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MEIOFAUNA AND FREE-LIVING AQUATIC NEMATODES FROM SOME STATIONS ALONG THE DRAVA RIVER (CROATIA)

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Sediment meiofauna and aquatic free-living nematodes were investigated at five littoral stations along the Drava River (11.0 – 77.0 stream km). Meiofauna abundance and diversity differ between investigation stations. In the sediment of station 5 (sandy sediment) only a few meiofaunal groups were found, with a high dominance of oligochaeta. The nematofauna of station 4 (sand-silt) differs from that recorded in the other stations investigated. In this station the lowest number of species was recorded, as well as the lowest diversity index (H', d), and a high dominance of species (D%) belonging to the genus *Rhabditis.* Cluster analysis based on nematode relative abundance resulted in only station 4 being distinct. Biological data indicate the influence of the discharge of untreated effluent into the Drava River.

Key words: river, sediment meiofauna, freshwater nematodes.

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Meiofauna i slobodnoživući slatkovodni oblići istraživani su u sedimentu pet litoralnih postaja duž rijeke Drave (11,0 – 77,0 riječnog km). Gustoća i sastav meiofaune postaje 5 (pjeskoviti sediment) razlikuju se od gustoće i sastava meiofaune postaja 1, 2, 3 i 4. Nematofauna postaje 4 (pjeskoviti silt) razlikuje se u odnosu na ostale istraživane postaje. Za tu je postaju utvrđen najmanji broj vrsta, najniže vrijednosti indeksa diverziteta (H', d), te visoka dominantnost (D%) vrsta koje pripadaju rodu *Rhabditis.* Klaster analiza učinjena na osnovi relativne zastupljenosti vrsta slobodnoživućih oblića ukazuje na razlike u odnosu na druge postaje, a što se može dovesti u vezu s ispuštanjem netretiranih otpadnih voda i blizine glavnog kanalizacijskog izljeva.

Ključne riječi: rijeka, meiofauna sedimenata, slobodnoživući slatkovodni oblići.

INTRODUCTION

Sediment meiofauna and freshwater nematodes of running and standing waters in Croatia remain unknown, especially from the ecological aspect. The primary aim of this investigation was to determine the sediment meiofauna and freshwater nematodes present at five stations along the Drava River in relation to different sediment types and different kinds of pollution.

According to HEIP *et al*. (1988) »meiofauna are in the 100 to 10000 m size range, and have been methodologically defined as passing through a 0.5 or 1 mm mesh sieve, but retained on a 63 m mesh (the silt/sand boundary sieve)«. Meiofauna was enumerated to the major taxon level: Acari, Chironomidae larvae (than 3 mm), Cladocera, Copepoda, Nematoda, Oligochaeta (than 3 mm), Ostracoda, Rotatoria, Tardigrada and Turbellaria (WASILEWSKA, 1973). Local abiotic conditions such as flows or velocity gradients, substrate structures, quality and/or quantity of organic matter and physical-chemical characteristics are important in the structuring of meiofauna and nematofauna communities, because they determine whether organisms can colonise and survive in stream habitats (PLENET & GIBERT, 1994). Some types of contamination may reduce abundance, biomass, and diversity, particularly among certain taxonomic groups e.g. insects and molluscs (ZULLINI, 1976). Meiofauna communities were also determined according to interactions, including predation and competition, with other organisms. Due to sediment quality, meiofauna and nematode abundance varied from a few individuals to 10^6 ind./m^2 of investigated area (PREJS & BERNARD, 1985; BRETSCHKO, 1992; NICHOLAS *et al*, 1992; BLOME & FAUBEL, 1996), and from unpolluted to polluted waters (WETZEL, 1975; ZULLINI, 1976; OCAÑA & PICAZO, 1991; PLENET & GIBERT, 1994). Most surveys recorded nematodes as the major meiofauna taxon, with oligochaetes second in dominance. Freshwater nematodes are widely distributed in the rivers, lakes and reservoirs, from eulittoral to sublittoral. NICHOLAS *et al*. (1992) investigated nematode fauna from coarse littoral sand in Mundoo. BLOME & FAUBEL (1996) studied eulittoral nematodes from the Elbe estuary, which includes limnetic, oligohaline-mesohaline, mesohaline and polyhaline regions. According to ZULLINI (1976) free-living nematodes can be used as indicators of river pollution because they are present even under the most extreme conditions of pollution. OCAÑA & PICAZO (1991) studied nematode species present in the Monachil River and their relation to organic pollution. Nematodes were grouped on a non-taxonomic functional basis according to the type of buccal cavity into five feeding types (NICHOLAS *et al*, 1992, after JENSEN).

