**International Journal of Ecosystems and Ecology Science (IJEES)** **Vol. 13 (2):** 71-76 (2023)
https://doi.org/10.31407/ijees https://doi.org/10.31407/ijees13.2

# RADIOPROTECTIVE AND SORPTION PROPERTIES OF BEESPINE

**Serhii Razanov[1,2*], Olha Koruniak[3], Andrii Dydiv[1], Tetiana Holubieva[4], Lyudmyla Symochko[5,6], Volodymyr Balkovskyi[1], Oleksiy Alekseev[2], Oksana Vradii[2], Halina Ohorodnichuk[2], Mikhaylo Polishchuk[2], Oleh Kolisnyk[2], Oleksandr Mazur[2], Olena Mazur[2]**

*[1*]Lviv National Environmental University, Dublyany, Lviv region, Ukraine;*
*[2]Vinnytsia National Agrarian University, Vinnytsia, Ukraine;*
*[3]Higher Education Institution "Podillia State University", Kamianets-Podilskyi, Ukraine;*
*[4]National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine;*
*[5]Uzhhorod National University, Uzhhorod, Ukraine;*
*[6]University of Coimbra, Coimbra, Portugal;*

[*]Corresponding Author Razanov Serhii, email: razanovsergej65@gmail.com;

Received December 2022; Accepted January 2023; Published February 2023;

DOI: https://doi.org/10.31407/ijees13.209

## ABSTRACT

The research aimed to study the radioprotective and sorption properties of beespine, which refers to the bodies of bees that die during the winter season. The goal was to expand the range of applications for beespine by exploring its potential as a source of radioprotective and sorption properties. The study found that beespine contains biologically active substances, including melitin, melanin, and sulfur-containing amino acids. These substances exhibited radioprotective and sorption properties, which could potentially be beneficial when ingested by quails as part of their feed mixture. Overall, the research suggests that beespine has the potential to be used in various applications beyond its current use as a waste product from beekeeping. Its radioprotective and sorption properties could have practical uses in areas such as animal feed and potentially even in human medicine. In particular, it was observed an increase in the lifespan of quails from 1.5 to 2.2 times with a content of 2 to 7.5% of beespine in the form of a powdered biological mass in the feed mixture after their fractional local irradiation of gamma rays with a total dose of 90 gr, compared to the poultry, in the diet of which there was no beespine. It was also determined that the removal from the bodies of quails with indigestible feed remains (droppings) of $^{137}$Cs by 6.6 p.p., $^{90}$Sr by 18.7 p.p., Pb by 15.3 p.p. and Cd by 34.6 p.p., respectively, was observed to be higher when adding the beespine (3%) in their diet. It was found a lower level of $^{137}$Cs by 32.7%, Pb by 53.2% and Cd by 20.0% in their muscle tissue, compared to their analogues that were not fed beespine.

**Keywords:** beespine, melanin, melittin, quail, radioprotective and sorption properties, feed mixture, biologically active substances.

## INTRODUCTION

The current ecological situation in some territories of Ukraine and in the whole world is characterized as unsatisfactory due to the high man-made load on the environment (Razanov et al, 2022). It especially concerns the areas with intensive use of industrial production, where the accidents due to various production violations cause a high level of environmental pollution with various toxic substances, including radionuclides and heavy metals (Bonazzola et al, 1997; Borawska et al, 2013; Ji-gen, Lu et al, 2006; Kryvyi et al, 2021). Territories have experienced a high man-made impact on the environment as a result of accidents at nuclear power plants, both in Ukraine and throughout the world. It is known that over 54 different types of accidents at nuclear power plants have been recorded during the period of use of nuclear energy in the world (Rose et al, 2016).

Ukraine suffered a significant impact on the environment in 1986 as a result of the accident at the Chernobyl nuclear power plant, during which a large area was contaminated with radionuclides and heavy metals (Yakymchuk, 2018). Among these toxic substances, $^{137}Cs$, $^{90}Sr$, Pb and Cd represent a high danger due to their intensive migration in the environment (Marciulioniene et al, 2004; Kohanoff et al, 2021). Accumulating in food products, these toxic substances enter living organisms, causing a whole series of changes, which are mainly accompanied by the occurrence of diseases. It is known that $^{137}Cs$ has a decay period of 60 years, accumulates mainly in muscle tissue and is removed from the body within 60 days. This radionuclide can replace potassium in living organisms; therefore it is concentrated mainly in those tissues that require a high content of this element. Such toxic substances as $^{90}Sr$, Pb and Cd accumulate mainly in cartilage and bone tissues, from where they are removed slowly; therefore they cause greater harm to the body.

