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Ionizing radiation long-term impact on biota in water bodies with diffferent levels radioactive contamination in belarusian sector of chernobyl nuclear accident zone

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Abstract. In 1986–2009 the dynamics of the radionuclide contamination of the Pripyat inlet ànd Perstok Lake and their biota as well as the set of biological test criteria reflecting impact of ionizing radiation on pulmonate mollusk *Lymnaea stagnalis* from these reservoirs have been investigated. The γ-activity of biota after the highest level in 1987 (up to 1100 kBq kg^{-1} wet mass) quickly decreasing. In 2005–2008 the activities of biota in the Pripyat inlet dropped to the natural level, but in the Perstok Lake they remained a rather high – up to 4000 Bq kg^{-1}. Alongside the increase of activity of transuranium α-isotope ^{241}Àm in bottom sediments of the Perstok Lake has been observed since 2006. In the *L. stagnalis* population in the Perstok Lake the obvious negative effect of chronic impact of radiation was noted. The share of cells with the micronuclei has considerably grown there if compared with the mollusks from the Pripyat inlet. The negative effects mentioned above did not influenced seriously on the viability on organism and populations levels. So, the embryonic mortality in both populations is low and they are capable to maintain sufficient level of reproduction despite the chronic radioactive impact.

1. INTRODUCTION

The Chernobyl disaster has led to meaningful radioactive pollution of adjoining regions of the Pripyat River drainage area and their biota. During the period after the disaster pollution of the Pripyat River and its inflows in the Chernobyl disaster zone (ChZ) has considerably decreased, however the pollution of stagnant inundated reservoirs even now remains very high. It has been revealed the versatility of the responses to chronic influence of rather low levels of ionizing radiation on aquatic biota in ChZ. The deterioration of organisms' physiological condition, the violations of their development and accumulation of the latent mutations can be shown up in the succeeding generation [1]. On the other hand, the process of radioadaptation expressing in increase of population radioresistance was noted in a number of species [2]. Therefore continuation of radioecological researches in aquatic ecosystems within ChZ will allow not only the trace of long-term dynamics of their radioactive pollution, but also give a more complete estimation of the effects of chronic impact of the radiating factor on their biota and trends of radioadaptive processes in it.

2. MATERIAL AND METHODS

In 1986–2008 we evaluated the activity of the most abundant radionuclides in bottom deposits, water with seston and in dominating biota species in model reservoirs of the Belarusian sector of ChZ – the Pripyat inlet, stagnant Perstok Lake and Borshchevka waterlogging. They are located at the distance of 14–20 km from Chernobyl atomic station. In 1991–2008 the set of biological test criteria reflecting impact of ionizing radiation on pulmonate mollusk *Lymnaea stagnalis* from the Pryipyat inlet and Perstok Lake have been defined too. Among them there are the structure of haemolymph cell population and the levels of its cell's cytogenetic damages, the relative weight of haemolymph, the survival of embryos, growth and reproduction of individuals, radioresistance of populations.

2.1 The characteristics of the model reservoirs

The Pripyat inlet is located on the left river bank, near to the settled out village Krasnoselye. The river Pripyat in lower reaches is characterized by a considerable (to 5–7 m) seasonal and perennial fluctuations of water level. The maximum annual water stage is usually observed during the spring flooding, and the minimum – late in summer or early in autumn.

The Perstok Lake is located in the Pripyat land, 2 km from the river channel, near the settled out village Masany. The lake is stagnant, only sometimes the Pripyat waters penetrate in it during very high floodings. The lake feeds mainly from the flood and ground waters, and atmospheric precipitation. In droughty 2002 the length of lake consisted of 1450 m, the maximum width – 98 m, the average width – 62 m; the area of the water surface – 0.9 $км^2$, and the maximum depth – 1.6 m.

Borshchevka waterlogging was generated in 1991 as a result of capacity reduction of hydromeliorative channel system in ChZ. The waterlogging begins near the settled out village Borshchevka and is extended to the southeast for 17 km. It is outlined by the isohyps with absolute height of about 110 m. In 2000 its average area was about 1100 ha. The maximum depths that reaches 1.5–2.1 m in low-water, are limited to ameliorative channels; in the flooded falls of the relief depth it does not exceed 0.5–1.1 m. During the floods the water level raises slightly – up to 0.8 m.

