HAZARD CLASSIFICATION OF DANUBE- DANUBE DELTA (WATER AND SEDIMENT) BASED ON ORGANISMS SENSITIVITY

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Abstract

A preliminary acute evaluation of toxicity in Danube River - Danube Delta Romanian sector (1075 – 0 km) was proposed including surface-water and sediment. The conventional classification of natural waters is based primarily on chemical and physical parameters. Recently the hazard classification have include the use of selected microbiotests based on organisms sensitivity. The spatial (11 control points) and temporal (in winter and spring of 2013) toxicity effects have been assessed using a biotests battery with representative species of producers (grean algae *Pseudokirchneriella subcapitata)* and consumers (rotifers - *Brachionus calyciflorus, crustaceeans – Daphnia magna, Heterocypris incongruent).* The physical, chemical, biological and microbiological characteristics of water and sediment also have been performed. The preliminary tests results revealed no acute to slightly toxic effect, respectively Class I and II of hazard. The sediment samples were more toxic than water. The toxicity results were directly influenced by organisms sensitivity, sampling locations, season and climate change issues. The water quality according to hazard classification was related to pollution classification and biological determinations (macrozoobenthos, phytoplankton and zooplankton).

Keywords: Danube River, Danube Delta, microbiotests, hazard classification

INTRODUCTION

In Romania the surface water and sediment control is based on physical, chemical and biological parameters (Water Law 107/1996; Ministry Order no. 161/2006 and Water Frame Work Directive CE 60/2000) [1, 2, 3]. In rivers exist a large number of potentially toxic substances for which the analytical determinations is became difficult due to diversity, novelty and small concentrations. To achieve a realistic estimation of pollutants hazard on aquatic ecosystems, it is necessary to know their toxic effects and their interactions in the environment. In the field of water and sediment toxicity assessment which is becoming increasingly current are little knowledge’s.
In some surface water and sediment studies, the toxicological studies complete the chemical monitoring. Some studies reveal different effects on aquatic

organisms such as fish, daphnia, algae or bacteria. Is known that each test species can be sensitive to different chemicals [4]. For this reason the environmental samples that include a variety of pollutants must be tested in a battery of bioassays with various species. This approach allow to threat test data as information about the whole aquatic ecosystem [5, 6].
The paper propose a preliminary study of the biological quality of Danube Delta surface water and sediment using a microbiotest battery consisting of the species belonging to different taxonomic groups (algae, planktonic and benthonic crustacean and rotifers). The information's were integrated in a toxicity classification system according to Persoone et al. (2003) [7].

MATERIAL AND METHODS

Study area and samples collection

The research area was Danube - Danube Delta, South – East sector. There were selected eleven sampling sites (located on the Danube River - Isaccea, Tulcea Branch - upstream and downstream of Tulcea and St. Gheorghe Branch – Nufaru, Balteni, Mahmudia, Murighiol, Uzlina, Ivancea, Sfantu Gheorghe and Black Sea Confluence). Geographical location of the study sites is shown in figure 1.

Figure 1 Geographical location of the study sites (Isaccea, upstream and downstream of Tulcea, Nufaru, Balteni, Mahmudia, Uzlina, Ivancea, Sfantu Gheorghe and Black Sea Confluence)

The surface water and sediment samples were collected monthly in January – March 2013 using specific equipments and a motorboat for transportation. The sampling and preservation was done according to specific international standards [8, 9, 10].

Major problems of Danube River in Romanian sector, especially in Danube Delta are untreated sewage, fertilizers and soil run-off. Also, the environmental impact of transport links, tourist developments, or new energy-producing facilities must be considered. According to national legislation the water quality is classified in 5 pollution classes (I - Very good quality, II - Good quality, III - Satisfactory quality, IV - Unsatisfactory quality, and V – Poor quality) [1]. In terms of ecological status in the last 5 years, the Danube - Danube Delta was a eutrophic system equilibrated for a good ecological status, class IInd according to the National Norms [11, 12].

Chemical and biological analyses

In all water and sediment samples were monitored different chemical indicators, such as nutrients, toxic metallic elements and organic compounds. The dissolved oxygen, pH, temperature and conductivity were analyzed *in-situ* using the portable devices from Environmental Monitoring Auto laboratory. Beside of chemical analyses some biological analyses (phytoplankton, zooplankton, macro zooplankton and bacterioplankton) were performed. The purpose of this paper was not to present a physical-chemical assessment of water and sediment quality in the area selected for study - more detailed data concerning the analytical and biological techniques were published in other papers [13, 14].

