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Effect of the Heavy Metals on Biological Characteristics of the Pond Snail (*Lymnaea stagnalis* L.) from the Water Bodies with Different Rate of Radionuclide Contamination[†]

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Effect of the heavy metal ions (Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}) of the aquatic environment in different concentration on biological characteristics of the pond snail (*Lymnaea stagnalis* L.) from the radioactively contaminated zone has been studied. Ionizing radiation is an aggravation factor for animals affected by toxic medium. Synergism of radiation and heavy metal ions impact on the reproductive system of the pond snail was noted. At this values of biological parameters were lower than those in the control group.

KEYWORDS: *Lymnaea stagnalis*, heavy metals, radionuclides, reproduction.

Introduction

Issue of joint effect of the ionizing radiation and other chemical agents, particularly heavy metals, has been paid attention, because heavy metals are found in abundance in the water bodies of Ukraine. Study of synergism of the small radiation doses impact and low concentration of chemicals is of special importance. Separately they can hardly reveal their harmful effect on the organism of the aquatic animals. It is also known that radioactive contamination of environment and accumulation of radionuclides in organs and tissues of hydrobionts can cause degenerative changes in gametal cells, disturbance of gametogenesis and in some cases to anomalies of gonads [1, 7]. Embryos can get internal irradiation as a result of radionuclides' penetration into the egg from the female during oogenesis [3]. Impact of radiation can cause morpho-functional disturbances of the embryonic development [16]. Until recently studies of the radioactive impact on biological characteristics were carried out mainly using fishes. So in order to reveal whether radiation complicates pathological process caused by the mollusks poisoning by the heavy metals (studied earlier [8–15]), peculiarities of reproduction under joint effect of radiation and different concentration of the heavy metal ion has been carried out.

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Mollusks dominate in the bottom communities in many water bodies of Ukraine and adjoining regions. These animals are able to accumulate heavy metals and radionuclides, that's why they play a key role in biological migration of the heavy metals and radionuclides in the aquatic ecosystems [17]. So the pond snail *Lymnaea stagnalis* Linnaeus (Gastropoda), the most common representative of hydrofauna of the Central (Zhytomyr) Polissia, was used as test-object in this study.

Material and Method

In study mollusks of the same size were used (average shell height 39.5 ± 1.1 mm), taken from the water body of the region suffered from the Chornobyl accident (the Loznitsa River, tributary of the Uzh River, nearby village Lubarka, the Narodichi district, Zhytomyr oblast', II zone[1]). Experiment duration – 70 days every year. Content of ^{137}Cs and ^{90}Sr in water, bottom sediments and animals was determined using spectrophotometer LP49900B AFORA № 905289 in Laboratory of radiology of the Polissia branch of the Ukrainian research institute of forestry and agroamelioration. Average content of ^{137}Cs in water of this water body amounted to 1.78 ± 0.02 Bq/l, and in bottom sediments (silt) – 350.2 ± 3.8 Bq/kg; content of ^{90}Sr in water – 0.12 ± 0.04 Bq/l, in bottom sediments – 68.7 ± 1.1 Bq/kg. Content of ^{137}Cs in water of the control water body amounted to 0.007 ± 0.004 Bq/l, and content of ^{90}Sr – 0.005 ± 0.002 Bq/l (in bottom sediments – 17.5 ± 2.1 and 4.9 ± 0.3 Bq/kg, accordingly). At the beginning of experiment content of ^{137}Cs in mollusks from the radioactively contaminated water body amounted to 64.0 ± 5.0 Bq/kg, and in mollusks from the conditionally clean zone[2] (the Teteriv River, Zhytomyr) – 12.0 ± 7.0 Bq/kg (content of ^{90}Sr – 554.0 ± 9.0 and 280.0 ± 12.0 Bq/kg, accordingly).

Totally 1 107 specimens of the pond snail were analyzed. Animals were adapted to the laboratory conditions during two days; then they were placed into solution with the heavy metal ions of the studied concentration. Animals were fed by green leaves of dandelion. As control were used mollusks of the same size from the Teteriv River kept in similar conditions.

