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THE RELATION BETWEEN CHIRONOMID (DIPTERA: CHIRONOMIDAE) ASSEMBLAGES AND ENVIRONMENTAL VARIABLES: THE KOLUBARA RIVER CASE STUDY

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Abstract: Chironomid larvae respond rapidly to environmental changes in aquatic ecosystems, with different species possessing different degrees of tolerance. Consequently, this group is considered an effective and reliable indicator of the ecological status. In this study, the relation between larval chironomid assemblages and environmental variables was examined at two sites on the Kolubara River. A nonparametric Mann-Whitney U test did not confirm significant seasonal differences between samples. Correspondence analysis indicated that the study sites are distinguished by their community composition. Based on forward selection analyses, 5 out of 28 analyzed water parameters and 3 out of 22 sediment parameters displayed the highest levels of correlation with chironomid assemblages. Forward selection analysis revealed that inorganic pollutants in the sediment (mercury, nickel and cadmium) exerted the greatest influence on the community. Results of canonical correspondence analysis indicated that the sediment characteristics have a more significant impact on chironomid communities than the analyzed water parameters. Our study confirmed that chironomids and sediment analyzes should be obligatorily included in the monitoring of ecological status, since chironomids are often a dominant component of benthic macroinvertebrate assemblages in freshwater ecosystems, with many species inhabiting the sediment with a proclivity for intake of toxic and persistent pollutants.

Key words: Chironomidae; Environmental parameters; Water; Sediment; Pollutants prioritization

INTRODUCTION

Chironomids (Insecta: Diptera, Chironomidae; non-biting midges) are an important component of freshwater ecosystems. They occupy a broad range of habitats and have a wide geographical distribution [1]. Chironomids are an important component of food webs, being the main prey for many invertebrates, fish and birds [2,3].

The abundance and taxonomic richness of chironomid larvae strongly correlate with environmental conditions and the physicochemical characteristics of the sediment and water [4]. The larval stages of non-biting midges show different degrees of tolerance [5-9] to changes in the environment, to which they can rapidly respond [10]. Therefore, they are suitable indicator organisms for the assessment of the ecological condition of aquatic habitats [11], and an important tool in ecological and monitoring studies [12,13]. For bioassessment programs, it is very important to estimate how environmental factors influence the chironomid community [11].

This study was undertaken to investigate which environmental variables of water and sediment have the most significant influence on chironomid assemblages, as a part of activities within the GLOBAQUA EU Seventh Framework Project [14]. The Kolubara River is one of the largest tributaries of the Sava River, and one of the largest rivers in the Belgrade area (Fig. 1). It is 86.5 km long, with a drainage area of 3,636 km^2 and an average altitude of 206 m a.s.l. [15]. According to national legislation [16], the Kolubara is classified as a type 2 watercourse, which refers to large rivers with predominantly medium-sized substrates in the riverbeds. The river along the investigated stretch is exposed to various pressures, including organic and nutrient pollution, from industry and due to hydromorphological degradation [17]. The protected zone of the Belgrade water supply begins 10 km downstream from the confluence of the Kolubara into the Sava River. This has imposed the necessity for effective measures for improving the ecological status of the Kolubara River.

MATERIALS AND METHODS

The study period was from 2007 to 2011. Samples were collected from 2 localities on the Kolubara River: near the village Ćelije (N44°22'36.17", E20°12'35.24") and the town of Obrenovac (N44°39'12", E20°13'27") (Fig. 1). These localities have been exposed to different types of anthropogenic pressure; the site near Ćelije is exposed to pollution from the rural area, while the site near Obrenovac to pollution from urban industrial and wastewaters.

Water sampling and analysis

Physical and chemical water parameters were measured monthly (March-October) at both sites. Water samples were taken in Friedinger bottles (volume = 3 L), at a depth of 0.5 m, using standard methods (APHA AWWA WEF 1995, SRPS ISO 5667/2008, SRPS ISO 7828/1997, SRPS ISO 5667-6: 1997, SRPSEN ISO 5667–3: 2007, SRPS EN ISO 5667–1: 2008).

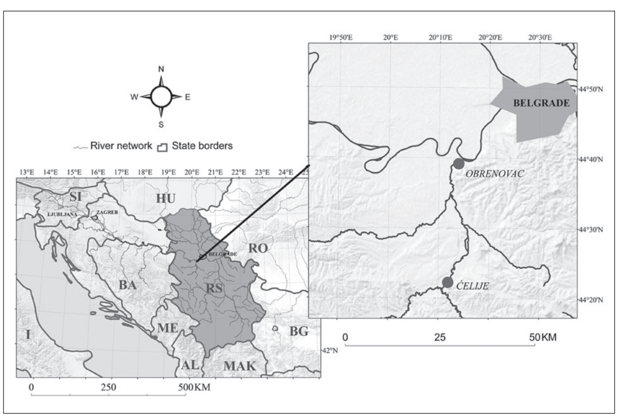

Fig. 1. Sampling sites on the Kolubara River.

