CONSEQUENCES OF RADIOACTIVE AND CHEMICAL CONTAMINATION FOR NATURAL POPULATION OF EARTHWORMS

[1]Rybak A.V., [1]Maystrenko T.A., [2]Geras'kin S.A.

[1] Institute of Biology of Komi Science Centre of Ural Branch of RAS, Russia
[2] Russian Institutes of Agricultural Radiology and Agroecology, Russia

ABSTRACT: *The reduced reproduction of A. caliginosaand population densityof lumbricidswas shown at the polluted site. The survival of A. caliginosa after acute γ-irradiation (2270 Gy) was higher at contaminated site ($LD_{50/10}$) than reference site ($LD_{50/6}$).The data obtained indicate the adaptation of inhabiting contaminated site earthworm population to the harsh environmental conditions.*

Key words: radionuclides, earthworms, survival, reproduction, population density

INTRODUCTION

Human industrial activities have left behind a legacy of ecosystems strongly impacted by a wide range of contaminants, including radionuclides. As a rule, the anthropogenic contamination is characterized by the presence of different types of pollutants in the environment. All pollutants, including radionuclides and heavy metals, are deposited in the soil that leads to changes in the state of natural populations of soil fauna. In particular, earthworms are excellent bioindicators of soil pollution because of low migration activity; close contact with contaminated environment.Due to such a set of properties earthworms was chosen as one of the basic reference species in the modern system of the radiation protection of the environment (Larsson, 2008). It is known that radioactive and chemical contamination of soil leads to a wide range of biological effects at different levels of organization of biosystems from molecules and cells to populations and communities. The data about changes in survival (Nakamori et al., 2009; Burgos et al., 2005), reproduction (Krivolutsky, 1987; Anderson et al., 2013), and population density of earthworms (Krivolutskii, 1994) under conditions of radioactive or chemical contamination are widely presented in the literature.

Neverthelessthe combined action of radiation and chemical factors on natural populations of earthworms is poorly studied (Lourenço et al., 2011a; 2011b; 2012; 2013; Mrdakovic Popic et al., 2012). The assessment of chronic radiation or chemical exposure to natural populations, as well as the identification of molecular and cellular mechanisms that allow organisms to sustainably exist in conditions of chronic low-dose exposure to genotoxicants is important challenge (Brechignac et al., 2016; Mothersill et al., 2007).

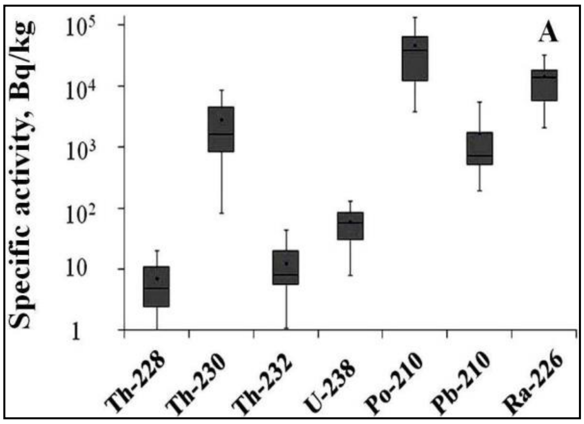
Therefore, the aim of our study was to investigate the consequences of chronic radiation and chemical exposure for earthworms populations.

MATERIALS AND METHODS

Study area

The earthworms Aporrectodea caliginosa were collected at experimental site near Vodny settlement (Russia) where previously the extraction and production of radium was carried out. This siteis characterized by increasedspecific activities of radionuclides (^{238}U,^{232}Th, ^{230}Th, ^{228}Th,^{226}Ra, ^{210}Po, ^{210}Pb) and concentrations of heavy metals (Cu, Pb, Cd, Zn, Ni, etc.) and As. The ambient dose rate in the air varied from 2.5 to 10.5 μSv/h. The reference sitewas chosen at area without radioactive and chemical contamination of soil. The value of γ–radiation dose rate at reference site was 0.08-0.12 μSv/h.The sites are characterized by similar natural and climatic conditions. The detailed characteristic of sites was presented in our previous articles (Geras'kin et al., 2007; Kaneva et al., 2015; Belykh et al., 2015; Maystrenko et al., 2018; Rybak et al., 2020).