MATERIALS AND METHODS

Meiofauna and free living aquatic nematode fauna were investigated during March and April of 1995 at five littoral stations along the main stream of the Drava River (11.0 – 77.0 stream km). The Drava River is a right-bank, 794 km long, tributary of the Danube River. In Croatia the Drava River is about 300 km long, from 140 to 370 m wide, and with a greatest depth of from 4 to 7 m. The investigated

Fig. 1. Investigated stations along the Drava River (11,0–77,0 stream km)

stations were (Fig. 1): Donji Miholjac – 1 (40 km away from Osijek, silty – sand sediment with great amount of fine organic detritus), Pampas – 2 (situated in Osijek area near the Hippodrome, sandy – silt sediment with great amount of coarser organic detritus), Zimska luka – 3 (in the middle part of Osijek, the sediment is gravel with silty-sand, rich in organic detritus, almost stagnant water), Zeleno polje – 4 (around the main sewage sludge of the city and near the sugar factory, sandy – silt sediment), Nemetin – 5 (situated 5 km from the city of Osijek to the east, sandy sediment).

The samples were taken from each station at the depth of about 10-cm below the water surface. Sediment samples were removed from 100 cm^2 surface, then transferred to plastic jars and preserved by 4% formalin. In the laboratory, meiofauna were removed from sediment by elutriation methods through a 75 m sieve (UHLIG *et al*, 1973). All material from the sieve was transferred to plastic jars and 4% formalin with Rose Bengal added. Meiofauna was counted using a binocular microscope. Permanent slides of nematodes were done according to the SEINHORST method (1959).

To assess the differences in the abundance of meiofauna between the investigated stations, t – test was used (PARKER, 1975). The abundance was expressed as number of individuals per 100 cm^{-2}. Data were analysed with both univariate and multivariate statistics. The univariate community measures number of species (S), species richness (SR), Shannon-Weaver diversity (H'), evenness (Pielou's J), taxonomic richness (Margalef's d) and dominance (D), while inverse values of evenness (J) were used to assess the differences between nematofauna from different stations (WARWICK, 1988). Multivariate statistical techniques were used to »indicate the de-

gree of similarity or dissimilarity in species composition between investigated stations. If station groupings can be related to measured pollutant loading or some indirect measure of pollution intensity, then this can provide strong correlative evidence of cause and effects« (WARWICK, 1988).

RESULTS

The investigated physical and chemical water parameters (from ČAČIĆ & HORVATIĆ, 1996) differed between the investigated stations. The highest NH_4, KMnO4, NO_2, PO_4, total N and total P were recorded for station 4, as well as the lowest values for dissolved oxygen (Table 1). Station 4 is situated just upstream of the main sewage sludge and close to the sugar factory which discharges untreated effluent into the Drava River.

In the sediment of the five investigated stations along the Drava River, nine meiofaunal groups were recorded: Nematoda, Rotatoria, Copepoda, naupli larvae, Oligochaeta, Tardigrada, Acarina, insects and larvae of insects (Table 2). During the period of investigation nematodes were dominant at all stations except for station 5. Nematode dominance varied from 67.1% for station 1 to 42% for station 3. Oligochaetes were dominant at station 5 with 55% of total meiofauna abundance, and second dominant at stations 2 and 3 (24.1% and 33.3% respectively). In the sediment of station 4 the insect species *Podura aquatica* was second in dominance with 26.2% of total meiofauna abundance. Other meiofaunal taxa were presented with a lower abundance. Different sample stations showed variation in meiofauna abundance and diversity. The statistical t-test used showed that there were no significant statistical differences in meiofauna abundance between station 1 and the other stations investigated. Differences in meiofauna abundance existed between station 2

Table 1. Mean values of the physical and chemical water parameters from five investigated stations (1. Donji Miholjac; 2. Pampas; 3. Zimska luka; 4. Zeleno polje; 5. Nemetin) (from ČAČIĆ & HORVATIĆ, 1996).