Experimental conditions were the follows: water temperature – 19–23°C, pH – 7.01–7.53, oxygen content – 8.39–8.87 mg/dm^3, free carbonic acid – 2.33 mg/dm^3, F – 0.24 mg/dm^3, Ca – 2.60 mg/dm^3. Background content of the heavy metals ions in water amounted to: Cu – 0.0002 mg/dm^3, Mn – 0.092 mg/dm^3, Zn – 0.0003 mg/dm^3, Ni – 0.0001 mg/dm^3, Co < 0.00001 mg/dm^3, Cd < 0.000001 mg/dm^3. Content of Sr in water amounted to 0.286 mg/dm^3. Needed concentration of the heavy metal ions was achieved by addition of chlorides of the considered metals into the settled tap water (chemicals of the enterprise "Reachim", grade "chemically pure" were used).

In order to avoid impact of exometabolites on mollusks and maintain needed concentration of the metal ions [2], water was replaced every other day and needed amount of toxicant was added. At every replacement walls of aquariums were cleaned by rigid brush.

Before the main experiment preliminary one has been carried out in order to choose concentration of toxicants to be studied [12]. At this fishery-toxicological approach was used, in order to

[1] Radioactive contamination of the soil surface $18.5 \cdot 10^{10}$–$55.5 \cdot 10^{10}$ Bq/km^2.

[2] Radioactive contamination of the soil surface below $3.7 \cdot 10^{10}$ Bq/km^2.

Table 1

Heavy metal ions concentration (mg/dm^3), considered in the experiment

Ions	Concentration			
	acute lethal	chronic lethal	sublethal	subliminal
Cu^{2+}	4	0.04	$4.0 \cdot 10^{-5}$	$4.0 \cdot 10^{-8}$
Cd^{2+}	5	0.05	$5.0 \cdot 10^{-4}$	$5.0 \cdot 10^{-6}$
Ni^{2+}	10	0.05	$5.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-6}$
Zn^{2+}	15	0.50	$5.0 \cdot 10^{-3}$	$5.0 \cdot 10^{-5}$
Co^{2+}	25	2.50	$2.5 \cdot 10^{-1}$	$3.0 \cdot 10^{-2}$
Mn^{2+}	110	30.00	$3.0 \cdot 10^{-1}$	$3.0 \cdot 10^{-2}$

mark out lethal (acute and chronic), sublethal and non-effective concentration [6]. In the main experiment four concentrations were considered, one of each concentration diapason (Table 1).

Egg sets were taken from substrate by soft brush. Syncapsules and their elements were examined and measured using light microscope MBS-9. Total number of egg capsules in a syncapsule and number of zygotes in a capsule were counted. Egg sets and all morphological abnormalities/deviations were sketched using the drawing device. Length of the egg capsules was measured by internal capsule membrane. Results of the experiment were processed by variation statistics methods [5].

Results and Discussion

Obtained results showed that considered concentrations of the heavy metals caused significant deviations of biological characteristics of the pond snail taken from the radioactively contaminated water body.

During 70 days of experiment control group of animals (taken from the conditionally clean water body) laid egg sets 1.5 times more than mollusks from the radioactively contaminated water body ($P < 0.05$). Pond snails from the radioactive zone, placed into solutions of Zn^{2+}, Co^{2+}, Mn^{2+} of all considered concentration (see Table 1), laid 1.8–2.0 times less ($P < 0.05$) (Fig. 1).

Statistically reliable differences in number of eggs were not noted only when mollusks stayed in solutions with subliminal concentration of Cd^{2+}. Minimal number of syncapsules was noted in solutions with subliminal concentration of Zn^{2+} (12 ± 1), where probably adaptation mechanisms become more active very slowly; and in solution of the chronic lethal concentration of Co^{2+} (9 ± 1), where poisoning reached its limits more quickly than in other solutions. Maximal number of syncapsules was laid in solutions of Ni^{2+} of all considered concentrations ($P < 0.05$). However it was lower than in the control group (see Fig. 1).

