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Original Article

Indirect assessment of internal irradiation from tritium decay on Lemna Minor duckweed

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A B S T R A C T

The response changes of the specific growth rate of Lemna minor duckweed was modeled using the logarithms of frond numbers on tritium activity concentration and gamma radiation dose from cobalt 60. The concept of average specific growth rate depends on the general exponential growth pattern, where toxicity is estimated based on the effect on the growth rate. One of the main questions of the effect of the radiation dose on duckweed is how to correlate the effect of beta radiation with the effect of any other radiation for modeling radiation on Lemna minor. Experimental data were extrapolated by utilizing the OECD guidelines.

A linear relationship of absorbed dose and activity concentration was obtained for the average dependency growth rate of Lemna minor as $D = (0.1257) \cdot A^{0.585}$. The dose rate of gamma irradiation from ^{60}Co increases with tritium activity dependence, on the specific growth rate of the Lemna minor duckweed. An increase in the tritium activity causes a decrease in the specific growth rate of the Lemna minor duckweed. It indicates that as the quantity of the beta radiation dose increase in Lemna minor duckweed, a higher quantity of gamma radiation will be required to cause the same effect in the specific growth rate of Lemna minor duckweed. The relation between the inhibition of the Lemna minor seedling growth and gamma and beta radiation dosage agrees roughly with that between the decrease of survival rate or fertility and dosage.

1. Introduction

External and internal emitters are the most significant sources of radiation exposure for man and its environment. There are some natural internal emitters in everyone's body, such as ^{3}H, ^{14}C, ^{40}K, and others. Fifty-five years after discovery of X-rays by W.C. Rontgen in 1895, International Commission on Radiological Protection (ICRP) established a committee on permission internal exposure. However the question is still open and radionuclides dosimetry is still at an early stage of development and effort in this direction is just beginning. Absorbed dose remains a critical variable, but still one of several likely to predict response in an individual organism. Tritiated water quickly enters into biological organisms as tissue free water tritium (TFWT). Its chemical properties are similar to that of normal water, the "natural" content of tritium in fresh underground and surface water in Russia is 2–6 Bq/l [1]. Water composition in living organs of plants is approximately about 80–95%, but this percentage strongly depends on the species and the stage of development [2].

Tritium, a naturally occurring radioisotope of hydrogen, and it is produced during the interaction of nitrogen with the cosmic ray in the atmosphere. It can also be formed as a result of the byproducts of a nuclear reactor [3]. Tritium is an essential fuel for controlled nuclear fusion in both magnetic and inertial confinement fusion reactor designs. The tritium, which is a neutron-activated radionuclide, is produced from activation by neutron generated in the fission process. There are some natural internal emitters in everyone body, such as ^{3}H and others. In recent years the nuclear power plants which annually release several tens of kilograms have become the primary source of technogenic tritium in the environment [1]. It has a half-life of 12.3 years and is transferred to the environment and the human body as tritium oxide (HTO) through inhalation, ingestion, and diffusion through the skin. The quality and content of tritium are complex to determine because it is a low energy beta emitter with a maximum energy of 18.6 Kev. Tritium, a unique radionuclide when dynamically enters into living organisms, disrupts the structure of biologically relevant molecules in

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cells not limited to internal beta-radiation, but also as a result of the following transmutation into

$$^3_1H \rightarrow ^3_2He + e - \ \ + \nu_e^-$$

Antineutrino in the reaction is biologically inactive [1]. Its transmutation into ^{3}He causes disruptions in the chemical bonding of the DNA, this leads to cell death and disruptions in the organism activity [4]. As radioisotopes elements, they are powerful tools, which gives a range of advantages as they offer ultrahigh sensitivity, and are easily detectable [5]. Increasing attention channeled toward their possible use as indicators of water quality [6]. Ref [7] demonstrated the effect of beta and gamma radiation on the survival and maturity of diploids wheat.

However, the problem of assessing the dose of internal irradiation of tritium on duckweed is still unsolved. One of the main questions of the effect of the radiation dose on duckweed is how to correlate the effect of beta radiation with the effect of any other radiation for modeling radiation on Lemna minor. This work aims to determine the effect of internal β-irradiation dose from tritium, and irradiated gamma radiation of ^{60}Co on Lemna minor duckweed specific growth rate. The response changes of the specific growth rate of Lemna minor duckweed was modeled using the logarithms of frond numbers (according to the requirement of ISO/DIS 20079. Water quality − Determination of the toxic effect of water constituens and waste water to duckweed (Lemna minor) − Duckweed growth inhibition test. Reference number ISO 20079:2005(E)) on tritium activity concentration and gamma radiation dose from ^{60}Co.

The concentration of tritium inside Lemna minor is difficult to measure. Therefore, it is assumed that the concentration of tritium inside the duckweed plant is equal to the concentration in the surrounding water. This was previously confirmed by other researchers. Authors indicate that as the quantity of the beta radiation dose increase in Lemna minor duckweed, a higher quantity of gamma radiation will be required to cause the same effect in the specific growth rate of Lemna minor duckweed. The relation between the inhibition of the Lemna minor seedling growth and gamma and beta radiation dosage agrees roughly with that between the decrease of survival rate or fertility and dosage.

