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COMBINED IMPACT OF TOXICANTS AND HELMINTHS ON GREAT POND SNAIL (*LYMNAEA STAGNALIS* L.) HOMEOSTASIS STABILITY

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Anthropogenic pressure on the hydrosphere has led to the progressive increase in its pollution by various components of both industrial and domestic effluents. The surfactants, which are the components of synthetic detergents, are among the most dangerous for aquatic organisms.

The effect of different concentrations of detergent "Ushastyi nian" produced by "Vinnytsiapobutkhim" was studied in concentrations of 2.5, 5, 10, 20, 40 mg/dm^3 on a number of physic-chemical parameters of the homeostasis of haemolymph of *Lymnaea stagnalis* (Linnaeus, 1758). The investigated great pond snails were intact or infected with various life cycle stages (maternal and daughter redia, cercariae) of the trematode *Echinoparyphium aconiatum* Dietz, 1909. The studied material includes 315 individuals of molluscs collected by hand in the reservoirs of the drainage and reclamation system in the basin of the Teteriv River (right-bank tributary of the Middle Dnieper river) in Central Polissya (Zatyshshia village, Zhytomyr Region). The toxicological experiment was conducted by standard scheme according to (Alekseev, 1981). It was preceded by a mandatory (Khlebovich, 1981) 15-day acclimation of animals intended for toxicological examination to aquarium conditions (volume of aquaria 20 l, density of molluscs 3–4 individuals/l, water temperature 20–22 °C, pH 7.9–8.4, oxygenation 8.1 to 8.5 mg O_2/dm^3).

The presence of molluscs in toxic environments was accompanied by the development of a responsive reaction such as poisoning. The most obvious symptoms were quantitative changes in the values of physic-chemical parameters of their haemolymph, which largely ensure its homeostasis. These parameters include the total protein level, the specific gravity, and pH. In these molluscs, about 90 % of the total haemolymph protein is represented by haemocyanin. It determines, due to its powerful buffering properties, both the level of oxygen capacity and water-osmotic pressure in the body of molluscs.

The pathological process, which develops as a result of combined toxicant and helminthic invasion impact on *L. stagnalis* expresses more clear, quick and higher toxicant concentration, helminthic invasion intensity and durability level of different lifecycle stages of trematodes. The experimental molluscs defend themselves against the affected values of the mentioned indicators of stability of homeostasis by the set of inherent protective and adaptive reactions (rapid behavioral, physiological and biochemical ones). They aimed at maintaining the viability of individuals.

The effectiveness of protective and adaptive reactions in trematode-infected molluscs was significantly lower compared to non-infected individuals. The degree of cumulative damaging effect of the toxicant and parasites was determined not only by the concentration of surfactants. To a large extent it depended on both the rate of the infection and the stage of the life cycle of parasites. The pathogenic effect of the latter on their mollusc hosts decreased as follows: "old" (maternal) redia > "young" (daughter) redia > cercariae.

Keywords: Lymnaea, Trematoda, surfactant solutions, homeostasis

In the second half of XX and the beginning of XXI centuries new artificial and toxic to freshwater organisms pollutants have emerged [6, 15–18, 23]. Now one of the most acute problems of hydroecology is the determination of the resistance levels of at least the most abundant and widespread hydrobionts to different concentrations of the contaminants. The levels of resistance characterize the possibility of formation and persistence of the majority of productive aquatic ecosystems. Hence the necessity of understanding the reactions of dominant species to different toxicity levels of aquatic environment. Such data are very important for choosing the biomarker species for testing and bioindication in the monitoring of natural waters [4, 6, 7, 9, 17]. They are also needed to create unified (ecological) qualifying system for natural land surface waters of Ukraine [22].

In the last few decades, the natural waters of Ukraine have become more and more polluted with various synthetic detergents [5, 7, 18, 20]. Usually they include surfactants (SA), both anion-active (alkyl sulfates, alkylbenzenesulfonates, alkylarylsulfonates, tetrapropylenebenzenesulfonates etc.) and non-ionic ethylene derivatives [13]. Together with municipal and industrial effluents they get into natural waters. The current MPC standards are 0.5 mg/dm^3 for anion-active and 0.1 mg/dm^3 of SA for non-ionic detergents [21]. However, in the waste dumping sites (both industrial and household) the concentrations of SA pollutants significantly exceed the MPC (20 mg/dm^3).

In water bodies and watercourses, SA usually concentrate at the air-water interface and severely inhibit the oxygen entry into the water through their ability to form surface foams. It negatively affects the aerobic hydrobionts, targeting their cutaneous and lung breathing. SA destruct very slowly, and persist in the environment for a very long time.

