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Dynamics of accumulation of heavy metals in *Lissachatina fulica* living in contaminated geographical area of East Kazakhstan

In the article presents the results of a study of the dynamics of the accumulation of heavy metals and macroelements in the body of snails in the vicinity of the Ulbi metallurgical plant in the city of Ust-Kamenogorsk, East Kazakhstan region, where heavy metals are found in large quantities. Experimental work was carried out on 40 *Lissachatina fulica* snails over a period of 48 days. During the work, the snails were fed with soil contaminated with heavy metals and macroelements, and the dynamics of the accumulation of chemical elements in the snails' bodies were studied every week. In the course of studying the accumulation of macroelements and heavy metals, experimental animals were prepared using the method of two-stage euthanasia in compliance with ethical standards. During the study, mass spectrometry (ISP-MS) analyzed the amount of elements Ca, K, Mg, N, V, Co, Pb, As, Sr, Cu, Zn in the bodies of intact animals, control and research groups. A large amount of heavy metals and macroelements was found in the body of the animals of the research group. The amount of heavy metals accumulated in the body of snails increased on the 32nd day and then decreased again (P<0.001). According to research results, it is known that heavy metals accumulate in the body in one month, which means that this environmental situation is one of the problems that requires an urgent solution. Although the accumulation of heavy metals in the soil of geographical area and their amount in the soil were studied, their accumulation in living organisms, including snails, and the dynamics of their accumulation as a result of many years of research have been studied for the first time.

Keywords: geographical area, heavy metals, macroelements, bioindicator, *Lissachatina fulica*, mass spectrometry, environmental problem, accumulation.

Introduction

According to the World Health Organization (WHO), human health is 20 % dependent on environmental conditions. Humanity is constantly exposed to the influence of environmental pollution factors. Radiation and chemical effects were especially harmful for people of ancient society. And now, the process of scientific and technological development has led to the fact that humanity is exposed to pollution factors from artificial sources. This stands for the reason of importance to study the impact of heavy metal production in the fields of medicine, ecology, radiobiology, and nutrition.

According to the data on soil pollution in the vicinity of the Ulba metallurgical plant in Ust-Kamenogorsk, the largest amount of uranium is concentrated in the snow cover located 300 meters north of the Ulba metallurgical plant, with a concentration of 1.7–19.7 mg/kg, the average content of uranium in the soil of Ust-Kamenogorsk was 2.9 mg/kg. The maximum concentration of thorium was determined in the soil located 2 km from the north-west of the Ulba metallurgical plant [1]. In addition, academician of the foundations of Russian medical science Buldakov L.A. presents the positive effects of heavy metals on the body in his articles in the world literature [2]. Furthermore, according to a study by Italian scientists of workers who are under the Daily influence of heavy metals, noticed such changes in the organs of workers as hardening of the carotid arteries. And it is known that these changes lead to diseases such as premature aging and coronary atherosclerosis [3]. In addition, there are works by Chinese scientists who studied the accumulation of heavy metals in the bodies of aquatic animals to study sea pollution. The results of the study showed that the content of CD, Cu and Zn in some samples of gastropods and oysters exceeded the permissible levels established by WHO. Scientists have concluded that due to its unique ability to bioaccumulate, *Rapana venosa* and *Ruditapes philippinarum* can be used as biomonitors to control water pollution by heavy metals [4]. Meanwhile, the study conducted by Yuyu Jia together with her colleagues determined that the values of the metal pollution index (MPI-MLI) for mollusk species were placed in descending order C. fluminea > A. woodiana > S. cancellata > P. eximius > P. Canaliculata, and the need to take into account the environmental parameters associated with the season when using double-framed mollusks as indicators is noted by Indonesian scientists [5, 6].

In addition, Middle Mexican scientists I. Gaso, N. Segovia and O. Morton determined the levels of accumulation of heavy metals in the body of snails by using soil samples and wild *Helix aspersa Muller* snail types as bioindicators of radioactivity and heavy metals in their research. Accordingly, the bioindicator property of snails is directly related to the ability of heavy metals to accumulate in their body, and this phenomenon has been fully proven due to metallothioneine proteins capable of forming bonds with metals in their body [7].

