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Risk assessment

ECOTOXICOLOGICAL TESTS USED AS WARNING SYSTEM FOR DANUBE DELTA QUALITY ASSESSMENT

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Abstract. The Danube Delta water and sediment toxicity effects have been assessed using a biotests battery based on representative species of primary producers (*Selenastrum capricornutum*, *Sorghum saccharatum*, *Lepidium sativum*, *Sinapis alba)* and consumers (rotifers – *Brachionus calyciflorus*, crustaceeans – *Daphnia magna*, *Heterocypris incongruens*). The ecotoxicological tests results revealed no acute to minimum toxic effect classified as Class I and II of hazard for water samples and Class II and III, in case of sediment samples. In order to control water and sediment-quality, these results indicated the need to integrate within a water and sediment-quality triad different tests such as toxicity tests, chemical analyses as well as field data from water phytoplankton, soft-sediment meio- and macro-invertebrates. Overall, the ecotoxicological results were modulated by organisms sensitivity, sampling locations, season and climate change issues.

Keywords: Danube Delta, ecotoxicological investigation, microbiotests.

AIMS AND BACKGROUND

The urban development, hydrotechnical works, navigation and anthropogenic disturbances have affected the rivers worldwide. It is the case of the Danube River and Danube Delta, the largest Romanian wetland, which are susceptible to those environmental changes leading to water quality degradation and reduction of biological resources which in turn affected the human well-being[1]. Moreover, in the past century, more than 100 000 ha (most of them temporarily flooded areas) were embanked and about 50 km of the southern Danube Delta branch (Sfantu Gheorghe) was affected by the meander cutoffs for navigation purposes. Moreover, the majority of the Delta ecological systems were subjected to inappropriate agricultural practices (which included fertilisers and pesticides)[2] and thus, the water quality was altered.

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Until recently, extensive monitoring programs of many European river basins[3] were conducted only for chemical contamination. Since the European Union (EU) Water Framework Directive (WFD) implementation, the assessment must be made by using biological elements due of their potential to retain the effects of environmental pressures[4,5]. Although, the chemical analysis identifies the source of toxic substances and their metabolites, the method fails to provide data about biological effects to organisms[6–8]. In contrast, ecotoxicological bioassay provides data about biological effects[9], making a realistic estimation and understanding of the pollutants hazard[10]. Moreover, the present European research is focused on toxicant identification in European river basins, to generate a strategy to reduce the toxic pressure on aquatic ecosystems, according to the WFD (Ref. 3).

The study aims to evaluate the toxicity of Danube Delta water and sediment using biological models, such as primary producers (*Selenastrum capricornutum*, *Sorghum saccharatum*, *Lepidium sativum*, *Sinapis alba)* and consumers (rotifers – *Brachionus calyciflorus*, and crustaceans – *Daphnia magna*, *Heterocypris incongruens*), which could be used as warning system for water body quality.

EXPERIMENTAL

To achieve an overview of the Danube Delta water quality, water and sediment samples were collected, according with the specific sampling guidelines, during 2013 (January, February, March, June, July, August and September) from eleven sites along the southern branch – Sfantu Gheorghe (Fig. 1). Bioassays performed to assess the water and sediment toxicity are presented in Table 1.

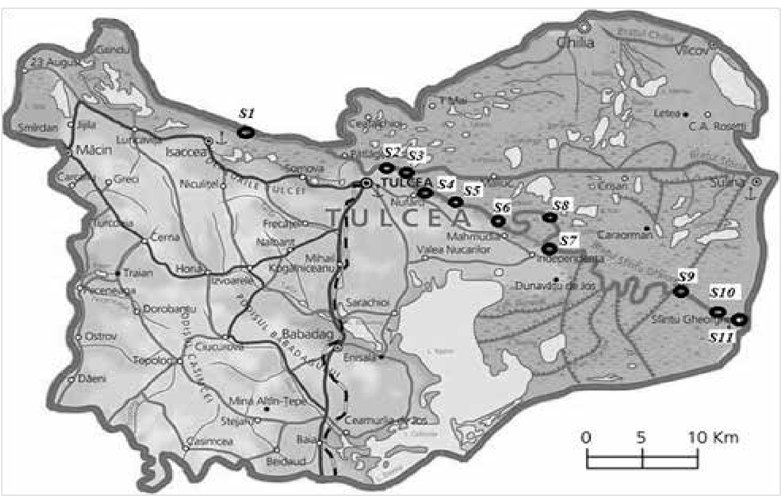

Fig. 1. Map of Danube Delta sampling sites (*S1* – Isaccea, *S2* – Tulcea Upstream, *S3* – Tulcea Downstream, *S4* – Nufaru, *S5* – Baltenii de Sus, *S6* – Mahmudia, *S7* – Murighiol, *S8* – Uzlina, *S9* – Ivancea, *S10* – Sfantu Gheorghe, *S11* – Black Sea confluence)