Toxicity tests

Toxicity assessment of water samples was perfomed using a battery test with 3 different species (green algae *Selenastrum capricornutum sp.* – 72h chronic growth inhibition test with Algaltoxkit F^{TM} [15], daphnia *Daphnia magna* sp.– 48h acute immobilization test with Daphtoxkit F^{TM} [16], and planktonic rotifers *Brachionus calyciflorus sp.* – 24h acute mortality test with Rotoxkit F^{TM} [17]). The sediment samples toxicity was assessed using benthonic crustacean (ostracods *Heterocypris incongruent sp.* – chronic 6 days growth inhibition test with Ostracodtoxkit F^{TM} [18]). The water samples were tested only undiluted. The preliminary sediment toxicity was assessed without any treatments using only the ostracods mortality / growth inhibition test and the effects were expressed in percentages. The effects were expressed as percentages (PE) of mortality / immobilization / inhibition according to the specific test procedures. All tests were performed comparatively with the control tests and reference tests (using potassium dichromate in daphnia, algae and rotifers tests or copper sulfate in ostracods test). For a relevant result each sample was tested in three replicates.

Toxicity classification system

The hazard of water toxicity was classified according to the hazard classification system for natural water [7]. The effect percentages for each type of organisms is transformed in toxicity units (TU) [gL^{-1}] using formula: TU =100 / PE. The current classification system is based on acute value which ranking in 5 hazard classes. The sample is classified as toxic when the PE was higher than or equal to 20%.
The acute hazard classes are: Class I – No acute hazard – none of the test shows a toxic effect; Class II – Slight acute hazard, 20%≤PE<50%, in at least on test; Class III- Acute hazard, 50% ≤PE<100%, in at least on test; Class IV- High acute hazard, PE=100%, in at least on test; Class V- Very high acute hazard, PE=100%, in all tests.

RESULTS AND DISCUSSIONS

Physical and chemical background of water and sediment

The present paper present only some basic informations concerning the physical, chemical and biological background of Danube Delta surface water and sediment in order to give a realistic and complete view about the aquatic environment hazard from this ecosystem.
During January – March 2013, the pH (7.54 to 8.07 pH units), dissolved oxygen (2.17 to 6.89 mgO_2/L), temperature (5 to 10^{o}C) and conductivity (361 – 483 µS/cm) of surface water were in the limits of Class I – Very good quality in all monitored locations. Organic loading (measured as chemical oxygen demand - COD and biochemical oxygen demand - BOD) was in the limits of Class I - Very good quality for COD and Class II – Good quality for BOD in the entire sampling period and for all control sections. The nutrients (ammonium, nitrites, nitrates, total nitrogen, total phosphorus, phosphates) and salinity (sulfates, sodium, calcium, magnesium and chlorides) were classified in Class I and Class II of quality. The toxic metals (Ni, Cd, Cr, Cu, Pb, As, Hg, Zn, Co, Ba) classify the water as very good and good in all control points. The analytical determinations of anionic surfactants and phenols shows undetectable concentrations. Concerning the hazardous chemicals such as polynuclear aromatic hydrocarbons, petroleum products, organochlorine, triaminic and ureic pesticides, and PCB, undetectable concentrations was obtained that classified the water in the admissible limits of Class I- very good quality.
Considering the national norms [1, 3], the chemical investigations of sediment samples revealed worrying increases of some toxic pollutants such as heavy metals Cu, Ni, Zn and Cr especially at Murighiol and Mahmudia. Also significant concentrations of organochlorine pesticides, DDT/DDE/DDD, HAP and petroleum products were permanently detected.

Biological background of water and sediment

In the surface water samples collected in the period of January – March 2013, were identified four phytoplankton species from *Bacillariophyta, Chlorophyta,* *Euglenophyta, Cyanophyta groups.* The saprobe index value classified the water quality in the good class, with two exceptions at Mahmudia and at Danube Delta confluence with Black Sea. There the saprobe index showed a very good quality of water. In all sampling periods the most dominant species were oligo-betamesosaprobe diatomeas (N*avicula gracilis, Cymbella ventricosa, Diatoma elongatum*), betamesosaprobe (*Amphipleura pellucida, Synedra acus, Nitzchia sigmoide*a) or betamesosaprobe clorophite (*Pediastrum duplex, Scenedesmus quadricauda*). Biomass of the phytoplankton had a linear dependency with chlorophyll "a" concentration.