The transparency of the water (m) was assessed using a Secchi disk. Temperature (°C), pH values, conductivity (μS cm^{-1}), dissolved oxygen (DO) (mg L^{-1} O_2) and oxygen saturation (O_2%) were measured with a Horiba W-23XD multiparametric probe (HORIBA Instruments Incorporation, USA) in the field. The biochemical oxygen demand for five days (BOD_5) (mg L^{-1} O_2) (SRPS ISO 5813: 1994, SRPS EN 1899-2: 2009), chemical oxygen demand (COD) (mg L^{-1} O_2) (SRPS ISO 6060: 1990), total organic carbon (TOC) (mg L^{-1} C) (SRPS ISO 8245:1994), total phosphates (mg L^{-1} P) (EPA 207. Rev 5, SRPS ENISO 6878: 2008), nitrites (NO_2) (mg L^{-1} N) (PRI P-V-32/A), nitrates (NO_3) (mg L^{-1} N) (EPA 300.1), suspended solids (mg L^{-1}) (SMEWW 19th method 2540 D), total nitrogen (N) (mg L^{-1}) (ISO 10048:1991, SRPS EN 12260:2008), alkalinity (Alk) (mg L^{-1} $CaCO_3$) (EPA method 310.1), total hardness (Tot H) (mg L^{-1} $CaCO_3$) (EPA 130.2), residues obtained after drying at 105°C (mg L^{-1}) (SMEWW 19th method 2540 B), ammonium ion (NH_4^+) (mg L^{-1} N) (PRI P-V-2A), chloride (mg L^{-1} Cl^-) (SRPS ISO 9297: 1994), sulphate (SO_4^{2-}) (mg L^{-1}) (EPA 300.1) and the following metals (mg L^{-1}): nickel (Ni), lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), arsenic (As), iron (Fe) (EPA 207. Rev 5, EPA 200.8), and mercury (Hg) (EPA 245.1) were measured. Detergents (mg L^{-1}) (SMEWW 16th method 512 B), phenols (mg L^{-1}) (SRPS ISO 6439:1997) and mineral oils (total hydrocarbons C_{10}-C_{40}) (mg L^{-1}) (SRPS ENISO 9377-2:2009) were measured at the Institute of Public Health, Belgrade.

Sediment sampling and analysis

Surface sediment samples were taken during the low water level regime in September/October at both sites. Sampling was performed according to the following methods: SRPS ISO 5667-2:1997, ISO 5667-15:1999, SRPS ISO 5667-12:2005, SRPS EN ISO 5667-3:2007, SRPS EN ISO 5667-1:2008. Sediment samples were prepared for analyzis using the wet fragmentation method by separation of fractions smaller than 63 μm. Sediment samples were analyzed at the Institute of Public Health in Belgrade. The following parameters were analyzed: moisture content (%) (SRPS ISO 11465:2002), pH value (SRPS ISO 10390:2007), metal content (mg kg^{-1}): As, Cu, Zn, Cr, Cd, Ni, Pb (EPA 3050

B (method A) 1996, ISO 11466: 2004, EPA 200.72001) and Hg (EPA 245.1); pesticides (μg kg^{-1}) DDT + DDE + DDD, lindane, aldrin, endrin, dieldrin, HCH (α, β, δ), heptachlor, heptachlorepoxid, alachlor, hexachlorobenzene, atrazine, simazine, propazine, trifluralin, dihlorbenil (ISO 10382 2002); polycyclic aromatic hydrocarbons (PAHs) (μg kg^{-1}) anthracene, benzo(a) anthracene, benzo(k)fluoranthene, benzo(a)pyrene, chrysene, phenanthrene, fluoranthene, indeno(1,2,3-c, d)pyrene, naphthalene, benzo(g, h, i)perylene (ISO 18287: 2006). The total PAH refers to the sum of the above-mentioned 10 polycyclic aromatic hydrocarbons. Acenaphthylene, acenaphthene, fluorene, pyrene, benzo(b)fluoranthene, dibenzo(a, h)anthracene were measured using standard methods (ISO 18287: 2006); polychlorinated bisphenyls (PCB) (mg kg^{-1}): PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180, and total PCB refers to the sum of the above-mentioned PCBs (ISO 10382: 2002); mineral oils were also measured (total hydrocarbons C10-C40) (mg kg^{-1}).

Sampling, identification and chironomid assemblages analysis

Samples of benthic fauna were collected during high (May/June) and low water level regimes (September/October) from 2007-2011, using a Van-Veen type of grab with a sample area of 270 cm^2. The organisms were separated on site from the sediment with a 200 μm-mesh-size sieve and preserved in 4% formaldehyde.

Preparation of chironomid larvae for identification was performed according to the method described by Epler [18] and Orendt and Spies [19]. Identification was done to the lowest possible taxonomic level (preferably species) using the following identification keys: Hirvenoja [20], Lellak [21], Wiederholm [22], Maschwitz and Cook [23], Epler [18], Moller Pillot [24], Orendt and Spies [19].

