Bioindication and biotesting of water and sediments of water bodies of the Danube biosphere reserve

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1 Introduction

Large amounts of toxic substances penetrate into the lower part of the river (Sheliah-Sosonko 1999; Aleksandrov 2001) demanding the continuous ecological monitoring of the Danube delta, especially in the area of the Danube biosphere reserve. Taking into account that sediments are the main sink of contaminants and can be a source of secondary pollution in aquatic ecosystems, the development of methods for rapid and cost-effective screening of bottom fauna status and sediment toxicity are urgently needed.

The tasks of the current study included: 1) characterization of benthic invertebrates of the Danube Delta and bioindication of the state of water bodies; 2) investigation of toxicity of the Danube Delta water and sediments (biotests); 3) comparison of bioindication and biotesting data.

2 Materials and methods

In June 2007 water and sediment samples were taken 3 m from the river bank at five monitoring stations in the Kilia part of the Danube Delta (see Figure 1): station 1 – the origin of Ochakivskiy arm, 17 km from the Black Sea (gray clayey silt, depth 0.8 m); station 2 – the end of Ochakivskiy arm, 6 km from the Black Sea (silty sand; depth 1.5 m); station 3 – the origin of Bistriy arm, 10 km from the Black Sea (gray clayey silt, depth 1.0 m); station 4 – the end of Bistriy arm, Danube outflow (sand, depth 0.5 m); station 5 – the origin of Vostochniy arm, 8 km from the Black Sea (gray clayey silt, depth 2.0 m). For sampling the sediments, a bottom-grab was used with the working surface of 100 cm^2. The samples were collected in triplicate.

Taxonomic diversity and abundance of benthic invertebrates were used for calculating biotic indices (Goodnight-Whitley, Mayer and Woodiwiss: Goodnight 1961; Woodiwiss 1977; Afanasiev 2004; Arsan et al. 2006).

Water samples were passed through a plankton net (mesh-size 320 µm) for separating small aquatic organisms. Water and sediment samples used for biotests were transported to the laboratory of the Institute of Hydrobiology in a portable refrigerated chamber (+4ºC).

For investigation of sediments toxicity water elutriates were prepared (Scherban' 1994). The procedure included 1-hour shaking of sediments with water (1:4 by mass), settling of suspended particles and further use of supernatant. The samples of water and elutriates of sediments were used for toxicity tests with water flea *Daphnia magna* and onion *Allium cepa* with end-points survival (48, 96 h) and root growth inhibition (120 h), respectively (Fiskesjo 1985; ISO 6341:1996).

We used a multimetric biological assessment system comparable to that developed by AQEM (Hering et al. 2004). To compare data on bioindication and toxicity we unified multi-metric indices and used 5-classes categorization, which was recommended by the European Water Framework Directive. It should be noted that only the way of presenting the results is in line with WFD, because this approach is not widely elaborated in Ukraine. Classes of categorization are known only for biomass and abundance indices (Oksiiuk et al. 1994), and for toxicity of waste waters. Therefore we based as well on our

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experience and recommendations of our colleagues (Romanenko et al. 2008). The five classes are presented in Table 1.

Table 1. *The scale for unification of bioindication and toxicity data and assessment of the ecological state of water body.*

State / Average mark	**High** 1.0–1.5	**Good** 1.5–2.5	**Moderate** 2.5–3.5	**Poor** 3.5–4.5	**Bad** 4.5–5.0
Survival (96 h), %	>90	90–80	80–67	67–50	<50
Growth inhibition or stimulation, %	<10	10–25	25–50	50–75	>75
Total abundance, ind/m^2	<500	600–2100	2100–10000	10100–40000	>40000
Total biomass, g/m^2	<5.0	5.1–50.0	50.1–300	300.1–1000	>3000
Woodiwiss index	10–9	8–7	6–5	4–3	2–1
Mayer index	>22	21–17	16–11	10–5	<5
Goodnight–Whitley index	1–45	46–70	71–80	81–90	91–100

2.1 Characterization of macrofauna

In total 24 taxa of benthic invertebrates were recorded (Table 2).