From the mentioned literature, we can determine that the accumulation of heavy metals and macroelements in the substrate in the body of snails has been studied. However, the study of the dynamics of the accumulation of chemical elements aggregated in the soil near the geographical area — Ulba metallurgical plant, which is engaged in the production of heavy metals in the city of Ust-Kamenogorsk, for a certain period of time in the snail's body was not carried out. Accordingly, the purpose of our research was to study the dynamics of the accumulation of heavy elements in the body of *Lissachatina fulica* — a bioindicator of environmental pollution in laboratory conditions. In order to achieve the goal set, the following steps were established:

1. Adaptation of Lissachatina fulica snails to laboratory conditions, determination of the amount of chemical elements in the soil, which is a substrate for them.
2. Study of the dynamics of accumulation of heavy metals in the substrate in the body of experimental animals using a 4-day interval.
3. Study of accumulation of macroelements in Lissachatina fulica organism placed in polluted soil.
4. Correlation study of accumulated heavy metals and macroelements in the body of bioindicators — snails living in the soil of Ust-Kamenogorsk.

Materials and methods

The research work was carried out in the laboratory at the Nazarbayev Intellectual School of Physics and Mathematics in Semey. 40 hermaphrodite *Lissachatina fulica* snails weighing 18±2 grams, 4±0.5 cm long and 2 cm high were taken as the object of study. 5 research objects were placed in each container with a length of 25 cm and a width of 15 cm. In order to adapt to the conditions of Experimental Research, bioobjects were fed for 14 days without soil, with a single sort of cucumbers (Table 1).

Table 1

Division of animals into experimental groups

Research series	Number of animals
1	2
Determination of the amount of chemicals in the body of biobjects before initial research — placement on the substrate — control group	5
Determination of the amount of chemical elements in the first 4 days of the organism of snails placed in the substrate (soil) — “4^{th} day, n=5” group	5
Determination of the content of chemical elements in the body of bioindicators 8 days after placement on the substrate — “8^{th} day, n=5” group	5

Continuation of Table 1.

1	2
Determination of the content of chemical elements in the body of bioindicators 16 days after placement on the substrate — “16th day, n=5” group	5
Determination of the content of chemical elements in the body of bioindicators 32 days after placement on the substrate — “32nd day, n=5” group	5
Determination of the content of chemical elements in the body of bioindicators 40 days after placement on the substrate — “40th day, n=5” group	5
Determination of the amount of chemical elements in 48 days of the organism of bioindicators placed on the substrate — “48th day, n=5” group	5
Determination of the amount of chemical elements in 48 days of the organism of bioindicators not placed on the substrate — “48th day without substrate, n=5” group	5
Total	40

In order to determine the content of heavy metals in the organism of snails, two-stage euthanasia was carried out before the analysis. The progress of euthanasia work was carried out through the research methodology proposed by Cody R. Gilbertson and Jeffrey D. Wyatt scientists in 2016. To determine the composition of the soil and the content of heavy metals and macroelements in the snail's body, mass spectrometry analysis was conducted in the Educational Center “Institute of Radiation Safety and Ecology” in Kurchatov, according to “General requirements for the competence of testing and Calibration Laboratories” to the requirements of Gost ISO/IEC 17025–2009 in the laboratory of elemental analysis accredited in the accreditation system of the Republic of Kazakhstan. During the analysis, the amount of about 14 chemical elements was determined: heavy metal group: V, Co, Ni, Cu, Zn, As, Cd, Pb, Cr, Cu, Sr, Cd, U. Group of macroelements: K, Ca, Mg, Na.

Statistical processing of the material was carried out by calculating the value of ±m, the average error of variational statistics according to the T-student method.

Results

In the course of the work, the dynamics of the accumulation of heavy metals and macroelements in the body of bioindicators was studied, the results of the study are shown in Tables 2–4 and Figures 1–3. According to the first and second graphs, heavy metals are absorbed by the snail's body between 0–4 days, as it can be observed that a given amount is higher ($p<0.001$) than the control group.