2.2 Radioactivity of the aquatic ecosystems

The tests of the bottom sediments, waters (together with seston) and the dominating taxa of biota – (gastropods *L. stagnalis and Viviparus spp.*, macrophytes and fishes) have been taken in model reservoirs in the summer period. The activity of the mass technogenic radionuclides (^{90}Sr, ^{137}Cs) in the selected samples was measured in the β/γ-spectrometer MKC AT-1315 (manufacture of Belarus), other radionuclides (^{40}K, ^{134}Cs, ^{154}Eu, ^{154}Eu, ^{241}Am) – in the β/γ-spectrometer Canberra.

2.3 Test criteria of the impact of the ionizing radiation

Adult snails with shell height of 25–35 mm were caught from the Pripyat inlet and Perstok Lake in the summer – autumn periods of 2002–2007 and transferred to the laborator*y*. There they were kept in groups at densities of 4–5 ind l^{-1} to obtain egg masses. They were cultivated individually at 20–22°C up to complete releases all newborns from them. For each egg mass the numbers of alive and dead embryos were recorded. Newborns were reared at the same densities during entire life span (7–8 months), they total fecundity of per life span were defined.

Hemolymph was obtained from adults captured from both reservoirs after 2–3 weeks of rearing in laboratory. The structure of cell population of hemolymph (percentage of different types of cells), the presence in these cells cytogenetic damages – with micronuclei, condensation and fragmentation of nuclear chromatin, pyknosis, budding (apoptotic bodies) has been determined too.

Radioresistance was defined for adults caught in summer period from the both reservoirs. Groups of 20–25 individuals after 2–3 week acclimations to laboratory conditions were subjected to acute irradiation of γ-rays in sub lethal dose of 500 Gy (period of irradiation – 15 min). The terms of elimination of each individual were recorded.

3. RESULTS

3.1 Perennial dynamics of radioactivity of bottom deposits and aquatic biota

The basic core of technogenic radionuclides that had come into the reservoirs had concentrated in the bottom sediments, and a considerable smaller one – in the water column and in the biota [3]. After

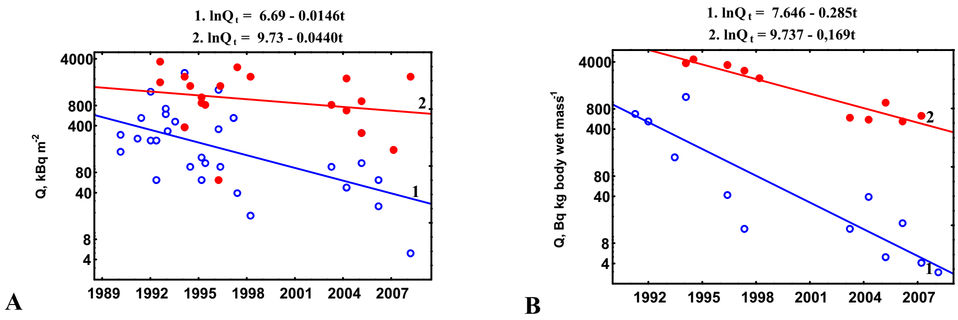

Figure 1. Long-term dynamics of γ-activity of bottom sediments (**A**) and *Lymnaea stagnalis*(**B**). 1. The Pripyat inlet; 2. The Perstok Lake. On the top – the equations characterizing decline of γ-activity (Q) with the time after disaster (t, years, counting from 01.05.1986).

Table 1. Radioactivity of bottom sediments (kBq m^{-2}) and water (Bq l^{-1}) in model reservoirs of ChZ in 2008.

Radio-nuclide	Borshchevka waterlogging		Perstok Lake	
	Bottom sediments	Water	Bottom sediments	Water
^{40}K	110.0 ± 26.6	-*	76.0 ± 40.8	-*
^{134}Cs	0.30 ± 0.05	-*	1.07 ± 0.24	-*
^{137}Cs	780.9 ± 45.2	0,54 ± 1,51	2149.0 ± 12.0	6.0
^{90}Sr	22.3 ± 12.7	4,77 ± 12,02	1016.6 ± 20.8	7.0
^{154} Eu	2.20 ± 0.75	-*	8.59 ± 0.64	-*
^{155} Eu	0.83 ± 0.29	-*	2.56 ± 0.79	-*
^{241} Am	12.8 ± 1.50	-*	40.0 ± 4.55	-*

*Lover than sensitivity of device.

1990 their irregular perennial fluctuations are distinctly seen on the back of the obvious tendency of the decrease of the γ-activity of bottom sediments in the Pripyat inlet and the Perstok Lake (Fig. 1A). They are caused by numerous formidably considered factors, the main of which is the changes of the atmospheric precipitation quantity which wash off radionuclides in reservoirs from adjoining territories and define the size of the reservoirs' annual drain and the position of their coastal line, the parity between the free and connected fractions of radionuclides in reservoirs, the physiological condition of organisms that influences the radionuclides' accumulation coefficients, etc.

