



EFFECT OF NICKEL IONS ON ECOTOXICOLOGICAL RESPONSES OF THE GREAT RAMSHORN *PLANORBARIUS CORNEUS* ALLOSPECIES (MOLLUSCA: GASTROPODA: PULMONATA: PLANORBIDAE) OF THE UKRAINIAN RIVER NETWORK

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ABSTRACT: This ecotoxicological study analyzed the impact of Ni²⁺ ions in different concentrations on the ecotoxicological and physiological features of the western and eastern vicariant allospecies of the great ramshorn superspecies *Planorbarius corneus* (Linnaeus, 1758) sensu lato in Ukrainian populations from rivers in two different nature-geographic zones: Forest (Polissia) and Forest-Steppe. The two allospecies differed from each other significantly ($p \leq 0.05$ – 0.001) in their responses to the main ecotoxicological indexes (LC₀, LC₅₀, LC₁₀₀) in terms of the duration of the latent period, lethal time and mortality rate. By all these indices, the western allospecies had significantly more tolerance than the eastern allospecies. The same was true also for respiratory responses. Under the MPC (maximum permissible concentration) of Ni²⁺ ions in the environment, no changes were registered compared to the control. Increase in toxicant concentration up to 2 MPC was followed by increase in the rate, number, volume and duration of inspirations. At 3 MPC of Ni²⁺, however, the indices of direct diffusive respiration progressively decreased. In all measures, the western allospecies appeared better able to adjust to, and to survive increasing concentrations of Ni²⁺ than that from the east.

KEY WORDS: Gastropoda; differential Ni²⁺; responses; Ukrainian hydrosphere

INTRODUCTION

The great ramshorn snail *Planorbarius corneus* (Linnaeus, 1758) was, at the beginning of the 21st century, one of the most widespread and abundant gastropod molluscs in Ukrainian fresh water habitats. It is a widespread species, nominally found from the Atlantic to the Urals and beyond. Recent work, however, based on genetic markers (GARBAR 2003, MEZHHERIN et al. 2005, GARBAR & GARBAR 2006) has shown that *P. corneus* is a superspecies complex, and that in Ukraine there are two vicariant allospecies, western and eastern, significantly differentiat-

ed by the centromere indexes of 12th chromosome pair (HARBAR et al. 2021). These allospecies are also distinguished by shell size, and by numerous conchological and anatomical variables (GARBAR 2003, BABYCH et al. 2021). The two allospecies are geographically separated by a zone approximately 100 km wide following the Dnipro river channel.

Toxicological studies by HARBAR et al. (2021) revealed the impact of different Ni²⁺ ions concentrations (0.001–1000 mg/L) on the responses of the two allospecies of *P. corneus*. The indices of mortality,



mean lethal time, adaptation coefficients of pulmonary and diffusive respiration appeared significantly different between allospecies ($p \leq 0.05-0.001$). In all cases, the eastern allospecies was more sensitive and suffered greater mortality than the western one at any given Ni^{2+} concentration.

Heavy metal pollution in Ukrainian rivers has increased greatly in recent decades, threatening the functioning of freshwater ecosystems (BABYCH 2022, BABYCH et al. 2022). While very low concentrations of some heavy metals are needed for by many freshwater animals, including molluscs (PIWONI-PIÓREWICZ et al. 2017, SANTORE et al. 2021, BABYCH et al. 2021, GANDZYURA et al. 2021, UVAYEVA et al. 2022), increased concentrations become toxic (BRIX et al. 2017, PETERS et al. 2019, PINKINA et al. 2022).

Ni^{2+} is among these heavy metals. In very low concentrations it is a necessary component, both in the process of hematopoiesis and of reproduction (ROMANENKO 2001). At higher concentrations, however, toxic effects become important. These may be complex. While very high concentrations (11.4 mg/L) are lethal to molluscs after 96 hours exposure, the effects are influenced by the age of the molluscs (HARANADHA et al. 2011), and by the hydrochemical conditions of environment, level of food consumption, their overall physiological state and genetic features of individuals (OROS & GOMOIU 2010, ZUYKOV et al. 2013).

The aim of the present study is to compare the ecotoxicological effects of increasing concentrations of Ni^{2+} ions on the vicariant allospecies of *P. corneus*.

