

УДК 574.52(58)

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TOXIC METALS IN FISH AND MOLLUSCS OF COASTAL WATERS OF ZMIINYI ISLAND

The aim of this paper is to determinate the levels of pollution in fish and molluscs mass species by Toxic metals (TM) such as Cd, Cu, Hg, Pb, Zn, Co, Ni, Cr, Fe, Mn for filling gaps in evaluation of descriptor 9 (contaminants in fish and other seafood).

Material for analysis comprised results of determination of TM in the tissues samples of mass species of fish and mollusks in the Zmiinyi Island coastal waters in 2012-2014. In total we sampled and processed 28 fish and 20 mollusks samples using national and international methods.

In the framework of the PERSEUS Project the data on TMs content in fish and molluscs mass species in the Zmiinyi Island area of the Black Sea (40 km from the Danube Delta) were collected for the first time.

Analysis of soft tissue samples of molluscs and fish mass species collected has revealed the periodically high TMs concentrations in the samples. It has been shown that the maximal concentrations of TMs were registered in samples of mussel soft tissues, as well as in the samples of mollusc-eating hydrobionts – rapa whelk and round goby. The concentrations of TMs in the samples of other bottom fish, such as grey wrasse and sole, feeding mainly on benthic crustaceans, were significantly lower, and in anchovy (pelagic migratory species) individuals' soft tissues was minimal. We concluded that the level of pollution in hydrobionts depends significantly on the feeding schemes of each particular species. The maximal pollution levels for practically all hydrobionts were observed in 2012 (the year of heavy precipitation and the highest runoff of the Danube River).

Keywords: Black Sea, Zmiinyi Island, PERSEUS, Toxic Metals, fish, molluscs

INTRODUCTION

As was shown in [3], the EU Marine Strategy Framework Directive aims at achieving or maintaining a Good Environmental Status (GENS) by 2020 in the territorial waters. To achieve the GENS, Member States have to develop marine strategies that contain programs of measures and that apply an ecosystem-based approach to the management of human activities. Achieving of the GENS requires that a wide range of pressures on marine ecosystems are addressed.

In the past decades qualitative and quantitative ichthyofauna composition of the north-western Black Sea suffered significant reorganizations due to a number of reasons (frequent and long-time fish-kills caused by eutrophication, arrival and spreading of aggressive invader species that undermined food base, intensive fishing and water pollution). The significance of short-cycle species decreased: sprat *S. phalericus* and anchovy *E. encrasicolus ponticus* [2, 13, 16, 18]. Recent studies of the Black Sea ichthyofauna and fishery state [6, 12] have shown the collapse of the larger, higher value fish species.

In the recent decades the studies of water, bottom sediments and biota pollution in the entire Black Sea were activated [1, 7-10, 20, 21]. Especially valuable for studies of pollution impact on fish and benthos are the areas of the Black Sea where untransformed, highly productive and at the same time the most vulnerable ichthyocoenoses are preserved. One of such unique parts of the NWBS is the Black Sea area adjacent to the Zmiinyi Island. There the Odessa National I.I.Mechnikov University is performing complex hydrological and biological studies, the results of which have shown [11, 13, 18, 19] that the Zmiinyi Island coastal waters are within the most productive Black Sea areas. That is why we have carried out in the framework of the PERSEUS Project the studies of fish and molluscs pollution in the Black Sea area where human impact is minimal. Especially valuable for studies of pollution impact to fish and benthos are the areas of the Black Sea where untransformed, highly productive and at the same time the most vulnerable ichthyocoenoses are preserved.

The objective of this paper is to determine the levels of pollution with toxic metals (TMs) such as Cd, Cu, Hg, Pb, Zn, Co, Ni, Cr, Fe and Mn in order to fill in the gaps in evaluation of descriptor 9 (contaminants in fish and other seafood) as the understanding and evaluation of the specific mechanisms of pollutants transfer into the matrix 'water – bottom sediments – biota' are very important and may help to illuminate potentially effective management strategies.

