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Bottom Fauna Qualitative Study of the Danube River in Belgrade Region

Nataša Popović*, Vladimir Jovanović, Maja Raković, Vladimir Kalafatić, Vesna Martinović-Vitanović

Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia; E-mail: natasa.popovic@ibiss.bg.ac.rs

Abstract: Limnological study of the Danube River in the Belgrade region was performed at five sites along 66 km of the river flow. Research was carried out during the high (May/June) and low (September/October) water level periods from 2007 to 2009. The qualitative and saprobiological analyses of meio- and macrozoobenthic communities with special emphasis on chironomid larvae, as well as investigations of basic physical and chemical water quality parameters were used for the water quality assessment, thus classifying the Danube water (in the Belgrade region) in the limits of II-III and III class based on modified National water assessment standards. Many species of chironomids manifested a distinct response to the presence of different pollutants in the water and bottom sediments, thus serving as indicators of the degree of the water body pollution. According to CCA, the environmental factors related to the assemblage composition were COD, BOD_5, percentage of oxygen saturation, TOC, total phosphates and nitrates.

Key words: meio- and macrozoobenthos, chironomid larvae, community structure, environmental parameters, Danube River, Serbian stretch

Introduction

The Danube River is the second largest European river, with a length of 2,857 km and a drainage area of about 817,000 km², and it is the only large European river that flows from west to east. The Serbian Danube sector (587.4 km) is a significant natural resource. In Serbia, the Danube River before its damming (in 1970 Djerdap I and in 1984 Djerdap II), was a typical large lowland river with characteristic potamobenthic communities. After damming, the Danube River was divided into the Upper part – Riverine zone, Middle part – Flow through reservoir, and Lower part – Reservoirs Iron Gate I and II (Martinović-Vitanović, Kalafatić 2002, Martinovic-Vitanovic *et al.* 2006). Two large cities, Belgrade (1.7 million inhabitants) and Novi Sad (300,000 inhabitants), lie on the Danube River, as well as many smaller towns and villages. None of them have a system for treating municipal waste waters (Veljković 2005). Although it receives great quantities of waste-water from large settlements and industrial installations on its banks, there is no worsening of water quality owing to the fact that the Danube is a large river with a great ability to absorb waste-waters and a considerable capacity for self-purification (Martinović-Vitanović *et al.*1995).

Limnological studies of the Danube River in the Belgrade region (belongs to the Middle part – Flow through reservoir) had a long tradition since 1984, as part of the Water Quality Monitoring Program designed by Belgrade municipal authorities (Martinović-Vitanović *et al.* 1999a, b, 2004a). Different species of chironomid larvae are used as lotic and lentic water quality indicators, because their distribution is closely related to the different degrees of water depth, dissolved oxygen, organic matters and temperature (SAETHER 1979). Therefore, their presence in habitats can be used as indicators in lake classification, river zonation and water quality (HART 1974). The family Chironomidae commonly known as non-biting midges constitutes more than 5000 described species worldwide, with still more to be described. It includes the most diverse group of aquatic insects, including many different feeding groups, habitat preferences, and tolerance levels to different environmental conditions (EPLER 2001). Chironomids are suitable for the ecological characterization of watercourses (DOMMERMUTH 1996, JANZEN 2003, ORENDT 2003).

The objectives of this study were to provide data about the state of the qualitative composition and distribution of bottom fauna communities during the period 2007-2009, with special emphasis on the Chironomidae family and to extend knowledge of the bottom community structure.

Saprobic Index	Water Quality Class	Saprobity Level
<1.25	I	oligosaprobic
1.25 to 1.75	I–II	oligo- to beta-mesosaprobic
1.76 to 2.25	II	beta-mesosaprobic
2.26 to 2.75	II–III	beta-meso- to alpha-mesosaprobic
2.76 to 3.25	III	alpha-mesosaprobic
3.26 to 3.75	III–IV	alpha-meso- to polysaprobic
>3.75	IV	polysaprobic

Materials and Methods

Study area

In order to investigate the Danube bottom fauna composition in the Belgrade region, five locations were selected along a stretch of the river measuring 66 km in length (Table 1): Stari Banovci, at the entrance into Belgrade region; Zemun, near the city's downtown; Višnjica, on the periphery of Belgrade, downstream from the mouth of the Sava River and the city dump on the right bank; Vinča, near the water intake of Vinča Waterworks; and Brestovik, at the exit from the Belgrade region (see Fig. 1).

Sampling procedure and techniques

Samples were taken near the right bank of the Danube in May/June and September/ October from 2007 to 2009. Limnological studies were performed using standard methods and techniques (APHA-AWWA-WEF 1995, SRPS-ISO 5667/1997, SRPS-ISO 7828/1997). In total, 30 samples for the meio- and macrozoobenthos analysis were taken from the river bottom with a Van-Veen type of grab (270 cm² grab area). The samples were gathered from water a depth varying between 1 and 7.5 m. Material for analysis of benthic fauna came from the different bottom facies. Substrate classification was performed by visual evaluation *in situ* and in the laboratory based on the diameter of sediment particles (WENTWORTH 1922), and according to the Serbian national classification (MARTINOVIĆ-VITANOVIĆ *et al*. 1995, LAKUSIĆ 2005). Specimens in each sample were separated from the sediment by washing and sieving (mesh size 200 µm). Part of the sampled material was examined on site and the rest was preserved with 4% formaldehyde and examined in the laboratory. A stereo zoom microscope with binocular magnifier (magnification 5–50x), Krüss, Germany and microscope (10x10 and 10x40), Opton, Germany were used for sorting and identification of organisms.

Physical and chemical water analysis was performed *in situ* or in the Institute of Public Health, Belgrade, using standard methods and techniques (APHA-AWWA-WEF 1995, SRPS-ISO /IEC 17025/2001). Water temperature (°C), pH, dissolved oxygen (DO, $mg.l^{-1}$), percentage of O_2 saturation, nitrites ($mg.l^{-1}$), nitrates ($mg.l^{-1}$) and conductivity ($\mu S.cm^{-1}$), were measured in the field with a Horiba W-23XD multiparametric probe. Biochemical oxygen demand for five days (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC) and total phosphates (total P) were measured in the laboratory in the Institute of Public Health, Belgrade. The transparency of the water was assessed using a Secchi-disc.

Appropriate keys were used for determining bottom fauna representatives either up to the species level or to the lowest possible taxonomic level (BRINKHURST, JAMIESON 1971, HIRVENOJA 1973, LELLAK 1980, WIEDERHOLM 1983; SLADEČEK, KOŠEL 1984, CROFT 1986, UZUNOV *et al*. 1988, HAMMOND 1997, TIMM 1999, MASCHWITZ, COOK 2000, PFLEGER 2000, KORNIUSHIN 2004). Bioindicator species were determined according to SLADEČEK, KOŠEL 1984, UZUNOV et al. 1988 and MOOG 1995, 2002. The Saprobic index (S), according to PANTLE, BUCK (1955) and classification of saprobity levels, according to MOOG (1995, 2002) were used to estimate the water quality at each site.