Nowadays the core radionuclides in bottom sediments of the Perstok Lake and Borschevka waterlogging are ^{90}Sr and ^{137}Cs with a period of half-decay of about 32 yr. Short-living γ-isotopes ^{134}Cs, ^{154}Eu and ^{155}Eu (periods of half-decay less than 5 yr.) are registrating in small quantities only in the bottom sediments (Table 1). Alongside an essential growth of activity of long-living α-isotope ^{241}Am (period of half-decay – 458 yr.) has been observed in this period of time (Table 1).

Significant differences of perennial dynamics of the sediment γ-activity in the both reservoirs are caused by the specificity of their hydrological regime. The decay of γ-isotopes in the inlet, characterized by a high flowage, is added by their constant washing away from the bottom sediments by the water flow. However in stagnant Perstok Lake decay of radio nuclides is compensated in certain degree by their constant carrying over to reservoir from coastal territories by melted and rain waters.

For both reservoirs of the ChZ significant positive correlation between the ^{137}Cs activity in mollusks and bottom sediments was established [2]. Therefore the character of mollusks' γ-activity dynamics in them in 1986–2008 is similar to that for bottom sediments (Fig. 1A).

The maximum values of γ-activity of the zoobenthos in the Pripyat inlet was noted in the summer of 1987, i.e. about a year after the disaster. It is caused by the fact that the life span of the majority of zoobenthos species does not exceed 1–2 years, therefore by the end of their lives they could save up a maximum quantity of radionuclides. In 1986–1987 the leeches living in the sludge deposits of reservoirs where the basic share of radionuclides concentrates had the highest γ-activity (up to 1100 kBq kg^{-1} wet mass). The predaceous water insects from the orders Coleoptera and Hemiptera occupying top trophic levels in aquatic ecosystems also had a high γ-activity – up to 300–600 kBq kg^{-1}. The slow-growing large bivalve mollusks were characterized by the lowest values – up to 35 kBq kg^{-1}.

After 1991 the rapid decrease γ-activity of biota as the result of decay of short-living "Chernonyl" isotopes have been observed (Fig. 1B; Table 2) If 15 radionuclides were detected in mollusks in 1986, then in 1987 – 7, in 1988 – 5 and in 1989 – 4 (^{134}Cs, ^{137}Cs, ^{144}Ce and ^{106}Ru). Since 2002 only γ-isotope ^{137}Cs, β–isotope ^{90}Sr (Table 2) are registrating confidently.

3.2 Cytogenetic inquires

On the molecular-genetic level one of the major test criteria of the influence of genotoxic factors, including ionizing radiation, is the finding of the micronuclei in the cells of living beings [4]. The proportion of cells with micronuclei in hemolymph cells of *L. stagnalis* was established to be higher in the most contaminated Perstok Lake (Table 3). Not all the DNA damages induced by radioactive irradiation are realized in cytogenetic lesion and formation of micronuclei. This often leads to the process of activation of the genetically determined program for elimination of affected cells (apoptosis), which can be observed at all stages of cell cycle. Apoptosis prevents from the accumulation of mutations in cell generations that is considered as adaptation to adverse factors. The level of apoptosis in *L. stagnalis* in both reservoirs depends on season and year. However there was not determined certain dependence between the share of cells with the signs of apoptosis and the level of radioactive pollution of reservoirs and molluscs.

3.3 The structure of hemolymph cell population

The cells of hemolymph are performing the function of immune system in *L. stagnalis*. They, exhibiting high proliferation and heterogeneity allow estimating the organism response to damaging factors by the changes of levels of their differentiation, mortality and cytogenetic injuries (see above). There is a single type of cells in hemolymph of pulmonates named as amoebocytes due to their specific shape. They can be divided into granulocytes and agranulocytes. The latters form the bulk of cell population, but granulocytes as functionally active cells are distinguishing by extreme sensitivity to changes of the physiological state of organism under impact of adverse factors.

In 2002–2006 the proportion of dead cells in *L. stagnalis* from the Pripyat inlet was irregularly changed within 7–10%, the those of granulocytes amounted up to 32–44%, and those for young cells – up to 5–13%. On the contrary, the proportion of young cells in mollusks from the Perstok Lake did not exceed 2–4% and the share of dead cells increased to 21–26%. These distinctions are determined by the levels of radioactive pollution of both water bodies and *L. stagnalis* populations inhabiting them (Fig. 1). If in 2002–2006 the activity of bottom sediments and biota decreased practically to the natural level in the Pripyat inlet, then in the Perstok Lake it remained on a very high level.