2. Methodology

2.1. Lemna Minor duckweed

In order to determine the dose of internal radiation from tritium to Lemna minor duckweed, the technique of assessing the degree of chemical contamination with Lemna minor was utilized [8]. Lemna minor a member of the Lemnaceae family, which can be found in a stagnant or slowly flowing waters across the universe, except in arctic and antarctic regions [9]. Lemna species have high sensitivity to both inorganic and organic substances; hence they are utilized as test organisms for water quality assessments and also for ecotoxicological researches regarding harmful effects of organic and inorganic elements, for example, heavy metals on aquatic plants [10,11]. Ref [4] used "Lemna minor as the bioassay of detecting natural and technical water contaminated with tritium as a part of tritium oxide (HTO) and organically bounded tritium(OBT)." Studies show that organically bounded tritium bound compounds behave differently from tritium oxide compounds [12].

2.2. Laboratory experiment

An experiment was conducted to study the effect of gamma radiation on a given plant to assess the dose level. The gamma irradiation on aquatic plants of the genus duckweed Lemna minor was carried out by the "All-Russian Research Institute of Radiology and Agroecology" (Obninsk). The exposure of the duckweed was performed on the Gamma installation "Gur-120 "(^{60}Co). The radiation dose was evaluated using the DKS-101 dosimeter (Polytech-M. passport relative measurement error 4%, verification certificate no. 41150.2 B 304). The plants were placed in sterilized plastic and glass containers with a volume of 100 ml filled with nutrient Steinberg's environment. Each experimental sample contained 30 individuals, two samples per dose.

A model was created showing the comparison of beta (tritium concentration) and gamma doses. A statistical interpolation was used on the data received from the laboratory experiment with the aid of the R program to estimate radiation doses on the minor Lemna change of specific growth rate dependence on the dose of gamma irradiation ^{60}Co.

2.3. Measured, calculated and analyzed duckweed growth indicators

During the development of the biotest, the technology of bio testing of chemical contaminants in water recommended by OECD guidelines for the testing of chemicals Lemna sp. Growth Inhibition Test was utilized.

Standard testing for growth inhibition was carried out for seven days, then the fronds (leaf blades) were counted to calculate the specific growth rate, the time of doubling the number of plates, and the percentage of inhibition. The doubling time of the frond number in control is less than 2.5 days (60 h). The average specific growth rate (growth rate) was calculated as a logarithmic increase in the growth rate − the number of fronds for each parallel of the experimental and control groups as stipulated in the OECD/OCDE guidelines [8].

$$\mu_{i-j} = \frac{\ln N_j - \ln N_i}{t} \tag{1}$$

μ_{i-j} : Average specific growth rate from the time i to j days^{-1}
N_i : Measurement variable in the test or control vessel at the time i, fronds number
N_j : Measurement variable in the test or control vessel at time j, fronds number
t: time from i to j, days

Equation (1) is the OECD standard equation for average specific growth rate of Lemna species.

For each treatment group and control group, a mean value for growth rate along with variance estimates was calculated.

The concentration of tritium inside the Lemna minor is difficult to measure. Therefore, it was assumed that the concentration of tritium inside the duckweed plant is equal to the concentration in the surrounding water. This was previously confirmed (established) by other researchers [13].

We have two experimental dependencies: μ(D) and μ(A). Estimating the coefficient of proportionality between dose and activity:

Where A is the tritium concentration activity, and μ(A) is the dependency of average specific growth rate of Lemna minor on the tritium concentration activity.

D is the absorbed dose from ^{60}Co, and μ(D) is the dependency of average specific growth rate of Lemna minor on the absorbed dose from ^{60}Co.

Suppose the dose depends on activity linearly:

$$D = \gamma_1 \bullet A \quad (2)$$

Modeling the experimental dependency as

$$\mu\ (D) = c_1 \bullet D^{b1} \quad (3)$$

Eqns. (2) and (3) gives

$$\mu\ (D) = c_1\ (\gamma_1 \bullet A)^{b_1} \quad (4)$$

Parameters $c_1$and b_1 in (4) could be computed from experimental data for μ (D) from experiments with gamma-rays from ^{60}Co, so we have to fit only γ_1 based on experimental data for μ (A).

Appropriate estimations were performed with R-program, and we have:

$$D = (0.1257) \cdot A^{0.585} \quad (5)$$

Validating the linear proportionality of equation (5), from our experimental data in Tables (1) and (2).

From experimental data in Table (1) and Table (2), the specific growth rate dependency on gamma radiation dose of ^{60}Co at 10 Gy is equivalent to the specific growth rate dependency on tritium concentration at 1774 Bq/l. Furthermore, it can be said that gamma radiation dose attain a saturation point at 10 Gy, which also correspond to the saturation point of the tritum concentration activity at 1774 Bq/l. The experimental data shows that there is a similar average specific growth rate response from tritium concentration and absorbed dose from ^{60}Co.