Stations	**1**	**2**	**3**	**4**	**5**
Depth, m	0,75	0,97	2,41	0,43	0,83
Turbidity, m	0,69	0,72	0,83	0,10	0,68
Dissolved O_2, mgL^{-1}	8,54	8,72	7,47	4,19	8,71
NH_4^+, mgL^{-1}	0,316	2,295	0,413	10,087	1,86
$KMnO_4$, mgL^{-1}	8,483	10,730	11,079	37,090	11,24
NO_2^-, mgL^{-1}	0,029	0,047	0,062	0,622	0,04
NO_3^-, mgL^{-1}	1,469	1,533	1,565	1,046	1,37
PO_4^{3-}, mgL^{-1}	0,062	0,060	0,080	0,939	0,05
Total N, mgL^{-1}	1,037	2,843	1,204	11,156	0,74
Total P, mgL^{-1}	0,238	0,565	0,776	1,997	1,14

Table 2. Meiofauna major groups (abundance and dominance structure) in the littoral sediment from five stations along the Drava River (1. Donji Miholjac; 2. Pampas; 3. Zimska luka; 4. Zeleno polje; 5. Nemetin).

Stations	**1**		**2**		**3**		**4**		**5**	
	x	**%**	**x**	**%**	**x**	**%**	**x**	**%**	**x**	**%**
Nematoda	174	67.1	57.5	64.6	60.5	42	30	46.1	25.5	27
Oligochaeta	23.5	9.1	21.5	24.1	48	33.3	7	10.8	53.5	55
Rotatoria	21	8.1	5.5	6.2	9	6.3	0.5	0.8	16	16
Copepoda	2.5	0.9	0	0	9	6.3	0	0	1	1
Tardigrada	21.5	8.3	1.5	1.7	4	2.8	1.5	2.3	1	1
Naupli-larvae	1	0.4	0.5	0.6	11.5	7.9	0	0	0	0
Insects	11	4.2	1.5	1.7	1	0.7	17	26.2	0	0
Insects larvae	5	1.9	1	1.1	1	0.7	1	1.5	0	0
Acarina	0	0	0	0	0	0	8	12.3	0	0
Total	**259.5**	**100**	**89**	**100**	**144**	**100**	**65**	**100**	**97**	**100**

Table 3. Free-living aquatic nematode fauna from five stations along the Drava River (1. Donji Miholjac; 2. Pampas; 3. Zimska luka; 4. Zeleno polje; 5. Nemetin). Values expressed in percentages (dominance < 3% not considered).

Stations	1	2	3	4	5
Mononchus aquaticus	25.7	40.9	17.6	8.3	8.7
Dorylaimus sp. 1	3.6	22.6	10.2	1.7	4.3
Dorylaimus sp. 2	5.0	13.9	7.4	3.3	2.2
Dorylaimus sp. 4	3.6	1.7	4.6	30.0	-
Monhystera sp.	40.7	-	4.6	-	13.0
Tobrilus gracilis	-	1.7	3.7	8.3	-
Diplogaster rivalis	-	4.3	6.5	-	13.0
Plectus sp. 1	2.9	3.5	8.3	-	-
Ethmolaimus pratensis	4.3	0.87	-	-	2.2
Plectus sp. 2	2.9	-	4.6	1.7	-
Monhystera stagnalis	-	3.5	12.9	-	19.6
Indet.1	-	-	-	3.3	4.3
Rhabditis sp. 2	-	-	-	38.3	-
Tobrilus sp. 3	-	-	-	1.7	13.0
Chromadora sp.	6.4	-	-	-	-
Dorylaimus sp. 6	-	-	6.5	-	-
Mermithidae	-	2.6	-	-	-
Mononchus sp. 2	-	-	4.6	-	-
Diplogaster sp. 2	-	-	-	3.3	-
Indet. 2	-	-	-	-	4.3
Number of genera	6	8	6	7	8