The following scheme shows the classification of the Saprobic Index (S), water quality classes and saprobity levels (modified National Water Assessment Standard, according to Martinovic-Vitanovic *et al*. 2009):

Correspondence analysis (CA), detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were used to check for the main changes in species composition of benthic fauna and with the chosen subset of ecological factors according to performed correlation tests (Pearson's r). All analyses were performed in PAST software, version 2.14 (Hammer *et al.* 2001). Environmental variables that did not meet the normality assumption (Shapiro-Wilk test, $p<0.05$) were log (x+1) transformed before the analyses. A Monte-Carlo permutation test (500 permutations, $p<0.05$) was performed to test the significance of the sum of all eigenvalues (Ter Braak, Prentece 1988, Ter Braak, Verdonschot 1995 Leps, Smilauer 2003).

Results

The majority of gathered samples were taken from fine substrates (silt and fine sand) with the presence of detritus. However, significant parts of gravel with pebbles and cobbles were present in some samples taken from Višnjica and Vinča localities (Table 1).

Results of the analysis of physical and chemical water characteristics obtained from depths of 0.5 m are shown in Table 2. The largest variations in analyzed parameter values were noted with dissolved oxygen concentration (5.2 to 9.3 mg $O_2.l^{-1}$), oxygen saturation (61 to 105%), total phosphate (0.04 to 0.16 mg $P.l^{-1}$), and BOD_5 (0.4 to 3.8 mg $O_2.l^{-1}$) while nitrites had varied in small amounts (0.01 – 0.03 mg $NO_2.l^{-1}$).

Qualitative composition of the bottom fauna is presented in Annex 1 as a list of all taxa recorded within the studied period (2007-2009) for each site. Alongside the 66 km of the Danube in the Belgrade region, a rich zoobenthic community was found with Chironomidae and Oligochaeta as dominant groups, followed by Bivalvia and Gastropoda. During the whole period of study, a total of 67 taxa were found; these taxa belonged to 14 taxonomic groups. Specific benthic groups had the following percentage of participation in the total bottom fauna list: Chironomidae – 23 taxa (34.33%), Oligochaeta – 17 taxa (25.37%), Bivalvia – 8 taxa (11.94%), Gastropoda – 5 taxa (7.46%), Hirudinea – 4 taxa (5.97%), Amphipoda – 2 taxa (2.98%). Nematoda, Polychaeta, Turbellaria, Isopoda, Odonata, Trichoptera, Ephemeroptera and Ceratopogonidae were represented by single taxa. The family Chironomidae, that had the highest diversity, was represented by four subfamilies: Chironominae (16 taxa), Orthocladiinae (4 taxa), Tanypodinae (2 taxa) and Prodiamesinae (1 taxon). Larvae of *P. scalaenum* and *Ch.* gr. *plumosus* were found in all sites while *Ch. riparius* was found in four out of five sites (absent from site 2). Co-dominant Oligochaeta were represented by three families, 13 genera and 17 species. Oligochaete species belong to the families of Tubificidae (10 species or 58.82% of the oligochaete community), Naididae (6 species – 35.29%) and Lumbricidae (1 species – 5.89%). *L. hoffmeisteri* and *L. claparedeanus* were found in all sampling sites. Molluscs were a subdominant component in zoobenthic communities, with 19.40% participation in the total bottom fauna. The groups of Gastropoda and Bivalvia were found during all investigated years and in all sites.

Calculated values of Pantle-Buck's saprobic index (S) of bottom fauna communities are given in Table 3. The values varied from minimum S = 2.24 (locality 1 in Sept 2009 = S22) to maximum S = 3.24 (locality 4 in June 2008 = S17), indicating the range from beta-meso- to alpha-mesosaprobic conditions. Seventeen out of twenty-three identified chironomid species had individual saprobic values in the range from 0.80 to 3.60. Nine of these species had individual saprobic values in the beta-mesosaprobity class,

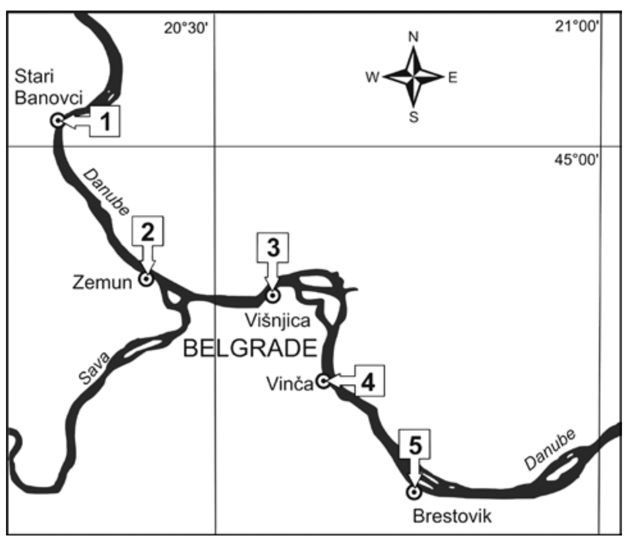

Fig. 1. Map of the Danube River in the Belgrade region

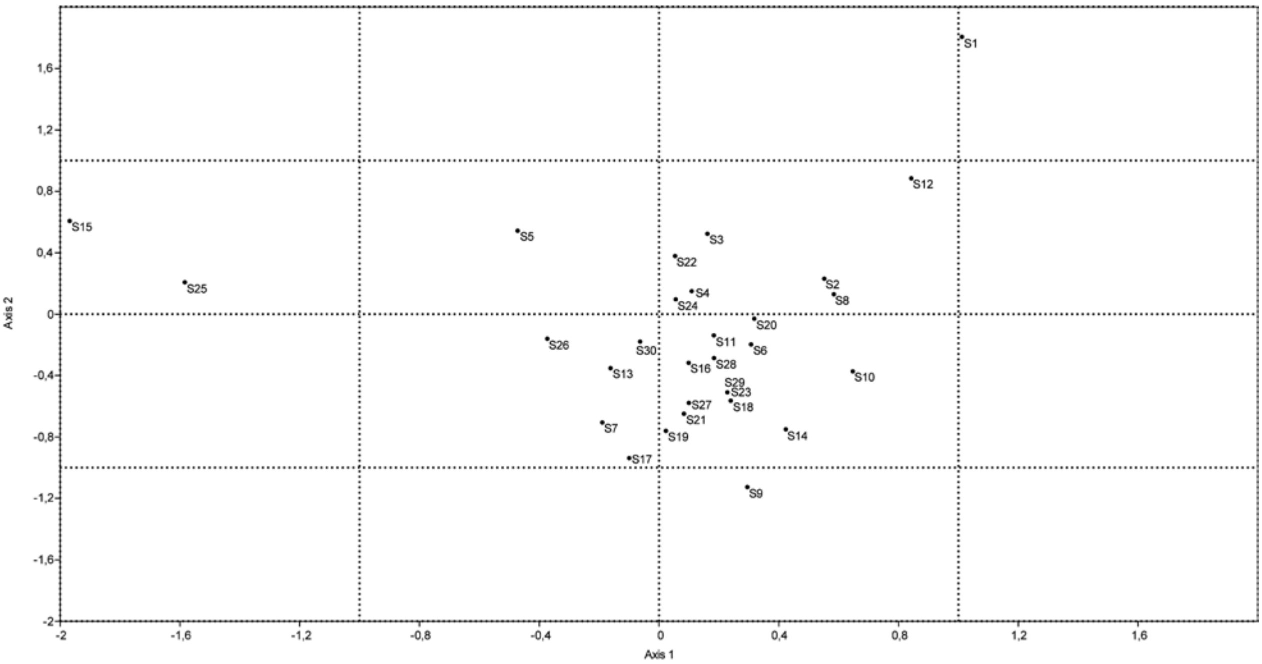

Fig. 2. Correspondence analysis of sampled meio- and macroinvertebrate communities. Abbreviations: S1 – S30 – samples (for details see Appendix 1)