Table 1. Bioassays used to assess the water and sediment toxicity in Danube Delta

Method/ microbiotest	Organisms	Test type	Examined behaviour	Test duration/ temperature	Sample matrix	Treatment
OECD 201 AlgaltoxKit F™ (Ref. 11)	*Selenastrum capricornutum*	acute/chronic	growth inhibition	72 h, 21–25°C	water	undiluted, without extra nutrients
OECD 202 DaphtoxKit F™ (Ref. 12)	*Daphnia magna*	acute	mortality	24–48 h, 20°C	water	undiluted
ASTM Standard Guide E1440-91 RotoxKit F™ (Ref. 13)	*Brachionus calyciflorus*	acute	mortality	24 h, 25°C	water	undiluted
ISO 11269-1 PhytotoxKit (Ref. 14)	*Sinapis alba (SIA), Lepidium sativum (LES), Sorghum saccharatum (SOS)*	acute	germination inhibition root growth	3 days, 25°C	sediment	untreated, 100% saturated
ISO 14371 OstracodtoxKit F™ (Ref. 15)	*Heterocypris incongruent*	chronic	mortality growth inhibition	6 days, 25°C	sediment	untreated, 100% saturated

The water toxicity was classified according to the hazard classification system for natural water[16]. The effect percentages (EP) of each type of organisms is transformed in toxicity units (TU) (g l^{-1}) using formula: TU = 100/EP.

The current classification system is based on acute toxic/hazard value, divided in five hazard classes, as follows: Class I – no acute hazard – no test did not reveal toxic effect; Class II – slight acute hazard, 20% ≤ EP < 50%, in at least one test; Class III – acute hazard, 50% ≤ EP < 100%, in at least one test; Class IV – high acute hazard, EP=100%, in at least one test; Class V – very high acute hazard, EP = 100%, in all tests.

RESULTS AND DISCUSSION

Water. The results showed a continuous algae growth during the analysed period (Table 2) with a few exeptions for: (i) February, no significant algae growth stimulation was recorded along the monitored stations, except S7 and S8 where it was observed a slight increase in growth inhibition, and (ii) August at S7 and S8, where the percentage of growth inhibition was higher than 40%. During the summer, in June (26.2% at S3, 28.2% at S7, 33.6% at S8 and 24.3% at S11) and July (34.8% at S7 and 48.9% at S8), but also September (24.8% at S4, 20.5% at S6, 35.8% at S7 and 36.1% at S8) the algae growth was stimulated in all sampling stations, due to high concentrations of nutrients (data not shown). The results were correlated with the chlorophyll 'a' values determined in the surface water samples, in the same time period. The highest chlorophyll 'a' concentrations were recorded in the sampling sites downstream Tulcea county in S3, S7, S8 and S10 (Ref. 17).

Table 2. Inhibition/stimulation percentage of green algae growth in the Danube Delta surface water samples after 72 h incubation period

Sample site	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	Control
February 2013	3.86	1.04	–1.16	–8.22	0.30	–0.07	9.87	6.26	–6.58	–7.27	–2.02	0
March 2013	12.70	10.6	6.28	2.84	12.60	4.66	22.20	7.43	33.00	0.80	6.67	0
June 2013	–12.20	13.10	–26.20	–10.80	4.94	–11.20	–28.20	–33.60	–1.56	2.27	–24.30	0
July 2013	–6.80	6.40	–21.40	–18.80	–0.46	–19.80	–34.80	–48.90	–24.10	1.32	–11.40	0
August 2013	–0.59	–11.60	–29.90	–20.30	12.80	–17.01	41.70	48.90	28.40	–5.99	15.90	0
September 2013	1.64	–9.84	–24.80	–21.80	4.85	–20.50	–35.80	–36.10	29.40	–3.66	9.48	0

The water samples toxicity collected along the Danube Delta systems showed no significant effects on *Daphnia magna*. Crustaceans mortality ranged from 0 to 30% with a peak in March and also, September. The S1, S3, S4, S6 and S9 sampling sites revealed the highest percentages of mortality in the range of 25–35% (Fig. 2).