Data analysis

The Shannon-Wiener diversity [25], density (ind. m^{-2}), and species richness indices were calculated to evaluate the structure of the chironomid assemblages. Classification based on the functional feeding groups and habitat type, was performed according to Moog [26], Schmedtje and Colling [27] and Mandaville [28].

Seasonal differences between chironomid community structures during regimes of low and high water levels were analyzed by the nonparametric Mann-Whitney U test ($p<0.05$), using the PAST statistical program, version 2.14 [29].

Correspondence analysis (CA) was used to compare the main faunistic features, while canonical correspondence analysis (CCA) was employed to evaluate the correlation between chironomid taxa and environmental variables of water and sediment at the researched localities [30-33], using the statistical program PAST, version 2.14 [29].

Forward selection analysis, based on Pearson's correlation test ($p<0.05$), and the Monte Carlo permutation test (999 permutations, $p<0.05$) was performed in order to identify the most influential environmental variables in the water and sediment, using FLORA software, version 2013 [32].

In the case of water parameters, the relation between chironomid assemblages and environmental variables was assessed using two data sets of physical and chemical parameters. The first data set included the values of the water variables that were measured at the time of fauna sampling from the riverbed. The second data set included three-month average values of water variables, two months before and on the month of sampling benthic fauna. The idea was to test the strength of the correlation in both cases.

RESULTS

Results of environmental variable analyses: water and sediment

The results of physicochemical analysis of the water and sediment at 2 sites (upstream – Ćelije and downstream – Obrenovac) on the Kolubara River during the five-year research period are shown in Tables 1. (for water) and 2. (for sediment).

Table 1. Minimal, maximal and average values of the physicochemical parameters of the water that were above the detection limit for the used method, measured during the 5-year period (2007-2011) at the study sites on the Kolubara River.

Localities	**Ćelije**			**Obrenovac**		
Parameters	Min	Max	Average ± SD	Min	Max	Average ± SD
Transparency of the water (m)	-	-	-	0.15	1.20	0.46 ± 0.24
Water temperature (°C)	3.70	27.70	15.99 ± 6.04	4.40	27.20	17.01 ± 6.23
pH value	7.60	8.40	8.12 ± 0.15	7.90	8.50	8.12 ± 0.12
Electrical conductivity (µS cm^{-1})	320	580	441.39 ± 68	250	600	471.71 ± 81.62
Alkalinity (mg L^{-1} $CaCO_3$)	153.50	289.50	230.56 ± 31.53	97	281	224.95 ± 38.63
Total hardness (mg L^{-1} $CaCO_3$)	176.80	298.90	241.85 ± 31.76	105.40	407.20	253.39 ± 49.71
Suspended solids (mg L^{-1})	2	428	73.44 ± 211.3	4	734	55.91 ± 132
Residue dried at 105°C (mg L^{-1})	224	390	300.79 ± 39.96	186	427	329.83 ± 63.28
Dissolved oxygen (mg L^{-1} O_2)	5.70	11.60	8.78 ± 1.41	6	15.50	8.41 ± 1.94
Oxygen saturation (%)	62	102	68.78 ± 9.16	67	185	86.88 ± 22.38
BOD_5 (mg L^{-1} O_2)	0.30	9.70	2.88 ± 1.58	0.20	12.21	2.85 ± 2.79
COD (mg L^{-1} O_2)	1.60	34.90	10.06 ± 8.09	2.1	42.2	13.73 ± 10.73
NH_4^+ (mg L^{-1} N)	0.05	0.85	0.30 ± 0.2	0.06	1.06	0.36 ± 0.24
Nitrite (mg L^{-1} N)	0.008	0.193	0.06 ± 0.04	0.014	0.23	0.08 ± 0.05
Nitrate (mg L^{-1} N)	0.70	6.10	1.92 ± 0.9	0.70	3	1.70 ± 0.47
Total nitrogen (mg L^{-1} N)	1.60	4.60	2.41 ± 0.7	1	3.68	2.33 ± 0.69
Total phosphate (mg L^{-1} P)	0.04	0.32	0.13 ± 0.08	0.04	0.30	0.12 ± 0.06
Total organic carbon (mg L^{-1})	1.88	36.21	4.22 ± 5.8	2.48	8.29	4.35 ± 1.26
Chloride (mg L^{-1} Cl)	5.60	23.70	11.18 ± 4.5	6.30	24.40	13.98 ± 5.26
Sulphate (mg L^{-1} SO_4)	16.70	36.90	21.79 ± 5.99	17.40	68.10	36.88 ± 17.33
Zn (mg L^{-1})	0.0008	0.0378	0.01 ± 0.009	0.001	0.21	0.03 ± 0.04
Cu (mg L^{-1})	0.002	0.02	0.004 ± 0.004	0.001	0.05	0.006 ± 0.01
Ni (mg L^{-1})	0.004	0.017	0.009 ± 0.008	0.004	0.018	0.01 ± 0.009
As (mg L^{-1})	0.001	0.007	0.002 ± 0.001	0.001	0.04	0.01 ± 0.01
Cd (mg L^{-1})	0.0006	0.002	0.0006 ± 0.0004	0.0006	0.002	0.0006 ± 0.0004
Fe (mg L^{-1})	0.008	2.58	0.25 ± 0.51	0.002	2.34	0.44 ± 0.66
Detergents (mg L^{-1})	<0.02	<0.02	-	0.02	0.13	0.02 ± 0.03
Phenols (mg L^{-1}l)	0.001	0.003	0.001 ± 0.0008	0.001	0.002	0.001 ± 0.0003