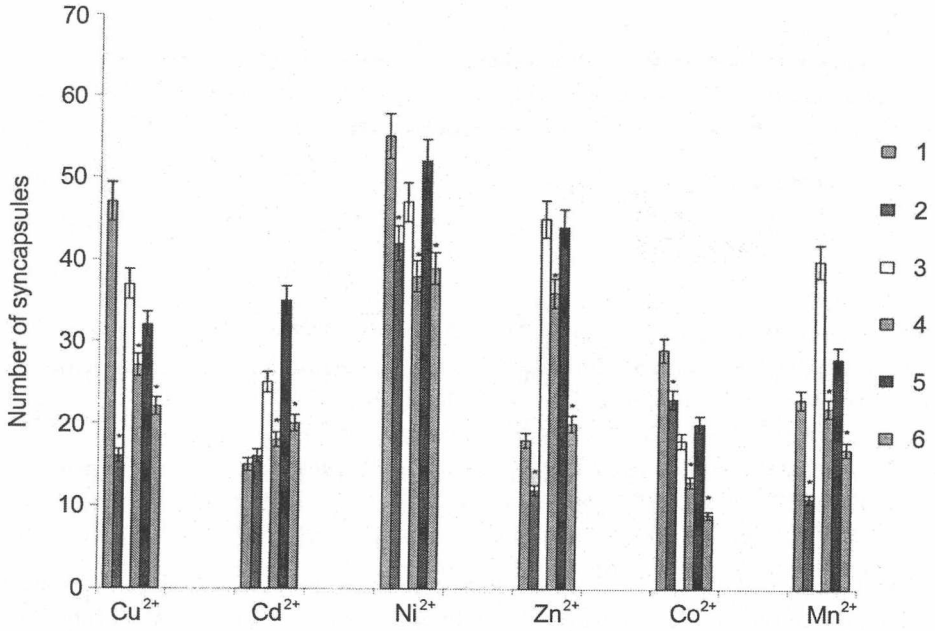

Fig. 1. Number of syncapsules, laid by the pond snail from the radioactive zones under the impact of heavy metals (period – 70 days). Here and in the Fig. 2–5: *1* – diapason of subliminal concentration (control – the Teteriv River); *2* – diapason of subliminal concentration (II zone – the Loznitsa River); *3* – diapason of sublethal concentration (control – the Teteriv River); *4* – diapason of sublethal concentration (II zone – the Loznitsa River); *5* – diapason of chronic lethal concentration (control – the Teteriv River); *6* – diapason of chronic lethal concentration (II zone – the Loznitsa River).

During the experiment mollusks from both radioactively contaminated and conditionally clean water body, placed into water without toxicants, were monitored. It was stated that during 70 days of the experiment mollusks from the Losnitsa River laid on average 50 ± 5 egg sets, that is 1.7 times less than number of syncapsules laid by the mollusks from the Teteriv River (84 ± 7 egg sets).

Length of syncapsules laid by mollusks from the radioactively contaminated zone varied insignificantly, though these syncapsules mainly were smaller than those of the control group (Fig. 2).

Similar regularity was observed in syncapsules' length laid by two animal groups kept in clean water (the Teteriv River – 33.75 ± 0.64 mm; the Loznitsa River – 32.86 ± 0.92 mm).

Statistically reliable differences in this parameter were observed only under the impact of sublethal and chronic lethal concentration of zinc ions ($P < 0.05$), at this under the impact of sublethal concentration length of syncapsules was more than in control group, and under the impact of chronic lethal it was less. Under the impact of toxicants egg capsules laid by mollusks from the Loznitsa River were mainly smaller as compared with control (Fig. 3).