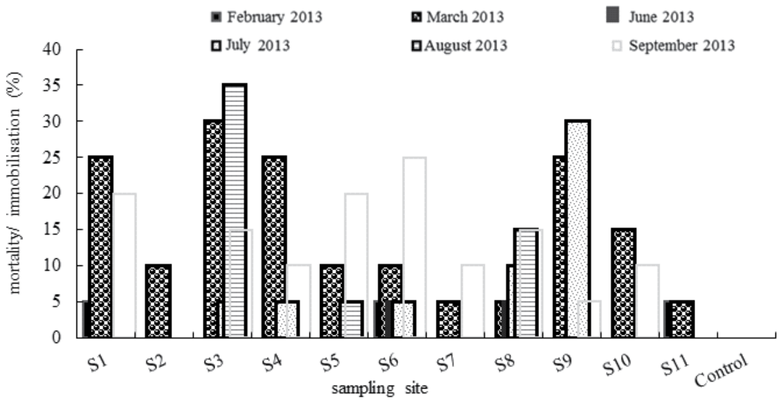

Fig. 2. Water samples toxicity on *Daphnia magna* along Sf. Gheorghe branch during 2013

Also, no significant effects on rotifers, the mortality percentages were lower than 13% in all sampling stations, except S9 (in March), when it was obtained 76% mortality (Fig. 3).

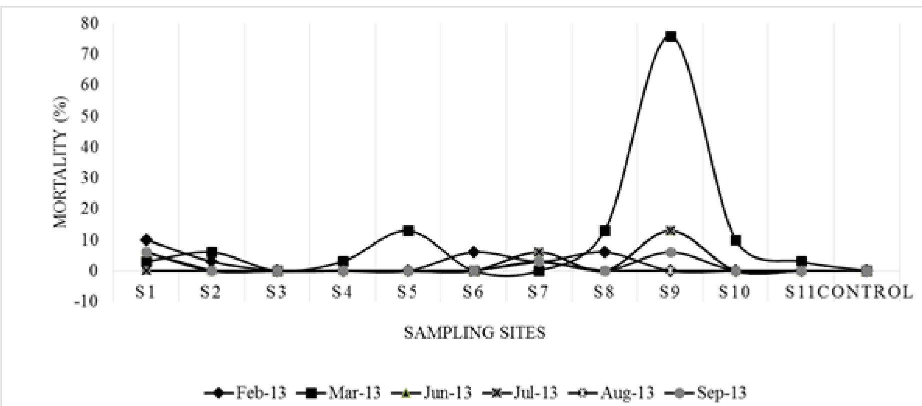

Fig. 3. Water samples toxicity on *Brachionus calyciflorus* along Sf. Gheorghe branch during 2013

Given the toxicity classification of natural waters[10], the Danube Delta water samples toxicity is centralised in Table 3.

Table 3. Classification of the Danube Delta surface water samples according to Persoone et al.[16]

Sample site	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	Water quality (the worst case)
						Toxicity class						
February 2013	I	I	I	I	I	I	I	I	II	I	I	II
March 2013	II	I	II	II	I	I	II	I	II	I	I	II
June 2013	I	I	II	I	I	I	II	II	I	I	II	II
July 2013	I	I	II	I	I	I	II	II	II	I	I	II
August 2013	I	I	II	II	I	I	II	II	II	I	I	II
September 2013	II	I	II	II	II	II	II	II	II	I	I	II

The data analysis showed that surface water in 2013 fits mainly in toxicity class I – no acute toxic effect (S1, S2, S5, S6, S10 and S11) for 59% of samples and toxicity class II – slightly acute toxic effect (S3, S4, S7, S8 and S9) for 41% of samples. The minimum acute hazard could be explained by the dilution effect due to incidence periods of heavy rainfall and flooding that may affect biotic or abiotic conditions throughout life environment. Therefore, was noted that the algae, as they are sensitive organisms, classified the water samples in the above mentioned toxicity classes.

Sediment. The Danube Delta sediment samples toxicity was assessed by direct exposure to benthic ostracods (*Heterocypris incongruens*) and by terrestrial plants seed germination (*Lepidium sativum* (LES), *Sorghum saccharatum* (SOS), *Sinapis alba* (SIA)).