Table 2. *Taxonomic composition and the abundance of bottom invertebrates.*

№	Groups of bottom invertebrates	Abundance, ind/m^2				
		Station 1	Station 2	Station 3	Station 4	Station 5
1	***Oligochaeta***	**1400**	**4900**	**2400**	**6300**	**2100**
	Limnodrilus sp.	800	2800	1300	3200	
	Limnodrilus hoffmeisteri (Claparede)	100	300	400		
	Isochaetides newaensis (Michaelsen)		300		400	300
	Isochaetides michaelseni (Lastockin)	500		300	2700	1400
	Potamothrix moldaviensis (Vejdovsky et Mrazek)		500			400
	Tubifex tubifex (O. F. Muller)		800	400		
	Branchiura sowerbyi (Beddard)		200			
2	***Corophiidae***					**600**
	Corophium curvispinum (Sars)					500
	Corophium robustum(Sars)					100
3	***Gammaridae***			**100**	**100**	
	Dikerogammarus haemobaphes (Ehrenberg)			100		
	Pontogammarus robustoides (Sars)				100	
4	***Coleoptera***	**100**				

	Hydrophilus flavipes (Steven)	100				
5	***Chironomidae***	**100**	**400**		**600**	**200**
	Cricotopus silvestris (Fabricius)	100			500	
	Fleuria lacustris (Kiffer)					200
	Psectrotanypus varius (Fabricius)		200			
	Polypedilum convictum (Walker)		200		100	
6	***Bivalvia:***	**100**		**100**		**100**
	Spheriidae sp.	100				
	Dreissena polymorpha (Pallas)			100		100
7	***Gastropoda:***	**1700**		**600**		**800**
	Fagotia esperi (Ferussae)	300				500
	Bithynia tentaculata (Linne)					200
	Physa fontinalis (Linne)			100		
	Lithogliphus naticoides (Pfeiffer)	1400		200		
	Theodoxux fluviatilis(Linne)			200		100
	Valvata pulhella (Studer)			100		
	Total taxa	**8**	**8**	**10**	**6**	**10**
	Total abundance, ind/m^2	**3400**	**5300**	**3200**	**7000**	**3800**
	Total biomass, g/m^2	**74.35±7.4**	**6.19±0.28**	**101.35±2.8**	**2.30±0.46**	**152.03±7.46**

Obtained values of Mayer and Woodiwiss indices are consistent in general, while the Goodnight-Whitley index deviated (Table 3). The reason for this disagreement may be the following: Goodnight-Whitley index takes into account only *Oligochaeta* that are not very sensitive to pollution, while Mayer and Woodiwiss indices are based on the diversity of indicator taxa. Moreover, some difficulties arose from transferring the original number of quality classes proposed by Goodnight-Whitley and Mayer (3 and 4, respectively) to the 5-class-system recommended by the European Water Framework Directive.

According to average marks determined from biotic characteristics (abundance; biomass; Woodiwiss, Mayer and Goodnight–Whitley indices) the ecological state at stations 1, 4 and 5 could be rated as “moderate” (class 3), while stations 2 and 3 were “poor” (class 4).

Table 3. *The values of biotic indices.*

Place of sampling	Goodnight–Whitley index, %	Woodiwiss index	Mayer index
Station 1	41	3	5
Station 2	92	2	4
Station 3	75	2	3
Station 4	90	4	5
Station 5	55	2	7

2.2 The toxicity assessment of water and sediments

According to the Daphnia test the water was not or weakly toxic (Table 4). High pollution was registered at station 1, where the sediment samples indicated acute toxicity.

Table 4. *Survival of Daphnia magna in water and water elutriates of sediments.*

Sampling stations		Survival, %	
		48 hours	96 hours
1	water	96.7	83.3
	sediments	66.7	45.4
2	water	100.0	93.3
	sediments	93.3	83.3
3	water	90.0	76.7
	sediments	90.0	83.3
4	water	93.3	80.0
	sediments	86.7	66.7
5	water	96.7	93.3
	sediments	93.3	89.7

Table 5. *Allium cepa roots growth inhibition in water and water elutriates of sediments.*

Sampling stations		Average length of roots, mm	Growth inhibition, %
1	water	47.8±0.2	0.4
	sediments	27.6±0.3	42.5
2	water	47.7±0.5	0.8
	sediments	30.1±0.4	37.4
3	water	32.6±0.2	32.2
	sediments	29.6±0.4	38.3
4	water	32.4±0.3	32.6
	sediments	39.9±0.2	17.0
5	water	40.1±0.5	16.6
	sediments	32.8±0.6	31.8
Control		48.0±0.3	

Sediments from most of the stations (except station 4) demonstrated statistically significant adverse effect to *Allium cepa* (Table 5). The toxicity of sediments is higher than the toxicity of water samples suggesting the accumulation of toxic substances in sediments. However, water in stations 3 and 4 (Bistriy arm) was more toxic than sediments, may be due to dredging.