Table 2. Minimal, maximal and average values of parameters for the surface layer of the sediment that were above the detection limit for the used method, measured during the 5 -year period (2007-2011) at the Ćelije and Obrenovac sites on the Kolubara River.

Localities	**Ćelije**			**Obrenovac**		
Parameters	min	max	average ± SD	min	max	average ± SD
Pb (mg kg^{-1})	24.10	41.20	31.97 ± 7.34	27.60	38.50	33.64 ± 3.94
Cd (mg kg^{-1})	0.20	0.70	0.35 ± 0.24	0.30	1	0.56 ± 0.29
Zn (mg kg^{-1})	43.20	86.90	63.82 ± 19.50	60.20	78.50	72.80 ± 7.56
Ni (mg kg^{-1})	45.90	179.40	117.57 ± 54.81	117.10	145.90	130.60 ± 12.48
Cr (mg kg^{-1})	22.20	779.90	242.57 ± 359.41	43.50	113.50	88.40 ± 26.99
Hg (mg kg^{-1})	0.10	0.20	0.12 ± 0.05	0.10	0.60	0.24 ± 0.21
As (mg kg^{-1})	8.80	10.90	10.05 ± 0.90	7.50	14	11.04 ± 2.59
Cu (mg kg^{-1})	21.90	34.60	25.50 ± 6.10	22.70	34.20	28 ± 4.87
Total PAH (µg kg^{-1})	85	251	133 ± 106.72	20	529	184.90 ± 227.72
Naphthalene (µg kg^{-1})	10	59	30.50 ± 21.42	10	28	12.60 ± 11.21
Fluoranthene (µg kg^{-1})	16	24	16.50 ± 8.35	29	72	23.70 ± 28.93
Phenanthrene (µg kg^{-1})	20	31	21±11.63	15	96	29.70 ± 38.61

Table 2 continued:

Benzo(a)anthracene (μg kg^{-1})	5	28	11.25 ± 11.21	5	34	11.80 ± 12.60
Chrysene (μg kg^{-1})	13	22	11.25 ± 8.10	26	58	18.80 ± 24.03
Benzo(a)pyrene (μg kg^{-1})	12	15	9.25 ± 5.06	26	140	35.20 ± 59.41
Indeno(1,2,3-cd)pyrene (μg kg^{-1})	5	10	6.25 ± 2.50	5	20	8 ± 6.71
Acenaphthene (μg kg^{-1})	5	22	9.25 ± 8.50	5	5	5
Fluorene (μg kg^{-1})	<10	<10	-	14	24	9.60 ± 9.34
Pyrene (μg kg^{-1})	15	28	16.75 ± 9.53	24	80	28.30 ± 31.24
Benzo(b)fluoranthene (μg kg^{-1})	17	35	15.50 ± 14.18	26	42	15.60 ± 17.75
Total hydrocarbons (C_{10} -C_{40}) (mg kg^{-1})	24.80	158	82.33 ± 55.40	52.82	189	86.184 ± 58.20

Table 3. Autecological characteristics of chironomid taxa recorded in the Kolubra River at the Ćelije and Obrenovac localities during 2007-2011. Abreviations: pel – pelal, ar – argyllal, ps – psamal, lit – lithal, ph – phytal; c-g – gatherers, c-f – filtrators, shr – shreders, scr – scrapers, pre – predators; S – saprobic valence (26]; T – taxon tolerance (28]; Hb – hemoglobin (24, 58, 59]; FT – feeding type according to Moog (26]; Schmedtje and Colling (27]; Mandaville (28]; HT – habitat type by Moog (26]; Schmedtje and Colling (27]; MT – type of microhabitat according to Schmedtje and Colling (27]; / – no data; Abbr – Abbreviation.

