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Preliminary Results about the Distribution of Macrozoobenthos along the Bulgarian Stretch of the Danube River with Respect to Loading of Nutrients, Heavy Metals and Arsenic

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Abstract: The Lower Danube, being an important navigable way, is recognized as a heavily modified water body according to the Water Framework Directive criteria. The riparian zone is subjected to various anthropogenic pressures, such as discharge of organic substances and nutrients, continuity interruption, sediments and gravel abstraction, rip-rap armoring, etc. All of these affect the aquatic communities. The present study represents the distribution of the main macrozoobenthic groups at 16 sites along the Bulgarian stretch of the Danube River, provisionally identified as influenced by different pressures and impacts. The relationship between the longitudinal distribution of macrozoobenthos and the content of some pollutants in both the water and the sediments were studied. Fourteen sampling sites were located downstream major tributaries, cities and harbours and two other were considered as relatively less influenced. The abundance of amphipods, oligochaets and chironomid larvae increased downstream along with the load of nutrients in the water and the sediments. Less abundant taxonomic groups like Ephemeroptera, Hirudinea and Diptera (varia) were also better represented in sampling sites situated at the lower part of the Bulgarian Danube stretch. The gastropods were more or less evenly distributed along the whole Bulgarian Danube section while Bivalvia seemed to be more abundant in its upstream part. As a whole, the content of Ni, Fe and Pb in both the sediment and the water samples were higher at the studied sites downstream the mouth of the Iskar River, while higher concentrations of Cu and As were found at the sites upstream the Iskar River mouthing.

Keywords: Lower Danube, macrozoobenthos, heavy metals, Water Framework Directive, nutrients

Introduction

The riparian zone of the Bulgarian part of the Danube River, situated from the confluence of the tributary Timok to the harbor of Silistra, is recognised as a heavily modified water body according to the Management Plan for the river basin in the Danube region (2010) and the Water Framework Directive (2000/60/EU) criteria. This zone is highly influenced by various anthropogenic pressures (discharge of organic substances and nutrients, continuity interruption, sediments or gravel abstraction, rip-rap armoring, etc.), which affect the distribution of the macroinvertebrate communities and cause changes in their composition and functioning. According to the Water Framework Directive requirements transposed also into the national legislation (Regulation N-4/2013), the benthic macroinvertebrates are determined as a one of the obligatory Biological Quality Elements to be used for the assessment of ecological state or potential due to their suitable characteristics as bioindicators (low mobility, long life-span and sensitiveness to water status alteration, Resh *et al.* 1996). Physical and chemical elements have supporting role for the biological elements. They are included also as necessary part of the ecological potential assessment as they are influencing biochemical processes in aquatic ecosystems.

Nutrient pollution has been recognized as one of the important water management issues in the Danube River Basin, also impacting on the Danube Delta and the Black Sea (Hamchevici, Craciun 2008). The water solubility of heavy metals and arsenic is limited in natural waters, and most of them are easily associated with the solid phase (particulate matter) either in suspension of after settling in the sediment (Litherathy *et al.* 2008). In certain concentrations heavy metals, arsenic and nutrients can affect the metabolism and reproduction of the aquatic communities and, therefore, may influence their distribution and abundance in the Danube River.

The present paper is aiming to represent the distribution of the main macrozoobenthic groups along the Bulgarian stretch of the Danube River in relation to the loading of nutrients, heavy metals and arsenic.

Material and Methods

The study was carried out at 16 sampling sites along the Bulgarian stretch of the Danube River (Table 1) during the period of low water in August-September 2013.