The decay period of $^{90}Sr$ is 59 years; this isotope can replace Ca in the body, which is accompanied by its displacement from bone tissue and its irradiation. The entry of $^{137}Cs$ and $^{90}Sr$ into living organisms leads to radiation above the norm, which reduces their protective functions, especially during their puberty, which is characterized by rapid cell division and growth of the organism as a whole. It has been determined that as a result of irradiation the cellular link is damaged, its biologically important structures are destroyed, the number of lymphoid cells decreases, which is evidence of immunodeficiency (Horalsky, 2003). It is known that because of the radioactive pollution of the environment, living organisms receive a radiation dose exceeding the annual average from natural sources that leads to a violation of the functions of the central nervous system (Tronko et al, 1995). It has also been experimentally proven that irradiation is accompanied by a violation of metabolic processes, a gradual decrease in the number of leukocytes in the blood, and other negative consequences. It has been proven that Pb is a weak migrant in environmental objects, so it is concentrated in high quantities in soils. As a result of various chemical reactions in the soil environment, Pb can change into an exchangeable form and its migration becomes higher. This toxic substance is a permanent component of body tissues. The entry and accumulation of Pb in living organisms significantly reduces the antioxidant activity of erythrocyte membranes and suppresses the activity of enzymes (Razanov et al, 2022).

Cd, unlike Pb, has a higher migration capacity. This element is slowly removed from the body. A high tendency to accumulate Cd was found in kidneys and liver. This chemical biomicroelement has high toxicity (Rose et al, 2016). It has also been determined that Cd also reduces immunity, negatively affects heredity, damages the kidneys, increases blood pressure, and increases the formation of oxygen free radicals (Solovyov et al, 1997).

Pb and Cd entering living organisms lead to a decrease in immunity, an increase in stress factors and, as a result, a whole series of diseases. The high toxic effect of heavy metals is observed during the period of intensive ontogenesis of organisms. To reduce the negative effects of $^{137}Cs$, $^{90}Sr$, Pb and Cd in practice, a number of measures are used, which are mainly aimed at reducing the level of their digestion in the gastrointestinal tract of living organisms and increasing their resistance to these toxic substances. In particular, substances with sorption and protective properties are widely used. Beekeeping products containing biologically active substances of radioprotective and sorption direction, in particular beespine as their wastes, are of great interest.

The biologically active substances with unique properties are formed from nectar and flower pollen in the body of bees (Al-Kahtani et al, 2020; Cornara et al, 2017; da Costa et al, 2021; da Silva et al, 2016; Estevinho et al, 2008). In particular, royal jelly, its chemical composition and quantity can affect the anatomical features of the structure of insects, as well as their lifespan (Donkersley et al, 2017). It is known that the body of a bee and the body of a bee queen can be formed from the same larva, which depends on the quantity and quality of the royal jelly that the bees feed the larvae with (Ibatullin et al, 2020; Nemo et al, 2021; Nurdin et al, 2021). Grown bees have a lifespan of 1.5 months to 7 months, while the queen bee lives up to 60 months or more. At the same time, it should be noted that grown individuals (queen and bees) have fundamentally different functions and anatomical structure. The body of bees contains a number of important substances, including protein, fat, minerals, vitamins, bee venom, heparin, melanin, etc. (Makarchuk, 2006). Chitin-melanin components and bee venom with their specific characteristics are of special value in the bee body.