3. Result and discussion

Figs. 1 and 2 shows the specific growth rate dependence of tritium activity and gamma dose on Lemna minor duckweed, respectively.

3.1. Tritium concentration and the specific growth rate

The lowest tritium activity at which the growth rate is lower than in the control is 444 Bq/l ($p \le 0.05$). Exposure to beta radiation in the activity of 444–1774 Bq/l causes a sharp drop in the growth rate of duckweed. After exposure in the range of activities from 1774 to 7333 Bq/l, the growth rate reaches a peak and does not change further. As an example, from tritium concentration of 21 Bq/l to 1774 Bq/l, there is about 67.7% percentage reduction in the specific growth rate. Whilst, for tritium concentration of 1774 Bq/l to 7333 Bq/l, about 16.7% percentage reduction of specific growth rate was recorded. On the first day of experiment, the number of duckweed is small, according to GOST 32426-2013 “testing methods for chemical products that pose a danger to the environment”. On the third day of the experiment, the water samples were monitored for duckweed with chlorosis, necrosis, or algae growth. Thus, in one of the water samples with an activity of 7333 Bq/l, chlorosis was observed in one of the duckweed individuals. Chlorosis – yellowing or complete discoloration of the frond as a result of loss of pigment, necrosis – localized dead areas of tissue (brown or white). On the seventh day of the experiment, control for the presence of chlorosis, necrosis and algae was also carried out, as well as counting duckweed leaves.

The amount of tritium concentration in water has a significant impact on the growth rate of the Lemna minor. The duckweed soaked in water with some level of tritium contamination; were able to survive with stunted growth, while others died off, depending on the number of days spent in the water. The higher the concentration of tritium in water, the less the specific growth rate and survival of the Lemna minor seed. The higher the dosage of beta and gamma-rays, the more delayed were the growth and elongation of the seedling. Typically, the germination of seeds was better; under more favorable conditions, the higher the concentration of tritium activity, the more distorted was the distribution in the direction of lower fertility.

Table 1
Dependence of the specific growth rate of duckweed feeds small depending on the radiation dose.

Dose (Gy)	Specific growth rate (μi-j), day^{-1}
Control	0.054 ± 0.002
5	0.048 ± 0.001
10	0.031 ± 0.004
15	0.031 ± 0.006
20	0.016 ± 0.001
50	0.012 ± 0.007
100	0.009 ± 0.002

Table 2
Dependence of the specific growth rate of duckweed feeds on the activity of tritium.

Tritium-containing samples, Bq/l	Specific growth rate (μi-j), day^{-1}
21	0.093 ± 0.002
222	0.085 ± 0.007
444	0.062 ± 0.01
917	0.045 ± 0.007
1774	0.030 ± 0.004
3548	0.029 ± 0.002
7333	0.025 ± 0.002

3.2. Gamma irradiation dose and the average specific growth rate

Acute irradiation was carried out in doses of 5, 10, 15, 20, 50, and 100 Gy. While control was applied by non-irradiated plants duckweed small. Seven days after irradiation, there are statistically significant differences in the specific growth rate between control plants and irradiated plants at doses higher than 10 Gy ($p \le 0.05$). The lowest dose of acute gamma radiation at which the growth rate is lower than in the non – irradiated control is 10 Gy. After irradiation in the dose range from 10 to 50 Gy there is a significant decrease in the average specific growth rate: at a dose of 10 Gy is 42%, and 15 Gy is 41%. The percentage of reduction in the specific growth rate, for example, at a dose of 10 Gy, was determined by the proportion.

In addition to chlorosis and necrosis after irradiation, there are other changes in duckweed colonies. One of them is the death of the roots. With daily measurements of the number of roots in minor duckweed, a reduction in their number was noticed. Fig. 3 – 9 show the dependence of the decrease in the number of roots on the time after irradiation. Data on changes in the number of roots at a dose of 0 Gy are shown in Fig. 3. The correlation is reliable ($p \le 0.05$) from this figure, it is clear that the decrease in the number of roots was almost uniform. As the number of days increases, the survival rate of the duckweed root decreases. For instance, for day 1 to day 7, the percentage reduction in the survival rate is about 51.6%.

Data on the change in the number of roots at a dose of 5 Gy are shown in Fig. 4 the Correlation is reliable at ($p \le 0.05$). According to Fig. 4, we can also say that the reduction in the number of roots occurred evenly, the jump in the number of roots falls on 6–7 days after exposure to minorduckweed. Fig. 5 shows the change in the number of roots depending on the gamma radiation dose of 10 Gy 12 days after irradiation. Visualizing data on the number of roots of minor duckweed, irradiated with a dose of 10 Gy (Fig. 5), we can say