The effects percentages (%) on ostracods growth after 6 days of direct contact with sediments ranged between 0 and 100% (Table 4). The highest inhibition percentage was recorded for S3 (in July and September), S4, S5 and S8 (in September), S7 (in July) and S9 (in July and September).

Table 4. Inhibition/stimulation percentages (%) on *Heterocypris incongruens* growth in 2013

Sample site	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	Control
January 2013	41	44	50	11	–4	28	47	–	34	25	3	–
February 2013	57	26	25	3	2	46	25	56	–	30	13	–
March 2013	–	2	1	–5	–5	6	19	35	15	2	7	–
June 2013	27	34	66	11	40	15	64	55	64	48	34	0
July 2013	22	43	**72**	29	43	29	**90**	45	**100**	67	66	0
August 2013	50	14	22	22	66	22	69	64	47	55	14	0
September 2013	43	64	**74**	**91**	**74**	50	36	**78**	**88**	55	59	0

Moreover, the mortality percentages were not significant, ranging from 3 to 20% (January–March), and from 10 to 20% (June–September) (Fig. 4). The preliminary study performed in 2012 (Ref. 18) compared to this survey showed a decreased level of toxicity on *Heterocypris incongruens*, especially at S7 and S11 sites.

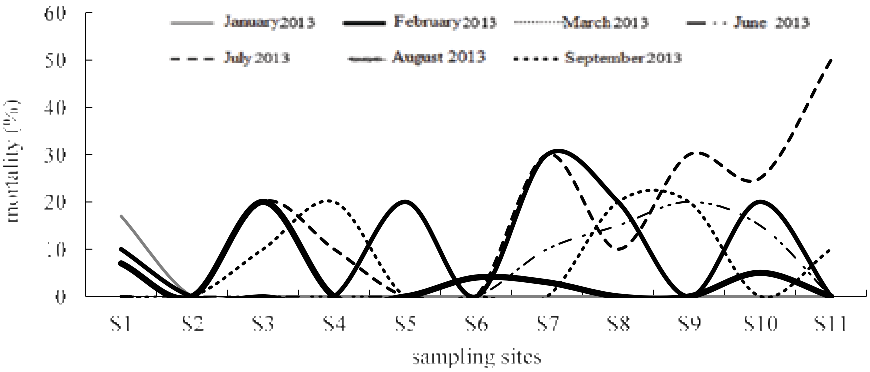

Fig. 4. Spatial and temporal representation of mortality percentages of benthic ostracods along the Danube Delta systems in 2013

In terms of terrestrial plants seed germination, it was found that sediment samples did not affect the germination process, all three plants having inhibitory effects from 2 to 30%. Although, it was observed inhibitory effect on root elongation of LES (45% in June, for S4 sediment sample) and SOS (31% for S3 sediment sample and 37% for S4 sediment sample, in June), and stimulation of root growth of SIA, in August, for S2, S7, S8, S9 and S10 sediment samples (Fig. 5).

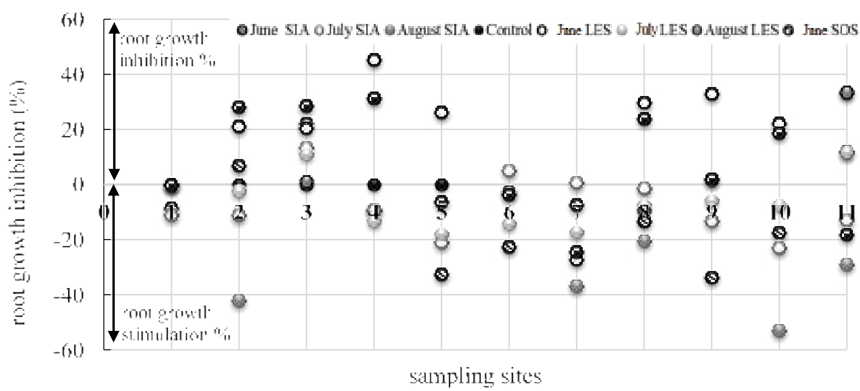

Fig. 5. Variation effects of inhibition/stimulation on terrestrial plants (SIA, LES, SOS) root growth exposed to Danube Delta (Sf. Gheorghe branch) sediment samples

Toxicity of the Danube Delta sediment samples is presented, in Table 5, according to toxicity classification[16]. The main toxicity classes for analysed sediment samples were: class II – slightly toxic acute effect (for S2, S4 and S6), Class III – acute toxic effect (for S1, S3, S5, S7, S8, S9, S10 and S11) and Class IV – high acute toxicity (for S9, in July).