Taxon name	**Abbr**	**S**	**T**	**Hb**	**FT**	**HT**	**MT**
Einfeldia pagana (Meigen, 1838)	Ein_pag	2.2	9	+	c-g	pot, rit	pel
Dicrotendipes nervosus (Staeger, 1839)	Dic_ner	2.7	8	+	c-g	pot, rit	pel, ps, lit
Cladotanytarsus mancus (Walker, 1856)	Cla_man	2.1	5	+	c-f	pot	pel
Harnischia sp.	Har_sp.	/	8	/	c-g, pre	/	pel, ps, lit
Polypedilum scalaenum (Schrank, 1803)	Pol_sca	2.3	6	+	shr	pot, rit	pel
Polypedilum albicorne (Meigen, 1838)	Pol_alb	0.8	6	+	shr	cre, rit	pel
Polypedilum convictum (Walker, 1856)	Pol_con	1.9	6	+	shr	rit, pot	pel
Polypedilum pedestre (Meigen, 1830)	Pol_ped	2.7	6	+	shr	pot, rit	pel
Polypedilum nubeculosum (Meigen, 1804)	Pol_nub	2.3	6	+	shr	pot, rit	pel, ps
Chironomus gr. *plumosus* (Linnaeus, 1758)	Ch_plu	3.6	10	+	c-g	Pot	pel
Chironomus riparius Meigen, 1804	Ch_rip	3.5	10	+	c-g	pot, rit	pel
Demicryptochironomus vulneratus (Zetterstedt, 1838)	Dem_vul	2.3	8	-	c-g	pot	pel
Cryptochironomus sp.	Cri_sp.	/	8	+	pre	pot	pel, ar, ps
Cryptotendipes sp.	Cry_sp.	/	6	/	c-g	/	pel, ar, ps
Glyptotendipes sp.	Gly_sp.	/	10	+	shr	/	ph, pel, ps
Virgatanytarsus arduennensis (Goetghebuer, 1922)	Vir_ard	2.0	/	/	c-g	pot, rit	/
Cladopelma lateralis (Goetghebuer, 1934)	Cla_lat	/	9	+	c-g	/	/
Procladius sp.	Pro_sp.	/	9	-	pre	/	pel, ps
Cricotopus triannulatus Macquart, 1826	Cri_tri	2.2	7	-	shr	pot, rit	lit
Cricotopus sylvestris (Fabricius, 1794)	Cri_syl	2.6	7	-	scr	Pot	pel, ph
Cricotopus bicinctus (Meigen, 1818)	Cri_bic	2.5	6	-	scr	pot, rit	/
Orthocladius sp.	Ort_sp.	/	6	-	c-g	/	/
Eukiefferiella claripennis (Lundbeck, 1898)	Euk_cla	2.3	8	-	c-g	rit, pot, cre	/
Prodiamesa olivacea (Meigen, 1818)	Pro_oli	/	8	/	c-g	rit, pot	ps, pel

In comparison to the values provided by the national Ecological Quality Standards – EQS [16,34], higher values of TOC, BOD_5 and nitrogen compounds were occasionally recorded. According to these parameters, ecological status of the Kolubara River can be classified as class IV for type 2 watercourses, whereas according to the concentration of suspended solids and based on the national legislation [16,34], it can be classified as class V.

The contents of Cr, Hg and Ni in the sediment exceeded the value of the given limit according to national legislation [34] and international standards [35, 36]. The content of Cr was double than the remediation value. The value of Ni exceeded the maximum allowable values, while Hg content exceeded the target value. Based on the results of the sediment parameters, the Kolubara River in the Belgrade area can be classified as a class IV watercourse [34].

Community analysis

At the Kolubara River study sites, 24 taxa of the family Chironomidae were recorded and their autecological characteristics are shown in Table 3.

The minimal density of the chironomid community was recorded at Obrenovac (5,594 ind. m^{-2}), and the maximal density (8,103 ind. m^{-2}) was observed at the Ćelije study site. Among chironomids, Chironominae was the dominant (61.70%) and the most diverse subfamily, with 17 taxa belonging to 12 genera. Tanypodinae (19.70%) and Orthocladiinae (17.32%) were subdominant subfamilies. Former was presented only by one, while the latter by 5 taxa within 3 genera. The chironomid community structure at the investigated sites is presented in Fig. 2.

Species richness and diversity (H') were higher at the Ćelije sampling site (20 taxa; 1.11 ± 0.74) compared to the Obrenovac site (12 taxa; 0.71 ± 0.54). At Ćelije the distribution of chironomid species was more homogenous, without any taxa predominating.

Results of the nonparametric Mann-Whitney U test ($p = 0.36$) showed that there were no statistically significant differences between the samples collected during regimes of low and high water levels at either site.

Based on community structure, CA showed marked separation of the study sites Ćelije and Obrenovac (Fig. 3). To perform the CCA, 5 out of 28 physicochemical water parameters were selected by forward selection (FS) analysis [32], both for 3-month average values, and for values recorded in the month community sampling. Different factors were selected from these two data sets. Factors with greater correlation strengths were obtained from the 3-month average value data set.