MATERIALS AND METHODS

The Zmiinyi Island area was indicated in the PERSEUS Project as **Study area 17** – North-Western part of the Black Sea. According to working plans in 2012-2014 we have performed studies of TMs accumulation in the mass species of fish and molluscs in the Zmiinyi Island coastal waters. In 2012-2014 fish and molluscs were sampled during five surveys (May-June 2012; May-June 2013; September – October 2013; May-June 2014 and October 2014) carried out in the Zmiinyi Island area and their tissues analysed for the following TMs: Cd, Cu, Hg, Pb, Zn, Co, Ni, Cr, Fe, Mn. The tissue samples were taken from such mass species of molluscs as mussels *Mytilus galloprovincialis* Lamarck, 1819 and rapa whelk *Rapana venosa* (Valenciennes, 1846) and such mass fish species as round goby *Neogobius melanostomus* (Pallas, 1814), rusty blenny *Parablennius sanguinolentus* (Pallas, 1814), grey wrasse *Symphodus cinereus* (Bonnaterre, 1788), sole *Pegusa lascaris* (Risso, 1810) and

anchovy *Engraulis encrasicolus* (Linnaeus, 1758). In July, 2014 were sampled mussels and round goby tissues from the Odessa Bay. In total we sampled and processed 28 fish samples and 20 mollusc samples using national methods [11, 14]. Analyses of biota (fish and mollusc) samples for TMs were performed according to the methodologies [4, 5]. To analyze samples for mercury content the MAS-50 Mercury Analyser was used. Analyses of total HMs were performed using atomic-absorption spectrophotometers with electrothermal atomization SpectrAA 880Z Varian and with flame atomization SpectrAA 220 Varian. The values of limiting permissible concentrations (LPCs) used were taken from [15]. TMs concentrations in biota samples were presented in mg/kg of wet weight.

RESULTS AND DISCUSSION

Fish. According to the results of presented studies the Zmiinyi Island coastal ichthyofauna is represented by 65 fish species. Demersal and bottom-dwelling species dominate: round goby *Neogobius melanostomus*, rusty blenny *Parablennius sanguinolentus* and grey wrasse *Symphodus cinereus*. Out of pelagic species, big aggregations of anchovy *Engraulis encrasicolus* are observed near the island.

Round goby (*Neogobius melanostomus* (Pallas, 1814)) is a typical mollusc-eating species. It accumulates toxic metals contained in large quantities in tissues of the mussels dwelling in the coastal waters of the island. The individuals of round goby of the age group 1-5 years were sampled in the Zmiinyi Island area in 2012 – 2014 and the individuals of age group 3+ in the Odessa Bay in 2014. Like with molluscs, TMs content in round goby tissues decreases with aging. Concentrations exceeding LPCs were found in fish tissue samples for arsenic (maximal value 19.30 mg/kg), copper (maximal value 19.50 mg/kg), lead (maximal value 3.42 mg/kg) and zinc (maximal value 56.70 mg/kg). Quite high concentrations of iron (maximal value 87.7 mg/kg) were also found. TMs concentrations in the individuals sampled in 2014 in the Odessa Bay did not exceed the LPC values. As round goby is a typical mollusc-eating species, it accumulates TMs contained in large quantities in tissues of the mussels dwelling in the coastal waters of the island. Like with molluscs, TMs content in round goby tissues decreases with aging. According to the results of analysis of the mean values of toxicants content in the period of studies, the highest level of pollution was revealed in the individuals of round goby sampled in 2012 (Table 1).

Rusty Blenny *Parablennius sanguinolentus* (Pallas, 1814). Total number of samples was 10. Average value of total length of all the individuals caught near the Zmiinyi Island and analysed made 14.6 ± 1.9 cm, weight – 54.2 ± 2.2 g, the biggest length and weight were 17.5 cm and 93.0 g respectively. Ages of species were 1-5 years. In the diet of the studied rusty blenny specimens 21 species of algae-macrophytes were found (Chlorophyta – 12, Rhodophyta – 8 and Phaeophyta – 1), algae prevailed in weight in the diet of rusty blenny (up to 90%). Individuals of the age 2-4+ years were selected for TMs content determination in the soft tissues of rusty blenny caught in the Zmiinyi Island coastal waters in 2012 – 2014. Concentrations exceeding LPCs