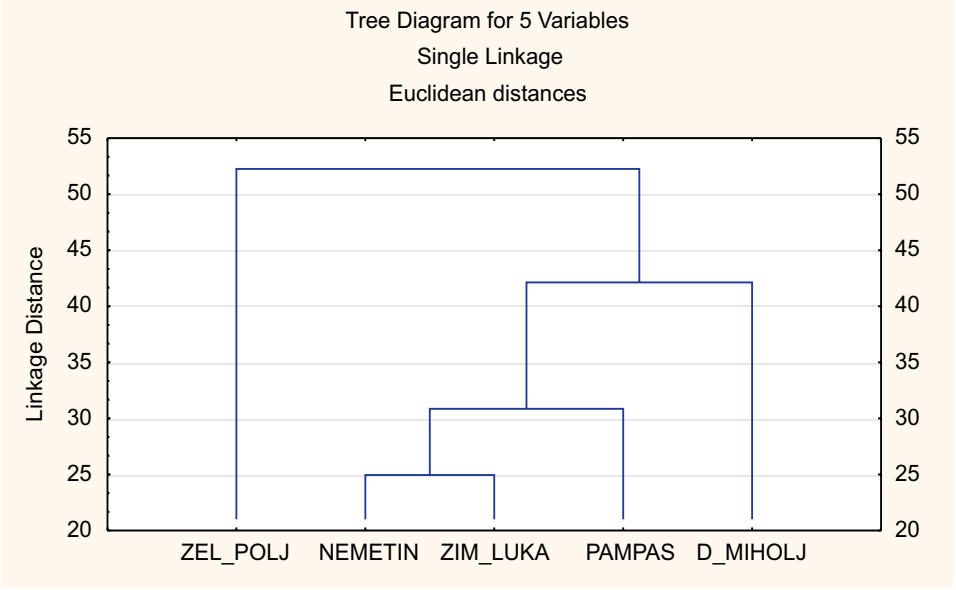

Fig. 2. Cluster analysis based on nematode relative abundance from five investigated stations along the Drava River.

and stations 3, 4 and 5; between station 3 and 5; as well as between station 4 and 5 ($p < 0.005$). The lowest total number of taxa was recorded at station 5, and the lowest total number of individuals at station 4.

A total of 33 nematode species or putative species were recorded during the survey (Table 3). 8 of the 33 species collected represented 74% of the nematofauna identified. 26 species belong to the subclass Adenophorea, and 7 species to the subclass Secernentea. At all the investigated stations, except for station 4, the dominant

Table 4. Univariate community measures number of taxa (S), Shannon-Weaver diversity (H'), evenness (Pielou's J), species richness (SR), taxonomic richness (Margalef's d) and dominance (D) for investigated nematode fauna.

Stations	1	2	3	4	5
Number of species (S)	13	13	17	10	15
Shannon-Weaver (H')	1,831	1,802	2,588	1,687	2,412
Species richness (SR)	1,014	1,119	1,540	1,162	2,064
Margalef's index (d)	2,428	2,529	3,417	2,198	3,656
H_{max}	3,701	3,701	4,088	3,322	3,907
Evenness (J)	0,495	0,487	0,633	0,508	0,617
Dominance (D)	0,505	0,513	0,367	0,492	0,383

Table 5. Feeding types as percentage at each station. 1. Donji Miholjac, 2. Pampas, 3. Zimska luka, 4. Zeleno polje, 5. Nemetin.

Stations	1	2	3	4	5
Predators	25.90	46.43	30.19	12.07	24.39
Plant feeders	12.23	41.07	30.19	36.21	9.76
Detritus feeders	48.92	9.82	35.85	41.38	46.34
Epistrate feeders	10.79	0.89	–	–	2.44
Scavengers	2.16	1.79	3.77	10.35	17.07

species belonged to Adenophorea. At station 4, the dominant species belonged to Secernentia: *Rhabditis sp*. 2, with 38.3% of the total number of nematofauna.

Differences in number of species, Shannon-Weaver diversity (H'), taxonomic richness (d), evenness (Pielou's J) appeared between some stations. The lowest community parameters were recorded for station 4 (Fig. 2). Also, classification based on nematode species relative abundance resulted in only station 4 being distinct (Table 4).