The results of soil radiochemical analysis were presented in figure1. Moreover the total indicator of soil contamination (Z_c) with heavy metals was 108 (dangerous level) and 2 (acceptable level) at contaminated and reference sites, respectively (Revich, 1982).



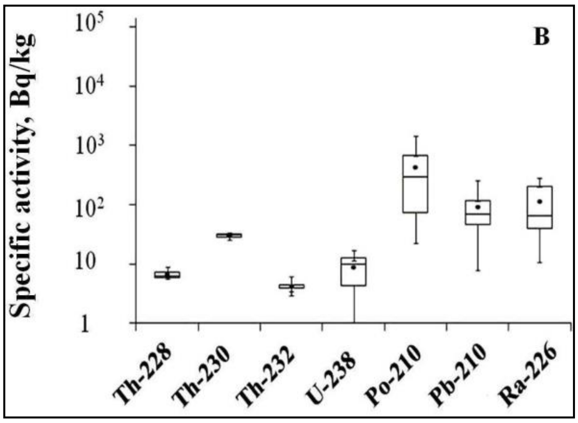


Figure 1 – The box plots of specific activities of radionuclides in soil of contaminated (A) and reference (B) sites

Cultivation of earthworms

The cultivation of earthworms was carried out in plastic containers (14×9×10 cm^3) with 1 kg of wet artificial soil, prepared in laboratory by mixing of natural sand, clay soil (Komi Republic) and neutralized peat (pH = 5.2–6.0) in proportion of 5:4:1, respectively. The acidity of artificial soil was measured before experiment: pH (KCl) = 7.70 ± 0.02. The humidity of soil was 26 ± 1 %(GOST 28268-89). The humidity and food availability were monitored during the experiment.

The specific activities of radionuclides in artificial soil were following: ^{226}Ra - 7.7 ± 1.2, ^{238}U - 94.5 ± 18.9, ^{228}Th - 0.52 ± 0.16, ^{230}Th - 0.38 ± 0.11, ^{232}Th - 0.18 ± 0.05, ^{210}Po - 11.9 ± 11.8, ^{210}Pb - 10.2 ± 1.5 Bg/kg (d.w.). The concentrations of heavy metals and As in artificial soil:Cu - 7 ± 2, Pb - 4 ± 1, Cd - 0.12 ± 0.06, Zn - 20 ± 4, Ni - 16 ± 6, Co - 5 ± 2, Mn - 220 ± 70, Cr - 13 ± 3, Ba - 33 ± 10, As - 3 ± 1, Hg - 0.008 ± 0.004 mg/kg (d.w.). The physical and chemical characteristics of artificial soil: P - 250 ± 80 mg/kg, N - 0.06 ± 0.02 %, C - 2.4 ± 0.4 %, organic matter - 2.8 ± 0.6 %, exchangeable Ca - 5.5 ± 0.4 mmol/100 g, exchangeable Mg - 3.6 ± 0.2 mmol/100 g, K_2O - 105 ±13 mg/kg, Fe_2O_3 - 0.18 ± 0.03 %, Al_2O_3 - 0.14 ± 0.03 %, N-NO_3- - 1.0 ± 0.2 mg/kg, HCO_3 - 170 ± 40 mg/kg, Cl- - 6.4 ± 0.5 mg/kg.

Survival

The survival of adult earthworms A. caliginosa was evaluated after γ-irradiation at 2270 Gy («Issledovatel», USSR, ^{137}Cs, $P_γ$ = 0.7 Gy/min). The distance between the source and biological object was 5 cm.Fiveearthworms were placed into plastic containers with 18 g artificial soil for γ-irradiation (2 replications). After irradiationinvertebrates were cultured inplastic containers with 1 kg of wet artificial soil. Non-irradiated individuals, collected from both sites, were used as control sample (1 replication).The number of surviving earthworms was registered during 30 days after irradiation. Then the cumulative survival curves were constructed using Kaplan–Meier estimator in the program STATISTICA 6.0. The significance of the differences was assessed bythe Mantel Cox test.

