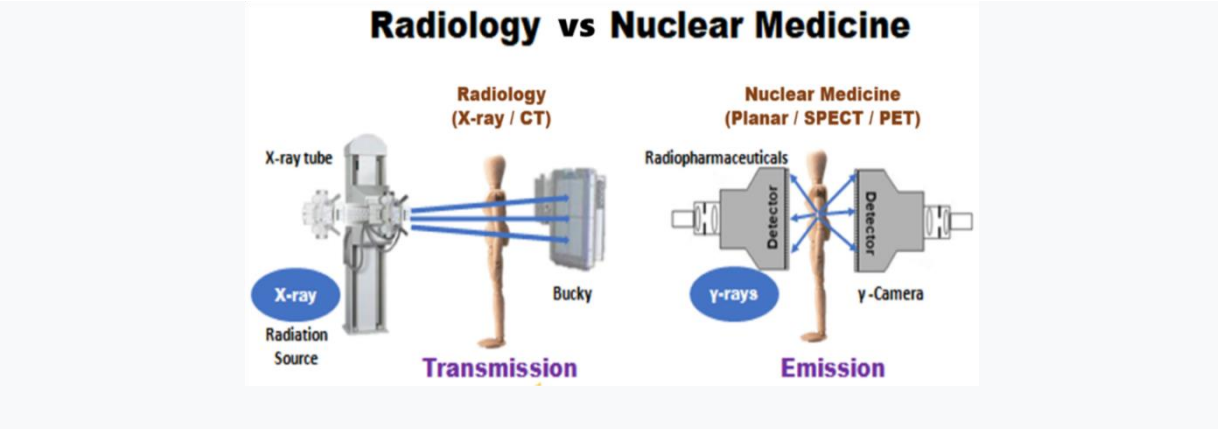
Recent advances in genomic analysis are inextricably linked with the use and development of molecular biology methods: various variants of DNA amplification (RFLP, PCR,) and cytological approaches (chromosome differential staining and in situ hybridization, etc [23] None of the above methods is comprehensive and infallible.

The effectiveness of methods is determined by the degree to which they solve the set tasks, in particular, they characterize directly or indirectly the similarity of DNA between species. Despite all these advances, chromosome analysis remains an important and, for some objects, the main part of genomic analysis. However, for more correct results, it is desirable to use a combination of methods simultaneously [26].

In the field of radiation therapy, everything is changing very quickly. A promising method is proton therapy. The method makes it possible to precisely target a tumor and destroy it at any depth of localization. Proton therapy is attracting attention as a method with high efficiency, characterized by a small impact on the body and a minimum number of side effects. Surrounding tissues receive minimal damage since almost the entire radiation dose is released into the tumor in the last millimeters of the particle path [19].

The perspective effect on healthy tissues with proton therapy compared to traditional radiation therapy allows for the reduction of side effects. If the parameters of irradiation with proton beams are set in accordance with the depth of the pathological focus, at the moment the pathological focus is reached, they are inhibited with the release of the maximum amount of energy without further penetration into the body. The calculation of the optimal irradiation for each patient makes it possible to accurately "remove" the tumor. Along with this, the advantage of the method is to reduce the harmful effects on healthy tissues [20].

Two major forms of radiation energy are employed in medicine: one is transmission radiation used in both radiology and radiation oncology treatment planning using the external beam, and the other is emission radiation used in nuclear medicine and brachytherapy planning. Therefore, radiation protection should be different between transmission and emission radiation.



Different radiation mechanisms of imaging formation between radiology and nuclear medicine departments are shown, for which ways of radiation protection could be different accordingly [3].

A newly emerged medical technology enabling advanced cancer patients to be treated precisely and effectively is diagnostics. With the help of it is possible to kill cancercells while sparing healthy tissue. Internal dosimetry on an individualized basis seems to be clinically needed [3]. So, the existing methods of biodosimetry are still limited in their capabilities.

Different aspects of biodosimetry and scenario-based options for clinical decision support in radiation accidents are presented. New external irradiation biodosimetry deviceDosiKit, based on the dose-dependent relationship between irradiation dose and radiation-induced H2AX protein phosphorylation in hair follicles [2].

According to the IAEA, extensive international multicenter studies are needed to improve the methodology for the clinical application of biodosimetry [23,29].

This was the basis for the launch of the IAEA Coordinated Research Project E35010 MEDBIODOSE: "Application of Biological Dosimetry Methods in Radiation Oncology, Nuclear Medicine, Diagnostic and Interventional Radiology".