The water physical and chemical parameters (pH, conductivity, temperature, dissolved oxygen and oxygen saturation) were measured *in situ.* Fifteen parameters were analyzed from the water and sediment samples – nutrients (NH_4-N, NO_2-N, NO_3-N, PO_4-P, TotP), heavy metals (Cu, Pb, Zn, Cd, Mn, Fe, Ni) and arsenic (As). The analytical methods used were as follows: water temperature(°C) - BDS 17.1.4.01 (Method Detection Limit-0.1); alkalinity (pH) - BDS ISO 10523: 2012 (MDL-0.00); conductivity (µS.cm^{-1}) - BDS EN 27888:2000 (MDL-0.0); dissolved oxygen/ oxygen saturation (mg.l^{-1}/ %) – BDS/EN/ ISO 5814:2012 (MDL-0.00); ammonia nitrogen – NH_4-N (mg.l^{-1}) - ISO 7150/1 (MDL-0.01); nitrite nitrogen – NO_2-N (mg.l^{-1}) - EN 26777 (MDL-0.002); nitrate nitrogen – NO_3-N (mg.l^{-1}) - ISO 7890-1 (MDL-0.2); P- orthophosphate – PO_4-P/ Total Phosphorus (TotP, mg.l^{-1}) - EN ISO 6878 (MDL-0.01); Cu, Fe, Mn, Zn, Ni, Pb (mg.l^{-1}-water; mg.kg- sediment), Cd (µg.l^{-1}- water, µg.l^{-1}- sediment)- ISO 11047; arsenic (As) (mg.l^{-1} - water, mg.kg - sediment) - BDS EN ISO 11969; alkalinity (pH) in sediments - BDS ISO 10390. Water and sediment samples were collected and preserved according to BDS 16777 and to BDS ISO 11466 respectively.

Macrozoobenthos was collected according to an adapted version (Cheshmedjiev *et al.* 2011) of the multi-habitat sampling methodology of AQEM/ STAR (Barbour *et al.* 1999). Benthic samples were taken in correspondence to the European standards EN ISO 10870:2012 using a hand-net (frame 30x30 cm, mesh size 500 µm) and were preserved according to BDS EN ISO 5667-3: 2012 in 4% solution of formaldehyde. Then the macroinvertebrates were separated and conserved in 70% ethanol. The following main taxonomic groups were identified: Hydrozoa, Nematoda, Polychaeta, Oligochaeta, Turbellaria, Hirudinea, Gastropoda, Bivalvia, Amphipoda, Ephemeroptera, Odonata, Coleoptera, Heteroptera, Trichoptera, Diptera: Chironomidae, Diptera (varia). The density of the taxa groups was calculated per 1 m². Statistical analyses were performed using Statistical software package PAST Version 3.0 (Hammer 2013). Pearson's linear coefficient was used to estimate correlations among environmental variables and macroinvertebrate groups. The distribution of the macroinvertebrate taxa among the studied sites was performed by Detrended Correspondence Analysis (DCA). The longitudinal distribution of the concentration of nutrients, heavy metals and arsenic is presented using Microsoft Office Excel 2007 package.

Results and Discussion

The obtained results of the concentrations of heavy metals and nutrients provisionally separated the Bulgarian stretch of the Danube in two parts, with a dividing point the Iskar River mouthing.

As a whole, the content of Ni, Fe and Pb in both the sediment and water were higher at the sites along the lower part (downstream the Iskar River mouth), while higher concentrations of Cu and As were found at the sites upstream the Iskar River. The concentration of nutrients in the water and the sediment, especially PO_4-P, TotP and NH_4-N, also increased at the downstream part.

Significant correlations were found among the environmental parameters conductivity (Cond), NH_4-N, PO_4-P, TotP, Ni, As, Fe. Cu and Pb in the water, and NO_2-N, NO_3-N, PO_4-P, TotP, Fe and Cu in the sediment and the taxa groups Oligochaeta, Hirudinea, Gastropoda, Bivalvia, Amphipoda, Ephemeroptera, Heteroptera, Trichoptera, Diptera: Chironomidae, Diptera (varia) (Table 2). Most of the

Table 1. Sampling sites (The names of the sampling sites is defined according to location of tributaries and harbours)