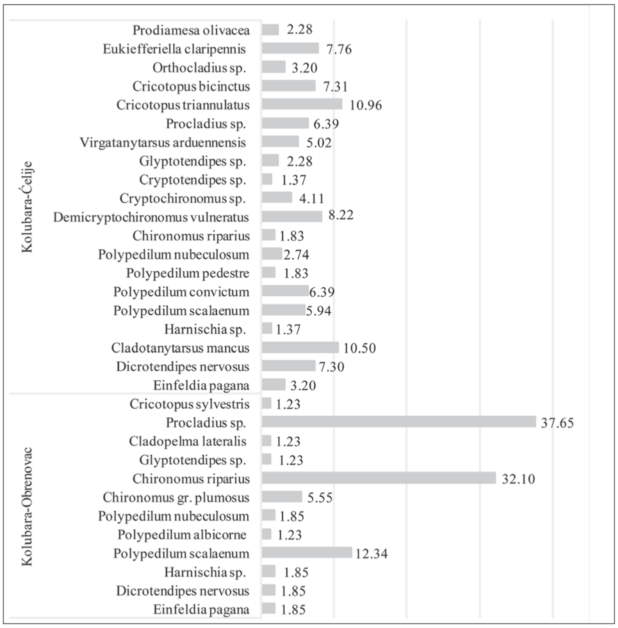

Fig. 2. Average percentage of chironomid taxa participation (%) in the community in the Kolubara River during 2007-2011.

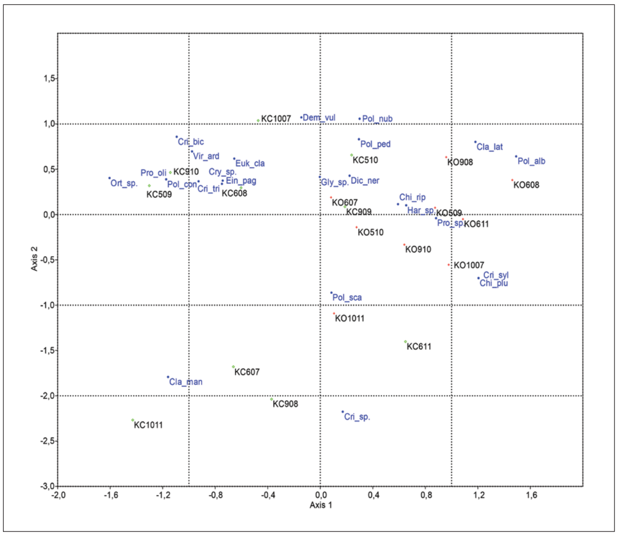

Fig. 3. Correspondence analysis of sampled chironomid communities (Ćelije – green; Obrenovac – red). The biplot is based on a matrix of 24 taxa and 20 samples displaying 27.73% of total variance (first axis 14.23%, second 13.50%). Abreviations for the names of taxa are given in Table 3. The samples were coded as follows: KO (Obrenovac) / KC (Ćelije) – month – year (e.g. KO0910 – Kolubara Obrenovac October 2010).

Table 4. The results of FS analysis. Selected environmental factors with the greatest influence on chironomid communities ($p < 0.05$). A – Water environmental factors for 3-month average values; B – water environmental factors for values recorded at the month of community sampling; C – sediment environmental factors.

Selected environmental parameters											
A				B				C			
Factor	**Eigen value**	**F statistics**	**p**	**Factor**	**Eigen value**	**F statistics**	**P**	**Factor**	**Eigen value**	**F statistics**	**p**
Fe	0.4768	1.7209	0.0000	NH_4^+	0.5969	2.2072	0.0000	Hg	0.3636	1.3637	0.0060
COD	0.4605	1.6566	0.0000	Total P	0.4203	1.4998	0.0000	Ni	0.4991	1.9842	0.0180
NH_4^+	0.4451	1.5962	0.0000	Tot H	0.3962	1.4071	0.0000	Cd	0.6178	2.5918	0.0300
BOD_5	0.4361	1.5612	0.0000	Tem	0.3936	1.3971	0.0000				
DO	0.4257	1.5208	0.0000	Alk	0.2500	0.8631	0.0380				

FS analysis was also carried out for the sediment variables; 3 out of 22 variables were selected as the most influential. Selected parameters are shown in Table 4. The first CCA was based on water environmental factors that were selected from the 3-month average values of variables. According to the first axis, the distribution of chironomids at both sites was the most influenced by the NH_4^+ concetration. NH_4^+ showed positive correlation with COD, BOD_5 and Fe (Fig. 4a). All of these variables were negatively correlated with DO. It is important to point out that COD, BOD_5 and NH_4^+, which are indicators of organic pollution, correlated highly with samples from the Obrenovac locality. NH_4^+ was positively correlated with following taxa: *Procladius* sp., *Polypedilum scalaenum*, *P. albicorne*, *P. nubeculosum* and *Cladopelma lateralis*. Positive correlations with BOD_5, COD and Fe were found for *Chironomus riparius*, *Ch. plumosus*, *Cricotopus sylvestris* and *Harnischia* sp. at the locality Obrenovac. The taxa *C. bicinctus*, *Glyptotendipes* sp., *Virgatanytarsus arduennensis*, *Prodiamesa olivacea* and *P. convictum*, recorded at Ćelije, displayed a negative correlation with these parameters and a positive correlation with DO.