were registered only for zinc (maximal value of content 120.00 mg/kg). Very high content of iron in soft tissues was also revealed (maximal value of content 112.00 mg/kg). As rusty blenny mostly feeds on macrophytes, the TMs content in its tissues is much lower than in the tissues of mollusc-eating round goby. Analysis of average annual TMs concentration has shown that maximal concentrations of copper, zinc and iron in soft tissues of rusty blenny were registered in 2012 (table 2). In 2014 maximal concentrations (not exceeding the LPC) were registered for arsenic.

Table 1

**Average Annual Concentrations of TMs in Soft Tissues
of Round Goby *Neogobius melanostomus* (Pallas, 1814)
in the Zmiinyi Island Coastal Waters in 2012-2014 and in the Odessa Bay in 2014**

TM concentration, mg/kg	Zmiinyi Island coastal waters			Odessa Bay	LPC [6]
	2012	2013	2014	2014	
As	19.30	2.11±1.36	0.87±0.66	0.61±0.16	5.00
Cd	0.17	0.03±0.01	<0.01	0.02±0.01	0.20
Cu	3.50	4.80±0.58	0.88±0.50	0.51±0.24	10.00
Hg	0.62	0.02±0.01	0.03±0.01	<0.01	0.40
Pb	3.42	0.06±0.04	0.15±0.06	<0.01	1.00
Zn	56.70	11.41±3.85	14.20±1.52	5.27±0.96	40.00
Ni	43.30	0.35±0.06	0.47±0.23	0.07±0.3	-
Cr	0.11	0.08±0.01	<0.30	0.07±0.2	-
Fe	87.70	12.86±6.52	10.88±2.78	3.95±0.76	-
Mn	14.00	3.16±1.04	2.47±1.30	2.04±0.46	-
Number, pcs	1	12	7	4	

Grey wrasse *Symphodus cinereus* (Bonnaterre, 1788), Sole *Pegusa lascaris* (Risso, 1810) and Anchovy *Engraulis encrasicolus* (Linnaeus, 1758). Overall length of the analysed individuals of grey wrasse and sole was 9.5-10.2 and 18.4 cm respectively, weight – 13.0 and 75 g respectively. The average overall length of anchovy individuals caught near the island in 2013 and 2014 made 10.5-11.6 cm, weight – 8.1-11.3 g.

According to the stomach content analysis, grey wrasse and sole are feeding mainly on benthic crustaceans, polychaeta and molluscs. The diet of anchovy individuals caught in the coastal waters of the island in 2013-2014 comprised organisms belonging to 15 taxa. Among them were organisms of planktobenthos, planktonic crustaceans and polychaeta. Under shortage of zooplankton anchovy have to feed on phytoplankton.

Table 2

**Average Annual Concentrations of TMs in Soft Tissues of Rusty Blenny
(*Parablennius sanguinolentus*, Pallas, 1814) in the Zmiinyi Island Coastal Waters
in 2012-2014**

TM concentration, mg/kg	Zmiinyi Island coastal waters			LPC [6]
	2012	2013	2014	
As	1.18	1.07±0.36	2.41±0.91	5.00
Cd	0.05	0.01±0.01	<0.30	0.20
Cu	4.09	0.90±0.33	3.14±0.50	10.00
Hg	0.31	0.03±0.02	0.03±0.02	0.40
Pb	0.78	0.05±0.02	0.19±0.02	1.00
Zn	120.00	31.83±9.95	11.23±1.55	40.00
Ni	1.01	0.59±0.38	<0.10	-
Cr	0.22	0.07±0.02	<0.30	-
Fe	112.00	12.94±4.30	15.43±6.61	-
Mn	11.90	3.48±1.42	0.95±0.01	-
Ind., pcs	1	18	7	