Our study aimed to conduct an acute test to determine the particularities of the impact effect of various SA concentrations on the great pond snail *Lymnaea stagnalis* (Linnaeus, 1758) homeostasis stability both intact individuals and infested with helminthes ones.

Material and methods

The study material is 315 specimens of *L. stagnalis* (height of shell 59.7±2.21 mm) collected manually on 03.05.2020 in small water bodies of drainage system at Central Polyssia in the basin of Teteriv river (farm Zatyshshia, Zhytomyr region). The animals were transported to the lab tightly wrapped in several layers of moistened burlap. In the lab the molluscs were acclimated for 15 days [10]. The conditions were as follows: aquarium volume 20–100 l, animal density 3–5 specimens/l, water temperature 18–20 °C, pH 7.9–8.4, oxygenation 8.1–8.5 mg O_2/dm^3. Every three days the water was refreshed. The animals were regularly fed by thin (2–3 mm) carrot, white cabbage slices, lettuce leaves macerated in water for 5–7 days.

Toxicological experiment was conducted according to standard methods [1]. The pollutant was modeled by detergent "Ushastyi nian" producted by "Vinnitsiapobutkhim" which contains 5–15 % anion-active and 5 % non-ionic SA. It also contains antifoam substances. In the tentative experiment, the LC_o was established as < 2.5 mg/dm^3, LC_{100} = 100 and LC_{50} = 50 mg/dm^3 as calculated by moving average. Between the LC_o and LC_{50} five concentrations were chosen for the main toxicological experiment (2.5, 5, 10, 20, 40 mg/dm^3). The exposure was set for three days. Water was refreshed daily. The animals were not fed. There were controls in all experiments.

After the molluscs were exposed to pollutant they were measured, weighted on WPS 1200/C weights, and dissected with full exsanguinations. The volume of haemolymph was estimated using insulin syringe, specific gravity was evaluated according to Fillips and Van Slyke [3], pH was tested using express method, total protein content was measured by refractometer RPL-3. The presence or absence of parasitic worms was determined using light microscopy (Biolam LOMO, x56, x280) of temporary histological preparations of hepatopancreas. Trematode species were identified according to Zdun [22]. Since total protein content and specific gravity of haemolymph are plastic features, CV – up to 50 % (p=95 %) was chosen for characterizing their changes.

Results and discussion

Body of great pond snails inside is filled with haemolymph and is called haemocoel. Stability of haemolymph homeostasis is one of the necessary requirements of homeostasis of the molluscs overall organism and its life activity. One of the most significant characteristics of the snail's physiological status is the total protein content in the gastropod's haemolymph. Nearly 90 % of all haemolymph protein in many molluscs is Cu-containing respiratory pigment haemocyanin (Hcy) [16], and the amount of Hcy determines the haemolymph oxygen capacity. The Hcy does not only transport oxygen, it also has significant buffer properties [2] and maintains pH balance of the haemocoel at the optimum level. Finally, its concentration regulates oncotic pressure of the haemolymph, which normally is 1.2–1.3 cm of water column [2, 15]. Similarly to haemoglobin, Hcy can inversely combine with oxygen, but its activity in this process is 3–5 times lower compared to haemoglobin. Still the role of Hcy in providing oxygen to these molluscs is really important, because the content of oxygen combined to Hcy is twice higher than that of oxygen otherwise dissolved in haemolymph plasma [2]. The rest 10 % of haemolymph protein metabolism-related substances include other proteins, sulfhydryl groups, products of nitrogen metabolism such as uric acid, ammonia, guanine, allantoin, purine, amino acids and non-protein (residual) nitrogen [21].

It is found out (Table 1) that neither intact nor infested with *Echinoparyphium aconiatum* Dietz, 1909 (mostly by "mature" (parent) and "young" (daugter) rediae, cercariae, and only occasionally – metarcercariae) *L. stagnalis* of control group had got significant changes in total protein content in haemolymph. We suppose that there are several combined reasons for this. Firstly, the infestation of molluscs was rather moderate, as only 4–7 % of overall volume of hepatopancreas (the organ which is preferable for the helmints) was infected. Also, the foci of parasitic infestation were both few (2–5 per specimen) and did not spread wide (concentrating on areas of 1.1–1.5 × 1.5–1.5 cm). Finally, the trematode life stages which are most harmful for molluscs ("mature" parent rediae), capable of simultaneous holozoic nutrition and parietal digestion [8] – were not numerous. The "young" rediae with underdeveloped pharynx, intestine and locomotive appendages could become a significant danger to their hosts only in large clusters and in connective tissue layers which bound hepatic ducts. The pressure of these peculiar "incrustations" on the adjacent tissue blocked the delivery of oxygen and nutrients from the haemocoel to the infestation foci, and hindered the removal of parasite metabolites toxic to their hosts.