MATERIAL AND METHODS

The positions of the sampling stations in relation to the biogeography of Ukraine is shown in Fig. 1. A sample (310 specimens) of the western allospecies was made in the Sapohivka river (Prypiat basin) near the Miropol settlement ($50^{\circ}6'27''N$, $27^{\circ}41'45''E$, Right

Bank Ukraine, Fig. 2), and a sample of 302 of the eastern allospecies from the Psel river near Bilotserkivka village ($49^{\circ}40'17''N$, $33^{\circ}45'39''E$) (Fig. 3). Snails were collected by hand from four types of biotopes in each: shallow water of 5–7 cm depth, with bottom covered

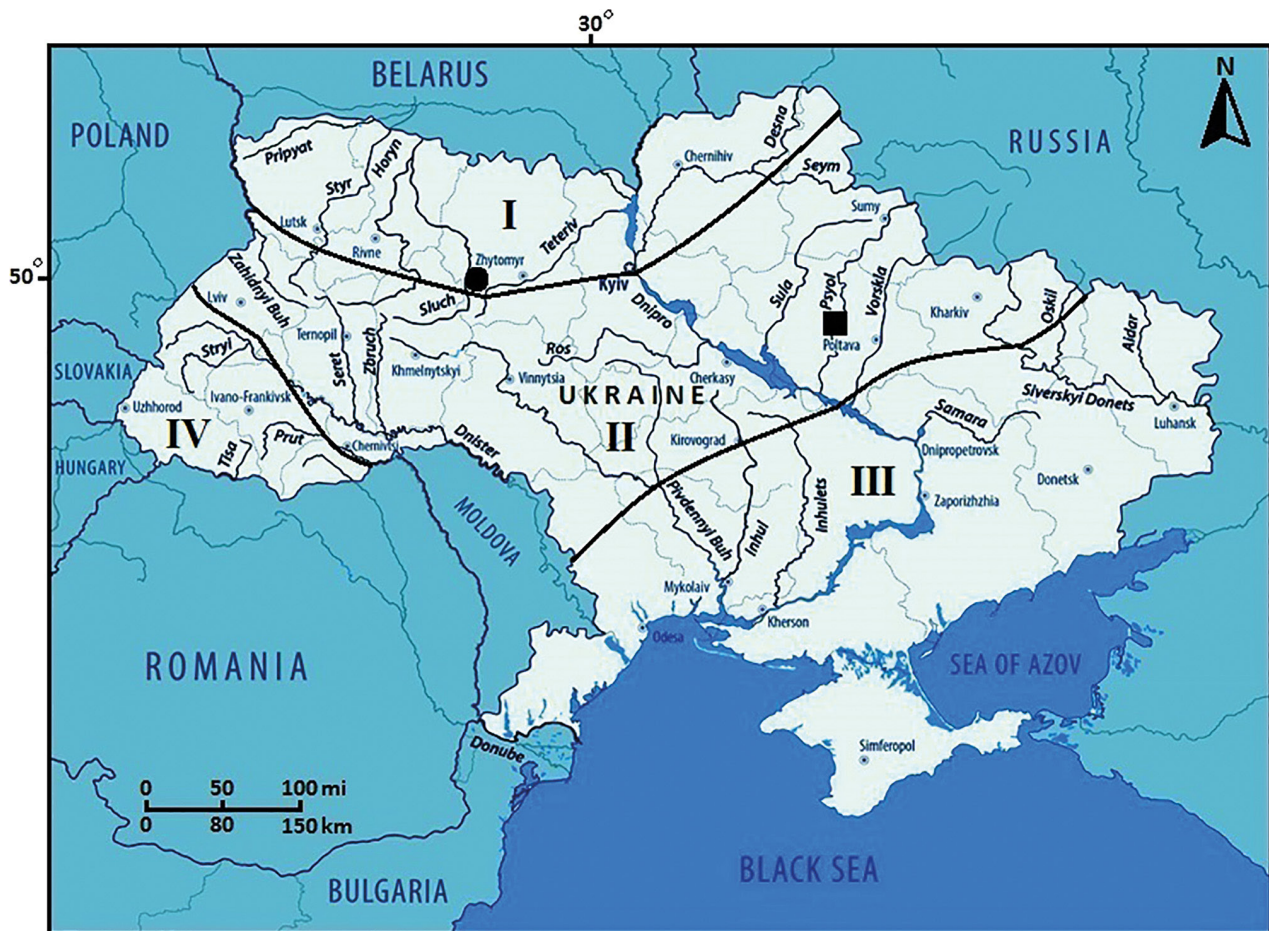


Fig. 1. Map showing the typical localities of *Planorbarius corneus* s. lato allospecies: I – Polissia; II – Forest-Steppe; III – Steppe; IV – Carpathian mountain region; black circle – “western”; black squares – “eastern”



Figs 2–3. Habitats of great ramshorn *Planorbarius corneus* s. lato: 2 – Sapohivka river (Miropol village, Zhytomyr region); 3 – Psel river (Bilotserkivka village, Poltava region)



by *Cladophora* sp. and with weak, variable flow; sites thickly covered with hemi-immersed water plants (*Typha* sp., *Scirpus* sp.) and depth of 15–20 cm, with unnoticeable flow; off the coast at 1–1.25 m depth with powerful development of soft-leaved plants (*Myriophyllum* sp., *Nymphaea* sp., *Lemna* sp.); in the benthic zone, with the bottom covered by plant detritus, and with 0.1–1 m/s flow. Species/allospecies identification was determined on conchological characters, following GARBAR (2003), MEZHHERIN et al. (2005) and GARBAR & GARBAR (2006).

In the laboratory, the snails were subject to a 15-day period of acclimation in aquariums of 30 l vol-

ume at a density of 5 snails/L. Water temperature was maintained at 20–23 °C, pH at 7.3–7.8, and oxygenation at 7.6–8.6 O₂ mg/L. The water was changed each day, and food, *Cladophora* sp. and *Myriophyllum spicatum* L., collected in the sampling sites, was renewed daily.