Bee venom contains peptides, enzymes, free amino acids, nitrogen, carbon, iron, magnesium, calcium, phosphorus, copper, zinc, sulfur, manganese, iodine, chlorine, amino acids, lipids, nucleic acids and other substances, as well as the polypeptide melanin. Bee chitin contains 20 to 30% of melanin. It contains carbon, nitrogen, hydrogen and other substances. Sulfur was also detected in melanin, the amount of which is up to 12%. Melanin has radioprotective and sorption characteristics. It has been determined that when it enters the body, there is a decrease in the formation of free radicals (under the influence of ionizing radiation, it is recognized as a highly effective antioxidant). Melanin also absorbs Pb, Zn, Cu and other ions. In particular, it is known that one melanin molecule binds 20,730 lead molecules. The bee colony is characterized by intensive growth of its biomass (number of individuals). Up to 200,000 bees are raised in families per year. Due to the short lifespan of bees, from 1.5 months to 7 months, these insects are constantly renewed (growing from eggs) in colonies. And on average, there are from 20,000 to 80,000 individuals in bee families during the year (Samuelson et al, 2020).

The most accessible for study and use is the biomass of bees, which is formed as a result of the death of bees (die-off) during their wintering. During the winter period, a certain part of the bodies of bees that die during this period accumulates at the bottom of the hive, which is called the "beespine". The amount of beespine can reach from 10 to 30%. That is, from 400 to 600 g of beespine is formed per 1 family after each winter period.

These beekeeping wastes contain a high level of protein, which is up to 58.5%. The content of fat is up to 12.7%; the content of carbohydrates is up to 13.5%; the content of minerals is up to 5.36%. In addition, they contain the chitin-melanin complex, bee venom and other biologically active substances. However, information on the preservation of radioprotective and sorption properties of biologically active substances in beespine is insufficient, which does not allow to use effectively these wastes in the national economy, in particular, in agricultural production in conditions of man-made load, where the issue of reducing the negative impact of toxic substances on the animal body and their products is of great urgency.

Therefore, the aim of our research was to study the radioprotective and sorption characteristics of beespine obtained after the winter period, as a result of the natural death of bees in bee colonies.

## MATERIALS AND METHODS

The studies on the radioprotective and sorption properties of beespine (bodies of bees that died during the winter period) were conducted on quails of the Japanese breed. Two groups of quails participated in the research. The first group of quails was a control group, the second was an experimental group, and each group of quails included three subgroups of birds with 50 heads each, which in total amounted to 150 heads in the control group and 150 in the experimental group. The conditions for keeping quails of the control and experimental groups were the same; the difference was only in the composition of feed mixture. The quails of the control group consumed a standard feed mixture (compound feed) during their rearing up to 60 days of age, while their analogues of the experimental group consumed a feed mixture, the part of which was replaced by beespine (2%, 3%, 5%, 7.5%) in the form of crushed biomass. During 40 days the quails of both control and experimental groups of 20 to 60 days of age were exposed to gamma irradiation with fractional local doses, which totaled 90 gr for the entire period. The study of the sorption properties of the biomass of beespine was carried out by evaluating the balance of $^{137}Cs$, $^{90}Sr$, Pb and Cd in the body of quails, which included the intake of these toxic substances with feed, their removal with droppings (indigestible remains of feed) and their retention in the body of the experimental poultry.

The experimental groups of quails were formed according to the principle of analogue groups. The ratio of females to males in each group was 50:50%. The specific activity of $^{137}Cs$ in the feed mixture, the muscle and fat tissue and the droppings of quails were determined by spectrometry. The activity of $^{90}Sr$ was defined by chemical way, the concentration of Pb and Cd by atomic absorption.

## RESULTS AND DISCUSSION

As a result of the conducted research, it was determined that the intake of the powdered mass of beespine after fractional local irradiation of gamma rays with a total dose of 90 gr into the body of quails as part of the main diet contributed to an increase in their lifespan compared to their analogues of the control group, in the diet of which there was no that biomass (Fig.1).

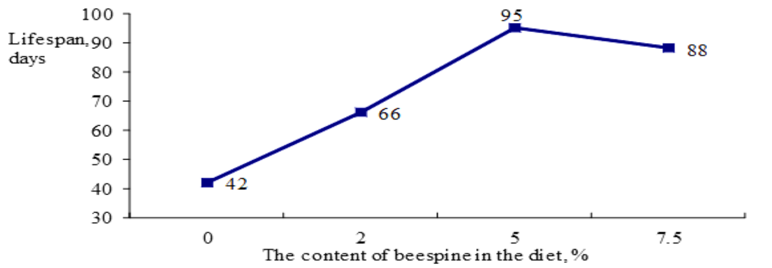


Figure 1. Lifespan of quails after their local-fractional gamma-irradiation, days