In the environment with 2,5 mg/dm^3 SA, total protein content in haemolymph of intact *L. stagnalis* remained at the normal level, while that of infested molluscs tended to decline. This may indicate the beginning of pathological process in the latter animal group, caused by SA intoxication. Clearly, the balance of $Hcy \leftarrow O_2$ system shifted to the increasing concentration of oxyhaemocyanin in the haemolymph which may be a sign of adaptive and protective processes against the combined harmful influence of toxicants and helminths infestation. Such changes in the $Hcy \leftarrow O_2$ system indicate the overall increased metabolism of *L. stagnalis* [12]. This is supported also by the simultaneous observations of two protective adaptation reactions in molluscs:

the behavioral reaction of avoidance and fast protective physiological reaction of mucus secretion by skin integument.

At 5 mg/dm³ SA in the environment, the gastropods lacking *E. aconiatum* were shown to have lower total protein content in haemolymph, possibly due to the additive shift of the Hcy←O_2 system. The snails infested with that helminths were unable to use this measure of protection against the toxicant in that concentration, as seen from the increased total protein content in their body ($p<0{,}01$). This concentration of SA caused progression of intoxication in the animals of the latter group to the next phase, which is depression. The intact animals in contrast, stayed at the stimulation phase. The depression phase is characterized by the beginning and fast development of increased water content in soft bodies of the snails. The first signal of this process is fine sparse adhesiveness over molluscs head and dorsally on foot, later the adhesiveness spreads and solidifies into extensive swelling.

Table 1

Effect of SA and trematode infestation on the some physic-chemical features of haemolymph homeostasis of *L. stagnalis*

Infestation	n	Tolal protein, %		Specific gravity, g/cm³		pH	
		lim	M±m CV, %	lim	M±m CV, %	lim	M±m CV, %
				Control			
Absent	28	1,2-2,2	1,82±0,01	1,13-1,25	1,14±0,01	7,0-8,0	7,64±0,13
Present	26	1,5-2,0	1,84±0,06	1,20-1,24	1,16±0,02	6,0-8,0	7,52±0,19
				2,5 mg/dm³			
Absent	19	1,3-2,1	1,79±0,05 91,00	1,10-1,17	1,11±0,01 91,00	7,0-8,0	7,60±0,16 92,50
Present	16	1,2-1,8	1,70±0,07 92,50	1,05-1,21	1,13±0,02 91,00	7,0-8,0	7,59±0,19 93,50
				5 mg/dm³			
Absent	17	1,5-1,7	1,62±0,03 94,50	1,09-1,25	1,17±0,04 91,00	7,0-8,0	7,57±0,17 93,50
Present	8	1,4-2,2	1,90±0,04 94,50	1,18-1,28	1,21±0,06 93,00	7,0-8,0	7,49±0,21 91,00
				10 mg/dm³			
Absent	36	1,4-2,1	1,91±0,05 94,00	1,11-1,73	1,16±0,02 90,00	6,0-9,0	7,67±0,26 91,00
Present	31	1,5-2,3	2,16±0,04 96,00	1,22-1,91	1,19±0,01 92,50	6,0-8,0	7,11±0,19 95,50
				20 mg/dm³			
Absent	37	1,6-2,3	2,10±0,08 96,00	1,13-1,36	1,20±0,01 92,00	6,0-7,0	6,88±0,34 97,60
Present	29	1,5-2,8	2,61±0,07 97,50	1,11-1,35	1,29±0,02 94,00	6,0-7,0	6,60±0,45 98,00
				40 mg/dm³			
Absent	33	1,6-2,4	2,02±0,06 95,50	1,22-1,41	1,28±0,02 94,00	5,0-7,0	6,18±0,28 98,70
Present	35	1,7-3,4	3,22±0,12 99,70	1,21-1,44	1,31±0,02 94,50	5,0-7,0	6,02±0,33 99,00

In the environment with 10 mg/dm³ SA, the negative influence of the toxicant is observed in both experimental groups, though differently. The intact specimens entered the depression phase, and the infested animals were deeply depressed. The latter is evidenced by the increase in total protein content by 17 % compared with control ($p<0{,}01$), intense mucus formation on the body surface and respiratory epithelium in the lung, loss of locomotion, highly increased water content of soft tissues (Table 2). The soft body of infested animals weighted significantly more than the soft body of intact molluscs (28.3 % compare to 8.8 %). Depressed lung and cutaneous respiration, or its lack can cause the development of hypoxic and anoxic stress in these animals. The molluscs that experience hypoxia and anoxia are known to revert to glycolysis [11, 13, 14]. The resultant energy is much less than in case of aerobic cleavage of glycogen. However, this allows the animals to survive for at least some time. It is obvious that the infested molluscs are unable to implement glycolysis as fully as the intact individuals because the tissues of hepatopancreas are destroyed in the foci of trematode infestation through mechanical damage and "biochemical mining" (one of the organ's functions is glycogen storage).

