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Distribution of epilithic diatoms in the Savinja River flowing through an urban landscape

Razširjenost epilitskih diatomej v reki Savinji, ki teče skozi mestno krajino

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Abstract: The catchment area in urban and agricultural landscapes is greatly influenced by human activities that reflect also in physical and chemical characteristics of water as well as in species diversity in waterbodies. The diversity and the species composition of epilithic diatom communities in the Savinja River, as well as basic environmental parameters were analysed. Sampling sites were selected in reaches subjected to different influences from the catchment area and with different physical and chemical characteristics. Samples were collected at the site where the Savinja River enters the urban area of the town Celje, at the end of urban landscape and downstream of the Celje waste water treatment plant outflow. The most common and dominant diatom species in the periphyton community was *Achnanthes biasolettiana.* Other common diatom taxa that were found in all samples and at least in one sample exceeded relative abundance of 10% were *Nitzschia fonticola, Amphora pediculus* and *Nitzschia dissipata.* The results of the redundancy analyses (RDA) revealed that the variance of the epilithic diatom community was explained by O_2 saturation (35%) and saprobic index (33% of TVE). Diatom species richness was positively correlated with O_2 saturation. Shannon-Wiener diversity index was positively correlated with saprobic index values based on all algae and trophic index calculated on the base of diatoms indicating a relatively low organic matter and nutrient input into the river system. The results showed that no significant changes in epilithic diatom species composition and no negative impacts on diversity of epilithic diatom community in the Savinja River were detected on its flow through the urban landscape. Moreover, changes between the seasons were more evident than changes between sampling sites, confirming the importance of sampling date for monitoring.

Keywords: Diatoms, microphytobenthos, periphyton, environmental factors, torrential river

Izvleček: Prispevno območje v mestnih in kmetijskih krajinah je pod močnim človekovim vplivom, kar se odraža v fizikalnih in kemijskih lastnostih kot tudi v raznolikosti vrst v vodnih telesih. Analizirali smo raznolikost in vrstno sestavo epilitonskih združb diatomej v reki Savinji, kot tudi osnovne okoljske parametre. Vzorčna mesta smo izbrali na odsekih, ki so izpostavljeni različnim vplivom iz prispevnega območja in z različnimi fizikalnimi in kemijskimi lastnostmi. Vzorci so bili nabrani na mestu, kjer reka vstopi v urbano območje mesta Celje, na koncu mestne krajine in dolvodno od iztoka centralne čistilne naprave Celje. Najpogostejša in prevladujoča vrsta kremenastih alg v perifitonski združbi je *Achnanthes biasolettiana*. Drugi pogosti taksoni diatomej, ki so bili najdeni v vseh vzorcih in so vsaj v enem vzorcu presegli 10 %, so bili: *Nitzschia fonticola, Amphora pediculus* in *Nitzschia dissipata*. Rezultati redundančne analize (RDA), so pokazali, da variabilnost epilitskih združb diatomej lahko pojasnimo z nasičenostjo vode s kisikom (35 %) in s saprobnim indeksom (33 %). Vrstna pestrost diatomej je bila v pozitivni korelaciji z nasičenostjo s O_2. Shannon-Wiener indeks je v pozitivni korelaciji z vrednostjo saprobnega indeksa, ki je izračunan na podlagi združbe vseh alg in z vrednostjo trofičnega indeksa, izračunanega na osnovi združbe kremenastih alg, kar kaže na relativno nizko vsebnost organskih snovi in hranil v rečnem sistemu. Glede na naše rezultate, nismo zaznali opaznih sprememb v vrstni sestavi in negativnih vplivov na raznolikost epilitske združbe diatomej iz reke Savinje pri njenem toku skozi mestno krajino. Poleg tega smo ugotovili, da so spremembe med sezonami bolj očitne, kot spremembe med vzorčnimi mesti, kar potrjuje pomembnost datuma vzorčenja pri monitoringu.

Ključne besede: diatomeje, mikrofitobentos, perifiton, okoljski dejavniki, hudourniška reka

Introduction

Monitoring of ecological status of aquatic ecosystems is essential for the estimation of human influence on aquatic environment as well as for the evaluation of aquatic environment management efficiency. Benthic diatoms are one of the biological quality elements used for the assessment of ecological status according to the European Water Framework Directive (WFD) (2000/60/EC). Benthic diatoms are used for the calculation of different metrics such as trophic and saprobic indices, measuring the extent of human impact to the rivers and lakes (emission of nutrients and dissolved organic matter, respectively). Diatoms are frequently used for the evaluation of the ecological status of running waters (Virtanen et al. 2011, Kelly et al. 2012), since they are various, dominant in phytobenthos, and since the ecological preferences of several diatom species are well known (Beltrami et al. 2012). Habitat and species diversity of biotic communities in running waters are strongly influenced by the properties of the catchment area, land use and pollution sources.