To analyse the content of toxic metals in the soft tissues of grey wrasse the individuals in the age of 2+ were selected; to analyse the content of TMs in the tissues of sole and anchovy the 3 years old individuals were selected. Only in the samples of grey wrasse and sole concentrations of zinc exceeding the LPC and high content of iron were registered. Significant content of manganese was found in grey wrasse caught near the island in 2012. Content of all the TMs in tissues of the analysed individuals of grey wrasse and sole, which are feeding mainly on small benthic crustaceans, was significantly lower than in the mollusc-eating round goby. Concentration of toxic metals in anchovy (pelagic migratory species) individuals' soft tissues was minimal. To analyse the content of toxic metals in the soft tissues of grey wrasse the individuals in the age of 2+ were selected; to analyse the content of TMs in the tissues of sole and anchovy the 3 years old individuals were selected. Only in the samples of grey wrasse and sole concentrations of zinc exceeding the LPC was registered and high content of iron. Significant content of manganese was found in grey wrasse caught near the island in 2012. Content of all the TMs in the tissues of the analysed individuals of grey wrasse and sole, which are feeding mainly on small benthic crustaceans, was significantly lower than in the mollusc-eating round goby. Concentration of toxic metals in anchovy (pelagic migratory species) individuals' soft tissues was minimal (Table 3).

Table 3

Concentrations of TMs in Soft Tissues of Grey Wrasse *Symphodus cinereus* (Bonnaterre, 1788), Sole *Pegusa lascaris* (Risso, 1810) and Anchovy *Engraulis encrasicolus* (Linnaeus, 1758) in the Zmiinyi Island Coastal Waters in 2012-2014

TMs concentration, mg/kg	Fish Species (sampling date; age, years)				LPC [6]
	<i>S. cinereus</i>		<i>P. lascaris</i>	<i>E. encrasicolus</i>	
	20.05.12	13.05.13	20.05.12	14.05.14	
As	1.64	<0.10	2.40	0.60	5.00
Cd	0.06	0.02	0.06	<0.01	0.20
Cu	4.66	0.67	4.95	0.27	10.00
Hg	0.42	<0.01	0.24	<0.01	0.40
Pb	0.69	0.06	0.83	0.15	1.00
Zn	56.70	5.30	53.30	4.71	40.00
Ni	<0.10	0.95	2.47	<0.10	-
Cr	0.11	<0.02	0.17	<0.30	-
Fe	87.80	3.90	55.00	1.24	-
Mn	37.80	4.45	7.53	2.04	-
Number, pcs	1	3	1	5	

Molluscs. Two species of molluscs were studied: mussels (*Mytilus galloprovincialis* Lamarck, 1819) and rapa whelk (*Rapana venosa*, Valenciennes, 1846).

Mussels (*Mytilus galloprovincialis* Lamarck, 1819). In 2012 – 2014 content of TMs was determined in the samples from 2.5 – 7 years old individuals. One sample of mussels was taken for comparison from the Odessa Bay in 2014. Analysis of the data (table 6) has shown that TMs concentrations exceeding LPCs were observed for arsenic (maximal value 8.76 mg/kg at LPC=2 mg/kg), cadmium (maximal value 3.17 mg/kg at LPC=2 mg/kg), mercury (maximal value 0.60 mg/kg at LPC=0.20 mg/kg) and zinc (maximal value 311.00 mg/kg at LPC=200 mg/kg). Significant concentrations of nickel (maximal value of content 85.10 mg/kg), manganese (maximal value of content 89.60 mg/kg), copper (maximal value of content 24.1 mg/kg) and iron (maximal value of content 878 mg/kg) were also found. LPCs for TMs were not exceeded in the mussels from the Odessa Bay, however high concentrations of zinc and iron were revealed (maximal values of content – 77.20 and 54.10 mg/kg, respectively).

Analysis of average annual TMs concentration values (table 4) in the samples of mussels during the period of studies revealed the highest level of pollution with TMs in the mussels sampled in 2012. That year was characterised by intensive precipitation and the impact of the Danube River runoff was the highest. In the low-water periods of 2013-2014 the levels of mussels' pollution with TMs decreased significantly.

Rapa whelk (*Rapana venosa*, Valenciennes, 1846). To analyse TMs content in soft tissues of rapa whelk caught in the Zmiinyi Island coastal waters in 2012 – 2014 samples of age group 2 – 5 years were selected.