In the first experimental group of quails, where the compound feed included 2% of the powdered biomass of beespine, the average lifespan was by 1.5 times (P<0.001) higher compared to their analogues in the control group, the diet of which did not contain that biomass. The increase of the powdered mass of beespine in the diet of quails up to 5% increased their lifespan by 2.2 times (P<0.001), while the increase of up to 7% increased the lifespan by 2.1 times (P<0.001) compared to the poultry of the control group. Thus, the increase in the lifespan of quails due to their gamma irradiation confirms the preservation of radioprotective properties of beespine biomass. Along with that, it was found that feeding quails with compound feed containing beespine biomass contributed to an increase in the removal of $^{137}$Cs from their bodies with droppings (Table1). It was found that $^{137}$Cs was removed with droppings from the body of quails of the experimental group by 6.7 p.p. more, while it retained in the body less, i.e. 38.4% compared to 45.1% (P<0.05) in the poultry of the control group.

Table 1. The balance of $^{137}$Cs and $^{90}$Sr in the body of quails (n=3, M+m).

| Groups of quails | Feed mixture | Intaken with compound feed, Bq | Removed with droppings | | Retained in the body | |
|---|---|---|---|---|---|---|
| | | | Bq | % | Bq | % |
| Balance of 137Cs | | | | | | |
| 1–control | compound feed | 0.1688± 0,043 | 0.0927± 0.0019 | 54.9 | 0.0761± 0.0028 | 45.1 |
| 2-experimental | compound feed + biomass of beespine | 0.1595± 0.021 | 0,0983± 0,0025 | 61.6 | 0.0612± 0.0031 | 38.4 |
| Balance of 90Sr | | | | | | |
| 1–control | compound feed | 0.0032± 0.00017 | 0.0016± 0.0005 | 50.0 | 0.0016± 0.00005 | 50.0 |
| 2-experimental | compound feed + biomass of beespine | 0.0032± 0.0004 | 0.0022± 0.0003 | 68.7 | 0.001± 0.00005 | 31.3 |

With the same total specific activity in the diet of quails of the experimental group, $^{90}$Sr was removed with droppings by 18.7 p.p. Bq more compared to their analogues of the control group. It was accompanied by a lower retention of $^{90}$Sr in the body by 18.7 p.p. (P<0.001). *A similar trend was observed for heavy metals (Pb and Cd) (Table 2).* Pb, which belongs to cumulative poisons, was removed from the body of quails of the experimental group by 15.3 p.p., while Cd by 34.6 p.p. more intensively compared to their analogues in the control group.

Table 2. The balance of Pb and Cd in the body of quails (n=3, M+m).

| Groups of quails | Feed mixture | Intaken with compound feed, mg | Removed with droppings | | Retained in the body | |
|---|---|---|---|---|---|---|
| | | | mg | % | mg | % from intaken |
| Balance of Pb | | | | | | |
| 1–control | compound feed | 0.0591± 0.0031 | 0.0218 ± 0.004 | 36.9 | 0.0373 ± 0.004 | 63.1 |
| 2-experimental | compound feed + biomass of beespine | 0.0598± 0.0012 | 0.0312 ± 0.006 | 52,2 | 0,0286 ± 0,01 | 47.8 |
| Balance of Cd | | | | | | |
| 1–control | compound feed | 0.0030± 0.0004 | 0,0017 ± 0,0002 | 56.7 | 0.0013 ± 0.0001 | 43.3 |
| 2-experimental | compound feed + biomass of beespine | 0.0023± 0.0007 | 0.0021 ± 0.0003 | 91.3 | 0.0002 ± 0.00003 | 8.7 |

47.8% of Pb and 8.7% of Cd, intaken with the feed, were retained in the body of quails of the experimental group. It was less compared to 63.1% and 43.3% in the poultry of the control group (P<0.001).
According to the research data, it was determined that the decrease in the intensity of digesting $^{137}Cs$, Pb and Cd in the gastrointestinal tract of quails had a positive effect on the level of accumulation of these toxic substances in their muscle and fat tissue (Table 3).