At 20 and 40 mg/dm^3 SA all experimental animals had demonstrated hypoproteinemia. The increased total protein content compared with that at 10 mg/dm^3 of toxicants is 9.9 and 16.2 % respectively in intact and 20.8 and 49.1% respectively in the infected individuals ($p<0,01$). This substantial difference in the discussed characteristic between these two animal groups indicates the much smaller endurance of infested molluscs against the combined damage of toxins at helminths. The changes in specific gravity of haemolymph are notably analogous to those of total protein content (Table 1).

Table 2

Effect of SA and trematode infestation on water content in soft body of *L. stagnalis*

Infestation	n	lim	M±m	CV, %
		Control		
Absent	28	2,17-3,99	3,51±0,19	–
Present	26	2,46-3,66	3,41±0,13	
		2,5 mg/dm^3		
Absent	19	2,45-3,80	3,63±0,21	93,00
Present	16	2,76-3,87	3,51±0,17	93,00
		5 mg/dm^3		
Absent	17	2,99-3,93	3,74±0,19	93,50
Present	8	3,14-3,97	3,79±0,15	94,00
		10 mg/dm^3		
Absent	36	2,85-4,99	3,82±0,11	94,00
Present	31	2,87-4,37	3,99±0,15	97,00
		20 mg/dm^3		
Absent	37	2,91-4,31	4,12±0,13	97,00
Present	29	2,98-4,66	4,56±0,23	98,50
		40 mg/dm^3		
Absent	33	3,10-4,52	4,25±0,30	98,00
Present	35	3,17-4,90	4,25±0,30	97,80

Simultaneously the water content of soft bodies of the animals increases, their head and foot do not fit in the shell cavity due to intense adhesiveness and fall out of it (the prolapse reaction). First singular instances of the latter reaction were observed at 10 mg/dm^3 SA in 20 hours after the beginning of experiment, and at the end of experiment it was seen in 100 % of experimental *L. stagnalis*. At the same time, the experimental molluscs develop degenerative and necrotic changes of cutaneous body surface and lung epithelium. The extreme cases had shown some ulcers and bleeding. Also, there was noticed one-time sharp massive intestinal emptying, and sometimes the abortion of underdeveloped egg batches was seen. At the end of exposure, morbidity at 20 mg/dm^3 SA was 5.4 % for intact and 13.8 % for infested animals. The latter still responded to mechanical stimulation (with injection of foot by the thin and sharp needle) meaning that they were able to survive sublethal stage of the pathological process. At 40 mg/dm^3 of toxicant, 29-hour exposure was lethal for 46 % of intact and 98 % of infested molluscs. Those few individuals who survived under the given exposure were in a state of true shock and soon died.

For molluscs it is known [2] that one of the most important characteristics of homeostasis stability is active reaction of their haemolymph regulated by buffer systems of their organisms. The haemolymph buffer systems of invertebrates are based on their proteins, primarily the respiratory pigments. In *L. stagnalis* this role belongs to Hcy which has got markedly high buffer ability. This protein maintains the somewhat stable acid-base balance in snail haemolymph. In our experiment, pH was used to characterize the acid-base balance (Table 1). It is determined that at 2.5, 5, 10 mg/dm^3 SA in the environment, the active reaction of the snail's haemolymph stays stable, weakly alkaline. It does not reflect the influx of acid products of metabolism into the haemocoel of the animals treated with SA in these concentrations (which exceed MPC in 5-80 times for anion-active and in 25-400 times for non-ionic SA) caused by the transition of the most affected animals to the anaerobic respiration under combined influence of toxicant and infestation. At 20 and 40 mg/dm^3 SA (exceeding MPC in 20–120 times for anion-active and in 100–200 times for non-ionic substances) Hcy buffer ability was insufficient to maintain the stability of acid-base balance, and it was acidified slightly. Acidification of the haemolymph was more expressed in the case of trematode infestation.

Hence, adaptation of *L. stagnalis* to various concentrations of SA in its environment in acute experiment is a complex process which combines behavioral, physiological and biochemical reactions aimed at maintaining stability of homeostasis of the molluscs body under the changing conditions of their environment.