The second CCA was based on the environmental factors of the water that were selected from the values recorded in the month of community sampling. According to the first CCA axis, the concentration of NH_4^+ had the greatest impact on the structure of the chironomid community (Fig. 4b). The concentration of NH_4^+ was positively correlated with *Ch. riparius*, *Ch.* gr. *plumosus*, *C. sylvestris*, *C. lateralis* and *Procladius* sp. from the Obrenovac site. Positive correlation with

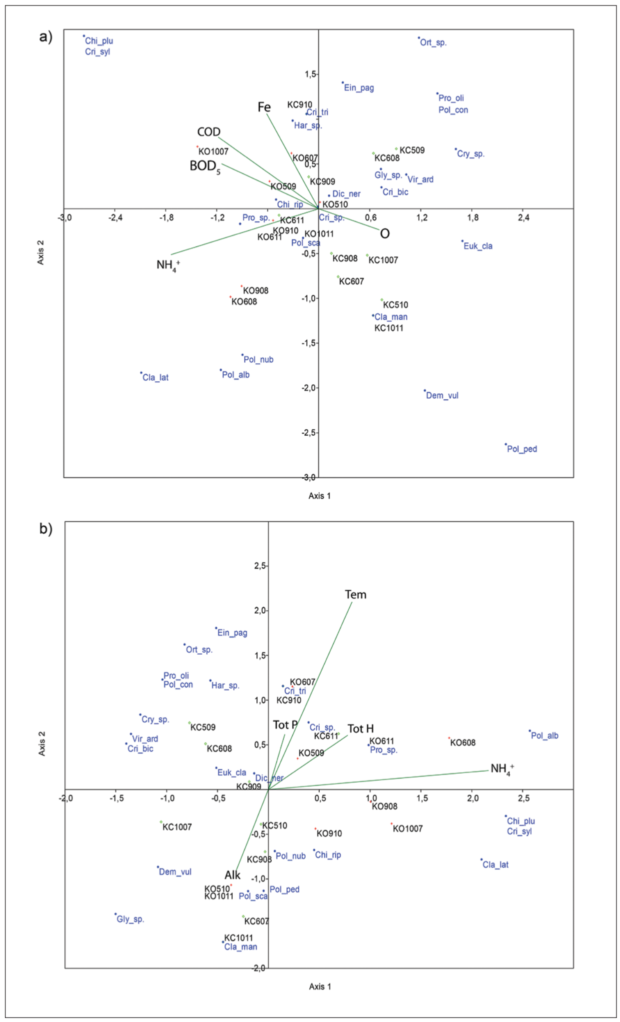

Fig. 4. CCA ordination triplot (matrix: 24 taxa × 20 samples × 5 environmental factors); a) selection of environmental factors was based on 3-month average values displaying 63.71% of total variance; b) selection of environmental factors was based on values recorded in the month of community sampling and which displayed 57.66% of total variance.

alkalinity was found for *P. scalaenum*, *P. pedestre*, *P. nubeculosum*, *D. vulneratus* and *Glyptotendipes* sp. Our results also revealed a positive correlation between temperature and *Cricotopus* sp. and *C. triannulatus*.

For the selected environmental factors of the sediment CCA was also performed (Fig. 5). The identified taxa indicate that Cd (according to the first CCA axis) and Hg (according to the second CCA axis) were variables with the highest influence. According to the first CCA axis, Ni also has a significant influence. At the Obrenovac site, *Ch. riparius* and *Procladius* sp. positively correlated with the content of Hg, and *Ch. plumosus*; *C. sylvestris* positively correlated with the content of Cd in the sediment. At the Ćelije site, chironomid species *P. nubeculosum, P. albimanus, C. lateralis* and *D. nervosus* positively correlated with the content of Ni in the sediment.

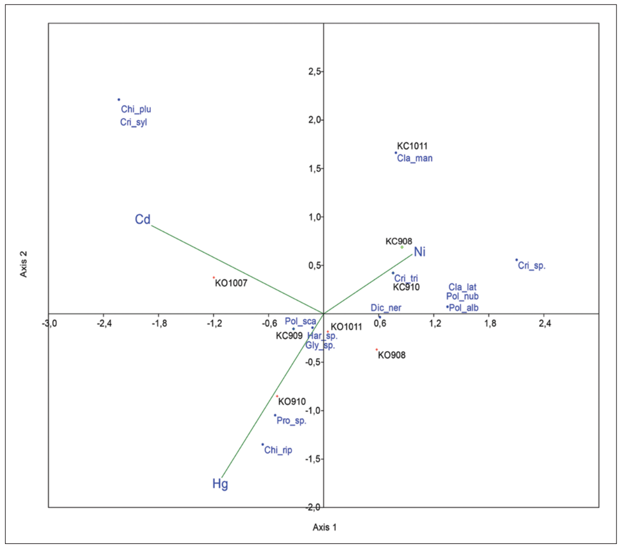

Fig. 5. CCA ordination triplot (matrix: 24 taxa × 10 samples × 3 environmental factors), displaying 98.80% of total variance. Analysis of the chironomid communities in Kolubara (Obrenovac – red, Ćelije – green) and of selected physicochemical parameters of the sediment.