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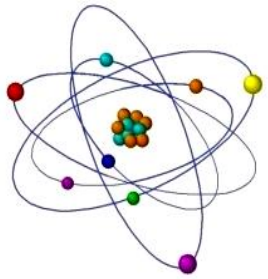
DETERMINATION OF RADON CONCENTRATIONS IN MTATSMINDA DISTRICTS OF TBILISI



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ABSTRACT**:** *Radon concentrations in the Mtatsminda region of Tbilisi was measured. Radon concentrations in the air inside buildings can vary greatly among different regions and different homes. In addition, they are characterized by a wide range of time variations. Due to the fact that radon eminence occurs from the soil under the house, the highest concentrations of radon in buildings are observed in basements and apartments located on the lower floor. Preliminary results of radon concentrations in residential and public areas in some districts of Tbilisi show that further investigations are needed that will involve larger-scale measurements and more extensive studies of this problem.*

Ionizing radiation ionizes the environment as it passes through it. In general, ionizing radiation is an integral part of scientific and technical progress. Nuclear and radioactive materials are used for medical, industrial and scientific-research purposes. There are natural (cosmic and terrestrial, building materials, radon, food) and artificial (X-ray machine, pharmacological and industrial radioisotopes) sources, the scope of which is gradually expanding and playing an important role in everyday life. However, radiation has a negative biological effect - it causes cell damage, and in some instances, cancer. it is necessary to protect humans from the harmful effects of ionizing radiation.

During Radon decay, non-evaporated radioactive products (Po, Bi and Pb isotopes) are formed, which are quite difficult to remove from the body. Thus, an increase in radon concentration in buildings poses an additional threat to the population and increases the potentially harmful risk to human health.

Key words: radon**,** exposure, radiation, carcinogen.

INTRODUCTION

By World Health Organization's International Agency for Research on Cancer (IARC) radon was classified as an A-class carcinogen [1]. Radon is responsible for approximately half of the average annual personal effective dose from all-natural sources of ionizing radiation [2]. The risk of lung cancer caused by radon exposure to the human body increases with increasing radon concentration. In particular, at a concentration of 100-200 Bq/m^3 in residential apartments and working buildings, the risk of lung cancer increases by 20%, at concentrations of 400-799 Bq/m^3 by 40%, and at concentrations above 800 Bq/m^3 by ~100%. Also, this risk depends on the duration of exposure to radon and its decay products.

Radon is a naturally occurring gas that is released from mountain rocks and soil. Radon is created from uranium, which is the part of the Earth's crust since its creation. The rate of its release from the soil is highly variable, partially because the uranium content in the soil significantly varies by location. Radon is released from the soil into the open atmosphere. Due to the movement of gases in the soil under the buildings, it reaches the air of the buildings. The air outside buildings usually contains very

small amounts of radon concentrations, but inside buildings where it has no way to dissipate, radon can be presented at quite high levels [3].

Although radon is chemically inert and electrically neutral, it is a radioactive substance, which means that its atoms spontaneously decayed and transform into other atoms in the air. The resulting atoms, called decay products, are electrically charged, so they can stick to dust particles in the building's air. By inhalation, these particles may enter the lung and settle on its inner surface. The settled atoms decay and transform into other atoms by emitting alpha particles that damage lung cells.

Radioactive gas radon enters public and residential buildings from the Earth's crust. Its penetration into the buildings occurs from cracks, building materials, water and as a result of gas combustion. In basements and lower floors, its rate is particularly high and decreases towards the upper floors. In a poorly ventilated room, it accumulates and increases the risk of lung cancer. Therefore, buildings should be constantly and well ventilated.

The problem is relevant in Georgia, mostly because the geologic formations of the country are characterized by a high content of uranium, and many buildings are constructed with local materials [4, 5, 6]. Exposure to radon inside the buildings in Georgia requires attention. In some districts of Tbilisi, we carry out measurements of radon concentrations in residential and public areas [7, 8].

Experimental

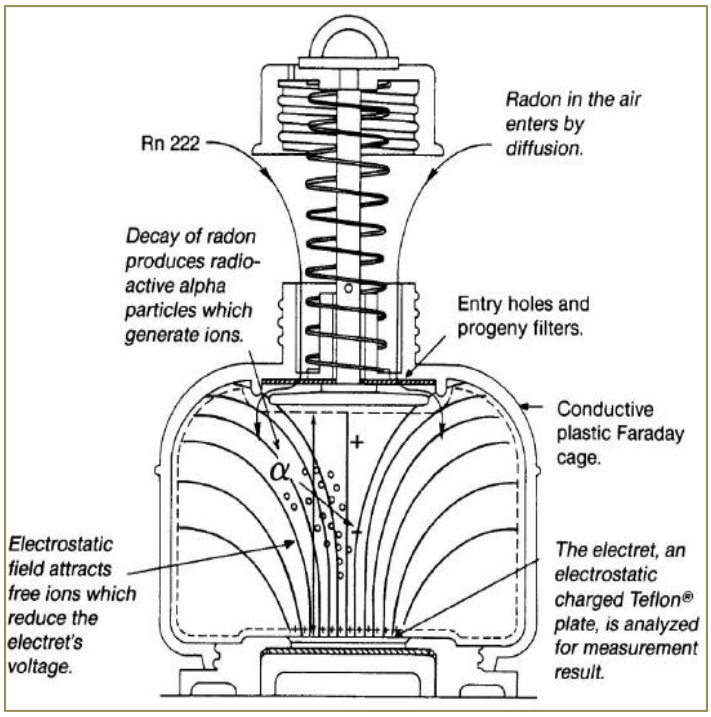

Fig. 2. E-PERM electret ion chamber.