Table 2

Content of chemical elements in the substrate, mg/kg

Chemical elements	V	Cr	Co	Ni	Cu	Zn	As
Substrate	83.00±13.00	130.00±20.00	14.00±2.10	40.00±6.30	47.00±7.00	190.00±29.00	10.60±1.70
	Cd	Pb	U	K	Na	Mg	Ca
	1.60±0.44	61.00±9.50	0.50±0.08	9400±1400	1100±160	9300±1400	14.0±2.10

Table 3

The content of heavy metals in the body of snails, mg/kg

Heavy metal	Control group	4th day	8th day	16th day	32nd day	40th day	48th day
V	0.06±0.01	2.60±0.40**	2.00±0.31**	2.00±0.30**	2.70±0.42**	0.81±0.13**	1.40±0.23**
Cr	0.32±0.05	1.40±0.22**	0.74±0.12**	0.60±0.09**	0.05±0.08**	0.03±0.05**	1.10±0.18**
Co	0.12±0.02	3.00±0.47**	1.80±0.27**	2.30±0.36**	2.50±0.38**	1.00±0.16**	2.40±0.37**
Ni	0.27±0.05	5.80±0.90**	2.90±0.45**	3.80±0.59**	3.70±0.57**	2.30±0.36**	4.30±0.68**
Cu	81.0±13.0	32.0±5.00**	36.0±6.00**	41.0±6.00**	48.0±7.00**	34.0±5.00**	59.0±9.00**
Zn	50.0±8.00	130±20.0**	110±17.0**	130±20.0**	180±27.0**	58.0±9.00**	110.0±17.0**
As	2.30±0.36	5.20±0.81**	4.30±0.67**	4.60±0.71**	6.30±0.97**	3.10±0.51**	6.10±0.98**
Cd	0.65±0.11	1.80±0.28**	1.60±0.24**	2.20±0.35**	3.20±0.05**	1.20±0.21**	2.60±0.42**
Pb	0.61±0.10	2.50±0.38**	2.00±0.30**	2.30±0.36**	3.60±0.55**	1.50±0.24**	3.10±0.48**
U	<0.003	0.41±0.06**	0.19±0.03**	0.17±0.03**	0.36±0.03**	<0.003**	0.17±0.03**
** – accuracy of difference according to the control group (p<0.001)							

Table 4

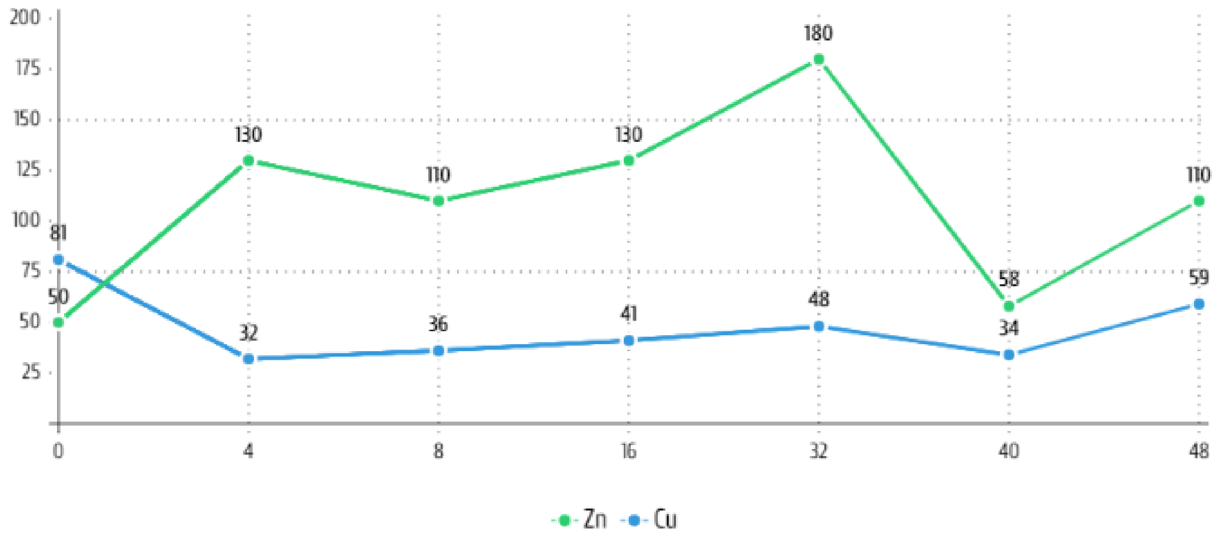
Content of macroelements in the body of snails, mg/kg

Macroelement	Control group	4th day	8th day	16th day	32nd day	40th day	48th day
K	6400±940	6900±1100	5300±790	4800±710	5200±770	3000±440	5000±730
Na	2500±370	3500±590	2600±380	2600±380	2400±400	2000±290	2600±380
Mg	2700±390	3300±580	3400±500	3200±480	3800±560	1900±290	3400±510
Ca	12.0±1.8	25.0±4.2**	8.8±1.3*	9.9±1.5	12.0±1.7	5.3±0.8*	14.0±2.1