The results of analysis of the data received have shown that the concentrations exceeding the LPCs were registered for arsenic (maximal value of content 11.30 mg/kg), copper (maximal value of content 131.00 mg/kg) and mercury (maximal value of content 0.55 mg/kg).

High levels of zinc and iron content were also revealed (maximal value of content 51.2 and 39.10 mg/kg, respectively). Rapa whelk is a predatory mollusc whose main diet near the Zmiinyi Island, like in the other Black Sea parts, are mussels.

Table 4

Average Annual Values of TMs Concentration in the Samples of Soft Tissues of Mussels *Mytilus galloprovincialis* Lamarck, 1819, Taken in the Zmiinyi Island Coastal Waters (2012-2014) and in the Odessa Bay (2014)

Concentration of TM, mg/kg	Zmiinyi Island coastal waters			Odessa Bay	LPC [6]
	2012	2013	2014	2014	
As	4.33±4.21	2.82±1.96	0.25±0.21	1.60±0.09	2.0
Cd	2.76±0.67	0.14±0.11	0.09±0.01	0.29±0.08	2.0
Cu	18.37±4.99	2.21±1.02	1.31±0.38	1.45±0.23	20.0
Hg	0.26±0.30	<0.01	0.04±0.03	<0,01	0.20
Pb	3.04±2.36	0.19±0.14	0.05±0.01	0.18±0.06	10.0
Zn	160.3±14.5	42.3±24.6	19.7±9.3	76.2±1.4	200.0
Ni	39.50±39.71	1.59±1.43	0.44±0.21	0.33±0.21	-
Cr	0.89±0.12	0.17±0.03	0.51±0.07	0.32±0.07	-
Mn	66.45±32.74	6.80±3.47	2.97±0.14	14.79±8.37	-
Fe	503.5±53.0	63.2±31.2	89.4±9.6	83.0±40.9	-
Ind., pcs	121	215	37	69	

It is apparent that toxic metals contained in the mussels tissues are accumulated by rapa whelk. It should be pointed out that according to the results received the content

of TMs in rapa whelk and molluscs tissues decreases with their age. Investigation of the dynamics of toxicants concentration in mollusc samples with age requires accumulation of statistically valid data for a longer period of studies.

Analysis of average annual TMs concentrations for the period of studies has shown that the maximal levels of pollution were revealed in the samples taken in 2012. It should be pointed out that during that period high concentrations of toxic metals were also found in mussels, which comprised the main diet of rapa whelk (Table 5).

Table 5

TMs Concentrations in the Samples of Soft Tissues of *Rapana venosa* (Valenciennes, 1846), Taken in the Zmiinyi Island Coastal Waters in 2012-2014

TM concentration, mg/kg	Zmiinyi Island Coastal Waters			LPC [6]
	2012	2013	2014	
As	11.30	3.84±0.08	3.34±1.60	2.00
Cd	0.42	2.13±3.10	0.18±0.10	2.00
Cu	131.00	14.91±1.55	4.40±±3.72	20.00
Hg	0.55	<0,01	0.03±0.03	0.20
Pb	2.65	0.05±0.02	0.50±0.54	10.00
Zn	51.20	19.60±5.46	14.64±4.44	200.00
Ni	<0.10	0.42±0.04	0.46±0.54	-
Cr	0.40	3.71±0.49	<0.02	-
Mn	6.69	1.19±0.21	1.63±0.52	-
Fe	146.00	29.10±10.69	11.29±1.50	-
Ind., pcs	2	15	14	

CONCLUSIONS

In the framework of the PERSEUS Project the data on TMs content in mass fish and mollusc species in the Zmiinyi Island area of the Black Sea (40 km from the Danube Delta) were collected for the first time. Analysis of soft tissue samples of molluscs (mussel and rapa whelk) and fish (round goby, rusty blenny, grey wrasse, sole and anchovy) mass species collected has revealed the periodically high TMs concentrations (arsenic, copper, zinc, nickel, iron, and manganese) in the samples, often exceeding the LPCs [6].