Detritus feeders (DF) were dominant at all stations except station 2 where predators dominated. Station 3 was characterised by an equal distribution of predators, plant feeders and detritus feeders (Table 5).

DISCUSSION

Sediment meiofauna diversity from the investigated stations along the Drava River differs from those of Sava River littoral sediments (unpublished data), the Narew and Bug backwaters (PREJS & BERNARD, 1985), in the uppermost parts of the Altenworth impoundment (BRETSCHKO, 1992), limnetic sediment from the Elbe estuary (BLOME & FAUBEL, 1996). Stations were distinguished by the absence of Chironomidae, Cladocera, Gastrotricha, Ostracoda and Turbellaria. The absence of ostracodes, and cladocerans can be due to sediment granulation and oxygen deficiency, especially for ostracodes (WETZEL, 1975).

The abundance of investigated sediment meiofauna at the five surveyed stations ranged from 6500 ind./m^2 (station 4) to 25900 ind./m^2 (station 1). Recorded meiofaunal abundance was somewhat lower than recorded by PREJS & BERNARD (1985), and by BLOME & FAUBEL (1996); somewhat higher than recorded by PLENET & GIBERT (1994) for polluted sites. At all investigated station the nematodes were the dominant group (from 42% to 67.1%), except for station 5 where oligochaetes dominated, with 55% of total meiofauna. According to many authors (PREJS & BERNARD, 1985; BRETSCHKO, 1992; NICHOLAS *et al*, 1992 PLENET & GILBERT, 1994; BLOME & FAUBEL, 1996) nematodes and oligochaetes were the eudominant groups at almost all investigated sites.

The number of nematode species recorded from diverse river sediments varied from 70 (Seveso River) to 8 (Narew and Bug backwaters); in most surveyed sited, a

few species accounted for more than 50% of the total number of nematofauna (ANDRASSY, 1959; ZULLINI, 1976; COLOMBA and VINCIGUERRA, 1979; EDER & KIRCHENGAST, 1980; PREJS & BERNARD, 1985; OCAÑA & PICAZO, 1991; BLOME & FAUBEL, 1996). The dominant species at all the sites investigated belong to a few genera: *Pareudiplogaster*, *Daptonema*, *Theristus*, *Paraigolaimella*, *Eutobrilus*, *Tobrilus*, *Mesodorylaimus*, *Mononchus* and *Rhabditis*. Polluted sites were characterised by the species *Rhabditis oxycerca*, *Mononchus aquaticus*, *Tobrilus gracilis*, *T. diversipapillatus* and *Aphelenchoides sacchari* (SCHIEMER & DUNCAN, 1974; ZULLINI, 1976; ARTHIGTON *et al*., 1986; OCAÑA & PICAZO, 1991). The most polluted station along the Drava River was characterised by rhabditis species. Species *Mononchus aquaticus* was abundant at stations 1, 2 and 3, and less abundant at the most polluted site, station 4. According to OCAÑA & PICAZO (1991) the species *Mononchus aquaticus* is an indifferent species when exposed to pollution. The species *Tobrilus gracilis* was recorded in low abundance at stations 2, 3 and 4, but at the site Crni fok (unpublished data) it was almost the only species found. The species was considered to be typical of eutrophic lotic and lentic environments and tolerant of low oxygen conditions (SCHIEMER & DUNCAN, 1974; PREJS, 1977a, b; EDER & KIRCHENGAST, 1982; PREJS & PAPINSKA, 1983). The lowest values of univariate community measurements recorded for station 4 indicate environmental alterations and instability, as recorded by other investigators (ZULLINI, 1976; OCAÑA & PICAZO, 1991; PLENET & GIBERT, 1994). Station 4 (Zeleno polje) along the Drava River differs from all the other stations. Biological and chemical data indicate the impact of the discharge of untreated effluent into the Drava River.

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SAŽETAK

Meiofauna i slatkovodni slobodnoživući oblići nekih lokaliteta duž rijeke Drave (Hrvatska).