In laboratory progeny from both populations, the proportion of dead cells has sharply decreased to 2–4%, and those for young cells has increased to 8% in the individuals from the Perstok Lake and to 13% – from the Pripyat inlet. Obviously, negative impact of radiation on immune system of *L. stagnalis* from reservoirs of ChZ remains even in their first laboratory generation.

Table 2. Radioactivity in biota (Bq kg^{-1} wet mass) in model reservoirs of ChZ in 2005–2008.

Reservoir	Radio nuclide	*L. stagnalis*	*Viviparus spp.*	Macrophites	Fishes
2005					
Pripyat inlet	^{90}Sr	168	125	62.8	56.6–89.3
	^{137}Cs	–*	15,5	54,1	257–318
Perstok Lake	^{90}Sr	17 665	13 844	2191	862
	^{137}Cs	979	1638	2634	6681
Borshchevka waterlogging	^{90}Sr	–**	–**	–**	–**
	^{137}Cs	–**	–**	–**	392–2772
2006					
Pripyat inlet	^{90}Sr	5.5	125–140,0	19.2	–**
	^{137}Cs	16.0	22.0–33.0	89.1	–**
Perstok Lake	^{90}Sr	16 272	295.6	< 687	–**
	^{137}Cs	518	22,5	456–8592	–*
Borshchevka waterlogging	^{90}Sr	–**	–**	–**	2497–2960
	^{137}Cs	–**	–**	–**	976–3387
2007					
Pripyat inlet	^{90}Sr	–**	–**	–**	–**
	^{137}Cs	–**	–**	–**	23–1855
Perstok Lake	^{90}Sr	18938	–**	9200–11148	–**
	^{137}Cs	625	–**	1224–4055	365–20013
Borshchevka waterlogging	^{90}Sr	–**	–**	–**	–**
	^{137}Cs	–**	–**	–**	835–6550
2008					
Pripyat inlet	^{90}Sr	–*	169,7	52,4	–**
	^{137}Cs	–*	17,3	6,3	–**
Perstok Lake	^{90}Sr	–**	–**	2841–3900	–**
	^{137}Cs	–**	–**	493–812	–**
	^{241}Am	–**	–**	1,0–3,7	–**
Borshchevka waterlogging	^{90}Sr	–**	–**	–**	–**
	^{137}Cs	–**	–**	–**	1068–2974

*Lover than sensitivity of device; ** Not determined.

3.4 Life history parameters

The average survival of embryos produced by mollusks from the Perstok Lake in laboratory (86.7%) was higher than in those from Pripyat inlet (74.4%). The average duration of embryonic development of mollusks from Pripyat inlet (20.4 ± 4.8 days) and the Perstok Lake (19.3 ± 4.9 days) was differed insufficiently (P > 0.05). The progeny from these egg masses also had a high level of viability. The average age of maturation in both populations was about 150 days. The total fecundity per life span

Table 3. Levels of cells with cytogenetic injuries in hemolymph cells of *L. stagnalis*.

Reservoir	Animals examined	Cells examined	Cells with micronuclei, %	Cells with the signs of apoptosis, %
Summer 2002				
Pripyat inlet	3	3300	0,94 ± 0,17	1,42 ± 1,02
Perstok Lake	4	4000	1,72 ± 0,21	0,68 ± 0,32
Autumn 2002				
Pripyat inlet	5	5200	1,54 ± 0,30	0,67 ± 0,24
Summer 2003				
Pripyat inlet	10	7309	0,24 ± 0 ,06	0,04 ± 0,02
Perstok Lake	10	7208	0,75 ± 0,19	0,28 ± 0,08
Summer 2007				
Perstok Lake	5	798	1,61 ± 0,42	1,25 ± 0,76

Table 4. The duration of survival (PS) in *L. stagnalis* after acute irradiation of γ-rays in dose of 500 Gy.

Reservoir, year	γ-activity of *L. stagnalis*, Bq kg^{-1}	PS_{min}– $PS_{max,}$, days	Average PS ± σ, days
Pripyat inlet, 1991	651	1–24	9.2 ± 7.05
Pripyat inlet, 1992	502	1–15	9.9 ± 4.50
Pripyat inlet, 2002	29.3	1–13	10.3 ± 2.67
Pripyat inlet, 2003	7.2	1–19	4.1 ± 4.26
Perstok Lake, 2002	1560	4–54	31.7 ± 15.4
Perstok Lake, 2003	585	1–50	11.9 ± 10.6
Perstok Lake, 2007	625	1–31	4.35 ± 9.23

in the progeny from specimen of the Perstok Lake (250 ± 49 eggs ind^{-1}) was rather higher, than from those of the Pripyat inlet (182 ± 89 eggs ind^{-1}), however these differences were doubtful ($P > 0.05$).