The analysis of zooplanktonic component showed the presence of ostracods, rotifers, copepods and cladocera. According to numerical abundance and biomass, have dominated oligo-betamesosaprobe rotifers species (*Keratella cochlearis*), betamesosaprobe (*Keratella quadrata frenzeli*, *Brachionus quadridentatus*), cladocere oligo-betamesosaprobe (*Bosmina longirostris*) and beta-alpha mesosaprobe copepodes (*Cyclops strennus metanauplius*).

The benthonic fauna was performed at species level, excepting the Oligochaeta and Chironomidae, which were determined at group level. In the sediment samples were identified species of gastropods, lamellibranchiate, oligochets, chironomids, nematodes, amphipods and isopods, very good represented in all control sections. The predominant species were gastropods, such as: *Bithynia tentaculata, Viviparus sp., Theodoxus fluviatilis, Theodoxus danubialis, Planorbis planorbis, Lithoglyphus naticoides,* and bivalve species such: *Dreissena polymorpha, Unio pictorum, Anodonta cygnea, Corbicula fluminea, Sphaerium corneum, Mya arenaria, Mytilus galoprovincialis.*

More detailed informations about chemical and biological evolution of Danube River - Danube Delta surface water and sediment were presented in other published papers [19, 20, 21].

To evaluate the microbial plankton of water, some bacteriological tests were performed for total coliforms, fecal coliforms, fecal streptococcus and *Salmonella s*p. The results interpretation was made according to Directive 2006/7/EC regarding the quality of bathing water [22]. Total coliforms and fecal streptococcus are permanently present in all control sections (predominant at Mahmudia, downstream of Tulcea and Ivancea) and in whole sampling period which indicated a low and moderate fecal pollution. Bacteriological analyses showed more than 1000 total coliforms per 100 mL for 27% of water samples (at Mahmudia and Sfantu Gheorghe). More than 1000 fecal coliforms per 100 mL were identified in 11% of samples collected from Murighiol, Mahmudia, Sfantu Gheorghe and Ivancea (figure 2).

Sediments samples were monitored for the same microbial parameters. The analyzed bacteriobenthos reveled an increase of total coliforms number (more than 2000 bacteria per gram dry weight) in the most control points, especially at Nufaru, Mahmudia, Ivancea and Sfantu Gheorghe (figure 3). The fecal coliforms were present only sporadically. In January a high density of streptococcus were present at Nufaru and Balteni.

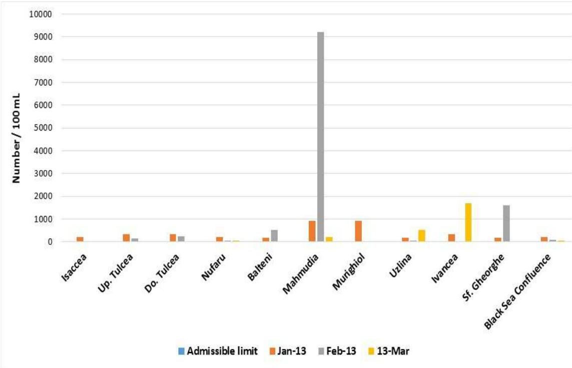

Figure 2 Total coliforms number in water samples

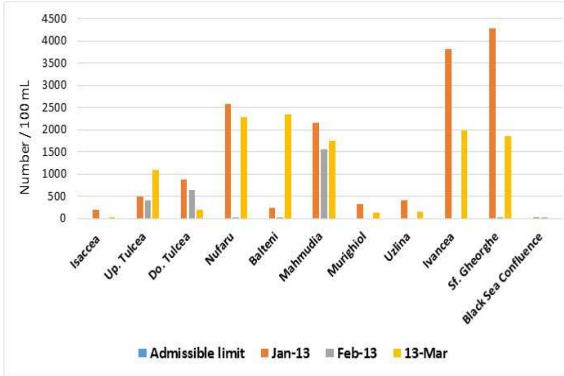

Figure 3 Total coliforms number in sediment samples

Toxicity assessment

The percentage of toxicity effects (PE) of water and sediment samples collected in winter – spring of 2013 and the toxicity class according to Persoone et al. (2003) [7] classification are presented in table 1. All samples belonged of I and II class of toxicity. In winter period the water does not have acute hazard. In spring, due to weather changes (abundant precipitations and floods), the water was classified as slight toxic. Most of samples revealed effects lower than 50%, excepting the sediment samples collected from Issacea and Mahmudia that have more than 50% growth inhibition on ostracods.