DISCUSSION

Many studies have examined the relations between physical and chemical parameters of water and sediment and the composition and abundance of chironomid communities [8,37-39]. Our results are in agreement with Janse et al. [40] who reported that increased anthropogenic pressure reduces species diversity. Higher number of chironomid taxa and higher diversity were recorded at the study site Ćelije than at the downstream site Obrenovac. This observation indicates that the latter site is exposed to greater anthropogenic influence. The higher number of polysaprobic taxa downstream (13.16% of total chironomid taxa), compared to upstream site Ćelije (2.86%) confirmed this statement.

A number of authors have highlighted the importance of temporal dimension and its effect on the seasonal pattern of chironomid distribution [41-44]. These authors mainly studied communities in mountainous rivers and/or habitats exposed to lower anthropogenic pressure. Based on the results of the Mann-Whitney U test, the seasonal dynamic of non-biting midge assemblages in investigated river type was not pronounced. In addition, there were no significant differences in community structure between two sampling seasons. This could be explained by the long larval period of common taxa or by appearance of new generation [45].

The characteristics of the substrate and the presence of pollutants in the water affect chironomid distribution. Literature data show that the characteristics of riverbed substrate, which are related to its physicochemical profile, are the principal factor influencing chironomid distribution [42,46,47]. According to the results of FS and CCA, the contents of Ni, Cd and Hg in the sediment displayed the highest correlation with the structure of chironomid assemblages. The samples from Obrenovac, where increased values of inorganic pollutants were observed, stood in significant correlation with the elevated contents of Cd and Hg.

As pelophilous species were dominant, the impact of sediment quality on chironomid communities was expected [48]. Pelophilous species mix the upper part of the sediment with water by bioturbation, thereby increasing the availability not only of nutrients, but also of other substances (including toxic matters) to other members of the food web. Contamination of the sediment with inorganic and persistent organic pollutants threatens the growth and survival of aquatic invertebrate communities [49].

Chironomids that live in the sediment and feed on detritus exhibit an adaptability to various pollutants in the sediment and can accumulate heavy and toxic metals [50,51]. The intensity of accumulation is proportional to the content of metals in the surroundings [52]. Michailova et al. [53] suggested that the chironomid genome is very sensitive to the presence of trace metals, but that heavy metals do not affect the diversity of chironomid species. Thus, it can be concluded that changes caused by metal pollution are not measurable by standard procedures at the community level. Most chironomids have hemoglobin in their hemolymph, which allows them to live in the suboxic sediment [54,55]. Studies have shown that the presence of Cd in the sediment can reduce the size of hemoglobin proteins in midges, and even its complete denaturation. This aspect should be examined further with the aim of developing a rapid bioassessment method for the detection of Cd in the environment [55,56]. Our results show that 54% of all 24 recorded taxa have hemoglobin in their hemolymph; 29% do not have hemoglobin (for 17% there are no data). At the Obrenovac locality, 9 of the 12 recorded species have hemoglobin. Based on these results we propose the use of chironomids for preliminary detection of Cd contamination of the sediment.

FS selected environmental variables (Fe, NH_4^+, BOD_5, COD, and DO) based on three month average data set showed better correlation with chironomid communities in comparison to variables (Alk, Tot H, Temp, NH_4^+ and Tot P) selected based on the values obtained at the time of community sampling). This indicates that in order to identify the influence of pollutants, a more reliable approach is to compare the mean values of longer time series of environmental parameters. In both cases (mean values and single measurements of environmental determinants), NH_4^+ was found to be the principal factor determining the structures of chironomid assemblages in the investigated river type. This variable showed significant correlation with the species *Cricotopus sylvestris*, *Polypedilum nubeculosum*, *Cladopelma lateralis*, *Chironomus plumosus* and *Procladius* sp, which were dominant in the investigated communities (except *C. lateralis*). BOD_5, COD, DO were also found to be defining factors of chironomid assemblages. These variables reflect the level of organic pollution, which together with nutrient input exert the greatest pressure on Serbian Waters [57](SRBMP, 2013). Milošević et al. [11] identified the same set of parameters as the most important determinant of chironomid distribution. These authors also described the significant influence of PO_4, NO_3 and electrical conductivity, albeit for a large river with a hard substrate, and for small mountain streams. Based on these findings, it can be concluded that NH_4^+, BOD_5, COD and DO have the most influence on the distribution of non-biting midges.

Multivariate analysis revealed that chironomid communities possess a high degree of correlation with habitat conditions. The results of our study indicate that the physicochemical characteristics of the sediment have a greater impact on chironomid communities than water parameters. Therefore, more effort should be devoted to sediment analyses in routine water-quality monitoring practice, especially to determine long-term pollution in an ecosystem. Since numerous chironomid species inhabit the sediment and absorb and retain toxic and persistent pollutants, they should be included in biomonitoring studies and in assessments of the ecological status of water ecosystems.

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