№	Name	Sampling site location on the Danube River	Rkm
1.	Timok	Downstream the mouthing of tributary Timok River (the state border)	844
2.	Novo Selo	Upstream the village of Novo Selo	834
3.	Vidin	Downstream the industrial zone of the town of Vidin	784
4.	Dolni Tsibar	Upstream the village of Dolni Tsibar, provisionally defined as less influenced sampling site	718
5.	Ogosta	Downstream the mouthing of the tributary Ogosta River	684
6.	Baykal	Upstream the village of Baykal and the mouthing of the tributary Iskar River	640
7.	Iskar	Downstream the mouthing of the tributary Iskar River	635
8.	Cherkvitsa	Downstream the Cherkvitsa village (downstream the left tributary Olt River)	601
9.	Svishtov	Upstream the town of Svishtov	559
10.	Vardim	Upstream the forehead of the Vardim Island, provisionally defined as less influenced sampling site	547
11.	Yantra	Downstream the mouthing of the tributary Yantra River	533
12.	u/s Ruse	Upstream the town of Ruse	500
13.	Rusenski Lom	Downstream the mouthing of the tributary Rusenski Lom	497
14.	d/s Ruse	Downstream the town of Ruse, Marten village	482
15.	Tutrakan	Downstream the town of Tutrakan and the mouthing of left tributary Ardzhesh River	431
16.	Aydemir	The village of Aydemir, the ferryboat harbor	382

Table 2. Pearson's correlation coefficients among the main taxa groups and measured parameters (bolded values are significant, $p<0.05$)

Taxa/ Chemistry	**Oligochaeta**	**Hirudinea**	**Gastropoda**	**Bivalvia**	**Amphipoda**	**Ephemeroptera**	**Heteroptera**	**Trichoptera**	**Diptera: Chironomidae**	**Diptera (varia)**
Cond	**0.521**	**0.515**	-0.121	-0.220	-0.181	0.406	0.217	-0.209	0.244	**0.662**
NH_4-N	0.065	-0.088	**0.508**	-0.122	0.261	**0.603**	-0.249	-0.119	-0.138	0.134
PO_4-P	**0.537**	0.479	0.152	-0.123	-0.106	**0.627**	-0.053	-0.085	0.239	**0.603**
TotP	0.422	**0.595**	0.051	-0.045	-0.283	0.263	-0.103	0.023	0.234	**0.567**
Ni	**0.749**	0.433	0.094	-0.290	0.169	**0.759**	**0.692**	-0.264	**0.640**	0.499
As	**-0.612**	0.199	0.347	-0.367	0.095	-0.550	-0.546	-0.358	**-0.671**	0.019
Fe	-0.565	0.248	**0.590**	-0.312	0.275	0.477	0.434	-0.357	0.234	0.225
Cu	0.329	0.145	-0.207	-0.370	**-0.689**	0.310	-0.127	-0.403	0.304	0.116
Pb	-0.565	0.140	0.157	-0.383	0.041	**-0.666**	-0.366	-0.381	-0.488	0.033
NO_2-Sed	-0.111	-0.130	0.061	-0.017	**0.614**	-0.126	**0.553**	-0.024	-0.091	-0.177
NO_3-Sed	-0.091	0.039	0.099	0.006	**0.653**	-0.124	**0.505**	- 0.007	-0.134	-0.054
PO_4-PSed	0.190	-0.222	-0.284	-0.122	**0.628**	-0.001	**0.663**	-0.157	0.157	-0.088
TotPSed	-0.015	-0.164	-0.134	-0.111	**0.716**	-0.116	**0.591**	-0.132	-0.058	-0.128
FeSed	0.117	-0.117	0.236	**0.513**	-0.278	-0.077	0.155	**0.498**	0.316	-0.217
CuSed	0.481	-0.170	-0.258	-0.112	-0.344	0.300	0.347	-0.111	**0.529**	-0.015

taxa groups had positive correlations with the concentration of nutrients and negative – with the concentration of the studied heavy metals. The measured concentrations of the nutrients provided probably a trophic resource to the representatives of the macrozoobenthic community, while the concentrations of some of the heavy metals had a negative influence on their development.

The primary aquatic macroinvertebrates (oligochaets, freshwater snails, clams and amphipods) along with the non-biting midges (Chironomidae) were well presented and abundant at all sampling sites, while the secondary water taxa groups (Trichoptera, Heteroptera, Ephemeroptera, Diptera, Coleoptera and Odonata) were presented with much smaller number of individuals, or were even absent at some of the studied sites. Nevertheless, higher diversity of insect larvae was observed at sampling sites Timok and Rusenski Lom (Fig. 1.), which was due to heterogeneity of the habitats, including an observed development of macrophytes.