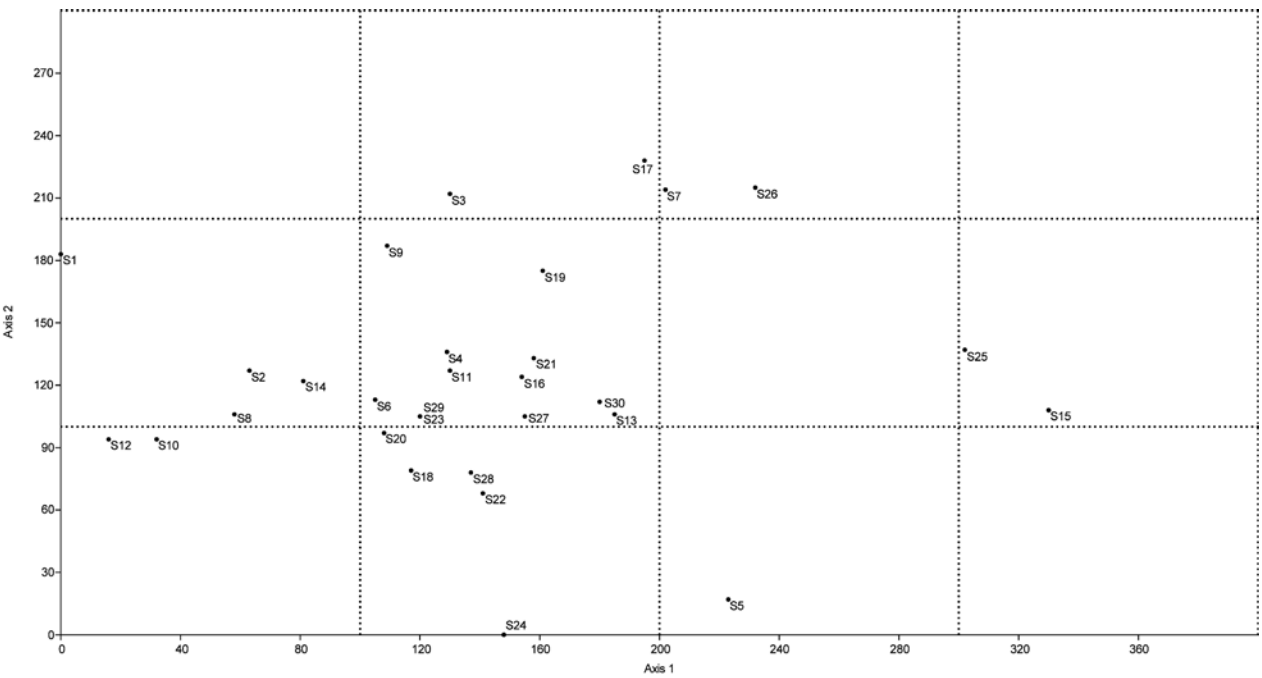

Fig. 3. Detrended correspondence analysis – plot of sample scores. Length of the first axis is 330 SD

and six bioindicators had saprobic valences in the alpha-mesosaprobity class. Hence, the majority of recorded chironomid bioindicators indicated mesosaprobic conditions.

The majority of samples were clustered in the CA graph (Fig. 2), with some samples significantly differing in species composition. The first CA axis explained 10.35%, while the second explained 8.79% of the total variation. Along the first CA axis the extremely divergent samples were S15 and S25, with the samples S5 and S26 nearly as divergent. These four samples were from the same locality (locality 3), which was characterized by the presence of intensive organic pollution originating from inadequately purified wastewaters and the city dumpsite on the bank. This locality differed signifi-