Table 3. Specific activity of $^{137}Cs$ and concentration of Pb and Cd in muscle-fat tissue of quails (n=3, M+m).

| Indicators | Groups | |
|---|---|---|
| | Control | Experimental |
| Activity of $^{137}Cs$, Bq/kg | 15.9 ± 0.12 | 10.7 ± 1.0 |
| Pb, mg/kg | 0.440 ± 0.01 | 0.206 ± 0.003 |
| Cd mg/kg | 0.11 ± 0 | 0.088 ± 0.001 |

In particular, the specific activity of $^{137}Cs$ in the muscle-fat tissue of quails of the experimental group was by 32.7% lower compared to their analogues of the control group. The accumulation of Pb and Cd in the muscle-fat tissue of quails of the experimental group was also lower by 53.2% and 20.0%, respectively, compared to the poultry of the control group. At the same time, it should be noted that Cd had a higher migration and deposition activity in the muscle-fat tissue of quails than Pb.

## CONCLUSIONS

The intake of 2 to 7.5% of beespine biomass into the compound feed contributed to an increase in the lifespan of quails by 1.5 to 2.2 times after their fractional-local gamma irradiation with a total dose of 90 gr compared to the poultry, the diet of which did not contain beespine.
3% content of beespine biomass in the composition of feed mixture contributed to an increase in the removal of $^{137}Cs$ by 6.7 p.p., $^{90}Sr$ by 18.7 p.p., Pb by 15.3 p.p. and Cd by 34.6 p.p., respectively, from their body with indigestible feed remains (droppings). It was found a lower level of $^{137}Cs$ by 32.7%, Pb by 53.2% and Cd by 20.0% in their muscle tissue, compared to their analogues of the control group, which were fed compound feed without the biomass of beespine.
That is, according to the research results, it has been determined the preservation of radioprotective and sorption properties of biologically active substances of beespine biomass when entering the body of quails in the composition of feed mixture. It gives the prospect of using these beekeeping wastes in feeding poultry (quails) with the aim of reducing the man-made load of radionuclides and heavy metals on their bodies and production (meat).

## REFERENCES

1. Al-Kahtani, S.N., Taha, E.K., Khan, K.A., Ansari, M.J., Farag, S.A., Shawer, D.M.B., Elnabawy, E.M. 2020. Effect of harvest season on the nutritional value of bee pollen protein. PLoS One. 15(12): e0241393;
2. Bonazzola, G.C., Ropolo, R., Patetta, A., Manino, A. Distribution of fallout radionuclides in soil, plants and honey. 1991. Health Physics. 60(4): 575-577;
3. Borawska, M.H., Kapała, J., Puścion-Jakubik, A., Horembała, J., Markiewicz-Żukowska, R. 2013. Radioactivity of Honeys from Poland After the Fukushima Accident. Bulletin of Environmental Contamination and Toxicology. 91(5): 489-492;
4. Cornara, L., Biagi, M., Xiao, J., Burlando, B. 2017. Therapeutic properties of bioactive compounds from different honeybee products. Front Pharmacol. 8: 412;
5. da Costa, I.F., Toro, M.J.U. 2021. Evaluation of the antioxidant capacity of bioactive compounds and determination of proline in honeys from Pará. Journal of Food Science and Technology. 58(5): 1900-1908. doi: 10.1007/s13197-020-04701-1;
6. da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C., Fett, R. 2016. Honey: Chemical composition, stability and authenticity. Food Chemistry. 196: 309-23;

7. Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C., Power, E.F., Wright, G.A., Wilson, K. 2017. Nutritional composition of honey bee food stores vary with floral composition. Oecologia. 185 (4): 749-761;
8. Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G., Pereira, E. 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food and Chemical Toxicology. 46: 3774–9;
9. Horalsky, L.P. 2003. Histomorphology and morphometric parameters of sheep exposed to radiation exposure. Problems of agricultural radiology: 17 years after the accident at the Chernobyl nuclear power plant. Zhytomyr. 203-205;
10. Ibatullin, I.I., Razanov, S.F., Nedaschkivskyi, V.M. 2020. Influence of soy flour and its processed products on the essential amino acids in the bees body. Ukrainian Journal of Veterinary and Agricultural Sciences. Vol. 3(3): 51-55. doi: 10.32718/ujvas3-3.10;
11. Ji-gen, Lu, Yanjun, Huang, Fang, Li, Lihua, Wang, Shuzhen, Li, Yuanfu, Hsia. The investigation of $^{137}Cs$ and $^{90}Sr$ background radiation levels in soil and plant around Tianwan NPP, China. Journal of Environmental Radioactivity. 2006. № 90. P. 89-99;
12. Kohanoff, J., Correa, A.A., Gribakin, G., Johnston, C., Saul, A. 2021. Radioactive decay of $^{90}Sr$ in cement: a non-equilibrium first-principles investigation. Eur. Phys. J. D. 75, 248. https://doi.org/10.1140/epjd/s10053-021-00202-8;
13. Kryvyi, M., Yushchenko, O., Dikhtiar, O., Lisohurska, D., Stepanenko, V. Quality of helianthus annuus honey obtained in the conditions of radioactive contamination. Food science and technology. 2021. № 15(2). C. 93-102;
14. Makarchuk, Z.V. What are dead bees worth? Apiary. Vol. 2. 2006. P. 26-27;
15. Marciulioniene, D., Montvydiene, D., Kiponas, D., Luksiene, B., Butkus, D. 2004. Toxicity to Tradescantia of technogenic radionuclides and their mixture with heavy metals. Environ Toxicol. 19(4): 346-350. doi:10.1002/tox.20040;
16. Nemo, R., Bacha, K. Microbial quality, physicochemical characteristics, proximate analysis, and antimicrobial activities of honey from Anfilo district. *Food Bioscience*. 2021. № 42. P. 101132
17. Nemtsov, S.V. Chitosan from bee hive is a new bee product. Beekeeping Vol. 5. 2001. P. 17-19;
18. Nurdin, A.S., Saelan, E., Nurdin, I.N., Dustan. Composition and nutritional content of Honey Trigona sp in the Tikep forest management unit (KPH) North Moluccas. IOP Conference Series: Earth and Environmental Science. 2021. Vol. 807. P. 1-6;
19. Razanov, S., Landin, V., Nedashkivskyi, V., Ohorodnichuk, H., Gucol, G., Symochko, L., Komynar, M. Intensity of $^{137}Cs$ transition into nectar-pollinating plants and beekeeping products during reclamation of radioactively contaminated soils. International Journal of Ecosystems and Ecology Science (IJEES). 2022. 12 (1): 291-298. https://doi.org/10.31407/ijees12.134;
20. Razanov, S.F., Ogorodnichuk, G.M., Komynar, M.F. 2021. Effect of soil treatment on $^{137}Cs$ accumulation in flower pollen and in its processing products by honey bees. Agriculture and forestry. 3 (22): 161-173. doi: 10.37128/2707-5826-2021-3-13;
21. Razanov, S.F., Shevchuk, V.V., Komynar, M.F. 2020. Accumulation of $^{137}Cs$ in honey produced by bees from nectar of agricultural honeybear in the northern Polisia. Agriculture and forestry. 19: 148-158.
22. Rose, T., Sweeting, T. 2016. How safe is nuclear power? A statistical study suggests less than expected. Bulletin of the Atomic Scientists. 72(2): 112–115. https://doi.org/10.1080/00963402.2016.1145910;
23. Samuelson, Ash E., Gill Richard, J., Leadbeater, E. Urbanisation is associated with reduced Nosema sp. infection, higher colony strength and higher richness of foraged pollen in honeybees. Apidologie. 2020. № 51. P. 46–762;
24. Seraglio, S.K.T., Silva B., Bergamo G. et al. 2019. An overview of physicochemical characteristics and health-promoting properties of honeydew honey. Food Res Int. 119: 44-66; doi:10.1016/j.foodres.2019.01.028;
25. Solovyov, A.V., Soroka, V.R. Cadmium and cardiovascular diseases. VII Ukrainian Biochemical Congress: abstracts of reports. K.: NAU. 1997. Part III. P. 77-78;
26. Tronko, N.D., Chaban, N.K., Oleinik, V.A., Enshtein E.V. Clinical aspects of the Chernobyl disaster: the endocrine system. Chernobyl disaster. 1995. P. 454-456;
27. Yakymchuk, R. A. 2018. Cytogenetic activity of radionuclide contamination of water reservoirs of the alienation zone of Chornobyl NPP. Regulatory Mechanisms in Biosystems. 9(2): 189-197. doi:10.15421/021828;