Reproduction

Two adult earthworms were placed at plastic container with artificial substrate (m = 1 kg). The estimation was provided for each site using 8-9 replications during 3 months of experiment. The number of produced cocoons was counted 2-3 times a week. The reproductive capacity of earthworms A. caliginosa was evaluated by distribution of the cocoons occurrence frequency per day of parameter registration in all replicationsfor each site (0 – no cocoons, 1-5 – one-five cocoons) and morphometric parameters of coccons (length, diameter, volume). The significance of the differences was assessed with the application of the Mann-Whitney test.

The volume of cocoon (ellipsoid) was calculated using followingequation:

$$V = \frac{4}{3}\pi ab^2,$$

where V – volume of cocoon (cm^3), a – length of coccon (cm), b – diameter ofcoccon (cm). Population density

The soil monoliths of 0.25×0.25×0.25 m^{3}size from contaminated and reference sites were used to determine the population density of earthworms of Lumbricidae family. The total number of earthworms, selected by hand from soil, was converted into a unit of "individuals per m^2" (ind./m^2). The analysis of relationship between population density and physical and chemical factors was assessed by correlation and regression analysis (Pearson test and linear regression).

RESULTS

Survival after additional acuteγ-irradiation

The long-term living of earthworms A. caliginosa in soil containing increased concentrations of chemicals including naturally occurring radionuclides, could led to pre-adaptation to ionizing radiation exposure and the development of adaptive traitsthat ensure the population existence in conditions of exposure to higher doses of ionizing radiation. Therefore, the survival of A. caliginosa earthworms was studied after acute γ-irradiation with dose 2270 Gy during 30 days of experiment.

The survival of non-irradiated individuals was 100%. The analysis of survival after acute γ-irradiation showed there were no statistically significant differences in resistance of earthworms from polluted and reference sites to high dose of ionizing radiation (Fig. 2A).

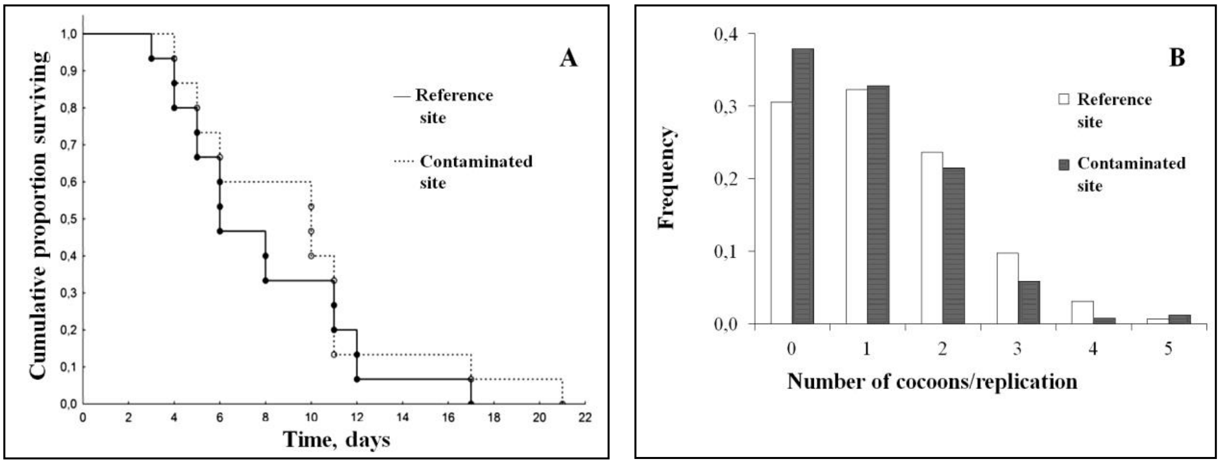


Figure 2 - Survival after γ-irradiation with dose of 2270 Gy (A) and distribution of the cocoons occurrence frequency (B) of A. caliginosa

Despite the lack of evident differences between cumulative survival curves of earthworms from both sites after γ-irradiation, higher survival rate of individuals from polluted site compared to invertebrates from reference site was identified – 21 and 17 days, respectively. After γ-irradiation with dose of 2270 Gy the mortality of 50 % of A. caliginosa from reference site was recorded at 6 day ($LD_{50/6}$), from polluted site – at 10 day of experiment ($LD_{50/10}$). This fact could also indicate the possible adaptation of A. caliginosa earthworms from contaminated site to high doses of ionizing radiation.