The first toxicological experiment (Tables 1–5) was conducted by the standard method (BABYCH et al. 2022, 2023): aquarium volume – 100 L, water temperature – 20–23 °C, pH – 7.3–7.8, oxygenation – 7.6–8.9 O₂ mg/L. Toxicant – NiCl₂·6 H₂O (p.a.) in concentrations between 0.001–100 in mg/L (per cation), expressed also in terms of MPC – maximum permissible concentration. In each of the experimental groups (Tables 1–5) there were 20 specimens of molluscs. Observations were made at 10, 30 min, 1, 2, 4, 6, 24, 48 hours after commencement. Renewing of environments – each day. The impact of toxicant on the molluscs was estimated by the visual observations of changes in their behavior, registering the integrity of the epithelial body covering, its level and functioning, locomotory activity, and intensity of their feeding and digestive processes.

The second toxicological experiment (Table 6) examined the impact of different concentrations of Ni²⁺ ions on the pulmonary and surface respiration of ramshorn allospecies, following the methods of BABYCH et al. (2023).

Analysis of results used the standard methods given by HILL & LEWICKI (2007) identifying any differences between western and eastern allospecies, using statistically significant differences at the p<0.05 and p<0.001 levels.

Table 1. The main toxicological characteristics of *P. corneus* s. lato allospecies under the exposure of Ni²⁺ (48 hours)

Indicator, mg/L	„Western” allospecies	„Eastern” allospecies
Threshold concentration	0.01	0.001
LC ₀	0.1	0.03
LC ₅₀ *	20	3
LC ₁₀₀	50	10

LC₀ – the highest concentration which caused no mortality of molluscs.

LC₅₀ – lethal concentration which caused in accordance 50% mortality of molluscs (* set graphically).

LC₁₀₀ – lethal concentration which caused in accordance 100% mortality of molluscs.

Table 2. Latent period (hours) of *P. corneus* s. lato allospecies under the exposure of Ni²⁺

Concentration, mg/L	„Western” allospecies	„Eastern” allospecies
	M±SE	M±SE
0.001	25.5±1.16	21.3±1.24*
0.01	21.2±1.23	18.1±1.05*
0.1	17.5±1.03	13.5±1.17*
1.0	2.1±1.12	1.4±1.28
10	1.1±1.08	0.6±1.15
100	0.1±0.98	0.04±1.11

* – statistically significant differences (p≤0.05) between *P. corneus* s. lato allospecies.