Radon measurement and data processing are carried out using the Electret Ionization Chamber, E-PERM. An electret ionization chamber is made of electrically conductive plastic and has a special shape that determines the chamber's preferred use for this or that type of ionizing imaging and for measuring the characteristics of its source. An electret (charged phone disk) is attached to the camera, which is both the source of the electrostatic field and the sensor (sensitive element) necessary for the operation of the camera. Penetrating the chamber or by the influence of ionizing radiation arising in it the ions are formed, which, under the influence of the electrostatic field, meet on the surface of the electret and change (reduce) its charge. Using the charge changes, exposure (irradiation time) and a number of additional parameters, the characteristic values of this or that type (α, β or γ) of ionizing radiation and its source are calculated (e.g., absorption dose, radionuclide concentration, etc.),(fig. 1) [9].

An electrostatically charged disk-shaped detector (electret) is placed inside a small container (ionization chamber). During the measurement period, radon penetrates into a hole covered by a filter via diffusion inside the chamber, where ionization occurs due to the fission of radon and its daughter products reducing the voltage applied to the electret. The calibration coefficient is determined by the relationship between the change in voltage and the radon concentration.

To determine the radon concentration in the environment, we take two values of voltage, namely the value of the electret (V_i) voltage before irradiation and (V_f) after irradiation. Using the voltage difference ($V_i - V_f$), time of irradiation and experimentally determined calibration factor (CF) for a given electret and ion chamber configuration, we obtain data on radon concentration.

Radon concentration in air is calculated by the formula:

$$C_{Rn,A} = \frac{V_i - V_f}{CF \times T_A} - 0.087 \times R.$$

WhereT_A-testing time; R - exposure dose strength at the test site and calibration factor (CF) is calculated by the formula:

$$CF = A + B \times \frac{V_i + V_f}{2}$$

A and B are the constants depending on the construction of the E-PERM system.
And the complete measurement error is calculated as follows:

$$\Delta C_{Rn,A} = \sqrt{E_1^2 + E_2^2 + E_3^2}$$

Where the E_1 error is related to the parameters of the system components, such as camera volume, electret thickness, etc. Experiments have shown that this error is equal to about 5%.

Error $E_2$is related to the reading of the voltage of the electret. Both the initial and final voltage reading error is 1 volt. The error of these two different readings will be the square root of the sum of their squares which is 1.4. The relative error is calculated by the formula: $100 \times 1.4/(V_i - V_f)$.

E_3 The error is related to the gamma background uncertainty, which is about 10%.

In order to eliminate any short-term effects on average concentration measurements caused by changes in weather conditions, we are taking these measurements over a period of several months. Typically, a three-month test. Short-term tests are also available (7-8 days) where long testing is impractical.

RESULTS AND DISCUSSION

For evaluation of the radon concentrations, the "action level" of 148 Bq / m^3 by the World Health Organization (WHO) was used. We carry out measurements in the Mtatsminda districts of Tbilisi. Radon concentration in the air varied within (12 - 712) Bq / m^3 in the air of the private apartments, and the residential and public areas (Fig. 1).

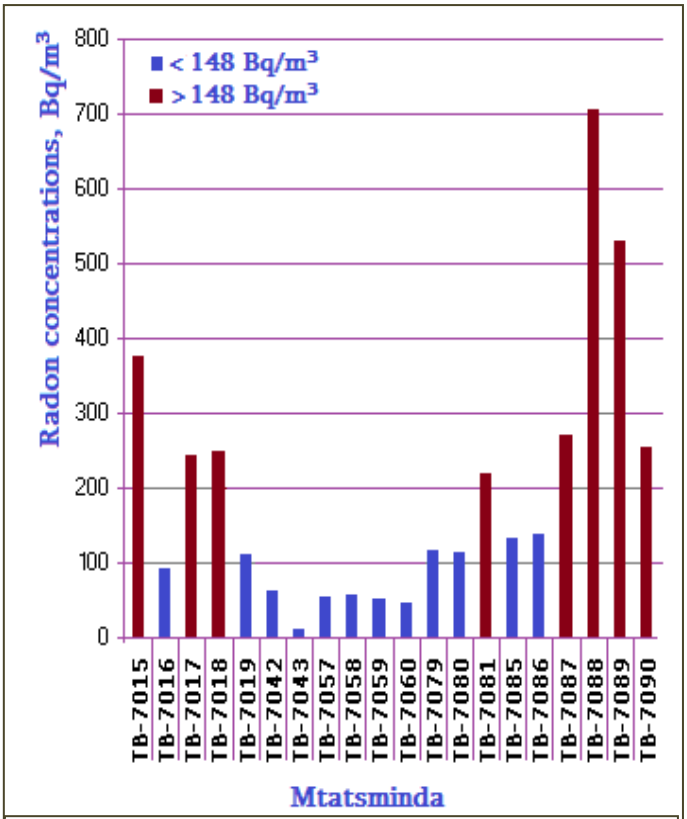

Fig. 1 Distribution of radon concentrations into the air of the private apartments, residential and public areas in Bq/m^3. (148 Bq/m^3 is the "action level").