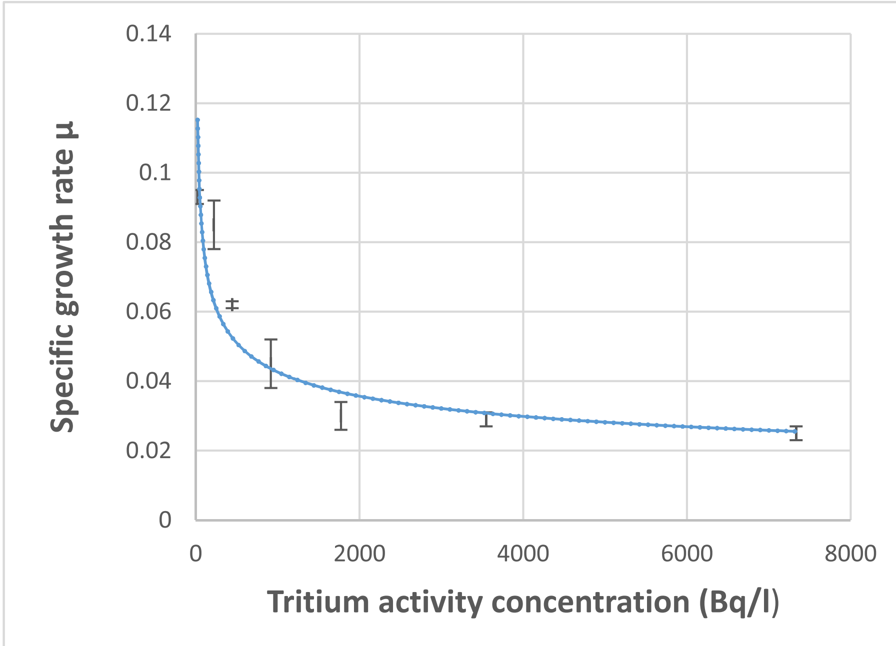

Fig. 1. Tritium activity dependency on the specific growth rate Lemna minor duckweed.

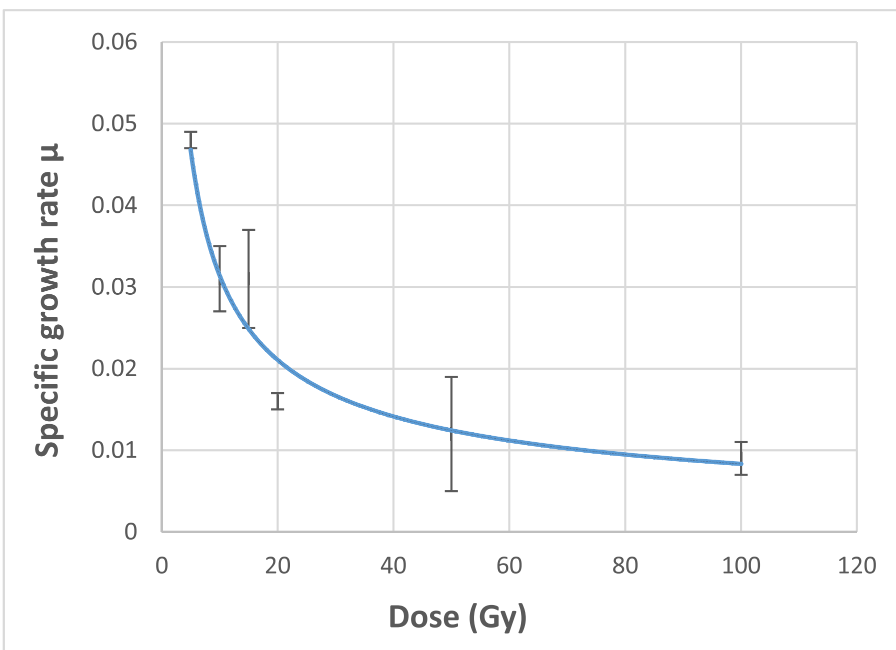

Fig. 2. Gamma dose dependency on the specific growth rate Lemna minor duckweed.

that from 3 to 5 days after irradiation, there is an increase in the number of roots. But from 5 to 8 days, a decline in the number was recorded. In Fig. 6, it can be seen that from 2 to 3 days after irradiation, there is an increase in the number of roots. However there was a gradual decrease in the number of roots on daily basis.

Fig. 7 is the change in the number of roots depending on the dose of gamma radiation 20 Gy 12 days after irradiation. The correlation is reliable ($p \leq 0.05$). It shows that there is a gradual decrease in the number of roots, except for the time interval from 2 to 4 days after irradiation. Based on the data in Fig. 8, it is difficult to affirm whether there was a gradual decrease in the number of roots, from day 1 to day 5 a decline in the number of roots of minor duckweed was recorded. But then, from days 5–7, there was an increase in the number of roots. On day 8, a decline was recorded again, and on day 9 an increase in rootlets, and later the number decreased again. According to the data of irradiation of the duckweed sample with a small dose of 100 Gy as shown in Fig. 9, a gradual decrease in the number of roots of duckweed is observed. But an increase in this number was also recorded from 2 to 4 days after irradiation.