Table 3. Mortality (%) of *P. corneus* s. lato allospecies under the exposure of Ni²⁺ (48 hours)

Concentration, mg/L	„Western” allospecies	„Eastern” allospecies
0.0001	0	0
0.001	0	0
0.01	0	0
0.1	0	20
1.0	20	50
10	80	100
100	100	100
1,000	100	100

Table 4. Time-to-death (hours) of *P. corneus* s. lato allospecies under the exposure of Ni²⁺

Concentration, mg/L	„Western” allospecies	„Eastern” allospecies
	M±SE	M±SE
0.01	0	0
0.1	48.3±1.13	39.3±1.18*
1.0	29.1±1.12	18.2±1.02**
10	8.1±1.23	5.1±1.11
100	2.1±1.13	0.5±0.02*

* – statistically significant differences (p≤0.05) between *P. corneus* s. lato allospecies.

Table 5. Rating of Ni²⁺ concentrations (mg/L) according to the effect on *P. corneus* s. lato allospecies

	„Western” allospecies		
	Subthreshold	Sublethal	Chronic lethal
0.01 and lower	0.1–2	6–13	10–40
„Eastern” allospecies			
10 ⁻⁴ and lower	0.001–0.01	0.1–0.4	1–10

RESULTS

The study of the impact of the set of different Ni²⁺ concentrations on the main toxicological and some physiological indexes of *P. corneus* sensu lato allospecies established (Tables 1 and 6) that all of them were higher in “western” allospecies than in “eastern” allospecies ($p \leq 0.05-0.001$). This confirms the higher level of viability of “western” allospecies: it has a longer latent period (Table 2, $p \leq 0.05$), and lethality at the higher doses of toxicants (Table 3) took longer to occur (Table 4, $p \leq 0.05$).

It was also established that the range of endurance (mg/L) of “western” allospecies under the im-

part of sub-threshold, sub-lethal, chronic lethal and acute lethal Ni²⁺ concentrations exceeded those of “eastern” allospecies (Table 5). The data in Table 5 indicates the higher level of sensitivity and lower level of endurance in “eastern” allospecies concerning all used toxicant concentrations.

The data in Table 6 proves the more intensive duration of pulmonary as opposed to surface diffusive respiration in the individuals of “western” allospecies comparing to “eastern” allospecies ($p \leq 0.05-0.001$).

Table 6. The impact of Ni²⁺ in different concentration on indexes of lung and direct diffusive respiration of *P. corneus* s. lato allospecies

Toxicant concentration	n	Indexes of lung respiration				Index of direct diffusive respiration, h
		Daily number of inspirations	Interval between inspirations, min	Duration of inspiration, min	Volume of inspiration, number of bubbles	
		M±SE	M±SE	M±SE	M±SE	
“Western” allospecies (Sapohivka river, Miropol)						
Control	20	12.99±1.17	47.98±1.16	16.99±1.38	14.95±1.27	42.82±2.83
0.5 MPC	20	13.06±1.13	46.95±1.14	19.04±1.11	15.08±1.23	44.94±2.59
1.0 MPC	19	14.78±1.20	41.65±1.15*	21.18±1.17*	19.76±1.10*	47.51±4.09*
2.0 MPC	19	16.70±1.18*	35.21±1.27**	24.09±1.11**	25.68±1.18**	32.09±3.40**
3.0 MPC	18	9.51±1.09*	63.11±1.18**	11.06±1.13**	7.95±1.15**	17.46±2.13**
“Eastern” allospecies (Psel river, Bilocerkevca)						
Control	20	10.13±1.01	57.71±1.19	14.76±1.18	12.43±1.16	37.78±2.74
0.5 MPC	20	11.34±1.26	55.88±1.16	16.17±1.20	16.09±1.11	42.21±2.12
1.0 MPC	19	12.25±1.21	55.14±1.31*	18.60±1.51*	22.05±1.06**	43.48±2.46*
2.0 MPC	18	14.09±1.22*	43.61±1.14**	22.05±1.07**	38.33±2.07**	31.04±2.05**
3.0 MPC	19	5.03±1.14**	96.01±1.15**	8.31±1.03**	4.27±1.08**	12.01±1.19**

n – number of individuals studied; MPC – maximum permissible concentration of ions in the water; M±SE – mean value of index and its standard error; * – statistically significant differences ($p \leq 0.05$); ** – highly significant differences ($p \leq 0.001$).