As we see the results of the research are important and require attention. In particular, a number of measures need for lowing these indicators to the "action level".

In addition to detailed measurements and determination of radon concentrations, it is necessary to carry out rehabilitation works. Rehabilitation measures are different and is depending on specific circumstances. Clearly, elevated radon levels in above-ground areas are caused by radon penetration through cracks and other openings in the floor due to pressure differences. Another way is through the diffusion of the soil in contact with the foundation of the building. Causes may be diffusion from building materials also. Rarely, as a result of radon content in the water. For all these cases, developed rehabilitation measures for residential houses can be used [10].

fig. 2 is presented Radon concentrations in the soil gas in Mtatsminda district of Tbilisi. As we see variation range of radon concentrations is from $1.8 \cdot 10^3 Bq/m^3$ to $12.7 \cdot 10^3 Bq/m^3$.

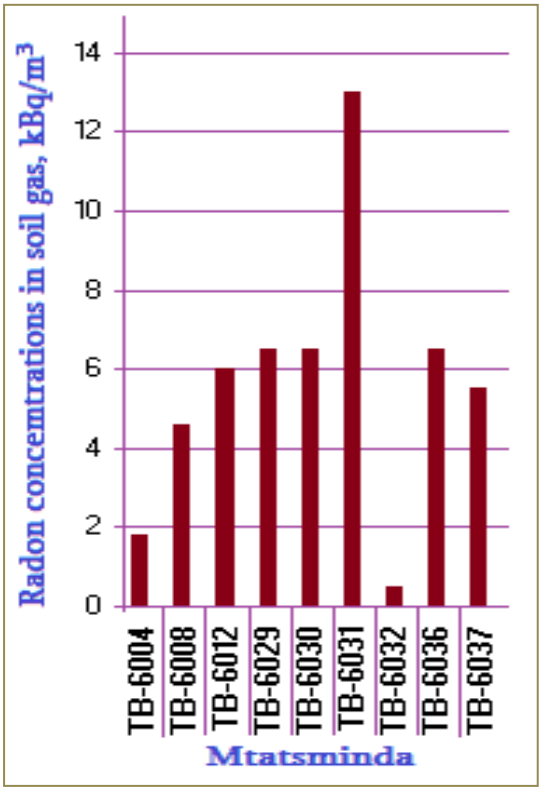

Fig. 2. Radon concentrations in the soil gas in Mtatsminda district of Tbilisi.

CONCLUSION

We measure the radon concentrations in the private apartments, the residential and public areas of the Mtatsminda district of Tbilisi– a total of 20 sites. 40% of them are with high content of radon concentrations. Also, we measure Radon concentrations in the soil gas in the same district. Mtatsminda is arranged at the end of the Trialeti ridge. The tectonic structure of its territory is quite complex. Radon, a by-product of the natural radioactive decay of Uranium, occurs widely in soil and rock. It can escape upwards to the shallow crust by diffusing and dispersing in permeable soils, or by migration upward along preferential pathways, such as cracks and defects.

High-risk areas based on our measurements require detailed investigations.

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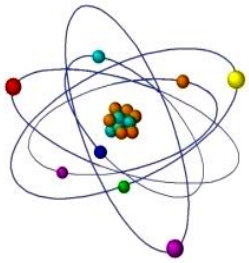
RADON IN SPRING WATER SOURCES IN THE TERRITORY OF KARTLI (GEORGIA)



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ABSTRACT**:** *In the present work it was studied the content of radioactive gas radon - Rn-222 in the number of sources of spring located in some settlements nearby to Tbilisi city (the capital of Georgia) in the territory of so-called Tbilisi and Kartli artesian basins. Research was carried out during the annual period (January-December); samples for measurements in some settlements were selected monthly. Radon detector RAD7 was used for determining radon content. It was established, that radon content in water considerably changes depending on the source location as well as on water type too. So, for example, radon content in various sources of spring water was within the limits from several units of Bq/L up to 100 Bq/L and more. The possibility of influence on various factors on the radon content was analyzed, for example, features of the soil-geological structure, features of water sampling, etc. A comparison with literary data has been carried out.*

Key words: Radon, spring water, activity concentration

INTRODUCTION

Control of a radioactive condition of the environment is one of the most actual problems of modern ecology. Radon is one of three main gases included in the so-called “terrestrial breath”, during which argon-40, helium and radon are constantly escaped from the bowels of the Earth in the atmosphere. Only radon is radioactive among these gases [1]. Radon gets in the water from environmental soil, and also granites, basalts, and sands to which aquiferous layers adjoin. Radon concentration in usually used water is small, but water from some deep wells and artesian wells can contain a lot of radon – from 100 pCi/L up to 1000000 pCi/L [2]. Radon dissolved in water operates in two ways. On the one hand, it together with water gets to the digestive system, and on the other hand, people inhale radon allocated from water by its utilization.