The level of effects, in case of water toxicity tests with *Daphnia magna*, are insignificant in February and March 2013 (0 to 30 % mortality). Mortality effects from 25% to 30% were observed at Isaccea, downstream of Tulcea, Nufaru and Ivancea in spring (figure 4).

Multigenerational tests with green algae *Selenastrum capricornutum* were performed without nutrient medium addition in order to evaluate the real toxicity of samples. An insignificant growth stimulation was observed in 60% of water samples in February. Contrary, in spring an inhibition from 2,84% to 33,2% was observed in all sections, especially at Murighiol and Ivancea (figure 5).

The *Brachionus calyciflorus* 24 h exposure test of water samples showed insignificant mortality effects lower than 15%. A special case was the water sample collected from Ivancea in March for which was obtained a 76% mortality effect (figure 6).

The chronic test with consumer organisms *Heterocypris incongruens* performed for sediment toxicity assessment in period of January – March highlight mortality effects from 3% to 20% in case of the most samples (figure 7). Concerning the growth inhibition some toxic effects were recorded for samples collected from the following locations: Isaccea (57% in February), downstream of Tulcea (50% in January) and Uzlina (56 % in February). Also the samples form Mahmudia and Murighiol could have a potential toxic effects on benthic crustacean.

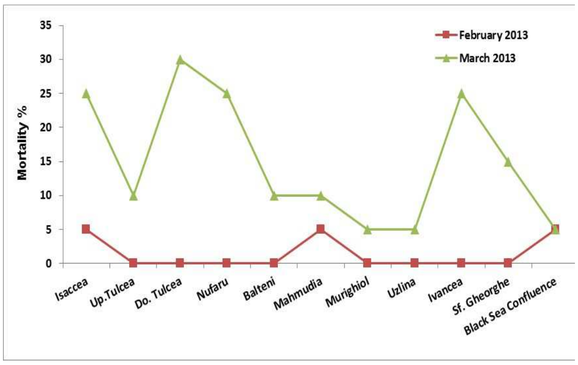

Figure 4 Effects of water samples on *Daphnia magna sp.* (expresed as mortality percentage)

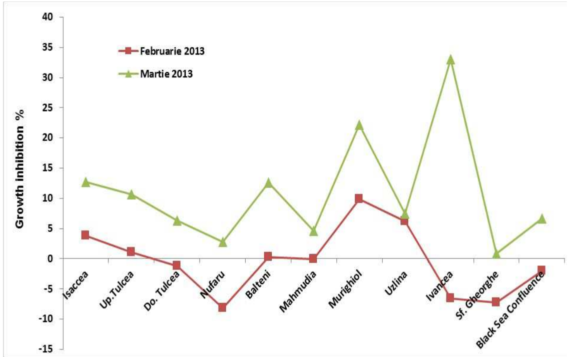

Figure 5 Growth inhibition / stimulation effects of water samples on *Selenastrum capricornutum sp.*

Table 1 Toxicity effects of water and sediment samples in battery tests

Sampling	**Isaccea**	**Upstream Tulcea**	**Downstream Tulcea**	**Nufaru**	**Balteni**	**Mahmudia**	**Murighiol**	**Uzlina**	**Ivancea**	**Sf. Gheorghe**	**Black Sea Confluence**
Surface water (*Daphnia magna)* – mortality %											
February 2013	5	0	0	0	0	5	0	0	0	0	5
March 2013	25	10	30	25	10	10	5	5	25	15	5
Surface water (*Selenastrum capricornutum*) – growth inhibition %											
February 2013	3,86	1,04	-1,16	-8,22	0,3	-0,07	9,87	6,26	-6,58	-7,27	-2,02
March 2013	12,77	10,61	6,28	2,84	12,61	4,66	22,17	7,43	33,02	0,8	6,67
Surface water (*Brachionus calyciflorus*) – mortality %											
February 2013	10	3,3	0	0	0	6,66	3,33	6,66	0	0	0
March 2013	3,33	6,66	0	3,33	13,3	0	0	13,33	76,66	10	3,33
Toxicity classification of surface water (Persoone et al., 2003)											
February 2013	I - No acute hazard								II – Slight acute hazard	I - No acute hazard	
March 2013	II – Slight acute hazard	I - No acute hazard	II – Slight acute hazard		I - No acute hazard		II – Slight acute hazard	I - No acute hazard	II – Slight acute hazard	I - No acute hazard	
Sediment (*Heterocypris incongruens)* – growth inhibition %											
January 2013	41	44	50	11	0	28	47	-	34	25	3
February 2013	57	26	25	3	2	46	25	56	-	30	13
March 2013	-	2	-	0	0	6	19	35	15	2	7