It is found out that at 2.5, 5, 10, 20, 40 mg/dm^3 SA in the environment, the stability of haemolymph homeostasis of *L. stagnalis* is disturbed: some changes in total protein content (which is 90 % respiratory pigment Hcy) and in its specific gravity are noticed. Simultaneously, water content of the snail body rises and destruction of the respiratory epithelial surfaces occur. High buffer ability of Hcy ensures the stability of acid-base balance in the snail haemolymph at 2.5, 5, 10 mg/dm^3 SA, and at 20 and 40 mg/dm^3 of the detergent it acidifies. Notably, all of the disturbances in homeostasis are more expressed in great pond snails infested with trematodes. The level of pathogenicity of these parasites for *L. stagnalis* depends both on the intensity of infestation and on their life cycle stages, decreasing from the "mature" (parent) rediae to "young" (daugter) rediae to cercariae.

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СУМІСНИЙ ВПЛИВ ТОКСИКАНТІВ І ГЕЛЬМІНТІВ НА СТАБІЛЬНІСТЬ ГОМЕОСТАЗУ СТАВКОВИКА ВЕЛИКОГО (*LYMNAEA STAGNALIS* L.)

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У сьогодення антропогенний пресинг на гідросферу спричинився до прогресуючого зростання забруднення її різними компонентами як промислових, так і побутово-господарських стоків. Серед них одними з найнебезпечніших для гідробіонтів є поверхнево-активні речовини (ПАР) – компоненти синтетичних миючих засобів.

Досліджено вплив різних концентрацій детергента «Ушастый нянь» (2,5, 5, 10, 20, 40 мг/дм^3) виробництва Вінницяпобутхім на низку фізико-хімічних показників стабільності гомеостазу гемолімфи – внутрішнього середовища *Lymnaea stagnalis* (Linnaeus, 1758) інтактних й інвазованих різними стадіями життєвого циклу (материнські та дочірні редії, церкарії) трематоди *Echinoparyphium aconiatum* Dietz, 1909. Матеріал: 315 екз. молюсків, зібраних вручну у водоймах осушувально-меліоративної системи у басейні р. Тетерів (правобережного допливу Середнього Дніпра) на Центральному Поліссі (хутір Затишшя Житомирської обл.). Методика постановки токсикологічного експерименту стандартна – за (Алексеев, 1981). Йому передувала обов'язкова (Хлебович, 1981) 15-добова аклімація призначених для токсикологічного досліду тварин до умов акваріумного утримання: об'єм акваріумів – 20 л, щільність посадки молюсків – 3–4 екз./л, температура води – 20–22 °С, водневий показник (рН) – 7,9–8,4, оксигенізація – 8,1–8,5 мг O_2/дм^3.

Перебування молюсків у токсичних середовищах супроводжувалось розвитком у них патологічного процесу – отруєння. Найвиразнішими симптомами його були кількісні зрушення значень фізико-хімічних показників гемолімфи піддослідних особин, котрі певною мірою забезпечують стабільність гомеостазу їхньої гемолімфи. До них належать вміст у ній загального білка, її питома маса і значення водневого показника рН. У цих молюсків близько 90 % загального білка гемолімфи представлено гемоціаніном, що визначає завдяки потужним його буферним властивостям рівень кисневої ємності й водно-осмотичного тиску в їх організмі.

Патологічний процес, який розвивається внаслідок комплексного впливу токсиканта і гельмінтної інвазії у *L. stagnalis*, проявляється тим виразніше і стрімкіше, чим вищими є концентрація токсиканта, інтенсивність гельмінтної інвазії та рівень витривалості щодо згаданих вище патогенних чинників різних стадій життєвого циклу трематод. Порушенням значень згаданих вище показників стабільності гомеостазу піддослідні тварини протиставляють сукупність притаманних їм захисно-пристосувальних реакцій – швидких поведінкових, фізіологічних і біохімічних, спрямованих на підтримання життєспроможності особин.

Ефективність таких зусиль у інвазованих трематодами молюсків виявилася значно нижчою порівняно з вільними від інвазії особинами. Ступінь сукупної ушкоджуючої дії токсиканта й паразитів зумовлювався не тільки концентрацією ПАР, але і значною мірою інтенсивністю інвазії і стадією життєвого циклу паразита. Патогенний ефект останніх щодо їхніх хазяїв-молюсків зменшувався у ряду "старі" (материнські) редії > "молоді" (дочірні) редії > церкарії.

Ключові слова: Lymnaea, Trematoda, ПАР, гомеостаз