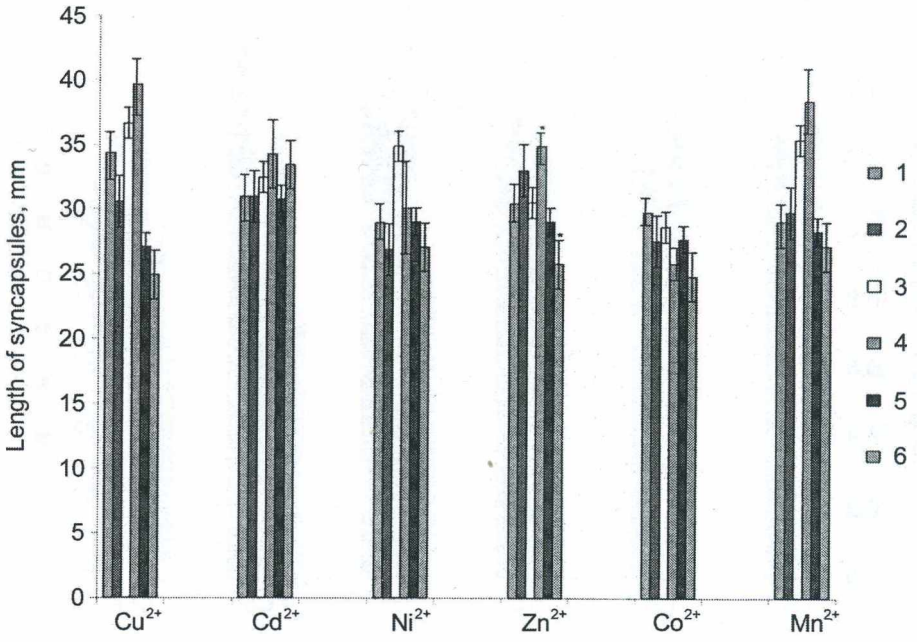

Fig. 2. Length of syncapsules (mm), laid by the pond snail from the radioactive zone under the impact of heavy metals.

In solutions with the cobalt ions of all considered concentration (see Table 1) dimensions of the egg capsules were reliably smaller than in control ($P < 0.05$). Similar pattern was observed under the impact of subliminal and chronic lethal concentration of Zn^{2+} and Cu^{2+} and subliminal concentration of Cd^{2+}. In solutions with manganese ions of all considered concentration length of egg capsules was less than in control, but this difference was not statistically reliable ($P < 0.05$). Under similar conditions average size of the egg capsules of the mollusks from the radioactively contaminated zone, kept in clean water, were somewhat more than capsules of the mollusks from the conditionally clean zone (1.37 ± 0.01 and 1.35 ± 0.01 mm, accordingly).

Abnormalities in structure of syncapsules laid by the pond snails from the radioactively contaminated zone were the same as in mollusks affected only by the heavy metal ions [15]. However it is worth noting, that almost all teratogenic abnormalities occurred 1.5 times more often. The most frequently occurred light spiralization of band with egg capsules, monozygotic egg capsules and presence of the egg capsules out of syncapsules. Doubling of the egg capsules and presence of eggs without capsules out of syncapsule also occurred quite frequently.

Teratogenic abnormalities partly caused decrease of vitality of youth of the mollusks affected by intensive radioactive impact, in turn this affected survival rate of young pond snails in the toxic medium.

Embryogenesis duration of the mollusk from the Loznitsa River at keeping in the solutions with the heavy metal ions increased on average by 1–2 days, but young animals left syncapsules more in-