Table 5. Classification of the Danube Delta sediment samples according Personne et al.[16]

Sample site	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	Sediment quality (the worst case)
						Toxicity class						
June 2013	II	II	III	II	II	II	III	III	III	II	II	III
July 2013	II	II	III	II	II	II	III	II	IV	III	III	IV
August 2013	III	II	II	II	III	II	III	III	II	III	II	III

The ostracods were the most sensitive organisms during the toxicity tests, which led to sediment samples classification into toxicity classes. *Heterocypris incongruens* registered significant growth inhibition during the studied period. Considering the worst cases, the Danube Delta lotic systems quality was evaluated on Class III of toxicity – acute hazard, except S9 (Ivancea) which can be considered as Class IV of toxicity – high acute hazard.

The toxicity tests for both water and sediment samples revealed a species-dependent feature. Thus, it was found a higher toxicity effect on algae compared to crustaceans and rotifers for water samples, and on the ostracods compared to the plants for sediment samples. It was also noted the accumulation of pollutants due to climatic factors, during summer drought periods, which changes more drastically the composition of water and sediment components. The largest number of water toxic responses was detected in case of bioassays conducted on *Selenastrum capricornutum* algae on various lotic waters systems, similar results were obtained from Polish rivers[19]. Toxicity tested on *Daphnia magna* crustaceans and *Brachionus calyciflourus* rotifers belonged to the second and third toxicity level. In case of sediment, the highest number of toxic responses was detected on *Heterocypris incrongruens* ostracods followed by *Lepidium sativum, Sorghum saccharatum* and *Sinapis alba*. The major variation of the toxicity results is based on the fact that each test organism is sensitive to differents toxins, but the most accurate information about a hazard effect of various pollutants to the environment[20] could be supplied by a pool of all these sensitive species. This research revealed that surface water caused insignificant toxic effects compared with the sediment. This observation is conforming to the fact that sediment offered considerable higher support for bioaccumulation of pollutants over long periods of time.

It is therefore recommended to control the sediment toxicity in areas where are regularly discharges of wastewaters, such as agro-industrial areas[21,22]. Nevertheless, it is recommended more use of chronic tests for surface water analyses, as they might be helpful to assess sublethal effects caused by pollutants, which are present even in low concentrations[10].

No significant dependence, as a general rule, was observed between toxicity results and sampling period, which can be explained by the fact that the main pollution sources of the studied lotic systems are municipal or industrial wastewater spills. In addition, there may be persistent compounds/mixtures in sediment and their mobility, bioavailability and solubility in water depends on numerous factors such as chemical reactions, organic compounds, oxidising and biological activities.

Overall, the assessment of Danube Delta water and sediment samples toxicity showed that S3 (Tulcea Downstream), S7 (Murighiol), S8 (Uzlina) and S9 (Ivancea) sampling sites are most likely on sediment and water toxicity. The use of different aquatic species (producers and consumers) to assess the toxicity effects on aquatic components, confirmed the utility of microbiots batteries in water bodies ecological classification[23,24].

CONCLUSIONS

Representative species of producers and consumers were used to evaluate the Danube Delta water and sediment quality according to hazard classification system of natural water. The water samples showed no acute toxic effect (Class I) or slightly acute toxic effect (Class II), respectively. The sediment samples were more toxic than water samples due to the pollutants accumulation. The main toxicity classes in which the sediment samples fit were: Class II – slightly acute toxic effect; Class III – acute toxic effect, and Class IV – high acute toxicity. The largest number of water toxic responses was observed in case of *Selenastrum capricornutum* algae, followed by *Daphnia magna* crustaceans and *Brachyonus calyciflourus* rotifers. In case of sediment, the highest number of toxic responses was obtained from *Heterocypris incrongruens* ostracods followed by *Lepidium sativum, Sorghum saccharatum* and *Sinapis alba*. The development of tools and strategies for an identification of key toxicants on a broader scale are a challenging task for the next years, so the need to integrate toxicity tests, chemical analyses and biological field data within a water and sediment-quality triad is of great importance.

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