Breathing radon in indoor air can cause lung cancer. Radon decays into radioactive particles that can get trapped in lungs at the inhalation. As they decay further, these particles emit energy impulses that can damage lung tissue and increase the chances of developing lung cancer over the course of a lifetime. Drinking water containing radon represents a risk of developing internal organ cancers, primarily stomach cancer [3].

Results of numerous pieces of research show, that radon content in natural waters in different countries fluctuates in the big ranges. Abdallah et al. showed [4] that radon activity concentration in samples of spring water selected directly in the spring zone (such water type is mentioned further as WSp-1) changes in the range 9.8-49.6 Bq/L with the average value of 29.0 Bq/L; radon concentration in similar samples studied in the work [5] varied from 12.62 to 20.65 Bq/L, and in the work [6] – from 0.15 to 1200 Bq/L with the average value of 98 Bq/L. Radon content in samples of spring water selected far from the spring zone (water type WSp-2) studied in the work [**Error! Bookmark not defined.**] changes in the range from 0.46 to 9.4 Bq/L (average value of 4.7 Bq/L), and in the work [7] – from 8 to 427 Bq/L.

The radon problem in Georgia was not given proper attention. Practically there were no data on radon content in drinking (tap) water. In some author's works [8] there are given data on radon content in drinking (including tap) water in Tbilisi, in the zone of Ureki-Shekvetili, and also radiological doses of radon from water ingestion have been estimated. Some results of radon content research in natural water and soil are resulted in the work [11].

Thus, regular research on the state of radon distribution in the water resources of Georgia is an actual problem. In the present work, some results on radon activity in waters of spring sources in the geographical area of Tbilisi city are given.

PROBLEM STATEMENT

There are several artesian basins in East Georgia some of which are used for reception and supply of the population by drinking water (in particular, Kartli basin is located the Natakhtari complex of the water supply of Tbilisi city by drinking water in which artesian waters are used). These artesian basins feed the numerous springs located on the whole territory of the region. Research of their natural radioactive activity represents doubtless interest from the scientific point of view as well as from the practical point of view. First of all, there are of interest waters of Kartli and Tbilisi artesian basins located to the north and to the south of Tbilisi (Figure 1). In this zone, there are springs that are often used by the population as drinking water.

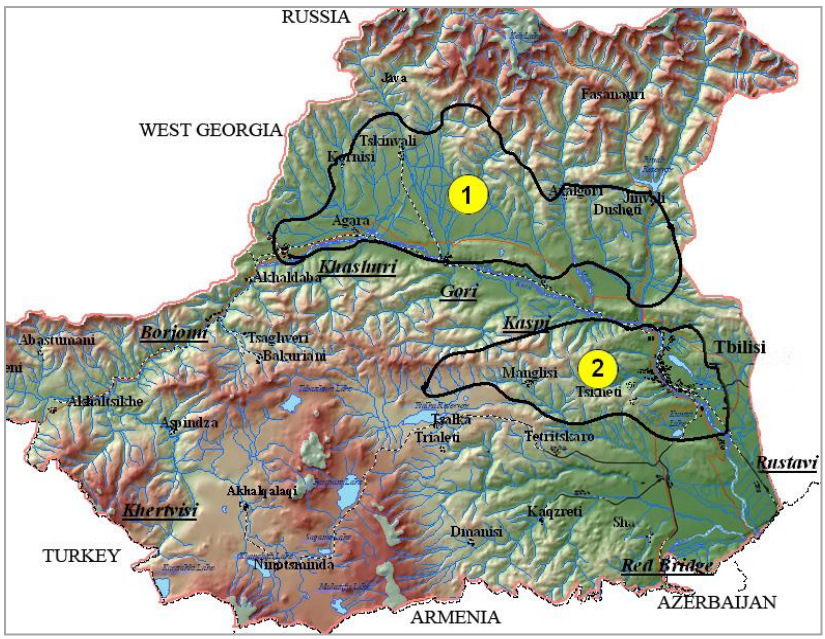


Figure 2. Kartli (1) and Tbilisi (2) artesian basins in East Georgia

The objective of the work was studying of features of radon content distribution depending on geographical factors in surface sources of water (spring), located in Tbilisi and Kartli artesian basins. The first results of carried out research on the water of spring sources are given in the work [12]. In the present publication, there are given and generalized all results of the carried out researches for the period of one calendar year – from January to December.