* – accuracy of difference according to the control group ($p<0.05$)
** – accuracy of difference according to the control group ($p<0.\ 0.001$)

However, it can be noted that up to the next 8 days, the amount decreases, and the snail's body tries to get rid of excess heavy metals. We find that heavy metals increase again up to the 16th day and accumulate in the snail's body. In the course of the study, according to the indicators of the content of heavy metals in the bodies of the biobject, you can find interesting data: according to the 32nd day, the indicators take the highest values on the graph.

Figure 1. Dynamics of accumulation of heavy metals



One of the most abundant accumulating heavy metals, Zn, showed a significantly higher value, reaching 50.0±8.00 mg/kg at 32 — day ($p<0.001$) to 180±27.0 mg/kg. Later, by the 40th day, the amount of heavy metals decreased again, but the amount of heavy metals was higher than the initial control group. If in the indicators of the control group the content of the element cobalt was 0.12±0.02 mg/kg, then within 40 days it increased by 0.88 mg/kg. However, when it comes to day 48, the size increases again, however, the size takes on a much lower value than on day 32. For example, the cadmium content on day 32 shows a maximum of 3.20±0.05 mg/kg, while on day 48 it is 2.60±0.42 mg/kg.

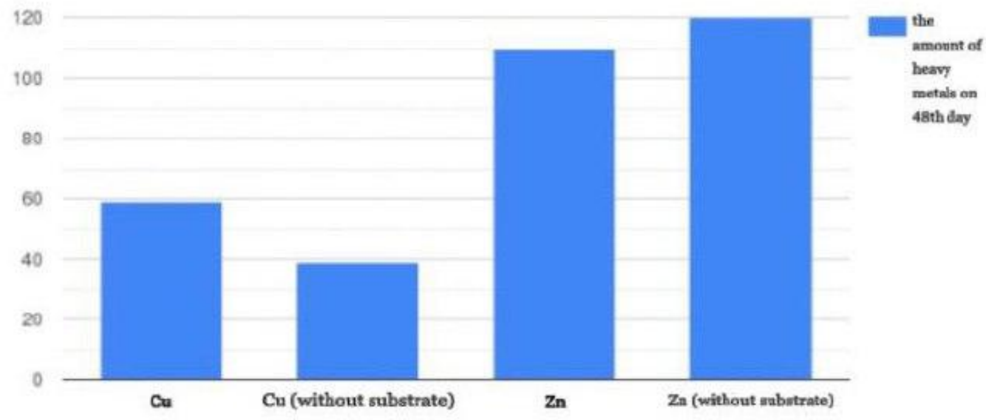


Figure 2. Differences in the dynamics of accumulation of heavy metals in the body of living and non-living snails on the substrate

From the graphs, we can observe a significant difference in the indicators of heavy metals in the body of snails that lived in soil and without soil until the 48th day. The content of heavy metals in the body of snails that lived without a substrate assumes a lower indicator than the amount of heavy metals accumulated in the body of snails that lived in the soil for 48 days. For example, if nickel is 4.30±0.68 mg/kg on Day 48, the nickel content in the body of snails living without a substrate is only 1.00±0.16 mg/kg, which is about 4 times less ($p<0.\ 0.001$).