Reproduction and population density

Radioactive contamination could inhibit the reproduction, growth and development of organisms. Therefore, we supposed that long-term habitation of A. caliginosa earthworms in soil under conditions of radioactive pollution in the presence of high concentrations of heavy metals can lead to decrease of reproductive potential of organisms as a protective mechanism to ensure a stable existence of population.

The reproductive capacity of earthworms A. caliginosa from reference site, assessed by distribution of the cocoons occurrence frequency in replication during experiment, was significantly higher than the value for invertebrates from contaminated site (p = 0.019) (Fig. 2B). It should be notedthat the absence of cocoons was observed with the greatest frequency in earthworms from polluted site compared to individuals from reference site. But the production of 2-4 cocoons was higher in soil invertebrates from reference site (Fig. 2B). This result indicates the suppression of reproductive function of A. caliginosafrom polluted site, which characterized by high activity concentrations radionuclides and concentrations of heavy metals in soil.

We also assume that the volume of cocoon could be linked with number of individuals in cocoon and the adaptation to living in harsh environment. However, the volumes of cocoons on polluted and reference sites did not significantly differ: 0.2153±0.0017 и 0.2147±0.0019 cm^3.

The population density of earthworms in polluted site was significantly lower than the value for reference site (p< 0.001): 51 ± 38 and 178 ± 72 ind./m^2, respectively. In addition, it was shown that the population density of Lumbricidae family linearly depends on pHof soil (r = –0.67; r^2 = 0.4509, F = 22.9919, p = 0.000049) and absorbed dose rate (r = –0.47; r^2 = 0.22, F = 7.9058, p = 0.0089).Therefore, the increasing values of these factors lead to reducing of population density (Fig. 3). It is important to notethat the influence of other physical and chemical factors (concentrations of heavy metals and main cations and anions) on that biological parameter was not detected.

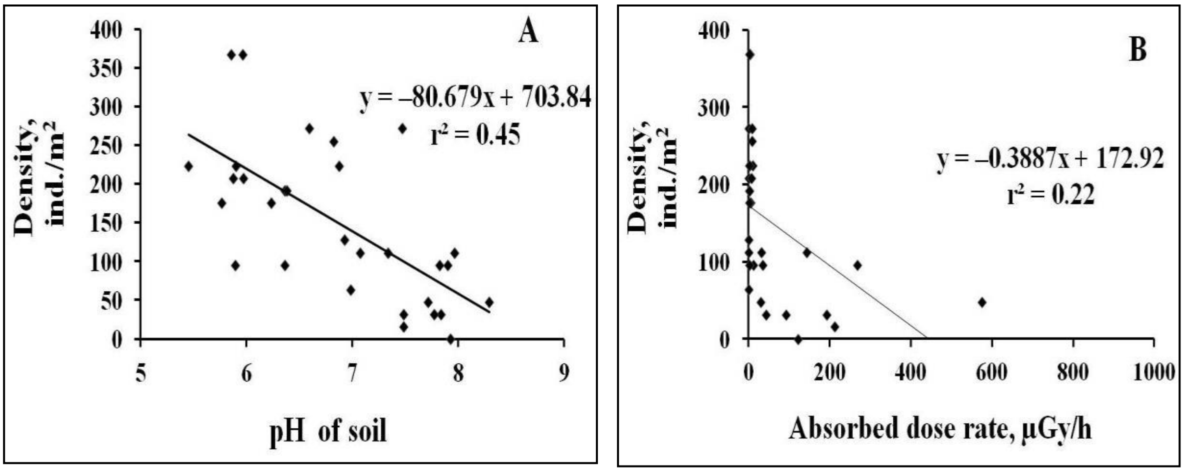


Figure 3- The relationship between population density and pH of soil (A) and absorbed dose rate (B)