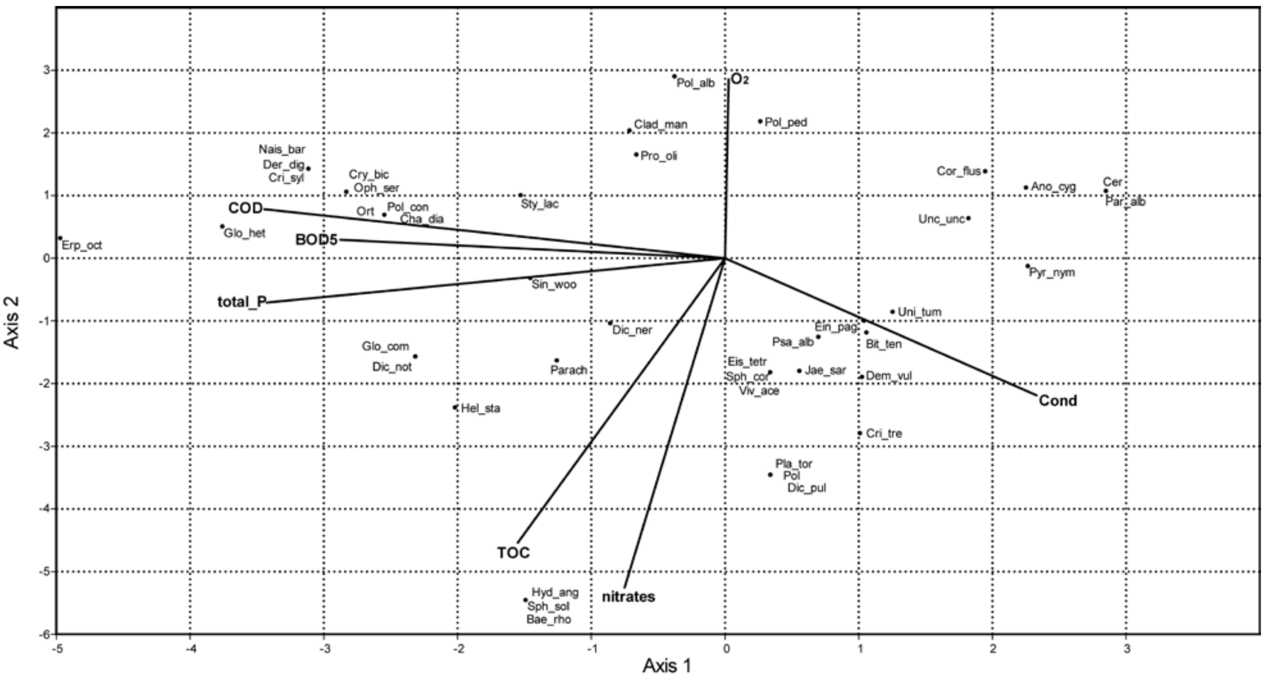

Fig. 4. CCA ordination plot of meio- and macroinvertebrate communities and environmental variables. Quantitative environmental variables are indicated by vectors (O_2 – percentage of oxygen saturation, BOD_5 – biological oxygen demand for five days, COD – chemical oxygen demand, TOC – total organic carbon, total P – total phosphates, cond – conductivity and nitrates. Codes of species showed on plot: Cha_dia – *Chaetogaster diaphanus*, Der_dig – *Dero digitata*, Nais_bar – *Nais barbata*, Oph_ser – *Ophidonais serpentine*, Unc_unc – *Uncinais uncinata*, Psa_alb – *Psammoryctides albicola*, Eis_tetr – *Eiseniella tetraedra*, Sty_lac – *Stylaria lacustris*, Erp_oct – *Erpobdella octoculata*, Glo_com – *Glossiphonia complanata*, Glo_het – *Glossiphonia heteroclita*, Hel_sta – *Helobdella stagnalis*, Pla_tor – *Planaria torva,* Jae_sar – *Jaera sarsi*, Bit_ten – *Bithynia tentaculata*, Viv_ace – *Viviparus acerosus,* Ano_cyg – *Anodonta cygnea*, Uni_tum – *Unio tumidus,* Sin_woo – *Sinanodonta woodiana,* Sph_cor – *Sphaerium corneum,* Cor_flus – *Corbicula fluminalis*, Sph_sol – *Sphaerium solidum,* Pyr_nym – *Pyrrhosoma nymphula*, Hyd_ang – *Hydropsyche angustipennis,* Bae_rho – *Baetis rhodani,* Cer – Ceratopogonidae, Dic_pul – *Dicrotendipes pulsus*, Dic_not – *Dicrotendipes notatus*, Dic_ner – *Dicrotendipes nervosus,* Pol_con – *Polypedilum convictum*, Pol – *Polypedilum* sp., Parach – *Parachironomus* sp., Par_alb – *Paratendipes albimanus,* Cri_syl – *Cricotopus sylvestris*, Cri_bic – *Cricotopus bicinctus*, Cri_tre – *Cricotopus tremulus*, Ort – *Orthocladius* sp., Pro_oli – *Prodiamesa olivacea*, Clad_man – *Cladotanytarsus mancus*, Pol_ped – *Polypedilum pedestre,* Pol_alb – *Polypedilum albicorne*, Ein_pag – *Einfeldia pagana*, Dem_vul – *Demicryptochironomus vulneratus*

cantly in BOD_5 and total phosphates content from all other sites. On the other extreme of the first CA axis some samples (S1, S12) from the least polluted locality (locality 1) were positioned. Results of DCA analysis (Fig. 3) showed great heterogeneity in the structure of studied communities, i.e. big overturn in species composition among the samples. This overturn in diversity did not follow river flow gradient, or seasons. To a certain extent, it could be described as the gradient of pollution. Since the highest score on the x-axis of DCA was notably higher than 4.SD, the CCA analysis was employed for the gradient analysis of the effect of ecological factors on community composition. At the community level, CCA analysis based on a presence-absence matrix was performed. Environmental variables included in the analysis were: BOD_5, percentage of oxygen saturation, nitrates, TOC, COD, total phosphates and conductivity. The first two CCA axes had eigenvalues of 0.31 and 0.26, explaining 51.19% of variation in the relationship between species and environmental factors (Fig. 4). COD and BOD_5 had the highest negative correlations with the first CCA axis and the percentage of oxygen saturation was positively correlated with the second CCA axis. Hirudinea species, *E. octoculata* and *G. heteroclita*, had the highest scores on the first CCA axis, and were found at sites with high total phosphates, BOD_5 and COD levels. Positive correlations with COD and BOD_5 were found for *C. bicinctus*, *C. sylvestris*, *P. convictum*, *Orthocladius* sp., *O. serpentina*, *D. digitata*, *N. barbata*, *S. lacustris* and *Ch. diaphanus*; while positive correlations with total

Table 1. Sites with river km (Rkm) of the Danube's course, GPS positions, description of the type of substrates (according to Martinović-Vitanović *et al.* 1995, Lakusić 2005) with dates of investigation. s – silt, sa – sand, d – detritus, cd – coarse detritus, p – pebbles, co – cobble, shz – shelly zone

Site	Rkm	GPS-position	Substrat type	Dates of sampling
S. Banovci	1192	44° 59' 08''N, 20° 17' 17''E	s; sa; d; shz	14.06.2007, 11.10.2007, 4.06.2008, 15.09.2008, 14.05.2009, 8.09.2009
Zemun	1171.5	44° 50' 50''N, 20° 24' 51''E	s; sa; d; cd; shz	14.06.2007, 11.10.2007, 4.06.2008, 25.09.2008, 14.05.2009, 6.09.2009
Višnjica	1162	44° 49' 54''N, 20° 32' 10''E	s; sa; d; cd; p	14.06.2007, 11.10.2007, 4.06.2008, 25.09.2008, 14.05.2009, 7.09.2009
Vinča	1144.5	44° 46' 09''N, 20° 37' 30''E	s; sa; d; p; co; shz	14.06.2007, 11.10.2007, 4.06.2008, 25.09.2008, 14.05.2009, 7.09.2009
Brestovik	1126	44° 39' 19''N, 20° 49' 26''E	s; sa; d	14.06.2007, 11.10.2007, 4.06.2008, 17.09.2008, 14.05.2009, 10.09.2009

Table 2. Physical and chemical Danube River water characteristics over a three year period (samples obtained from depths of 0.5 m)

Year	2007										2008										2009									
Sites	S. Ba-novci		Zemun		Višnjica		Vinča		Brestovik		S. Ba-novci		Zemun		Višnjica		Vinča		Brestovik		S. Ba-novci		Zemun		Višnjica		Vinča		Brestovik	
Month	Jun	Oct	Jun	Oct	Jun	Oct	Jun	Oct	Jun	Oct	Jun	Sept	Jun	Sept	Jun	Sept	Jun	Sept	Jun	Sept	May	Sept	May	Sept	May	Sept	May	Sept	May	Sept
Depth (m)	2	1.5	2.5	1.5	1	1.5	2	1.5	2	3	1	5	2	1	1.5	1	1	1	1.5	3	1	4	1.5	3	1	1	2	2.5	2	7.5
Water temperature (^0C)	24.2	16.3	24	16.4	24	17.2	24.1	17.2	24.1	17.3	21.1	15.2	21.5	15.6	22.5	16.2	22.7	16.2	22.8	16.1	20	20.3	20	20.1	20	20.5	21.2	20.6	21.5	20.8
pH	8.2	7.9	7.9	7.9	7.9	7.9	7.9	8	8	7.9	8	8.1	8	8	8	8	8	8.1	8.1	8.1	8.3	8.1	8.2	8.1	8.4	8.1	8.2	8.1	8.2	8.2
DO ($mg.l^{-1}$) O_2	7.4	7.0	5.2	6.7	5.3	6.8	5.4	6.8	5.8	6.9	6.9	8.9	5.9	8.5	6.7	7.5	6.7	9.2	7.1	8.4	9.6	8.0	9.1	7.3	8.1	7.5	8.2	8.2	9.3	7.9
O_2 saturation (%)	87	71	61	68	62	70	63	70	68	71	77	88	66	84	76	75	77	92	82	84	105	88	99	78	90	83	91	90	104	88
BOD ($mg.l^{-1}$)	2.1	0.8	1.2	1.2	2.6	2.4	1.5	1	1.4	1.4	1.2	1.6	0.4	1.8	2.3	3.8	1.4	2.1	1.4	1.7	2.4	1.4	2.4	0.9	3	3.7	2.7	1.3	2.3	1
TOC ($mg.l^{-1}$)	2.7	2.42	2.77	2.34	2.87	2.33	2.43	2.4	2.54	2.46	1.89	2.34	2.09	2.19	2.37	2.41	1.96	2.1	2.01	2.24	2.31	2.41	2.15	2.32	2.49	2.86	2.43	2.42	2.31	2.29
Conductivity ($\mu S.cm^{-1}$ at 20^0C)	360	330	340	330	350	340	340	340	340	350	260	390	270	360	300	380	300	370	300	370	320	330	300	330	320	350	310	340	310	340
Transparency (m)	0.6	0.4	0.6	0.4	0.7	0.4	0.75	0.8	0.7	0.7	0.7	0.9	0.75	0.8	0.75	0.8	0.75	0.75	0.7	1	0.5	0.7	0.5	0.6	0.7	0.6	0.75	0.8	0.7	0.6
COD ($mg.l^{-1}$) O_2	3.2	2.6	2.5	3.1	1.9	3.6	1.9	2.1	2.4	3.7	3.3	2.6	2.7	3	1.9	2.8	2	2.5	2.9	2.7	2.6	2.9	3.4	2.4	3.4	2.4	2.9	2.8	3.5	2.7
Total phosphate ($mg.l^{-1}$)	0.09	0.09	0.09	0.09	0.08	0.07	0.08	0.07	0.07	0.06	0.06	0.09	0.06	0.08	0.07	0.1	0.08	0.14	0.08	0.07	0.07	0.09	0.04	0.07	0.12	0.07	0.07	0.07	0.16	0.12
Nitrites ($mg.l^{-1}$)	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.03	0.01	0.02	0.01	0.02	0.01	0.02	0.02	0.03	0.01	0.02
Nitrates ($mg.l^{-1}$)	1.2	1	1	1.1	0.9	1.33	1.2	1	1.48	1.22	1.24	1.53	1.2	1.2	1.1	1.1	1.2	1.1	1.49	1.3	1.42	1.5	1.26	1.2	1.26	1.6	1.7	1.8	1.44	2.1