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U meiofaunu ubrajaju se životinje čija se veličina kreće od 100 do 10000 m. Metodološki je meiofauna definirana prolaskom kroz sito veličine pora od 0.5 ili 1 mm, a zadržavanjem na situ veličine pora 0.63 m (HEIP *et al*., 1988). Meiofauna sedime-

nata slatkovodnih ekosistema uključuje nekoliko skupina beskralježnjaka: Acarina (grinje), Chironomidae (ličinke trzalaca < 3 mm), Cladocera (rašljoticalci), Copepoda (veslonošci), Nematoda (oblići), Oligochaeta (maločetinaši < 3 mm), Ostracoda (ljuskari), Rotatoria (kolnjaci), Tardigrada (dugoživci), Turbellaria (virnjaci), nauplij ličinke rakova, te juvenilne oblike mekušaca (WASILEWSKA, 1973). Lokalni abiotički čimbenici kao što su tip sedimenta, brzina strujanja vode, sadržaj kisika, hrane kao i stupanj zagađenja određuju sastav i brojnost meiofaune te vrsni sastav i brojnost slobodnoživućih vodenih oblića (PLENET & GIBERT, 1994; ZULLINI, 1976; OCAÑA & PICAZO, 1991). Zajednice su meiofaune, također, određene interakcijom s drugim organizmima, kao što su predatorstvo i kompeticija. Različiti oblici zagađenja mogu smanjiti gustoću, biomasu i raznolikost bentoskih organizama, a naročito pojedinih taksona kao što su kukci i mekušci (ZULLINI, 1976). Tijekom ožujka i travnja 1995. godine na pet litoralnih lokaliteta duž rijeke Drave istraživan je kvalitativni i kvantitativni sastav meiofaune i nematofaune. Uzorci sedimenta obrađeni su standardnim metodama (SEINHORST, 1959; UHLIG *et al.*, 1973). Za utvrđivanje razlike u kvantitativnom sastavu meiofaune između istraživanih lokaliteta podaci su statistički obrađeni t-testom (PARKER, 1975). Univarijantne i multivarijantne statističke metode korištene su za utvrđivanje mogućih razlika u nematofauni istraživanih postaja, a s obzirom na utjecaj zagađenja i različitih svojstava sedimenta (WARWICK, 1988). Prema građi usne šupljine oblići su podijeljeni u pet hranidbenih skupina (NICHOLAS *et al.*, 1992; poslije Jensena).

U sedimentima istraživanih lokaliteta nisu ustanovljeni Chironomidae, Cladocera, Ostracoda i Turbellaria, inače skupine prisutne u sedimentima vodenih ekosistema (PREJS & BERNARD, 1985; BRETSCHKO, 1992; PLENET & GIBERT, 1994; BLOME & FAUBEL, 1996). U sedimentu postaja 1, 2, 3 i 4 dominantna su skupina bili oblići, a na postaji 5 maločetinaši, također, eudominantna skupina na postajama 2, 3 i 4. Na istraživanim su postajama utvrđene 33 vrste slatkovodnih slobodnoživućih oblića. Vrsta *Mononchus aquaticus* bila je zastupljena relativno visokom gustoćom na postajama 1,2, i 3. OCAÑA & PICAZO (1991) označili su je kao indiferentnu vrstu prema različitim tipovima zagađenja. Vrsta *Tobrilus gracilis* značajna je za sedimente stajaćica i tekućica te se smatra da može duže proboraviti u uvjetima anoksije (SCHIEMER & DUNCAN, 1974; PREJS, 1977a, b; EDER & KIRCHENGAST, 1982; PREJS & PAPINSKA, 1983). Ta je vrsta bila zastupljena relativno niskom relativnom gustoćom na postajama 2, 3 i 4. Nije zabilježena na postajama 1 i 5. Prema rezultatima univarijantnih statističkih analiza (mali broj vrsta, male vrijednosti indeksa raznolikosti i ravnomjernosti) postaja 4 razlikuje se od ostalih istraživanih postaja. Klaster analiza je, također, potvrdila razlike značajne za tu postaju. Ako se uzmu u obzir kemijski i biološki čimbenici utvrđeni za vodu i sediment postaje 4, može se sa sigurnošću reći da je fauna dna pod utjecajem znatnog zagađenja.