RESEARCH OBJECTS

Research objects were spring water sources located in the territory of Tbilisi and Kartli artesian basin – in total 24 control points.

During the period from January to December there were selected samples of spring water sources, in particular:

- springs (WSp-1) in which water was selected directly in the spring zone - 10 points;
- springs (WSp-2) in which water was selected far from spring zone – in pipelines, on sufficiently remote distance - from hundred meters up to several kilometers) - 14 points;

In total 134 samples of spring waters were selected and analyzed (*note:* samples from some control points were selected monthly).

METHODOLOGY

Sampling

Sampling was carried out in special glass containers; the volume of the container is 250 mL. Containers were filled with water up to the top and densely closed by a cover. Then the selected water samples were transported to the laboratory for analysis.

Of the three isotopes of radon, the subject of research is Rn-222, because the half-lives of Rn-220 and Rn-219 are much shorter, and they decompose before migrating into soil and rocks, and their amount in the air is insignificant.

Electronic radon detector RAD7 was used for the determination of radon content in water. The RAD7 device uses a method for the registration of radon decay products, namely alpha particles Po-218, Po-2014 and Po-210 (which are formed as a result of decay), based on the use of a solid semiconductor sensor.

The radon measurement process is as follows:

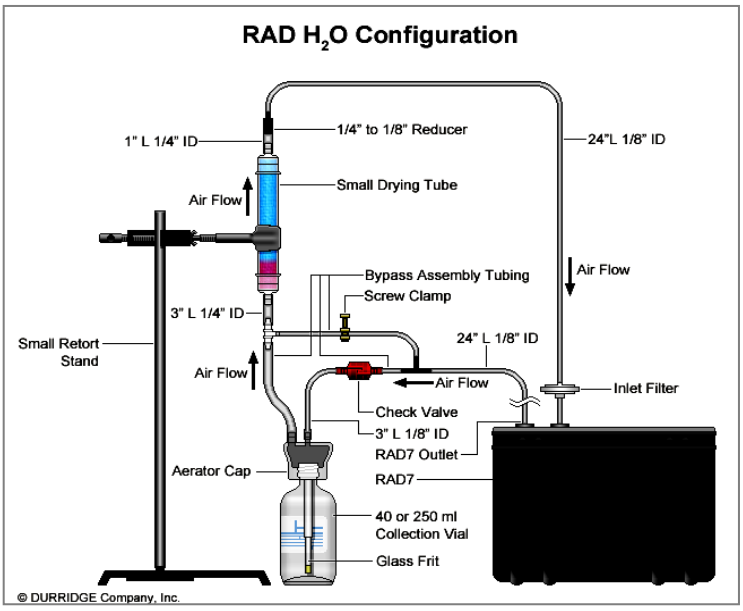


Fig.3. RAD H_2O configuration diagram

A water sample glass container is placed in a circle of closed air pipes (see figure 3). The circle also includes a small desiccant tube, the purpose of which is to dry the air in the closed circle during the process. The duration of the measurement process is specified in the protocol as 30 minutes.

The measurement error of the method does not exceed ± 5%. The device allows the measurement of radon activity in water from 0.2 Bq/L to more than $3.7 \cdot 10^3$ Bq/L [13].

Special multiple measurements were performed to assess the radiation background, using distilled water as a sample. The results obtained on two different detectors showed that the background activity varied in the range of 0.03 - 0.22 Bq/L (average 0.09 Bq/L).

Use of Software CAPTURE v. 4.4.10

Radon concentration measurement data is transferred from the RAD7 device to a computer by using the Capture program. Software CAPTURE v. 4.4.10is a specialized program developed by the manufacturer of RAD7 detectors. CAPTURE is designed to analyze data registered by the RAD7 detector (see figure 4).