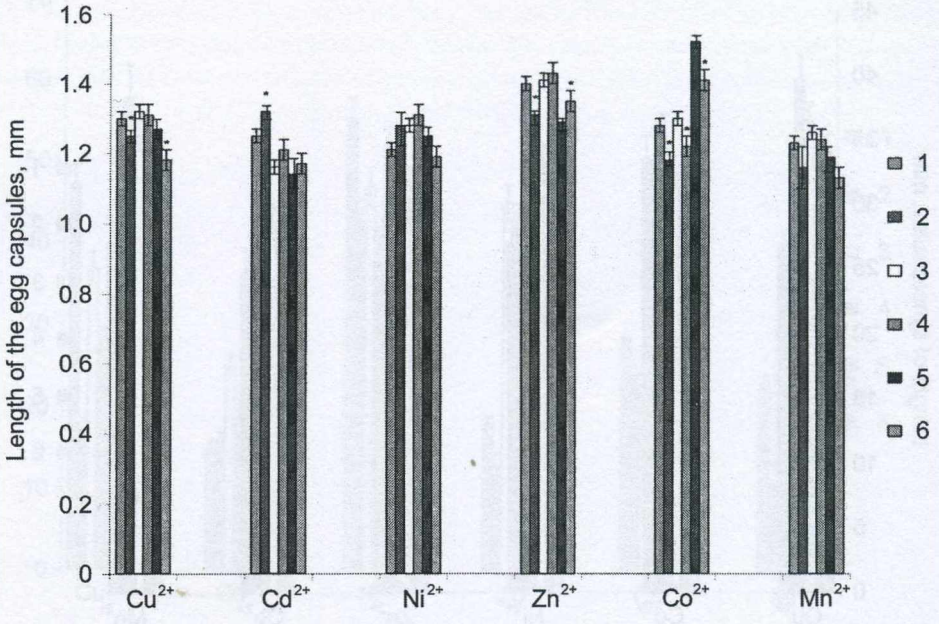

Fig. 3. Length of the egg capsules (mm), laid by the pond snail from the radioactive zone under the impact of heavy metals.

tensively (hatching time amounted to 3–5 days). Similar pattern was observed in control variant only under the impact of the highest considered concentrations of the heavy metal ions.

We also studied number of the egg capsules laid into syncapsules and portion of young mollusks successfully completed embryonic development and hatched. These data were compared with data obtained in experiments were mollusks from conditionally clean water body were affected by toxic impact. It is worth noting that all obtained values were lower than in control. Number of the egg capsules in a syncapsule of the mollusks from the radioactively contaminated zone kept in considered solutions reliably decreased 1.5–2.3 times ($P < 0.05$) (Fig. 4). In turn, this decreased hatching of youth.

Reliable differences were not found only under the impact of subliminal and sublethal concentration of zinc ions ($P < 0.05$). One of the reasons of the egg capsules number in syncapsules decrease can be disturbance of spiralization, when egg capsules were laid loosely. By this reason the syncapsules of the same length contained twice less egg capsules. Hatching rate of young mollusks was on average 1.5 times less than in control ($P < 0.05$). Minimal values were registered in solutions with Co^{2+}, where they were 1.7–1.8 times less (Fig. 5).

Stimulating effect of sublethal concentration of the heavy metal ions, noted in the control experiment, was not registered in the main experiment. Subliminal concentration of the heavy metal ions in this case can not be considered not-effective: portion of the hatched juveniles in these solutions was 1.3–1.5 times less than in control ($P < 0.05$). At this limits of response to the sublethal and

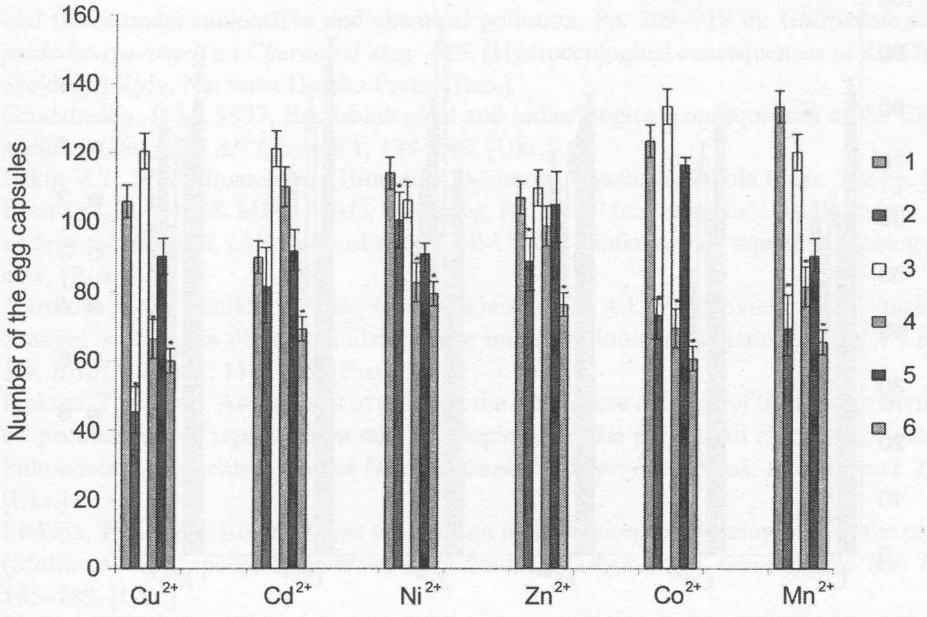

Fig. 4. Number of the egg capsules in a syncapsule of the pond snail from the radioactive zone under the impact of heavy metals.

chronic lethal concentrations were smeared – the last did not obviously depress youth hatching (see Fig. 5).