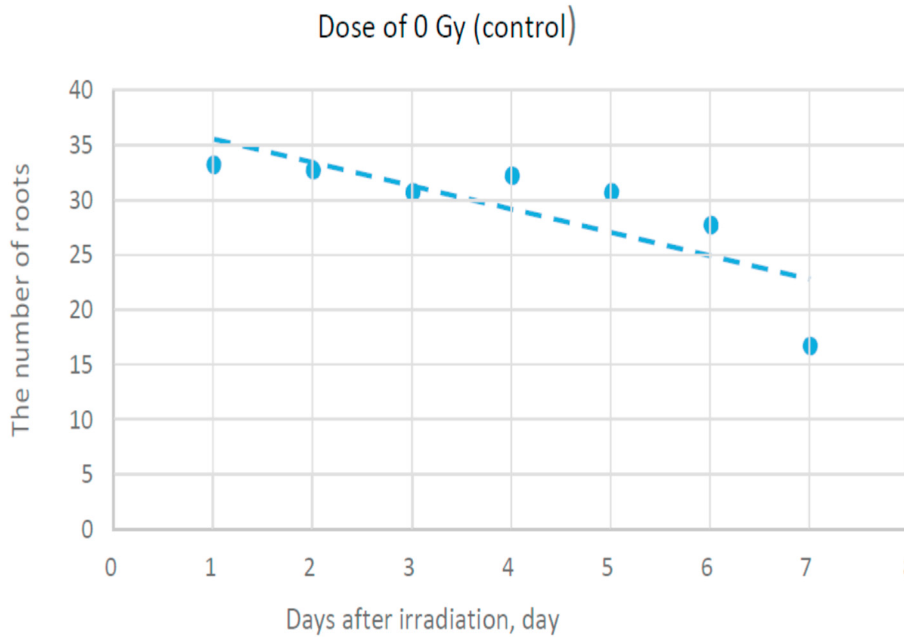

Fig. 3. Change in the number of roots depending on the dose of gamma –radiation of 0 Gy for 7 days after irradiation.

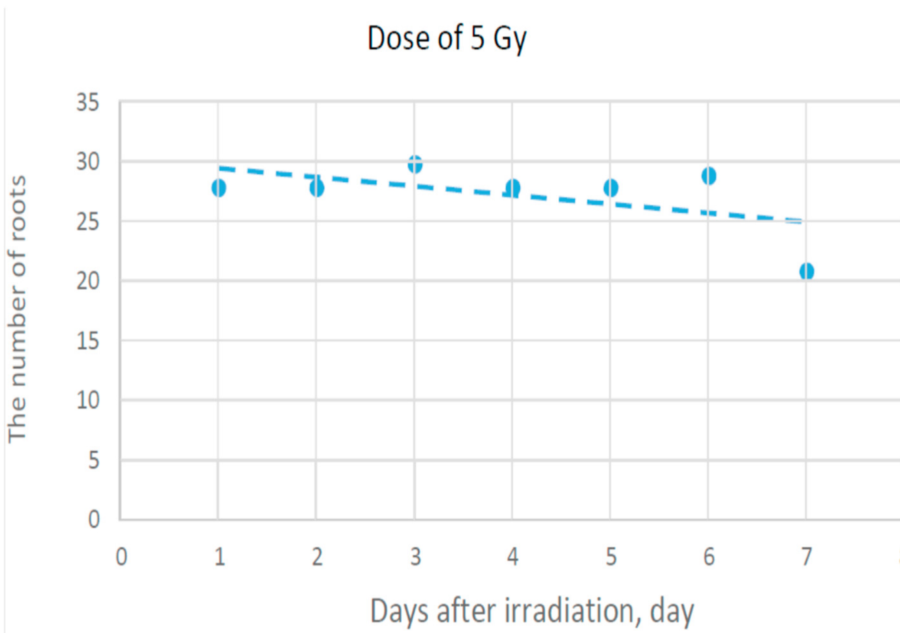

Fig. 4. Change in the number of roots depending on the dose of gamma –radiation of 5 Gy for 12 days after irradiation.

Drawing a conclusion from all data on the number of roots of small duckweed, we can say that almost all samples showed an increase in the growth of roots for the first week after irradiation, as which on the average occurred on the fourth day. Furthermore, in most cases, there is a gradual reduction in the number of roots. The radiation dose might have contributed to the death of the roots. Again, it can be said that the maximum reduction in the survival rate of the duckweed with gamma radiation occurs between 15 Gy and 20 Gy. Going from day 1 to day 7, the percentage reduction in the survival rate for 15 Gy and 20 Gy are 65.4% and 50%, respectively. Furthermore, increasing the gamma dose beyond 20 Gy, have no significant effect on the survival rate of the duckweed root.

The result shows as the dose rate of gamma irradiation from ^{60}Co increases with days, the specific growth rate of the Lemna minor duckweed decreases. The highest rate of growth is on the control level within the first few days. The amount of gamma irradiation duckweeds are exposed to have a significant impact on the growth rate of the plant. The inhibition of the seedling growth and the survival rate of the plant depends significantly on the amount of gamma irradiation dose.