DISSCUTION

The maintenance of reproductive capacity of organisms on reduced level is one of the mechanisms of acclimation and population stability to adverse environmental conditions (Sowmithra et al., 2015). In our work the decrease of reproductive potential of A. caliginosa earthworms inhabited contaminated site corresponds to results of investigation (Lourenço et al., 2012). The authors showed that soil contaminated by high concentration of radionuclides (^{234,235,238}U = 163–3696 Bq/kg, ^{230,232}Th = 324–10682 Bq/kg, ^{226}Ra = 1506 Bq/kg, ^{210}Pb = 2318 Bq/kg) and heavy metals (Al = 26440 mg/kg, Ba = 8.5 mg/kg, Be = 50 mg/kg, Cd = 2.6 mg/kg, Fe = 13383 mg/kg, Mn = 3711 mg/kg, Ni = 91.4 mg/kg, Pb = 9.7 mg/kg, Sr = 19.3 mg/kg, U = 215.7 mg/kg, Zn = 511.7 mg/kg) causes negative biological effects associated with the suppression of the growth and reproductive ability of earthworms E. fetida. Ultimately, the reduced reproduction (number, weight, size of cocoons, and number of offspring) is crucial in the survival and stability of the population as a whole (Nakamori et al., 2009; Sowmithra et al., 2015). The increased sensitivity of reproduction capacity compared to other parameters can be explained by the fact that mutations appear immediately after genotoxic exposure and lead to the death of germ cells, thereby reducing the population density of viable individuals in the F1 generation (Nakamori et al., 2009). In addition, the suppression of reproductive function under chronic radioactive and/or chemical contamination also has been shown in studies (Krivolutsky, 1987; Spurgeon et al., 1996; Hertel-Aas et al., 2007; Lourenço et al., 2012; Anderson et al., 2013). This process is usually characterized by a decrease in number of cocoons and juveniles or their complete absence, slowdown in the processes of achieving maturation of juveniles, changes in the size and hatchability of cocoons, etc. In our investigation we identified a statistically significant decrease in the reproductive capacity of A. caliginosa from the contaminated by radionuclides and heavy metals site according to the frequency distribution of a cocoons number ($p = 0.019$). At that, the absence of cocoons was most frequent in individuals from contaminated site, while the number of cocoons from 2 to 4 was more often recorded in earthworms from reference group.

Not only chronic (as was shown above), but also acute radiation exposure causes changes in number, morphological parameters and hatchability of earthworms's cocoons. For example, the acute γ-irradiation in doses of 1-60 Gy did not lead to influence of cocoon number of E. fetidaearthworms: the number of cocoons varied from 34 to 49 during the experiment (Sowmithra et al., 2015). Nevertheless, the doses of 20-60 Gy induced the decrease of cocoon weight, which became the reason of statistically significant decline of hatchlings number of individuals compared to values for non-irradiated earthworms. Therefore, we expected that combined action of chronic irradiation and chemical pollution on A. caliginosa earthworms from natural population can reduce its reproductive function through volume of cocoons. However, the differences between volumes of A. caliginosa's cocoons were not found: 0.2153 ± 0.0017 и 0.2147 ± 0.0019 cm^{3}from contaminated and reference sites, respectively. Perhaps, the given result can be explained by the fact that acute irradiation has a more negative impact on the reproductive function than chronic exposure as, for example, it is shown in investigation (Hertal-Aas et al., 2007), where the reproductive function of E. fetida earthworms after acute γ-irradiation turned out to be much more sensitive to radiation stress than after chronic exposure at the same doses. Also morphological changes of cocoons is less sensitive characteristic of organism compared to the level of DNA damages (Kaneva et al., 2015), therefore, much larger data samples are required to record statistically significant differences.

On the contrary, the intensification of reproductive process (increase in the proportion of females and their fertility) of Alexandromys oeconomus from natural populations, lived for 100 generations under conditions of chronic low-intensity exposure of radionuclides on the territory near settlement Vodny, was revealed that is also one of the types of adaptation to stressful conditions (Ermakova et al., 2020). The relationship between reproductive function of earthworms A. caliginosaand population density under radioactive and chemical contamination of soil was shown in our study. The population density of earthworms was significantly ($p< 0.05$) lower in soil of contaminated site than in reference site with suppressed reproductive function of A. caliginosa earthworms. The same changes in populations density was shown in invertebrates populations inhabiting territories affected by an underground nuclear explosion in the Northern Urals, and areas with increased natural radioactivity (Kolesnikova, Taskaeva, 2005; Kolesnikova et al., 2015).It should be remembered that not only technogenic but also natural factors affect the natural populations of living organisms. Natural factors often play animportant role enhancing the effect of anthropogenic factors. We have shown that soil radioactive contamination and pH value has a negative influence on population density of Lumbricidae family. As an example, the study of impact of copper smelters emissions in the Sverdlovsk region on population density of earthworms showed that there was the effect of “Lumbricidae desert” on the plots near smelters - complete absence of earthworms. The author explains this result by the synergistic effect of increased concentrations of heavy metals (Cu, Cd, Pb) and reduced soil acidity (pH 4.7–5.2) (Vorobeichik, 1998).