DISCUSSION

Burning the deposits of fossil energy sources, disposing the wastes of coal and oil refining industry, and the emissions of Ni-Cd batteries production into the hydrosphere components are the main modern sources of Earth freshwater ecosystems pollution by nickel and compounds, and their harmful impact on the hydrobiota (EL-ENANY & ISSA 2000, BINET et al. 2018, WANG et al. 2020). Average yearly emission of Ni²⁺ into the atmosphere is 55.65×10^3 kg, into the aquatic ecosystems – 113×10^6 kg, and into the soil – 416.5×10^6 kg (SHUKLA et al. 2009).

Ni²⁺ ions enter aquatic molluscs mainly from the environment through the body’s ectodermal epithelial coverings, and much less from their plant food (WONG & PAK 2004, OROS & GOMOIU 2010,

YEVTUSHENKO & DUDNYK 2019). These ions accumulate first in the hepatopancreas, subsequently distributed among different organs and tissues, maintained by the ongoing circulation of hemolymph.

Ni²⁺, in very low concentrations, is essential to freshwater gastropods to maintain hematopoiesis at the optimal level (ROMANENKO 2001), which is one of the crucial conditions for maintenance of their internal homeostasis, and hence their viability and maximum population size and density (AFANASYEV & GRODZINSKY 2004, BAEVA & CHERNIKH 2020). Exceeding the optimal Ni²⁺ ion concentrations in the water, however, is progressively more lethal for them (BABYCH & PINKINA 2021, HARBAR et al. 2021). The maximal permitted concentration of Ni²⁺



for hydrobionts from Ukrainian river network is 0.01 mg/L (DECREE OF THE CABINET OF MINISTERS OF UKRAINE 2013). Exceeding this concentration drives a sequence of phasic pathological processes, whose duration is divided into 1 to 5 phases (dependent on the dose acquired), each having a characteristic set of symptoms. Under the MPC of Ni²⁺ in the environment (0.01 mg/L) the experimental molluscs developed the first intoxication phase – latent, without symptoms, the most secure and the longest. Under 2 MPC, the latent phase changed into the stimulation phase, an increase in physiological activity that maintained viability in experimental conditions. However, the impact of these ions under 3 MPC induced a rapid onset of the three final phases of intoxication – the first and longest being depressive (linked with progressive decrease of all the vital processes), followed by acceleration of these in the sub-lethal phase, and terminating in death.

The “eastern” allospecies moved through these phases more rapidly in relation to ionic concentrations than the “western” allospecies, and its traits developed earlier. The “western” allospecies has evolved more tolerance. The index of direct diffusive respiration in the “western” allospecies is higher than in the “eastern” one when pulmonary respiration is prevented ($p \leq 0.05-0.001$); the total amount

of oxygen consumed by these molluscs during diffusive surface respiration is almost the same as consumed during pulmonary respiration (BABYCH 2022). The data in Table 6 also indicates that pulmonary respiration in “western” allospecies is more effective comparing to that in “eastern” allospecies; the “western” allospecies had a greater number of performed “inspirations” (daily) and their duration and volume along with much shorter intervals between them follow the same pattern ($p \leq 0.05-0.001$).

It is noteworthy that our results supplement those obtained earlier by HARBAR et al. (2021). However we collected material in different period of year. The studied molluscs were studied in different parameters, therefore received different results. This comparison of “western” and “eastern” allospecies of *P. corneus* sensu lato in terms of their response to increasing concentrations of Ni²⁺ raises the question of how such differences arose. A possible reason for such a difference may lie in the different nature-geographic conditions in which they have lived (LOGVINOV & SHCHERBAN 1984, STADNYCHENKO et al. 2020). These conditions are much harder for “eastern” allospecies, which is distributed in drought climate of the Left-Bank Ukraine and the extreme South of Ukraine’s Steppe Zone.

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