Table 3. Values of the Pantle-Buck saprobic index based on saprobiological analysis of the benthic communities of the Danube River in the Belgrade region, Serbia in the years 2007-2009 with saprobity level (sl.): o – oligosaprobic; β – beta-mesosaprobic; α – alpha-mesosaprobic; p – polysaprobic and water quality class (cl.): I-IV

Sites		**S. Banovci**		**Zemun**		**Višnjica**		**Vinča**		**Brestovik**	
Year	Month	S	sl., cl.	S	sl., cl.	S	sl., cl.	S	sl., cl.	S	sl., cl.
2007	June	2.48	β-α, II-III	2.50	β-α, II-III	3.04	α, III	3.16	α, III	3.00	α, III
	October	2.70	β-α, II-III	3.11	α, III	3.20	α, III	2.95	α, III	2.79	α, III
2008	June	3.03	α, III	2.91	α, III	2.94	α, III	3.24	α, III	3.18	α, III
	September	2.37	β-α, II-III	2.77	α, III	3.17	α, III	3.09	α, III	3.05	α, III
2009	May	3.11	α, III	2.97	α, III	2.81	α, III	3.07	α, III	2.95	α, III
	September	2.24	β, II	2.74	β-α, II-III	3.09	α, III	2.79	α, III	3.23	α, III

phosphates and TOC were found for the remaining Hirudinea species and chironomids: *D. notatus*, *D. nervosus*, *Parachironomus* sp. Among chironomids, affinity toward the sites with higher conductivity levels was shown by *D. vulneratus*, *C. tremulus, P. albimanus* and *E. pagana*, showing at the same time the negative correlations with total phosphates, BOD_5 and COD. Chironomids *P. albicorne*, *P. pedestre*, *C. mancus* and *P. olivacea* were positively associated with the percentage of oxygen saturation.

Discussion

The invertebrate benthic assemblage of 67 taxa from 14 faunistic groups, recorded in the Danube River in the Belgrade region, may be considered as a rich one. In the previous paper (Martinovic-Vitanovic *et al*. 2006) the number of benthic species recorded in the Belgrade region was the same, although the total of 136 taxa were recorded from the whole Danube course in Serbia. Chironomidae and Oligochaeta represent the main components of benthic communities in the Danube as a large lowland river.

In our present paper five recorded taxa are denoted as invasive species: *H. invalida*, *B. sowerbyi, L. naticoides*, *D. polymorpha* and *C. curvispinum*. Also new species (Neozoa) of the Danube's invertebrate fauna, *C. fluminea* and *C. fluminalis*, were recorded in three and seven samples, respectively (Martinovic-Vitanovic *et al*. 2006). Distribution of the oligochaete species *I. michaelseni* in Serbia is linked with the Danube River (Martinovic-Vitanovic *et al*. 2006). Among Gastropoda, only *V. acerosus* was known as an endemic species in the Danube River (Martinovic-Vitanovic *et al.* 2006). The core of the benthic community in the investigated part of the Danube River can be inferred from taxa common at all sampling sites. This group includes: oligochaetes (*B. sowerbyi*, *T. tubifex*, *L. hoffmeisteri*, *L. claparedeanus*, *L. udekemianus*, *P. hammoniensis*, *P. albicola*), chironomids (*P. scalaenum*, *Ch. plumosus*, *Ch. riparius*), gastropods (*T. fluviatilis* and *L. naticoides*), bivalves (*C. fluminea* and *C. fluminalis*), with the occurrence of nematodes, crustaceans (Gammaridae and *J. sarsi*) and polychaete *H. invalida*. The identified bottom fauna community is common for the Danube in the Belgrade region (Martinović-Vitanović *et al*. 1999a, 2004a). Comparing the obtained results with those of other authors for the Danube River (Illies 1978, Janković 1978, Moog 1995, 2002, Russev *et al.* 1998, Martinović-Vitanović *et al*. 1999a, b, 2004a, b, Martinovic-Vitanovic *et al*. 2006, 2008, Fesl 2002) we noticed a similarity and correspondence in terms of community composition. This community-type was also reported from the middle lowland sector of the Volga River (Zinchenko 2006).

In the present study, the highest species richness in the fauna of Chironomidae was recorded for the subfamily Chironominae (16 taxa), while species richness among three other subfamilies (Orthocladiinae, Tanypodinae and Prodiamesinae) was significantly lower (4, 2 and 1 taxa, respectively). Previously, Janković (1978) recorded 73 species of Chironomidae in the Serbian stretch of the Danube River, 47 species have been identified in the Bulgarian section of the Danube River (Russev *et al.* 1998), while Fittkau, Reiss (1978) stated that the total number of chironomids in Europe's inland waters was 1,404. Larvae of dominant chironomid species (*P. scalaenum*, *Ch. plumosus* and *Ch. riparius*) were found in almost all samples. Some chironomid species can tolerate low oxygen and strongly polluted conditions (Curry 1961), e.g. *Ch. riparius* larvae have a wide range of ecological tolerance to low pH values (Havas, Hutchinson 1982) and low oxygen saturation of water (Heinis 1993). Larvae of the genus *Polypedilum* are also known for inhabiting all types of freshwaters (Lenz 1962).