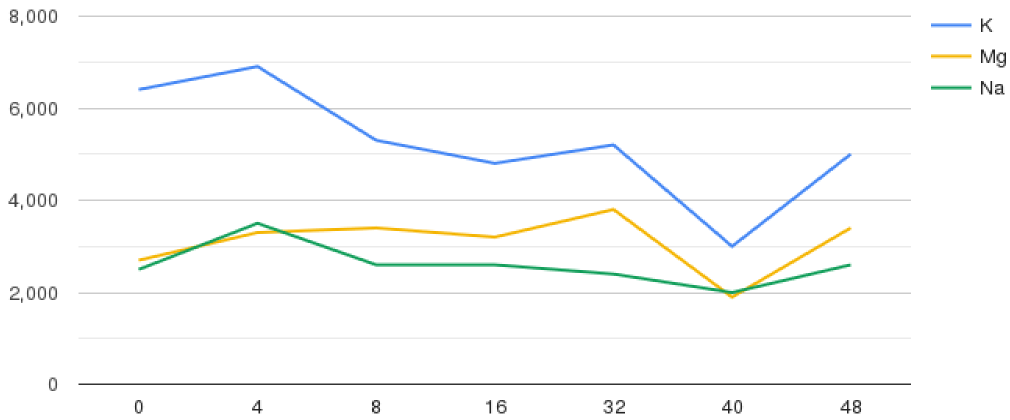


Figure 3. Dynamics of accumulation of macroelements

Indicators of changes in the accumulation of macroelements are similar to the graph of the dynamics of the accumulation of heavy metals (Fig. 3). Macroelements are absorbed by the snail's body between 0–4 days, as it can be seen that a given amount is higher than the control group ($p<0.001$). During the 4th day of the experiment, it can be seen that the content of macroelements in the bodies of the biobject has the highest values. One of the most abundant accumulating macroelements, potassium, increased from 6400±940 mg/kg to 6900±1100 mg/kg on Day 4 ($p<0.05$), showing a significantly higher value. It can be noted that up to the next 8 days, its amount decreases, and the snail's body tries to get rid of the excess accumulated amount of macroelements. It can be seen that the content of macroelements in the snail's body increases in small quantities again at intervals of up to the 16th day and accumulates throughout the biobject. On day 40, the amount of heavy metals decreased again compared to day 32, but the amount of macroelements was lower than the initial control group. In the last 48 days, the amount of macroelements increased again, showing significantly more values than in the 32nd day. If in the indicators of a group of snails that lived in the substrate for 32 days, the content of the most necessary and important element calcium in snail frogs was 12.0 mg/kg, then by the 48th day it increased to 14 mg/kg.

It can be said that during the period of one month, the amount of heavy metal and macroelements decreases at first, and then reaches the greatest values (Fig. 1–3). However, after a period of time of one month, we can observe that the amount of heavy metals continues to decline. For example, if the most dangerous cadmium content in the snail's body under normal conditions is 0.65±0.11 mg/kg, on the 32nd day the maximum value is 3.20 mg/kg. The most interesting thing is that it drops to 2.60 mg/kg between 48 days. And the content of important macroelements in the organisms of snails, on the contrary, increases. For example, the content of calcium, the main forming component of the snail shell, under normal conditions was 12.0±1.8 mg/kg, and during 48 days increased by 14.0±2.1 mg/kg due to a decrease in the content of heavy metals. In addition, if we pay attention to the results of research by world scientists, it has been proven that an excess of some heavy metals, namely Pb and Cd heavy metals, negatively affects the amount of macroelements [3, 7]. For example, an excess of the element cadmium disrupts the absorption of calcium ions, and a lack of iron and calcium elements increases the toxicity of lead. According to the control group, taking into account the intervals of day 32 and day 48, we can conclude that the amount of heavy metals gradually decreases after day 40, and the amount of macroelements tries to accumulate in the body. In the organism of snails, the dynamics of chemical elements increases between the initial interval and then decreases sharply, however, this size indicates a value higher than normal, that is, the control group. For example, a strong relationship between CR and Na elements was established, and a decrease in Cr content between 8–16 and 16–32 days also led to a decrease in sodium in the macroelement dynamics figure. However, after the 40th day, it can be determined that the snail's body is trying to get rid of excess heavy metals by increasing the amount of macroelements.

This corresponds to the “triad” of stress, which was emphasized by G. Selye as the total force of external influences affecting the body and its response to the body, that is, the period of alarm to 32 days and resistance after 40 days.

Conclusions

1. The content of heavy metals in the soil, which is a substrate for Lissachatina fulica snails, had a significantly high value.

2. During a 48-day study of the dynamics of accumulation of heavy metals in the body of experimental animals using a 4-day interval, the amount of heavy metals showed the highest value on the 32nd day.

3. In the study of the accumulation of macroelements in Lissachatina fulica, which was placed in polluted soil, their amount was on the contrary increased.

4. It was found that the amount of heavy metal and macroelements accumulated in the body of bioindicators — snails living in the soil of Ust-Kamenogorsk depends on time. After 40 days in the environment of a new geographical object, the process of adaptation begins in the body of snails.