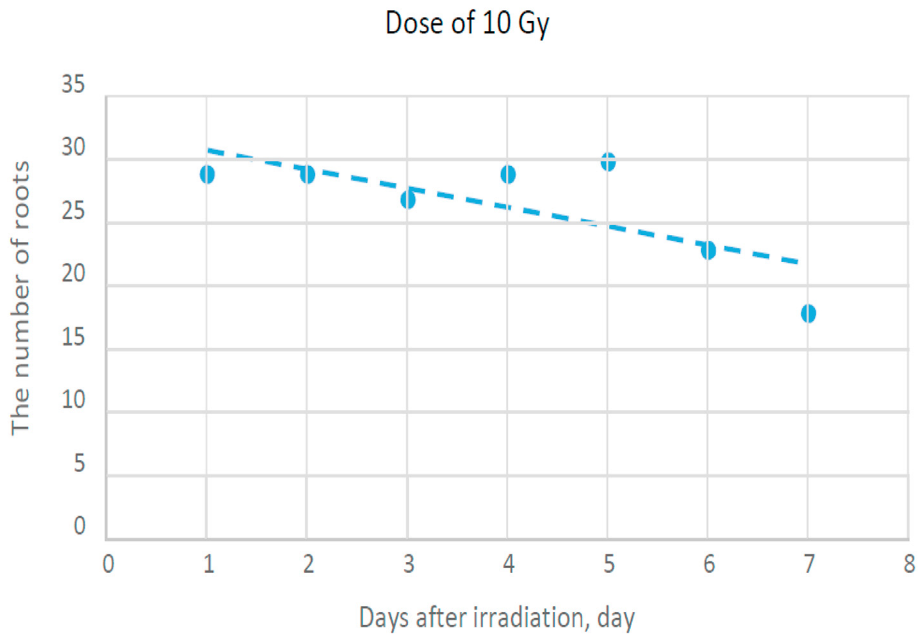

Fig. 5. Change in the number of roots depending on the dose of gamma –radiation of 10 Gy for 12 days after irradiation.

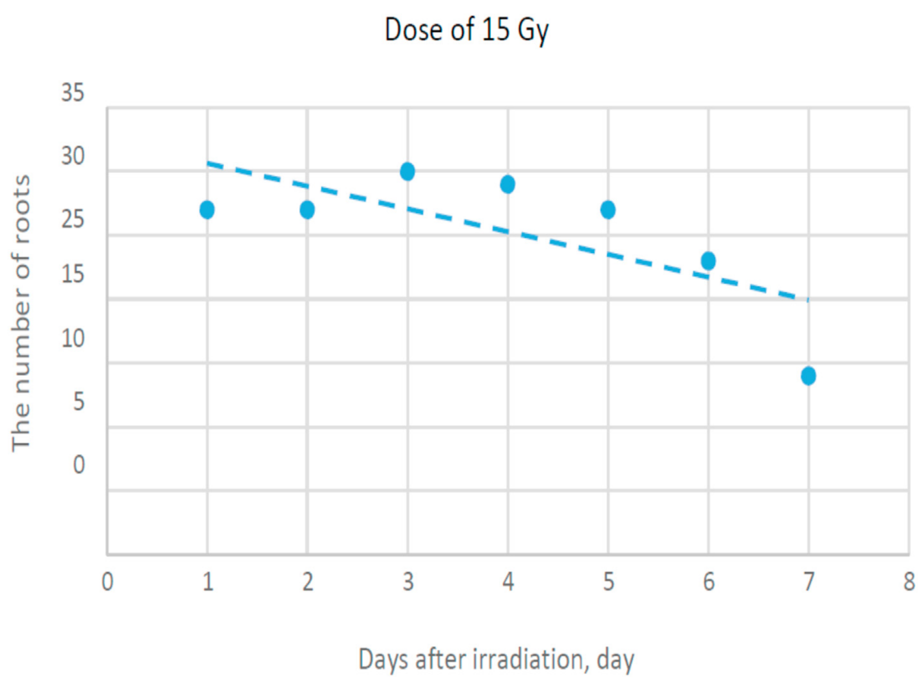

Fig. 6. Change in the number of roots depending on the dose of gamma –radiation of 15 Gy for 12 days after irradiation.

3.3. Absorbed dose and Tritium Concentration Dependency on Lemna Minor

From Fig. 10 as a result, the dose from tritium according to the formula: $D = (0.1257) \cdot A^{0.585} \cdot$ An increase in the tritium activity causes a decrease of the specific growth rate of the Lemna minor duckweed. The highest level of the specific growth rate was obtained at the control level, i.e., the absence of gamma irradiation dose. It indicates that as the quantity of the beta radiation dose increases in Lemna minor duckweed, a higher quantity of gamma radiation will be required to cause the same effect in the specific growth rate of Lemna minor duckweed. To illustrate, to produce the same amount of specific growth rate of 0.031 at gamma radiation dose of ^{60}Co at 10 Gy, a higher quantity of tritium concentration at 1774 Bq/l is required. Hence, there is a linear relationship between the absorbed dose of gamma radiation and the tritium concentration activity on Lemna Minor, with squared value of correlation coefficient of 0.9579.