The 30 analyzed samples were taken from prevailing soft bottom substrates (silt, sand and detritus). The distribution and diversity of potamobenthos communities (expressed by species richness) are affected by the nature of substrates. In soft, muddy sediments, we recorded dominance of ubiquitous taxa of Chironominae and Tubificidae. Substrate type is also assumed to be an important abiotic factor governing the species richness of aquatic invertebrates. Species richness may be affected by habitat stability, being lower under less stable conditions (Fesl 2002). Differentiation in the composition of the zoobenthic community of the Danube River in the Belgrade region was mostly driven by the following environmental factors: COD, BOD_5, total phosphates and oxygen saturation percentage (Fig. 4), all of them strongly related to water pollution. Also, the changes in overall characteristics of the Danube River in investigated riverine, transitional (Flow through reservoir – Danube in the Belgrade region) and lacustrine zones could be the result of river damming (Martinovic-Vitanovic *et al.* 2006, Chapman 1997). Conductivity was generally as expected for Danube-type rivers (SRPS-ISO 7828/1997), so it was not stressful for the communities, as well as for the levels of nitrates and TOC. Oxygen parameter variations (dissolved oxygen, percentage of oxygen saturation and BOD) are effects of waste water inflow bringing abundance of easily degradable organic matter and the inability of water plants and algae to recuperate, through photosynthesis, oxygen used for its decomposition (Martinović-Vitanović *et al.* 2004a, b). The distribution and diversity of potamobenthos communities in the Danube River in Serbia are mostly affected, apart from the presence of different habitat types, by the permanent presence of biodegradable organic pollution (received from tributaries such as Tisa and Sava rivers) and poorly treated industrial or communal wastes (Martinovic-Vitanovic 2006). This type of anthropogenic influence on the Danube bottom can be easily seen in the community structure of Višnjica samples. These samples differed from both upstream and downstream localities (Fig. 2), which suggests that there is a huge impact at this locality. This impact is brought by communal waste, which changes the BOD_5 and total phosphates content at the site. Phosphate becomes available following the biological decomposition of domestic sewage. These changes in the chemical composition of the water are followed by significant changes in the structure of the biota, some of which exploit the increased nutrients and others which can tolerate reduced oxygen concentrations. Positive correlation with total phosphate, COD and BOD_5 showed pollution tolerant organisms (*E. octoculata*, *G. heterolitica*). However, there was also a huge capacity for self-purification: communities downstream from Višnjica restored the standard structure.

Positive correlation with percentage of oxygen saturation showed species which are pollution sensitive (e.g. *P. albimanus*, *C. mancus*). The genus *Cladotanytarsus* may colonize all kinds of freshwater and brackish environments, but shows a preference for those with rather coarse sediments and low organic matter contents (McGarrigle 1980, Bass 1986). A sufficient supply of dissolved oxygen is essential for the integrity of river ecosystems and vital to the self-purification process within rivers (Oliver 1983).

Our results are consistent with the results of other authors dealing with benthic community composition of large lowland rivers (Russev *et al.* 1998; Zinchenko 2006). This is a confirmation of the statement that large lowland rivers are characterized by a typical potamobenthic community in which the highest species richness was recorded for subfamily Chironominae.

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References

APHA-AWWA-WEF 1995. Standard Methods for the Examination of Water and Wastewater. – 19th ed., Eaton, A. D., Clesceri L. S & Greenberg, A. E. American Public Health Association, Washington, DC.

Bass D. 1986. Habitat ecology of Chironomid larvae of the big Thicket streams. – *Hydrobiologia*, **135**: 271-285.

Brinkhurst R. O., B. G. M. Jamieson 1971. Aquatic Oligochaeta of the World, Oliver & Boyd, Edinburgh, UK. 860p.

Chapman D. 1997. Water Quality Assessments, A Guide to the Use of Biota, Sediments and Water in Environmental Monitoring, 2nd ed., Chapman & Hall, London. 609 p.

Croft P. S. 1986. A Key to the Major Groups of British Freshwater Invertebrates. – *Field Studies*, **6**: 531-579.

Curry L. L. 1961. A key for the larval forms of aquatic midges (Tendipedidae: Diptera) found in Michigan, Manuscript presented at the Midwest Benthological Society, Whitewater, Wis., Biology Dept. Central Michigan Univ., Mt. Pleasant, Michigan. 163 p.

Dommermuth M. 1996. Die Wied: Limnologische Untersuchung eines Fließgewässersystems im Westerwald (Rheinland-Pfalz) mit einem Beitrag zum Indikationswert der Chironomidae (Diptera). Schriftenreihe naturwissenschaftliche Forschungsergebnisse Hamburg, 309 p.

Epler J. H. 2001. Identification Manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC, and St. Johns River Water Management District, Palatka, FL. 526 p.

Fesl C. 2002. Biodiversity and resource use of larval chironomids in relation to environmental factors in a large river. – *Freshwater Biology,* **47**: 1065-1087.

Fittkau E. J., F. Reiss 1978. Chironomidae. – In: Illies, J. (Ed.): Limnofauna Europaea. A Checklist of the Animals Inhabiting European Inland Waters with Accounts of their Distribution and Ecology (except Protozoa). G. Fischer Verl., Stuttgart/New York, Swets and Zeitlinger B.V., Amsterdam: 404-440.

Hammer Ø., D.A.T. Harper and P. D. Ryan 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. – *Palaeontologia Electronica*, **4** (1).

Hammond C. O. 1997. The Dragonflies of Great Britain and Ireland. Harley Books. Colchester, Essex, England. 116 p.

Hart C. W. 1974. Pollution ecology of freshwater invertebrates. New York, Academic Press, Inc. 389 p.

Havas M., T. C. Hutchinson 1982. Aquatic invertebrates from the Smoking Hills, N.W.T.: effect of pH and metals on mortality. – *Canadian Journal of Fisheries and Aquatic Sciences* **39**: 89-903

Heinis F. 1993. Oxygen as a factor controlling occurrence and distribution of chironomid larvae. PhD Thesis University of Amsterdam.

Hirvenoja M. (1973): Revision der Gattung *Cricotopus* van der Wulp und ihrer Verwandten (Diptera, Chironomidae). – *Annales Zoologici Fennici* **10**: 1-363.

Illies J. 1978. Limnofauna Europaea. A Checklist of the Animals Inhabiting European Inland Waters with Accounts of their Distribution and Ecology (except Protozoa), G. Fischer Verl., Stittgart/New York, Swets and Zeitlinger B.V., Amsterdam. 533 p.

Janković M. 1978. Fauna Chironomidae (Diptera, Nematocera) on Yugoslav part of Danube and its flodearegion and caracteristics of communities which their larvas organize of different domiciles. – *Proceedings on the Entomofauna of SR Serbia, Serbian academy of science and arts* **2**: 29-89 (in Serbian, English summary)

Janzen L. 2003. Typisierung und Bewertung von Flieβgewässern mit Hilfe der Chironomidae (Zuckmücken) – Fauna anhand des Aquem – Datensatzes. Diplomarbeit Universität Essen.

Korniushin A.V. 2004. A revision of some Asian and African freshwater clams assigned to *Corbicula fluminalis* (Müller, 1774) (Mollusca: Bivalvia: Corbiculidae), with review of anatomical characters and reproductive features based on museum collections. – *Hydrobiologia*, **529**: 251-270.