Influence of the concentration of heavy metals and nutrients in sediments on the distribution of the macroinvertebrates

Tolerant chironomid larvae were the most abundant at the sampling site Timok (Fig. 1.) which is also the second of the most contaminated by copper (Fig. 2). This result comports with the data of Mothersill et al. (1980) that the non-biting midges commonly occur in sediment, heavily loaded with cooper. De Haas et al. (2005) also observe high densities of chironomid species at the river stretches

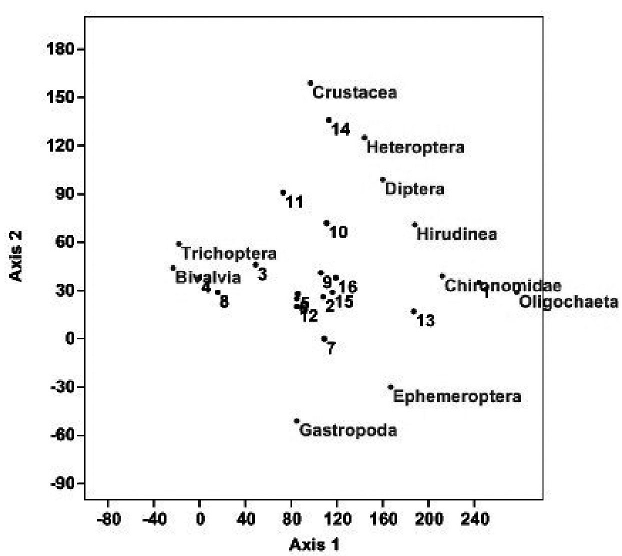

Fig. 1. DCA ordination plot with sampling sites and abundance of the taxonomic groups

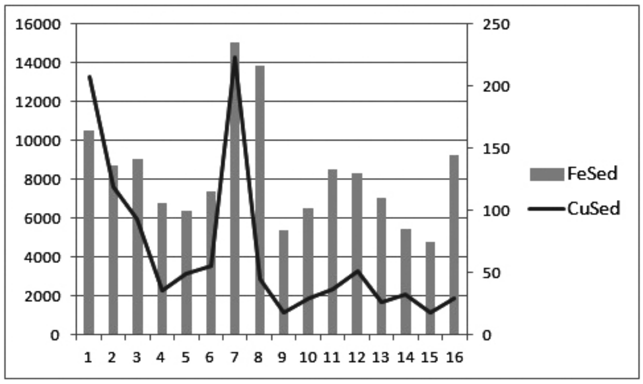

Fig. 2. Longitudinal distribution of the concentration of Fe and Cu in the sediments

characterised with higher levels of sediment contaminants. The abundance of Bivalvia and Trichoptera increased along with the concentrations of iron in the sediments at the sampling sites Cherkvitsa, Vardim (only Trichoptera taxa), Yantra, and Aydemir (only Bivalvia) (Table 1, Figs. 1 and 2). The most abundant within the clams was *Corbicula* spp. which was also found by Hubenov et al. (2013). Its abundance ratio to the total macroinvertebrate abundance was reported to increase along with the increasing of the environmental perturbation (Kerans, Kaar 1994 after Nguyen, de Pauw 2002), although the cited authors did not find any correlation between the abundance of *Corbicula* spp. and the sediment quality.

Almost all of the caddisflies, observed within the sampling sites were presented by *Hydropsyche spp.* This corresponds with the assumption that the *Hydropsyche spp.* could be more tolerant to elevated trace-element concentrations in the sediment (Mize, Deacon 2002). The abundance of amphipods increased at the sampling sites Cherkvitsa, Vardim, Yantra, d/s Ruse and Tutrakan (Table 1 and Fig.1) along with concentration of nutrients in sediments (Fig. 3).