The chemistry of water and aqueous solutions is very different after irradiation with ^{3}H beta particles and high-energy ^{60}Co gamma rays. The higher the linear energy transfer (LET) of the medium for ^{3}H beta particles compared to high-energy electrons or

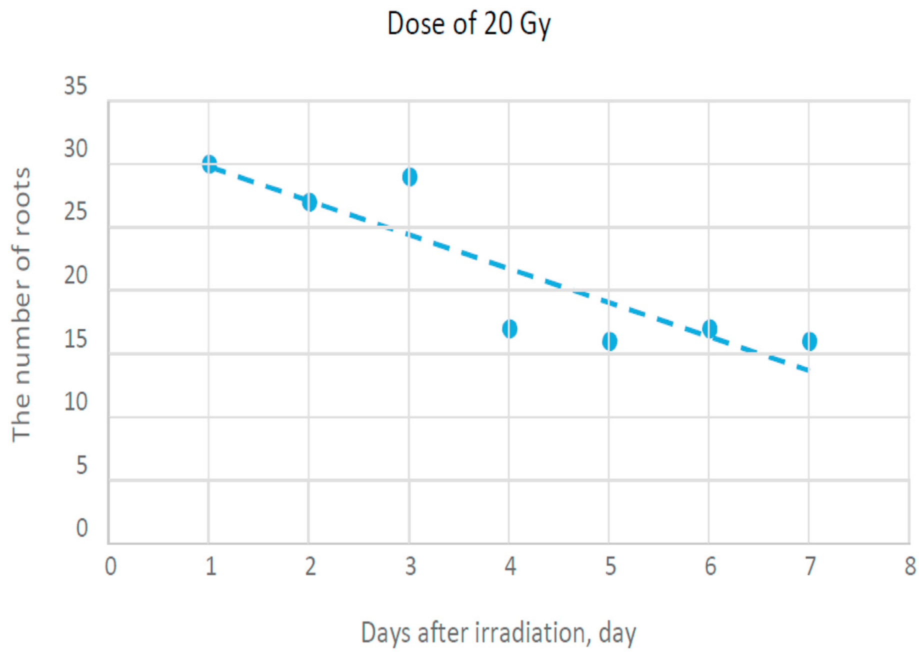

Fig. 7. Change in the number of roots depending on the dose of gamma –radiation of 20 Gy for 12 days after irradiation.

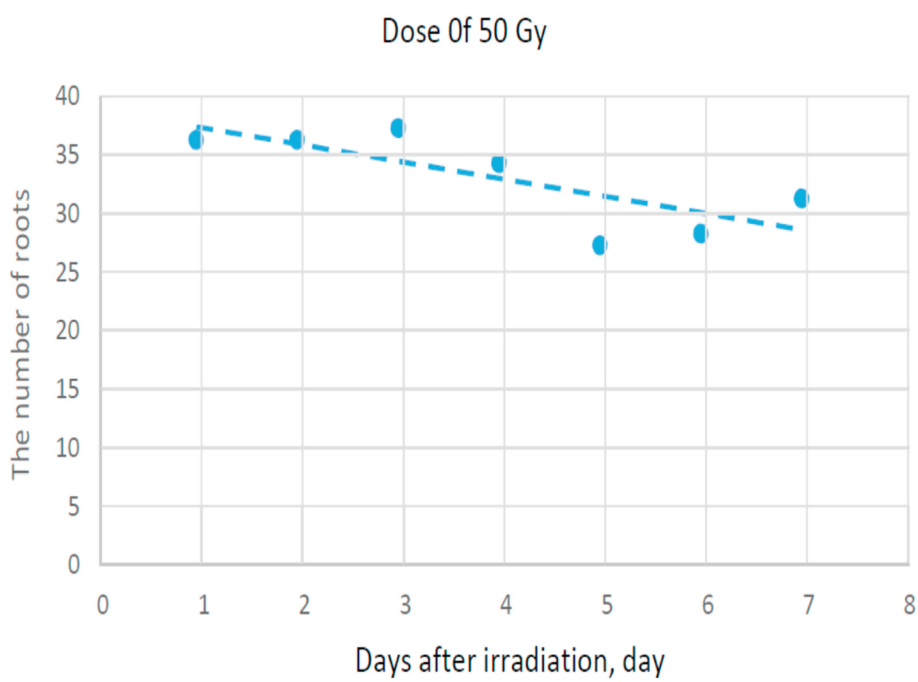

Fig. 8. Change in the number of roots depending on the dose of gamma –radiation of 50 Gy for 12 days after irradiation.

^{60}Co gamma rays leads to an increased local concentration of reactants [14].

It also validates the claim by Matsumura from is research work [7], which says, "the higher the dosage of beta- and gamma-rays, the more delayed were emergence and growth of seedlings, and the lower was survival rate, the height of mature plants, and fertility. The relation between the inhibition of seedling growth and dosage of beta- and gamma radiations coincides roughly with that between the decrease of survival rate or fertility and dosage". After the estimation of the coefficient of proportionality between gamma dose and tritium activity dependencies on the specific growth rate of Lemna minor duckweed, the result shows a linear increase of dose with increasing activity.