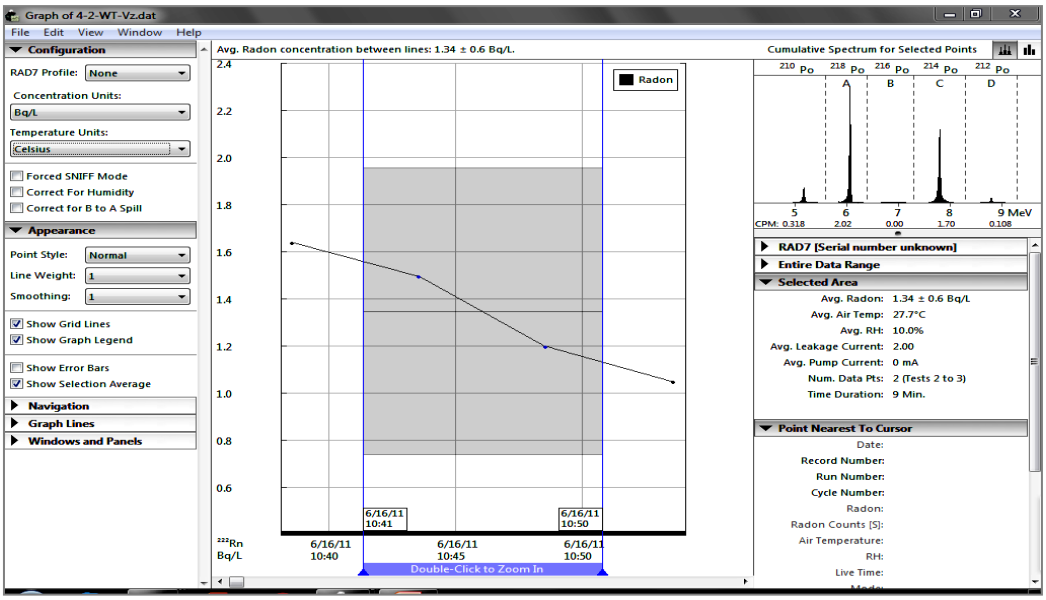


Figure 4. Graphic representation of the results obtained with the RAD7

Processing of the results

For the investigated points taking into account reference level of 11 Bq/L recommended by US EPA [12] and the received results 7 conditional groups of radon activity level in water samples selected in various control points have been established, in particular, in the 1st group (I) there were included control points in which value of radon concentration is very low - did not exceed 0.3 Bq/L (i.e. close to the background), in 2nd group (II) – control points in which it is possible to consider that value of radon concentration is low – in the range of 0.3 - 1.0 Bq/L, in 3rd group (III) – control points in which value of radon concentration can be designated conditionally as typical - in the range of 1.0 - 3.0 Bq/L, in 4th group (IV) – control points in which it is possible to consider that value of radon concentration is above typical – in the range of 3.0 - 10.0 Bq/L, in 5th group – control points in which it is possible to consider that value of radon concentration is high – in the range of 10 - 30 Bq/L, in 6th group (VI) – control points in which it is possible to consider that value of radon concentration is very high – in the range of 30 - 100 Bq/L, in 7th group (VII) – control points in which it is possible to consider that value of radon concentration is ultrahigh – more than 100 Bq/L.

For the characteristic of time history (stability) of radon activity in control points depending on the period (month) of measurements the value of the standard relative deviation of the results received during the year was used. For values of relative standard deviation, no more than 50 % corresponding average value conditionally was accepted as a stable (constant) value of activity concentration (and, accordingly, group of activity) for the given control point. If the value of relative standard deviation exceeded this size, then the value of activity concentration (and, accordingly, group of activity) for the given control point was considered as unstable.

RESULTS

Results of carried measurements are given in Table 1.

Table 1. Generalized monthly activity of radon (A, Bq/L) in spring water, their average (A_{av}), minimal (A_{mn}), maximal (A_{mx}) values, relative standard deviation (RSD), and in the column (Prm) also their averaged values (*aver* – average, *min* – minimal, *max* – maximal)

#	ST	Prm	A, Bq/L												A_{av}	A_{mn}	A_{mx}	RSD
			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Bq/L	Bq/L	Bq/L	%
1	TAB-WSp-1	aver	96.3	54.1	33.1	98.8	79.2	75.5	86.3	45.7	63.7	65.9	99.9	68.8	72.3	33.1	99.9	29
2		min		16.7	2.7	49.8	29.8	18.1	19.0	21.9	27.6	9.2	17.2	16.1	20.7	2.7	49.8	59
3		max		110	73	148	129	144	139	91	134	108	163	123	124	72.9	163	21
4	TAB-WSp-2	aver		13.4	11.7	6.0	12.0	8.3	5.4	4.8	7.3	6.5	9.6	7.5	8.4	4.8	13.4	34
5		min		4.0	3.3	0.1	1.1	1.9	0.9	0.3	0.4	0.3	2.9	1.1	1.5	0.1	4.0	92
6		max		27	36	10	21	20	7	8	13	11	13	12	16.2	7.3	35.8	55
1	KAB-WSp–1	aver		7.5	11.1	8.4	6.9	13.3	7.6	8.5	11.1	6.8	12.5	10.9	9.5	6.8	13.3	25
2		min			9.1	5.7	3.7	9.8	5.0	6.6	9.3	5.1	8.1	3.3	6.6	3.3	9.8	36
3		max			13.0	9.8	9.1	16.6	10.3	10.7	12.1	7.9	14.9	14.8	11.9	7.9	16.6	24
4	KAB-WSp–2	aver		9.4	3.8	4.8	2.4	7.7	3.5	5.2	6.3	4.6	7.3	4.2	5.4	2.4	9.4	39
5		min		9.4	3.1	1.7	0.5	5.1	1.6	2.0	3.3	1.8	4.3	3.5	3.3	0.5	9.4	73
6		max		9.4	4.6	10.1	3.7	10.3	5.3	8.3	9.3	7.3	10.2	4.9	7.6	3.7	10.3	33