The survival is less sensitive biomarker of technogenic exposure in invertebrates than reproduction. It is confirmed by the fact that the values of effective doses (ED_{10} and ED_{50}) are tens to hundreds times lower than the half-lethal doses (LD_{50}). After acute γ-irradiation LD_{50} was 825 Gy for E. fetida earthworms, but effective doses to reduce reproduction were much lower LD_{50} (ED_{10} = 3.3 Gy, ED_{50} = 11.1 Gy) (Nakamori et al., 2009). Generally, the half-lethal doses of ionizing radiation for different species of Lumbricidae family vary from 20 to 1600 Gy and depend on their stage of ontogenesis (cocoons, juveniles, adults) (Table 1). The most sensitive stage of ontogenesis is a stage of cocoon. The half-lethal doses of irradiation for cocoons and juveniles can be 10% of LD_{50} for adults (Krivolutskii, 1988). For instance, LD_{50} for cocoons of Dendrobaena octaedra was 20 Gy (Table 1).

The analysis of survival of A. caliginosa adult earthworms after acute γ-irradiation did not allow confirming the results of preliminary laboratory experiments that the dose ~ 2000 Gy is $LD_{50/30}$, that is, the causing mortality of 50% of individuals during 30 days. However, it should be mentionedthat dose 2270 Gy is half-lethal for A. caliginosa earthworms from reference site after 6 days of exposure, for invertebrates from polluted site – 10 days (Fig. 2A, Table 1). A. caliginosa earthworms from contaminated site showed neither statistically significant increased sensitivity nor resistance for this indicator after additional γ-irradiation. However, we observe a tendency to greater survival after additional acute exposure in invertebrates previously lived in conditions of chronic radiation exposure (Fig. 2A).

CONCLUTION

Finally, soil radioactive and chemical contamination induces the biological effects in earthworms of family Lumbricidae such as reduction of reproduction of individuals and associated population density, but also can lead to higher survival rate of A. caliginosa after additional acute γ-irradiation with dose of 2270 Gy. This dose was $LD_{50/10}$ for earthworms from contaminated by radionuclides and heavy metals site and $LD_{50/6}$ – from reference site. This may be sign of adaptation for higher doses of ionizing radiation invertebrates that previously lived under soil contamination with radionuclides and heavy metals.

This study was performed within the frameworks of thebudget federal program of the Russian Federation (the state task number is AAAA-A18-118011190102-7).

Table 1 – LD_{50} for earthworms after irradiation

Species	LD_{50}, Gy	Time of survival	Publications
Eisenia fetida	20	30 days	Viktorov, 1999
	600	30 days	
	650	4 weeks	Suzuki, Egami, 1983
	825	2 weeks	Nakamorietal., 2009
Lumbricus rubellus	100	30 days	Viktorov, 1999
	<600	30 days	
	>1024 (β)	30 days	
Lumbricus terrestris	1000	35 days	Hancock, 1962
	678	30 days	Reichle et al., 1972
	600	5 days	Viktorov, 1989
Aporrectodea caliginosa Kursk population Ural population	 1120 ± 30 1340 ± 60	30 days	Viktorov, 1989
Aporrectodea rosea	570 ± 60	30 days	Viktorov, 1989
Dendrobaena octaedra (cocoons)	20	30 days	Viktorov, 1989
Earthworms*	600–1600	30 days	Krivolutskii, 1988; Krivolutsky, 1994
Earthworms *	~1000	–	Krivolutskii, 1988
Aporrectodea caliginosa Reference site Contaminated site	2270	 6 days 10 days	our study

* – the species were not identified.