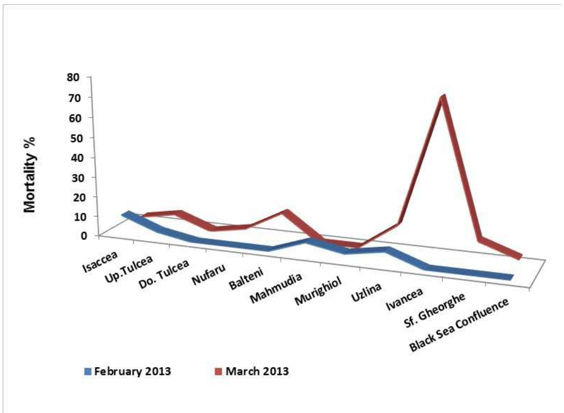

Figure 6 Effects of water samples on *Brachionus calyciflorus sp.* (expresed as mortality percentage)

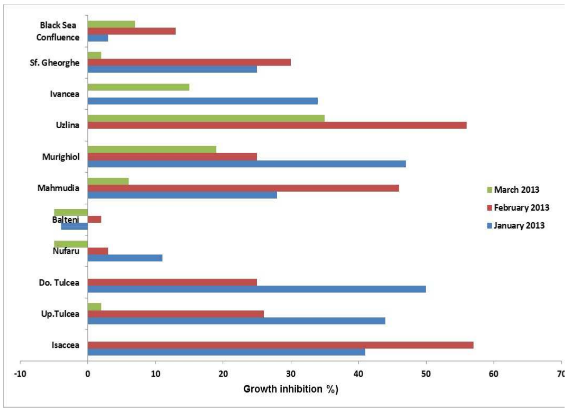

Figure 7 Effects of sediment samples on *Heterocypris incongruens sp.* (expresed as mortality percentage)

Slight differences in chemical compounds and toxicity effects monitored in the period January – March 2013 were observed. The reasons could be municipal and industrial wastewater discharges which can have different pollution levels, some persistent substances cumulated in sediments like metals and pesticides, mobility and bioavailability of pollutants, unknown or unmonitored chemicals.

The toxicity tests performed for water and sediment samples showed a species dependent response, which confirm the necessity of toxicity battery tests for environmental sample hazard assessment. The *Daphnia magna* toxicity test carried on water sample and *Heterocypris incongruens* test carried on sediment samples were the most sensitive. This aquatic consumers (crustacean) could be easily affected by abiotic and biotic changes from their environment.

Also the effects were influenced by climatic changes, pollutant concentrations and locations. The toxicological effects could be correlated with the chemical and biological results above mentioned. Preliminary tests showed that Isaceea, Murighiol, Uzlina and Ivancea were the most susceptible control sections concerning the toxicity of water and sediment. In this sections were detected important concentrations of toxic pollutants probably occurred as a result of significant waste water discharges and shiping activities.

Considering the national norms and the monitoring studies performed in period of January – March 2013 (including abiotic and biotic parameters), the ecological status of Danube Delta water was classified in Class II – Good quality which indicates a good correlation with the toxicity classification of water samples.

CONCLUSIONS

The toxicity battery tests used to evaluate the quality of surface water and sediment and hazard classification system of natural water are suitable to assess the rivers pollution in addition to chemical investigations required by norms. The spatial and temporal toxicity effects have been assessed using a biotests with representative species of aquatic producers and consumers. The preliminary tests results obtained for water samples revealed no acute to

slightly toxic effect, respectively Class I and II of hazard considering Persoone et al. (2003) classification system. The sediment samples were more toxic than the surface water samples as a result of pollutant accumulation. The toxicity results were directly influenced by organisms sensitivity, sampling locations, season and climate change issues. The water quality assessment according to hazard classification was related to pollution classification and biological determinations.
Moreover, diversified toxic responses indicated and confirmed the need of microbiotests battery application consisting in acute and chronic tests with the organisms representing different trophic levels.
The presented results are preliminary and the monitoring toxicity studies will be continued especially in sediment assessment in order to classify the hazard on the entire aquatic life and estimate the negative changes in river systems.

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