Lakusić D., J. Blazencić, V. Randjelović, B. Butorac, S. Vukojičić, B. and Zlatković *et. al*. 2005. Habitats of Serbia – Manual with descriptions and basic data. In: **Lakusić** D. (ed.): Habitats of Serbia, Project Results: Harmonization of national nomenclature in the classification of habitats with standards of International Community. Ministry of Science and Environmental Protection of Serbia, (http://habitat.bio.bg.ac.rs/)

Lellak J. 1980. Pakomárovití – Chironomidae, In: Rozkošný R. (Ed.): Klíč vodních larev hmyzu. (Identification key to aquatic larvae of insects), Academia Praha, 310 – 392. (In Czech).

Lenz F. 1962. Tendipedidae (Chironomidae) Subfamilie Tendipedinae (Chironominae). – In **Lindner** E. (ed.): Die Fliegen der palaearktischen Region, Schweizerbartsche, Stuttgart, 139-260.

Leps J., P.Smilauer 2003. Multivariate analysis of ecological data using CANOCO, Cambridge University Press, Cambridge. 269 p.

Martinović-Vitanović V., V. Kalafatić 1995. The basic hydrobiological characteristics of inland waters in Yugoslavia. – In: Stevanović V. and V. Vasić (Eds.): The Biodiversity of Yugoslavia with a survey of species of International Significance. Publ. Fac. of Biology, Univ. of Belgrade and Ecolibri, Belgrade: 97-115. (in Serbian, English summary).

Martinović-Vitanović V., V. Kalafatić 2002. Limnological investigation of the Danube River in Yugoslavia – Joint Investigation of the Danube River on the Territory of the FR Yugoslavia within the International JSD-ITR Program, pp. 75-105. In: Publ. Rep. of Serbia, Ministry for Protection of Nat. Res. and Env. & Federal Hydrometeorol. Inst., Belgrade (also on CD).

Martinović-Vitanović V., V. Kalafatić, J. M. Martinović, M. Paunović and D. Jakovcev 1999a. The Saprobiological Analysis of Benthic Communities in the Danube in Belgrade Region. – In: Special issues of the Macedonian Ecological Society (Skopje) – *Section V Ecological Monitoring*, **5** (2): 504-516.

Martinović-Vitanović V., V. Kalafatić, J.M. Martinović, D. Jakovcev and M. Paunović 1999b. Benthic fauna as an indicator of the Sava River water quality in Belgrade region, In: Special issues of the Macedonian Ecological Society (Skopje) – *Section V Ecological Monitoring*, **5** (2): 517-529.

Martinović-Vitanović V., D. Jakovcev-Todorović, V. Djikanović and V. Kalafatić 2004a. Water quality studies of the River Danube in Belgrade region based on benthic fauna saprobial analysis. *Limnological Reports* 35 IAD-SIL, Novi Sad, 289-295.

Martinović-Vitanović V., D. Jakovcev-Todorović, V. Djikanović and V. Kalafatić 2004b. The saprobiological analysis of benthic communities in the Sava River in Belgrade region. – *Limnological Reports*, IAD-SIL, Novi Sad, **35**: 341-347.

Martinovic-Vitanovic V., D. Jakovcev-Todorovic and V. Kalafatic 2006. Qualitative study of the bottom fauna of the River Danube (R.km 1433 – 845.6), with special emphasis on the oligochaetes. – *Archiv für Hydrobiologie*, **158** (*Large Rives* 16): 427-452.

Martinovic-Vitanovic V., S. Obradovic, V. Milankov and V. Kalafatic 2008. Bottom fauna communities of the Sava River (R.km 61.5-0.5) in Serbia. – *Archiv für Hydrobiologie,* **166** (Large Rivers 18): 209-241.

Martinovic-Vitanovic V., N. Popovic, S. Ostojic, M. Rakovic and V. Kalafatic 2009. Spreading and ecology of Manayunkia caspica Annenkova 1928 (Polychaeta) in the Serbian Danube stretch. – *Transylvanian review of systematical and ecological research,* **8**: 137-160.

Maschwitz, D.E., E.F. Cook 2000. Revision of the Nearctic species of the genus Polyp*edilum* Kieffer (Diptera: Chironomidae) in the subgenera *P. (Polypedilum)* Kieffer and *P. (Uresipedilum)* Oyewo and Saether. – *Bulletin of the Ohio Biological Survey*, *New Series*, **12**(3): 1-135.

McGarrigle M. L. 1980. The distribution of chironomid communities and controlling sediment parameters in L. Derravaragh, Ireland. Paper presented at 7th International Symposium on Chironomidae, Dublin. 275-282.

Moog O. 1995. Fauna Aquatica Austriaca, Katalog zur autökologischen Einstufungaquatischer Organismen Österreichs. Teil III, B, Metazoa. Bundesministerium f. Land-u. Fortwirtschaft, Wien.

Moog O. 2002. Fauna Aquatica Austriaca, Wasserwirtschaftskataster – Bundesministerium f. Land-u. Forstwirtschaft, Umwelt u. Wasserwirtschaft, Wien.

Oliver R. D., E. M. Roussel 1983. The Insects and Arachnids of Canada, Part II, The Genera of Larval Midges of Canada. Diptera: Chironomidae. Biosystematics Research Institute, Ottawa, Ontario. 263 p.

Orendt C. 2003. A classification of semi-natural northern prealpine river stretches based on chironomid communities. – *Annales de Limnologie – International Journal of Limnology* **39**: 219-237.

Pantle R., H. Buck 1955. Die biologische Überwachung der Gewässer und die Darstellung der Ergebnisse. – *Gas-Wasserfach*, **96**: 604-620.

Pfleger V. 2000. Molluscs. The English edition, Blits Ed. 216 p.

Russev, B., A. Petrova, I. Janeva and S. Andreev 1998. Diversity of zooplankton and zoobenthos in the Danube river, its tributaries and adjacent water bodies. – In: Bulgaria's Biological Diversity: Conservation Status and Needs Assessment, Vol. I, II (Curt Meine- ed.), Washington, 261-292.

Saether, O. A., 1979. Chironomid communities as water quality indicators. – *Holarctic Ecology* **2**: 65-74.

Sladeček V., V. Košel 1984. Indicator Value of Freshwater Leeches (Hirudinea) with a Key to the Determination of European Species. – *Acta Hydrochimica et Hydrobiologica*, **12**: 451-461.

Ter Braak C. J. F., I. C.Prentece 1988. A Theory of Gradient Analysis. – *Advances in Ecological Research*, **18**: 271-317.

Ter Braak, C. J. F., P. F. M. Verdonschot 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. – *Aquatic Sciences*, **57**: 255-289.

Timm T. 1999. A Guide to the Estonian Annelida. Estonian Academy Publishing Tartu/Tallinn. 208 p.

Uzunov, Y., V. Kojъel and V. Sladeček 1988. Indicator Value of Freshwater Oligochaeta. – *Acta Hydrochimica et Hydrobiologica,* **16** (2): 173-186.

Veljković N. 2005. Modern technical procedures in seweage, Belgrade: *Association for water technology and sanitary engineering*. 11 p.

Wentworth C. K. 1922. A scale of grade and class terms for clastic sediments. – *Journal of Geology,* **30**: 377-392.