REFERENCES

Anderson C.J., Kille P., Lawlor A.J. Life–history effects of arsenic toxicity in clades of the earthworm Lumbricus rubellus // Environmental Pollution. – 2013. – V. 172. – P. 200–207.

Belykh E.S., Maystrenko T.A., Gruzdev B.I., Vakhrusheva O.M., Kaneva A.V., Trapeznikov A.V., Zainullin V.G. Species diversity of plant communities from territories anthropogenically contaminated with natural radionuclides // Russ J Ecol. – 2015. – V. 46. – P. 425–430.

Brechignac F., Oughton D., Mays C. et al. Addressing ecological effects of radiation on populations and ecosystems to improve protection of the environment against radiation: Agreed statements from a Consensus Symposium // J. Environ. Radioact. . – 2016. – V. 158–159. – P. 21–29.

Burgos M.G.,Winters C., Stürzenbaum S.R. et al. Cu and Cd effects on the earthworm Lumbricus rubellus in laboratory: multivariate statistical analysis on relationships between exposure, biomarkers and ecologically relevant parameters // Environ. Sci. Technol. – 2005. – V. 39. – P. 1757–1763.

Viktorov A.G. Ecology, karyology, radiosensitivity of earthworm races with different ploidy. PhD thesis: 03.00.16. – Moscow. – 1989. – 23 p. (in Russian)

Viktorov A.G. Radiosensitivity and radiopathology of earthworms, their use in bioindication of radioactive contamination. – Moscow: Nauka. – 1999. – P. 213–217. (in Russian)

Vorobeichik E.L. Population of earthworms (Lumbricidae) in forests of the middle Urals in conditions of pollution by discharge from copper works // Russian Journal of Ecology. – 1998. – V. 29. – N. 2. – P. 85-91.

Krivolutskii D.A., Tikhomirov F.A., Fedorov E.A., Pokarzhevskii A.D., Taskaev A.I. The effect of ionizing radiation on biogeocenosis. – Moscow: Nauka. – 1988. – 240 p. (in Russian)

Ermakova O.V., Bashlykova L.A., Raskosha O.V. et al. Effects of chronic low–intensity irradiation on reproductive parameters of the root vole (Alexandromys oeconomus): Responses of parents and offspring // Russ. J. Ecol. – 2020. – V. 51. – P. 242–249.

Geras'kin S.A., Evseeva T.I., Belykh E.S., Majstrenko T.A., Michalik B., Taskaev A.I. Effects on non-human species inhabiting areas with enhanced level of natural radioactivity in the north of Russia: a review / J. Environmental Radioactivity. – 2007. – V. 94. – P. 151-182.

GOST 28268-89 Soils. Methods of determination of moisture, maximum hygroscopic moisture and moisture of steady plant fading. – M.: Standartinform. – 2008. – 8 p.

Hancock R.L. Lethal doses of irradiation for Lumbricus // Life Sciences. – 1962. – V. 1. – Iss. 11. – P. 625–628.

Hertel-AasT., Oughton D.H., Jaworska A., Bjerke H., Salbu B., Brunborg G. Effects of chronic gamma irradiation on reproduction in the earthworm Eisenia fetida (Oligochaeta) // Radiation research. – 2007. – V. 168. – P. 515–526.

KanevaA.V., Belykh, E.S., Maystrenko, T.A., Shadrin, D.M., Pylina, Y.I., Velegzhaninov, I.O., 2015. The level of DNA damage and DNA reparation rate in cells of earthworms sampled from natural populations for numerous generations inhabited territories with anthropogenically enhanced levels of radionuclides in soil / Radiation Biology. Radioecology.– V. 55. – P. 24 –34. (in Russian)

Kolesnikova A.A., Kudrin A.A., Konakova T.N., Taskaeva A.A. Composition and abundance of soil fauna in an area with an increased level of radioactivity (Komi Republic, settlement Vodny) / Radiation Biology. Radioecology. – 2015. – V. 55. – N. 3. – P. 282-292. (in Russian)

Kolesnikova A.A., Taskaeva A.A., Krivolutskii D.A., Taskaev A.I. Condition of the Soil Fauna near the Epicenter of an Underground Nuclear Explosion in the Northern Urals / Russian Journal of Ecology. – 2005. – V. 36. – N. 3. – P. 150-157.