3.5 Radioresistance

One of the most important integral indices of radioadaptation is the average period of survival (PS) of specimen at additional acute doses of ionizing radiation. Assessment of perennial variations of PS allows determining the trends of radioadaptation processes at population level. Considerable individual variability on PS in both *L. stagnalis* populations has been established (Table 4). In 2002 average PS for population from the Perstok Lake (31.7 days) was significantly higher ($P < 0.05$) than those for population from the Pripyat inlet for the whole period of investigations. The individuals from both water bodies may be divided into two groups according to the terms of elimination after irradiation. The first group has already eliminated at the first 1–4 days. The second one (so-called "radio mutants") eliminated noticeable later and the individuals with intermediate terms of elimination were practically absent. The proportion of "radio mutants" in investigated groups amounted to 30–60%. Furthermore in 1992–2002 some "radiomutants" from both populations after irradiation were able to produce eggs from which quite viable newborns emerged. Evidently, prolonged impact of ionizing radiation on *L. stagnalis* (more than 10 generations) in the Perstok Lake have led to essential increase of their populations' radioresistance. However afterwards the essential decline of average PS for both populations have been noted in parallel with the decrease of γ-activity of mollusks. The decrease of PS has been caused firstly by the reduction percentage of "radio mutants" below to 30–35%.

4. DISCUSSION

During the period of 1986–2006 absorbed dose from external γ-radiation in *L. stagnalis* in the most contaminated Perstok Lake decreased from 4,2 to $0,2\,\mu$Gy h^{-1}. Obviously, in considerably less polluted Pripyat inlet the absorbed dose for this species is even lower. These values are much lower than screening level for aquatic biota (algae, macrophites, zoo benthos), which according to recommendations of IAEA (1992), UNSCEAR (1996) and ICRP (2007) lie within the limits 4–40 μGy h^{-1}. Project PROTECT recommends for generic screening level for aquatic biota equals to 10 μGy h^{-1} [5]. Nevertheless, in *L. stagnalis* population in the high contaminated Perstok Lake the obvious negative signs of long-term influence of radiation were noted. Firstly they are resulted in the lesions of the DNA structure that leads to increasing of the share of dead cells in hemolymph cells' population and the shares of cells with the interphase destruction and cytogenetic aberrations (micronuclei) has considerably grown if compared with the population from the Pripyat inlet.

Nevertheless, these negative effects do not influence much the viability of organisms and reproduction of populations. It might be caused by the destruction of a considerable part of the damaged cells by means of apoptosis. So, the mortality of embryos (the most vulnerable life span stage) in both populations is low, and the fecundity of individuals is high enough. Obviously, both populations at the conditions of chronic impact of radiation factor have kept high level of reproduction. Therefore the principal cause of elimination in aquatic biota in water bodies within the ChZ is not radioactive pollution of reservoirs, but the seasonal fluctuations of water level that leads to periodic drainage of the coastal zones of reservoirs where the highest number of zoo benthos is located. In parallel with the decrease of radioactive pollution of reservoirs of the ChZ, both investigated populations have lost their higher radio resistance that they got earlier.

By this time radioactive pollution of bottom sediments and biota in the Pripyat inlet has decreased practically to natural level. In contrary, the Perstok Lake and its biota remain the most polluted in Belarusian sector of the ChZ and these raised levels will be observed there during the next 30–40 years. Alongside with the activity decrease of ^{90}Sr and ^{137}Cs in the ChZ the activity of α-isotope ^{241}Am – the descendant of ^{239}Pu, thrown out from the destroyed reactor, has grown. ^{241}Am, as α-isotope, has a considerably higher damaging effect, than ^{90}Sr and ^{137}Cs, especially at internal irradiation. Since 2008 ^{241}Am has been noticed in the reservoirs within the ChZ, whence it will inevitably pass in biota. According to forecasts, ^{241}Am activity will increase in the ChZ approximately till 2070, and after 20–30 years it will be the principal dose making factor for its biota. It makes possible the approach of the third, after "iodine" and "caesium", stage of radioactive pollution evolution of the ChZ. Thereupon the further radio ecological researches on aquatic ecosystems in the ChZ may be of great interest.

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