According to Branstetter *et al.* (2010) Gammaridae prefers the sediment with relatively high organic matter content and considering the amphipods are tolerant taxa, they could be found on a more loaded, organic rich sediments (Kilgour *et al.* 2008). Vos *et al.* (2004) discovered that the abundance of detritiherbivorous species (*Gammarus* spp. and *Corophium* spp.), in shallow euthrophic lakes strongly correlated with the biochemical parameters in the sediments. The authors did not find correlation between the occurrence of Heteroptera and the quality of the detritus, but between them and the total

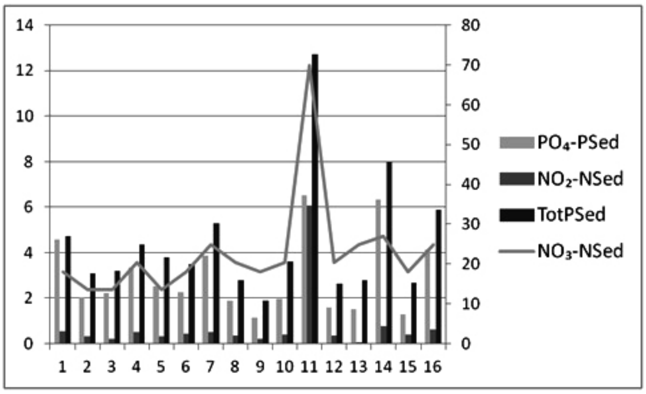

Fig. 3. Longitudinal distribution of the concentration of nutrients (PO_4-P, TP, NO_2-N, NO_3-N) in the sediment

number of invertebrates, *i.e.* density of prey, as they are carnivores.

Influence of concentration of the measured environmental parameters in water on the macrozoobenthos distribution

The abundance of Oligochaeta increased at the sampling sites Timok, Iskar, Rusenski Lom and Tutrakan (Fig. 1) in correspondence with the high values of nickel (only for Timok and Rusenski Lom), conductivity (Timok, Iskar, Rusenski Lom) and PO_4-P (Timok, Iskar, Rusenski Lom, Tutrakan (Figs. 4 and 5). In addition, the highest concentrations of arsenic, iron, copper and TotP in the water samples were registered on these stations.

The above mentioned studied sites are located downstream the confluences of the tributaries that are highly polluted from industrial and communal untreated waste waters (Hamchevici, Craciun 2008). The concentration of As seemed to act as a suppressor of the development of oligochaets, since their abundance elevated at the sampling sites Timok, downstream Ruse and Aydemir, in correspondence with the low values of As. Exceptions were Iskar and Rusenski Lom sites, where both the As concentrations and the aquatic oligochaets abundance correlated positively and probably the measured environmental parameters had multidirectional influence (Fig. 4). The abundance of chironomid larvae was increasing along with the high concentration of Ni at the sampling sites Timok and Rusenski Lom. The low concentration of As at Timok and at the river stretch downstream the Iskar sampling site corresponded also with higher density of chironomid larvae. The concentration of nutrients increased downstream the sampling site Iskar along with the increasing of Oligochaeta and Chironomidae abundance. Most of the species of these taxa groups belonged to the trophic group of deposit feeders, which demonstrated a preference to sites or stretches, loaded with organic pollution (Varadinova et al. 2013, Pavlova 2013).

The relatively increasing abundance of mayflies at the sampling sites Timok, Iskar, Rusenski Lom and Tutrakan (Fig. 1) corresponded to the elevated values of Ni (only Timok and Rusenski Lom), PO_4-P and NH_4-N (only Tutrakan) (Figs. 4 and 5). This abundance of mayflies correlated also to the lowest concentration of lead at these sampling sites, as well as at the sampling sites upstream and downstream Ruse. At the other sampling sites mayflies were not found. This, on the other hand, may be due to the sampling period (late summer), when the mayflies may have emerged already. Higher levels of nutrients were registered at Timok, Iskar, Rusenski Lom and Tutrakan sites and could cause a growth of periphyton, which is a trophic resource for mayflies and freshwater snails (most of them belong to the trophic groups of the grazers or scrapers).