4. Conclusion

The statistical interpolation result obtained from a laboratory experiment was utilized to model the dependence of tritium activity and gamma dose on the average specific growth rate of Lemna minor. The specific growth rate of Lemna minor depends on

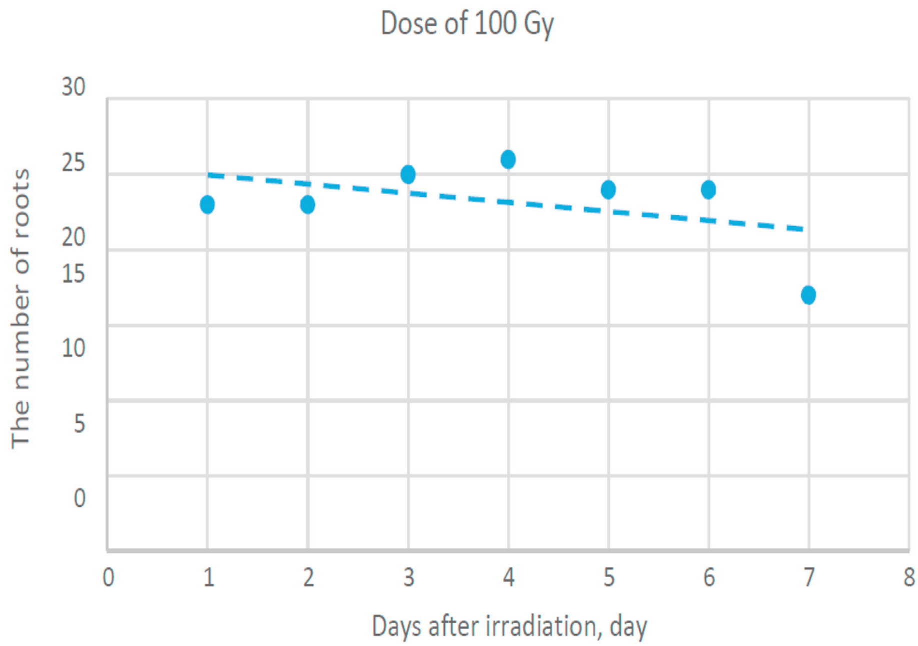

Fig. 9. Change in the number of roots depending on the dose of gamma –radiation of 100 Gy for 12 days after irradiation.

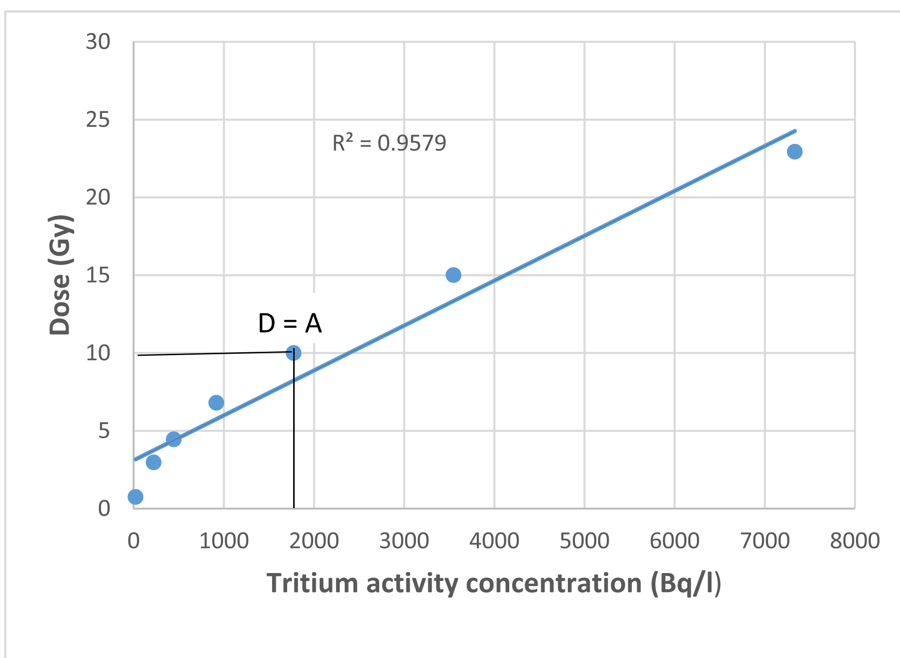

Fig. 10. The linear relationship of the Absorbed Dose and Tritium Concentration Dependency on Lemna Minor.

the amount of tritium concentration activity present in water, the quantity of gamma radiation dose exposure, and the number of days in which the Lemna minor has been exposed to tritium concentration and gamma dose. The beta radiation and gamma radiation dependencies on the specific growth rate shows a proportionality curve; it implies that the higher tritium activity, the higher the dosage of gamma radiation, and the more delayed the growth of seedlings and the lower the survival rate, the height of mature plants, and productivity. The relation between the inhibition of the Lemna minor seedling growth and gamma and beta radiation dosage agrees roughly with that between the decrease of survival rate or fertility and dosage. More advanced studies are needed for the estimation of tritium concentration activity and gamma dose radiation from the environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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