Average number of the egg capsules in a syncapsule and portion of the hatched juvenile of the mollusks from the radioactively contaminated water body were significantly less than of the mollusks from the Teteriv River at keeping of both groups in water without toxicants. Number of the egg capsules laid by mollusks from the Loznitsa River amounted to 94.88 ± 4.7, and by mollusks from the Teteriv River – to 106.6 ± 4.37. Portion of the hatched juveniles of the mollusks from the Loznitsa River under such conditions was on average 1.3 times less as compared with mollusks from the Teteriv River (the Loznitsa River – 68.9 ± 3.67%, the Teteriv River – 87.9 ± 1.35%) ($P < 0.05$).

Conclusion

Reproductive system of the pond snail from the radioactively contaminated zone was found to be more sensitive to the toxic impact of the heavy metal ions as compared with other physiological systems of the organism [13], it responded more sharply than reproductive system of the mollusks from the conditionally clean zone. Even subliminal concentrations of toxicants caused response of the reproductive system. Mollusks depressed by radiation were not able to resist intoxication by intensification of the vital processes. Thus, impact of sublethal and chronic lethal concentrations significantly suppressed functioning of the mollusks' reproductive system. Under the impact of all

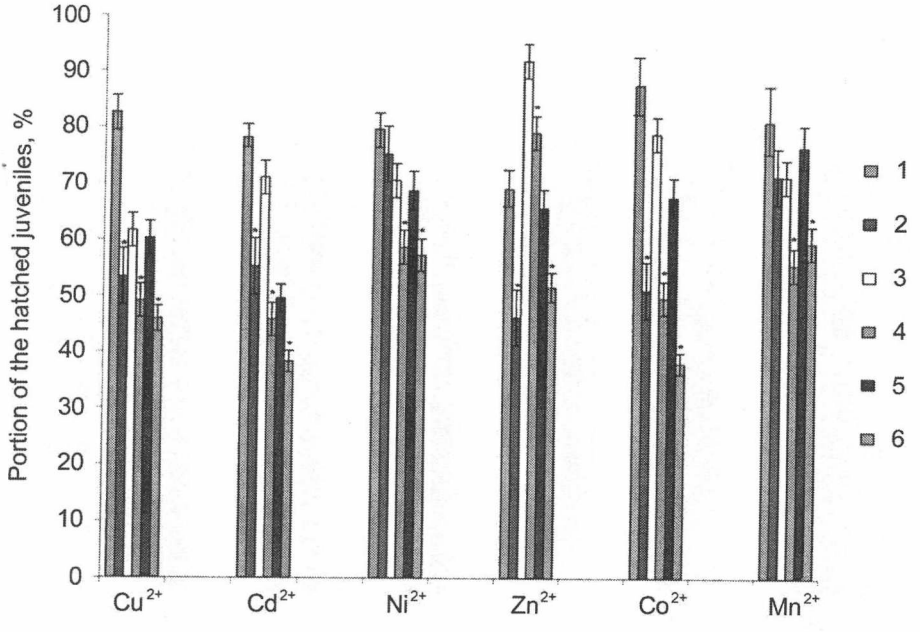

Fig. 5. Portion of the hatched juveniles of the pond snail from the radioactive zones under the impact of heavy metals.

considered concentrations of the heavy metal ions studied biological characteristics were lower than those of the animals not affected by radiation.

By dynamics of the considered biological parameters it may by concluded about synergism of radiation factor and heavy metal ions. It was stated that ionizing radiation is an aggravation factor for the animals, affected by the heavy metal ions impact. At this organism of the adult mollusks became depressed, and this fact inevitably led to negative changes in organism of juvenile specimens.

In connection with intensification of the hydrosphere pollution by the heavy metal ions and radionuclides, data on the mollusks' fertility changes can be used in development of ecological monitoring of the surface waters of Ukraine, and for prediction of changes in the aquatic communities diversity.

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