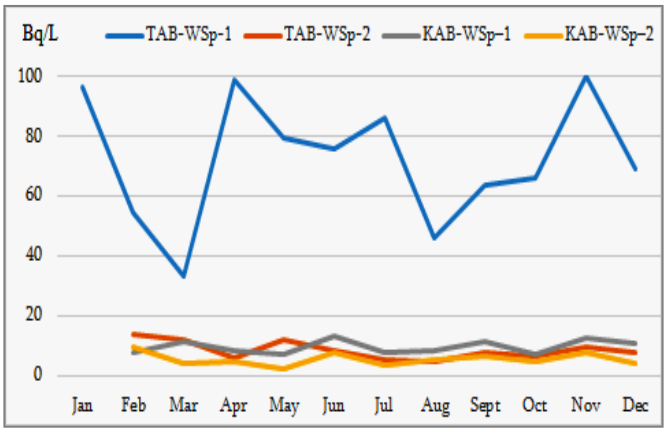
Note: TAB – Tbilisi Artesian Basin; KAB – Kartli Artesian Basin

Apparently from the received results, it is possible to note the following features:

- in Tbilisi artesian basin the highest activity was observed in WSp-1 (163 Bq/L), and the lowest activity (closely to the background) was in WSp-2 (0.1 Bq/L);
- in Kartli artesian basin the highest activity was observed in WSp-1 (16.6 Bq/L), and the lowest activity (closely to the background) was in WSp-2 (0.1 Bq/L);
- values of activity in the same control point depending on the period of measurements are sufficiently unstable; any laws in seasonal dependence were not observed.

ANALYSIS

Apparently from the received data, in the geographical area of Tbilisi city in the territory of Tbilisi and Kartli artesian basins, there was observed a sufficiently big variety of radon activity in spring waters (which covers a range of values from very low 0.1 Bq/L up to ultrahigh – 163Bq/L) (see Table 1, Fig.2).



Observable much lower values for spring water of the second type (selected sufficiently far from the spring site) in comparison with water of the first type (selected directly in the spring location) are, of course, connected with the sufficiently intensive process of radon decontamination in by-pass pipelines (and also, in accumulator tanks).

Rather high values of radon activity observed in some cases in river water, apparently, are connected with their mixing with water from nearby located springs. At a considerable distance from a spring (some kilometers) radon activity in river water is close to background values.

Table 4 shows a comparison of the received results with some literary data. Apparently from the data, the results received in the present work lay within the values received in other publications.

Table 4. Radon content in surface water in different countries.

#	Country (site)	Water type	A, Bq/L			Ref.
			av	mn	mx	
1	Lebanon (Beirut, Mount Lebanon, Beqaa, etc)	WSp-1	29.0	9.8	49.6	[4]
2	Iran (Mashhad)		-	12.62	20.65	[5]
3	Spain (Extremadura)		98	0.15	1200	[6]
4	Georgia (Kartli artesian basin)		9.5	3.3	16.6	Present work
5	Georgia (Tbilisi artesian basin)		72.3	2.7	163	Present work
6	Lebanon (Beirut, Mount Lebanon, Beqaa, etc)	WSp-2	4.7	0.46	9.4	[4]
7	Poland (Walbrzych)		131	8	427	[7]
8	Georgia (Kartli artesian basin)		5.4	0.5	10.3	Present work
9	Georgia (Tbilisi artesian basin)		8.4	0.1	35.8	Present work

CONCLUSIONS

1. It was established, that radon content in spring water in Tbilisi artesian basin varies in a wide range, in particular:
   - in spring water of the first type (samples were selected directly in the zone of spring location - WSp-1) is in the limits from several units of Bq/L (2.7 Bq/L) up to 100 and more (163 Bq/L), with an average value of 72.3 Bq/L;
   - in spring water of the second type (samples were selected from the pipeline at a sufficiently big distance from the zone of spring location - WSp-2) is in the limits from 0.1 Bq/L up to 35.8 Bq/L, with the average value of 8.4 Bq/L;
2. Radon content in spring water in the areal of Kartli artesian basin varies in a wide range:
   - in spring water of the first type (samples were selected directly in the zone of spring location - WSp-1) is in the limits from 3.3 to 16.6 Bq/L, with the average value of 9.5 Bq/L;
   - in spring water of the second type (samples were selected from the pipeline at a sufficiently big distance from the zone of spring location - WSp-2) is in the limits from 0.5 to 10.3 Bq/L, with average value of 5.4 Bq/L;
3. It was carried out analysis in which it was shown that the received results for spring waters can be connected with features of geology of these territories; comparison with literary data has been carried out.

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