Wiederholm T. 1983. Chironomidae of the Holarctic region. Keys and diagnoses. Part I. Larvae – *Entomologica Scandinavica*, **19**: 457.

Zinchenko T. D. 2006. Results and prospects of bioindication researches of the reservoirs and channels in the Volga river basin (by the exemple of chironomids, Diptera: Chironomidae). – *Izvestiya Samarskogo nauchnogo centra Rossiyskoy akademii nauk*, **8** (1): 248–262. (In Russian).

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Appendix 1. Qualitative composition of the bottom fauna in the investigated part of the Danube (r-km 1192 - 1126) during the period 2007 - 2009.

Year	2007										2008										2009									
Sites	S. Banovci		Zemun		Visnjica		Vinca		Brestovik		S. Banovci		Zemun		Visnjica		Vinca		Brestovik		S. Banovci		Zemun		Visnjica		Vinca		Brestovik	
Month	*jun*	*oct*	*jun*	*oct*	*jun*	*oct*	*jun*	*oct*	*jun*	*oct*	*jun*	*sept*	*jun*	*sept*	*jun*	*sept*	*jun*	*sept*	*jun*	*sept*	*may*	*sept*	*may*	*sept*	*may*	*sept*	*may*	*sept*	*may*	*sept*
Samples	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
Nematoda			*	*			*								*										*	*				*
Polychaeta																														
Hypania invalida (Grube, 1860)			*										*									*		*				*		
Oligochaeta																														
Branchiura sowerbyi Beddard, 1892		*	*	*		*		*	*	*		*	*	*				*	*	*			*	*			*	*	*	
Chaetogaster diaphanus (Gruithuisen, 1828)															*															
Dero digitata (Müller, 1773)																									*					
Eiseniella tetraedra (Savigny, 1826)	*																													
Isochaetides michaelseni (Lastockin, 1937)																								*						
Limnodrilus claparedeanus Ratzel, 1868	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Limnodrilus hoffmeisteri Claparede, 1862	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Limnodrilus profundicola (Verril, 1871)																						*				*				*
Limnodrilus udekemianus Claparede, 1862		*		*	*	*		*			*			*		*	*	*	*	*	*		*		*	*	*	*	*	*
Nais barbata Müller, 1773																									*					
Ophidonais serpentina (Müller, 1773)															*										*					
Potamothrix hammoniensis (Michaelsen, 1902)					*			*	*		*	*	*		*	*		*			*	*	*	*	*	*	*	*	*	
Psammoryctides albicola (Michaelsen, 1901)			*		*															*		*		*				*		*
Psammoryctides barbatus (Grube, 1861)																						*		*						
Stylaria lacustris (Linnaeus, 1767)													*		*							*			*					
Tubifex tubifex (Müller, 1774)	*	*	*	*	*	*	*	*	*		*		*	*	*	*	*	*	*	*	*	*			*	*		*		*
Uncinais uncinata (Ørsted, 1842)		*										*																		
Hirudinea																														
Erpobdella octoculata (Linnaeus, 1758)																										*				
Glossiphonia complanata (Linnaeus, 1758)			*																							*				
Glossiphonia heteroclita (Linnaeus, 1761)															*											*				
Helobdella stagnalis (Linnaeus, 1758)					*										*															
Turbellaria																														
Planaria torva (Müller, 1773)			*																											
Amphipoda																														
Gammaridae	*	*	*		*			*			*	*	*	*	*	*				*		*			*	*		*		
Corophium curvispinum Sars, 1895	*	*						*				*								*		*						*		
Isopoda																														
Jaera sarsi Valkanov, 1936	*		*		*		*					*										*								
Gastropoda																														
Bithynia tentaculata (Linnaeus, 1758)	*		*					*				*																		
Lithoglyphus naticoides C. Pfeiffer, 1828		*	*	*					*	*	*		*	*		*					*		*						*	
Theodoxus fluviatilis (Linnaeus, 1758)	*	*	*		*	*		*		*	*	*										*								
Viviparus acerosus (Bourguignat, 1862)	*																													
Viviparus viviparus (Linnaeus, 1758)	*			*																										
Bivalvia																														
Anodonta cygnea Linnaeus, 1758														*				*												
Corbicula fluminalis (Müller, 1774)				*								*								*										
Corbicula fluminea (Müller, 1774)								*		*		*						*				*		*						*
Dreissena polymorpha (Pallas, 1771)	*	*	*					*			*	*										*								
Sinanodonta woodiana (Lea, 1834)			*														*									*				
Sphaerium corneum (Linnaeus, 1758)	*																													
Sphaerium solidum (Normand, 1844)					*																									
Unio tumidus Retzius, 1788								*	*	*				*																

Appendix 1. continued.

Year	2007										2008										2009									
Sites	S. Banovci		Zemun		Visnjica		Vinca		Brestovik		S. Banovci		Zemun		Visnjica		Vinca		Brestovik		S. Banovci		Zemun		Visnjica		Vinca		Brestovik	
Month	*jun*	*oct*	*jun*	*oct*	*jun*	*oct*	*jun*	*oct*	*jun*	*oct*	*jun*	*sept*	*jun*	*sept*	*jun*	*sept*	*jun*	*sept*	*jun*	*sept*	*may*	*sept*	*may*	*sept*	*may*	*sept*	*may*	*sept*	*may*	*sept*
Samples	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
Odonata																														
Pyrrhosoma nymphula (Sulzer, 1776)														*																
Trichoptera																														
Hydropsyche angustipennis (Curtis, 1834)					*																									
Ephemeroptera																														
Baetis rhodani (Pictet, 1843)					*																									
Diptera																														
Ceratopogonidae												*																		
subfam. Chironominae																														
Einfeldia pagana (Meigen, 1838)							*																							
Dicrotendipes pulsus (Walker, 1856)			*																											
Dicrotendipes nervosus (Staeger, 1839)			*	*	*										*									*	*					
Dicrotendipes notatus (Meigen, 1818)			*																							*				
Cladotanytarsus mancus (Walker, 1856)													*																	
Polypedilum (Tripodura) scalaenum (Schrank, 1803)									*		*		*				*		*	*		*				*	*			
Polypedilum albicorne (Meigen, 1838)																					*									
Polypedilum (Uresipedilum) convictum (Walker, 1856)															*															
Polypedilum pedestre (Meigen, 1830)																	*													
Polypedilum nubeculosum (Meigen, 1804)								*		*								*												
Polypedilum sp.			*																											
Chironomus gr. plumosus (Linnaeus, 1758)							*				*		*		*		*	*	*						*					
Chironomus riparius Meigen, 1804					*		*														*					*	*	*		*
Demicryptochironomus vulneratus (Zetterstedt, 1838)							*		*																					
Cryptotendipes sp.											*											*								
Paratendipes albimanus (Meigen, 1818)												*																		
Parachironomus sp.					*										*							*								
subfam. Tanypodinae																														
Tanypus sp.																												*		
Procladius sp.							*		*								*		*									*		
subfam. Orthocladiinae																														
Cricotopus tremulus (Linnaeus, 1758)									*																					
Cricotopus (Isocladius) sylvestris (Fabricius, 1794)																									*					
Cricotopus bicinctus (Meigen, 1818)															*										*					
Orthocladius sp.															*															
subfam. Prodiamesinae																														
Prodiamesa olivacea (Meigen, 1818)											*																			