Krivolutsky D.A. Radiation ecology of soil animals / Biol Fertil Soils. – 1987. – V. 3. – P. 51–55.

Krivolutskii D.A. Soil fauna in ecological control.–M.: Nauka. – 1994. – 272 p. (in Russian)

Larsson C.-M.An overview of the ERICA Integrated Approach to the assessmentand management of environmental risks from ionising contaminants / J. Environ. Radioact. – 2008. – V. 99. – P. 1364–1370.

Lourenço J.I., Pereira R.O., Silva A.C. et al. Genotoxic endpoints in the earthworms sub–lethal assay to evaluate natural soils contaminated by metals and radionuclides // Journal of Hazardous Materials. – 2011a. – V. 186. – P. 788–795.

Lourenço J., Silva A., Carvalho F. et al. Histopathological changes in the earthworm Eisenia andrei associated with the exposure to metals and radionuclides // Chemosphere. – 2011b. – V. 85. – P. 1630–1634.

Lourenço J.,Pereira R., Silva A. et al. Evaluation of the sensitivity of genotoxicity and cytotoxicity endpoints in earthworms exposed in situ to uranium mining wastes // Ecotoxicol. Environ. Saf. – 2012. – V. 75. – P. 46–54.

Lourenço J., Pereira R., Gonçalves F. SSH gene expression profile of Eisenia andrei exposed in situ to a naturally contaminated soil from an abandoned uranium mine // Ecotoxicology and Environmental Safety. – 2013. – V. 88. – P. 16–25.

Maystrenko T., Gruzdev B., Belykh E. et al. The succession of the plant community on a decontaminated radioactive meadow site // J. Environ. Radioact. – 2018. – V. 192. – P. 687–697.

Mothersill C., Salbu B., Heier L.S. et al. Multiple stressor effects of radiation and metals in salmon (Salmo salar) // J. Environ. Radioact. . – 2007. – V. 96. – P. 20–31.

Mrdakovic Popic J., Salbu B., Skipperud L. Ecological transfer of radionuclides and metals to free–living earthworm species in natural habitats rich in NORM // Science of the Total Environment. – 2012. – V. 414. – P. 167–176.

Nakamori T., Kubota Y., Ban'nai T. et al. Effects of acute gamma irradiation on soil invertebrates in laboratory tests // Radioprot. – 2009. – V. 44. – P. 421–424.

Reichle D.E., Witherspoon J.P., Mitchell M.J., Styron C.E. Effects of beta–gamma radiation of earthworms under simulated–fallout conditions // Survival of food crops and viveslock in the event of nuclear war Oak Ridge. – 1972. – P. 527–534. (USAEC Symp. Ser.; Conf–700909).

Revich B.A., Saet Yu.E. Smirnova R.S., Sorokina E.P. Methodological recommendations for the geochemical assessment of pollution of urban areas with chemical elements. – M.: IMGRE. – 1982. – 112 p. (in Russian)

Rybak A.V., Belykh E.S., Maystrenko T.A., Shadrin D.M., Pylina Y.I., Chadin I.F., Velegzhaninov I.O. Genetic analysis in earthworm population from area contaminated with radionuclides and heavy metals // The Science of the Total Environmental. – 2020. – V. 723. – P. 137920.

Sowmithra K., Shetty N.J., Harini B.P, Jha S.K., Chaubey R.C.Effects of acute gamma radiation on the reproductive ability of the earthworm Eisenia fetida // Journal of Environmental Radioactivity. – V. 140.– 2015. – P. 11-15.

Spurgeon D.J., Hopkin S.P. Effects of metal–contaminated soils on the growth, sexual development, and early cocoon production of the earthworm Eisenia fetida, with particular reference to zinc // Ecotoxicology and environmental safety. – 1996. – V. 35. – P. 86 – 95.

Suzuki J., Egami N.J. Mortality of the earthworms Eisenia fetida after γ–irradiation at different stages of their life history // Radiat. Res. – 1983. – V. 24. – P. 209–220.