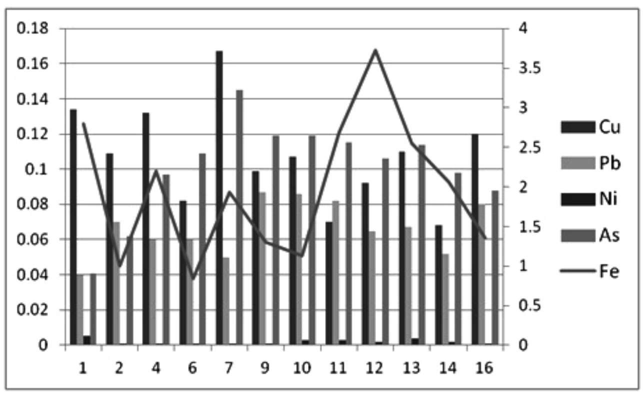

Fig. 4. Longitudinal distribution of the concentration of heavy metals (Cu, Pb, Ni, Fe) and As in the water

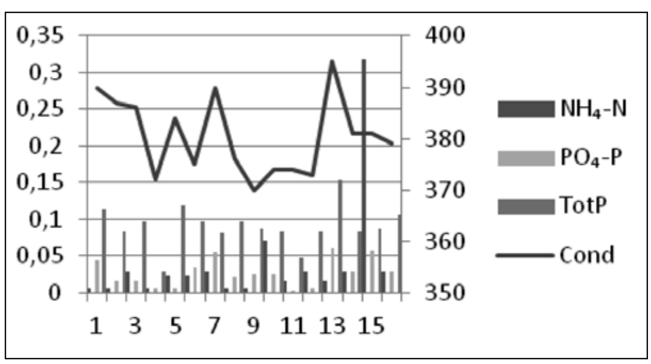

Fig. 5. Longitudinal distribution of the values of measured physical and chemical parameters and nutrients (NH_4-N, PO_4-P, TotP, Conductivity) in water

The freshwater snails were observed to become more abundant at the studied sites Iskar, u/s Ruse, Rusenski Lom and Tutrakan (Fig. 1) in correspondence with the concentration of iron and NH_4-N (only Tutrakan) (Figs. 4 and 5).

The abundance of Heteroptera was elevated also in correspondence with the values of Ni at the sampling points Timok and Rusenski Lom (Figs. 4 and 5). The highest concentrations of PO_4-P, TotP and conductivity were measured at the sampling site Rusenski Lom, where the only specimens of Hirudinea and the highest number of dipterans (other than chironomids) larvae were found (Figs. 3 and 4). The abundance of Amphipoda decreased at all of the studied sites that were located upstream Svishtov and elevated at the sampling sites Rusenski Lom and Aydemir contrariwise to the values of Cu.

Conclusions

The Bulgarian stretch of the Danube River is divided into two parts – upstream and downstream the Iskar sampling site. The upstream part could be considered as less loaded with nutrients and heavy metals than the downstream one. An exception is the sampling site Timok, located the most upstream within the investigated stretch and also highly affected by the right tributary, the Timok River, which is polluted from the mining industry in the Republic of Serbia.

The obtained results revealed that the studied sites Timok, Iskar, Rusenski Lom and Tutrakan were the most loaded among the investigated in the present study sites along the Bulgarian stretch of the Danube River. The environmental conditions, formed at the pointed sites and characterised with permanent sources of pollution favoured the development of tolerant and more resistant benthic taxa. This corresponded to the abundance of the taxonomic groups Oligochaeta and Chironomidae. On the other hand, the sampling sites which were more loaded with nutrients, were also rich of macrophytes and periphython. The heterogeneity of the habitats created a diverse environment favouring for the development of abundant benthic community.

The distribution pattern of the studied main benthic groups in relation to the environmental parameters (concentration of heavy metals and nutrients) is a result of their tolerance to the level and the type of human impact. Displayed correlations should not be considered only in the context of direct interactions between benthic groups and parameters of the environment. The relationship could be expressed trough the feeding preferences and the effect could be transported through the trophic chain.

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