Research recommendation

By analyzing the dynamics of the accumulation of studied heavy metals, snails can be used as bioindicators in order to determine the level of pollution of the environment under the influence of antropogenic factors. The accumulation of heavy metals reaches its peak on day 32. The results of the research obtained are fundamental research for ecologists, biologists and scientists interested in environmental issues.

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Шығыс Қазақстанның ластанған географиялық объектісінде мекендейтін *Lissachatina fulica* организмінде ауыр металдардың жинақталу динамикасы

Мақалада ауыр металдар аса көп кездесетін Шығыс Қазақстан облысы Өскемен қаласындағы Үлбі металлургиялық зауытының маңайындағы ауыр металдар мен макроэлементтердің ұлулардың ағзасына жинақталу динамикасын зерттеу нәтижелері берілген. Эксперименттік жұмыс 40 *Lissachatina fulica* ұлуларына 48 күн аралығында жүргізілген. Жұмыс барысында ұлулар ауыр металдар мен макроэлементтермен ластанған топырақпен коректендіріліп, апта сайын ұлу ағзасындағы химиялық элементтердің жинақталу динамикасы зерттелген. Макроэлементтер мен ауыр металдардың жинақталуын зерттеу барысында эксперименттік жануарларды препараттау этикалық нормаларды сақтай отырып, екі сатылы эвтаназия әдісі арқылы жүзеге асырылған. Масс-спектрометрия (ISP–MS) әдісімен зерттеу барысында бақылау және зерттеу топтарының бұзылмаған жануарларының денелеріндегі Ca, K, Mg, N, V, Co, Pb, As, Sr, Cu, Zn элементтерінің санын талдады. Зерттеу тобының жануарларының денесінде көптеген ауыр металдар мен макронутриенттер табылды. Ұлулардың денесінде жинақталған ауыр металдардың саны 32-ші күні өсті, содан кейін қайтадан төмендеді (p<0,001). Зерттеу нәтижелері бойынша ауыр металдар денеде бір айда жиналатыны белгілі, яғни бұл экологиялық жағдайда шұғыл шешуді қажет ететін мәселелердің бірі болып саналады. Топырақтағы ауыр металдардың жиналуы және олардың топырақтағы мөлшері зерттелгенімен, олардың тірі ағзада, соның ішінде ұлуларда жиналуы және олардың көпжылдық зерттеулерден жинақталу динамикасы алғаш рет зерттеліп отыр.

Кілт сөздер: географиялық объект, ауыр металдар, макроэлементтер, биоиндикатор, *Lissachatina fulica,* масс-спектрометрия, экологиялық мәселе, аккумуляция.

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Динамика накопления тяжелых металлов у *Lissachatina fulica*, обитающей в загрязненном географическом объекте Восточного Казахстана

В статье представлены результаты изучения динамики накопления тяжелых металлов и макроэлементов в организме улиток в окрестностях Ульбийского металлургического завода города Усть-Каменогорска Восточно-Казахстанской области, где тяжелые металлы встречаются в большом количестве. Экспериментальная работа проводилась на 40 улитках *Lissachatina fulica* в течение 48 дней. В ходе работы улиток кормили почвой, загрязненной тяжелыми металлами и макроэлементами, и каждую неделю изучали динамику накопления химических элементов в организме улиток. В ходе изучения накопления макроэлементов и тяжелых металлов подготовку экспериментальных животных проводили методом двуэтапной эвтаназии с соблюдением этических норм. В ходе исследования методом масс-спектрометрии (ISP–MS) анализировали количество элементов Ca, K, Mg, N, V, Co, Pb, As, Sr, Cu, Zn в телах интактных животных контрольной и исследовательской групп. В организме животных исследовательской группы обнаружено большое количество тяжелых металлов и макроэлементов. Количество тяжелых металлов, накопленных в организме улиток, увеличивалось на 32-е сутки, а затем снова снижалось (P<0,001). По результатам исследований известно, что тяжелые металлы накапливаются в организме за один месяц, а это означает, что данная экологическая ситуация является одной из проблем, требующих безотлагательного решения. Хотя накопление тяжелых металлов в почве и их количество в почве изучены, накопление их в живых организмах, в том числе улиток, и динамика их накопления в результате многолетних исследований изучаются впервые.

Ключевые слова: географический объект, тяжелые металлы, макроэлементы, биоиндикатор, *Lissachatina fulica*, масс-спектрометрия, экологическая проблема, аккумуляция.

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