The maximal concentrations of TMs were registered in samples of mussel soft tissues, as well as in the samples of mollusc-eating hydrobionts – rapa whelk and

round goby. The concentrations of TMs in the samples of other bottom fish, such as grey wrasse and sole, feeding mainly on benthic crustaceans, were significantly lower. The level of rusty blenny's soft tissues pollution was even lower, as rusty blenny feeds mainly on macrophytes. Concentration of toxic metals in anchovy (pelagic migratory species) individuals' soft tissues was minimal. Thus, we can make the conclusion that the level of hydrobionts pollution depends significantly on the feeding schemes of each particular species. According to the results of the TMs average annual concentrations analysis in 2012-2014, the maximal pollution levels for practically all the studied hydrobionts were observed in 2012 (the year of heavy precipitation and the highest runoff of the Danube River). During the low-water periods of 2013-2014 the levels of hydrobionts pollution with TMs decreased significantly. It was shown that the TMs concentrations in the tissues of mussels, round goby and rusty blenny decrease with the hydrobionts' age. Study of dependence of the TMs concentration level in those species on age requires accumulation of statistically valid data for a longer period of studies. To understand the processes of pollution influence on benthic and pelagic communities of the coastal ecosystem the further in-depth studies and modelling of the TMs impact on hydrobionts under the natural conditions and in the areas suffering intensive anthropogenic impact are required. At that, measurements of TMs in bottom sediments and marine water should be taken simultaneously with the studies of hydrobionts pollution because water and sediments are the hydrobionts' habitat. TMs should also be measured in such biological objects as phytoplankton, zooplankton etc., which are food resource for the studied fish and molluscs. We propose to use the Zmiinyi Island Marine Research Station as one of basic sites for future Reference Monitoring network in the Black Sea open waters.

ACKNOWLEDGEMENTS

The work has been done in the framework of the FP7 PERSEUS Project No. 287600 (2011-2015) and the National projects funded by the Ministry of Education and Science of Ukraine.

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Надійшла 10.12.2015

УДК 574.52(58)

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ТОКСИЧНІ МЕТАЛИ В РИБІ ТА МОЛЮСКАХ ПРИБЕРЕЖНИХ ВОД ОСТРОВУ ЗМІЇНИЙ)

Резюме

Представлені і проаналізовані результати визначення токсичних металів (ТМ) в масових видах риб та молюсків в 2012-2014 роках, що були виконані в рамках проекту PERSEUS поблизу острова Зміїний, розташованого в північно-західній частині Чорного моря в 40 км від дельти Дунаю. Показано, що у зразках м'яких тканин масових видів молюсків (мідія та рапана) і риб (бичок-кругляк, морська собачка, зеленушка, морський язик і анчоус), зібраних в прибережних водах острова, періодично реєструвались високі концентрації токсичних металів (миш'як, мідь, цинк, нікель, залізо та марганець), які часто перевищували гранично дозвалені концентрації.

Ключові слова: Чорне море, острів Зміїний, PERSEUS, токсичні метали, риби, молюски

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ТОКСИЧНЫЕ МЕТАЛЛЫ В РЫБЕ И МОЛЛЮСКАХ ПРИБРЕЖНЫХ ВОД ОСТРОВА ЗМЕИНЫЙ

Резюме

Представлены и проанализированы результаты определения токсических металлов (ТМ) в массовых видах рыб и моллюсков в 2012-2014 годах, которые были выполнены в рамках проекта PERSEUS вблизи острова Змеиный, расположенного в северо-западной части Черного моря в 40 км от дельты Дуная. Показано, что в образцах мягких тканей массовых видов моллюсков (мидия и рапана) и рыб (бычок-кругляк, морская собачка, зеленушка, морской язык и анчоус), собранных в прибрежных водах острова, периодически регистрировались высокие концентрации токсических металлов (мышьяк, медь, цинк, никель, железо и марганец), которые часто превышали предельно-допустимые концентрации.

Ключевые слова: Черное море, остров Змеиный, PERSEUS, токсические металлы, рыбы, моллюски.