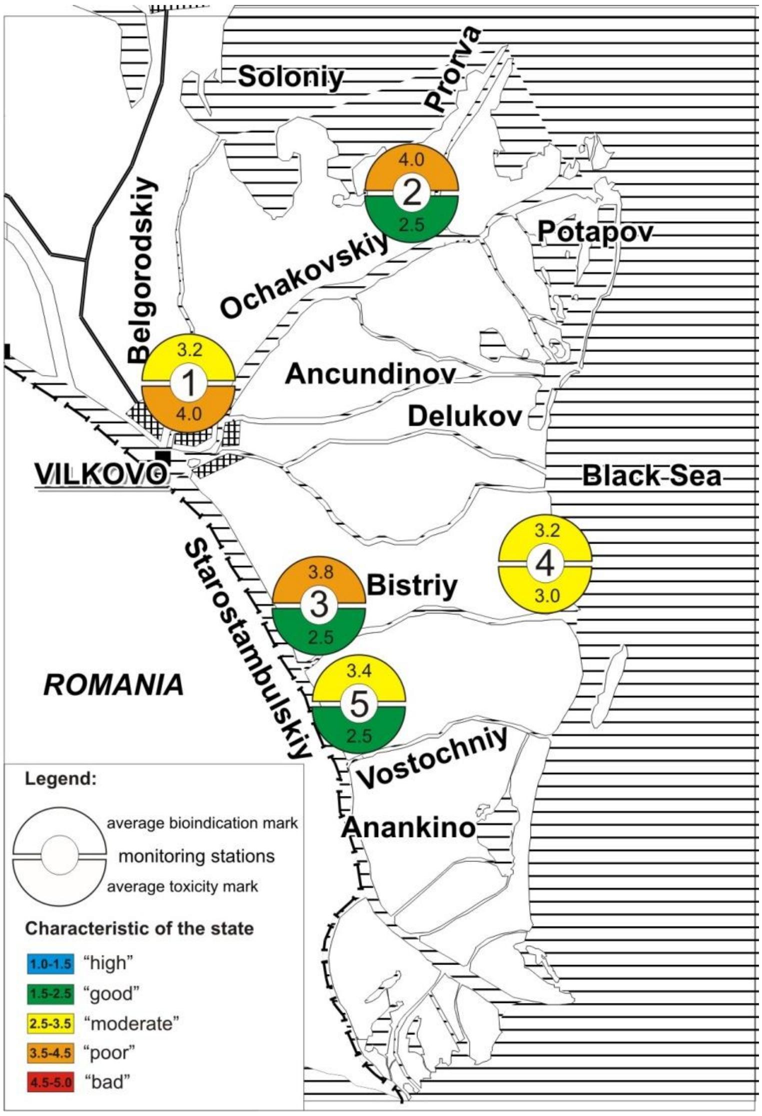

Figure 1. *Mapping of ecological state of investigated water bodies according to bioindication of benthic communities and sediments toxicity testing.*

The toxicity and bioindication data have no close correlation (Figure 1). Possible explanation of this phenomenon is that biotic indices characterize the general ecological state of water bodies that depends on many environmental factors; however, contamination of water bodies by toxic substances is only one of these factors. The poor state of benthic communities at sampling stations 2 and 3 may not be caused by sediment contamination and additional investigations are required.

3 Conclusion

Our data are indicative of moderate state of benthic communities of water bodies of the Danube biosphere reserve. The toxicity data suggest that the sediments of Ochakivskiy arm were most toxic (station 1), most likely caused by anthropogenic impact of Vilkovo city (Kharchenko et al. 1993). Sediments from the Bystriy arm were less toxic; those from Vostochniy arm (station 5) did not show statistically significant adverse effect. As expected, the toxicity of sediments was higher than the toxicity of water samples, and *Allium cepa* was more susceptible than *Daphnia magna*. This could indicate that sediments were contaminated by agricultural chemical substances, but supplementary investigations are required.

In Ukraine there is a need for developing methods allowing for the rapid and cost-effective screening of bottom fauna status and sediment toxicity. Moreover, improvement of the approach for assessment of ecological state is required taking into account peculiarities and individuality of water bodies.

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