Diatoms are a taxonomically diverse group of organisms with high sensitivity to chemical stressors (Martínez De Fabricius et al. 2003, Frankovich et al. 2006, Almeida and Feio 2012, Várbíró et al. 2012), and vary spatially and temporally (Passy 2007, Soininen 2007). The relationships between diatoms and environmental variables were shown by many authors (Passy 2007, Soininen 2007, Lange et al. 2011, Virtanen and Soininen 2012, Toman et al. 2014).

Diversity and abundance of diatoms are controlled by environmental factors like nutrients, temperature, light intensity, grazing pressure, substrate stability and discharge (Izagirre and Elosegi 2005). Major environmental determinants for diatom distribution in streams, as reported by Soininen (2007) are pH, conductivity, total phosphorus, temperature, alkalinity, altitude, nitrates, calcium, biological oxygen demand (BOD), chlorophyll *a* and substrate type. Lange et al. (2011) found that light availability, nutrient concentrations and grazing pressure determined the stream diatom community composition. Biggs and Close (1989) suggested that disturbances such as spates reduce the effect of grazing pressure, because re-colonization of invertebrates is usually slow compared to periphyton growth.

In depth studies dealing with the relationships between environmental parameters and algal communities in Slovenia have been performed only in extreme environments (Krivograd Klemenčič et al. 2010, Krivograd Klemenčič and Toman 2010), while the distribution of diatoms along the environmental gradients in running waters of Slovenia is poorly known. Similar study as is present one on environmental effects on diatom communities in rivers in Slovenia was conducted for Kamniška Bistrica River (Toman et al. 2014) together with a preliminary study on the Savinja River (Koren 2009, Čatorič 2013).

On the studied section the Savinja River flows through the urban landscape of the town Celje and is subjected to numerous influences from the catchment area i.e. emissions from different kinds of industry, intensive agriculture and Celje wastewater treatment plant.

The main goal of this research was to investigate the influence of various environmental and temporal factors on the species composition and diversity of epilithic diatom assemblages and possible longitudinal changes. We hypothesized that there will be greater differences in species composition and diversity of epilithic diatom assemblages between the seasons than between the sampling sites due to general degradation of environment within the research area. We also hypothesized that the loading of the river will be higher at the sites downstream of the major part of the settled area and emissions of the wastewater than at the reference site upstream the urban landscape of Celje.

Materials and methods

Study area

The Savinja River is a left tributary of the Sava River and an important part of the Danube catchment area, collecting water from the southern belt of the limestone Alps. The length of the river is 101 km, drainage area 1848 km^2 and the average (monthly) discharge near the sampling site S3 is from 25 to 56 m^3 s^{-1} (data available on: http://www.arso.gov.si/vode/podatki/amp/H6200_g_1.html) Hydrological conditions in the Savinja River are extremely variable; at the highest water levels the flow can increase more than 300- fold compared to the basal flow. The source (GKX: 140697, GKY: 472458) is at an altitude of 734 m a.s.l. The riverbed in the studied reach consists of different types of rock (sandstones, conglomerates), but limestone and magnesium limestone (dolomite) of Middle to Early Jurassic age is the most common rock type (Buser 2009). The catchment area in the middle and lower Savinja valley is characterized by agricultural land, farms and numerous settlements.

Samples were taken at three sampling sites (Fig. 1). The sites S1 was chosen as a reference site in this study, since it is situated on the Savinja River before it enters into densely populated urban landscape of the town Celje. Sites S2 and S3 where the influences of human activities was to be detected are downstream of the urban area of Celje with all its emissions. The distance from the upper sampling site S1 to the lowest site S3 is approximately 10 km. The catchment between S1 and S2 is the urban area of the town Celje with population over 40.000 inhabitants, where an urban landscape can be found with different types of settlements and different kinds of industry, all of which are potential sources of inorganic and organic compounds (Fig. 1). The sampling site S3 is downstream the Celje wastewater treatment plant, which influences the river with its effluent.

Sampling and laboratory analyses

Samples were taken in different seasons in the years 2011 and 2012. The sample from the beginning of September is considered a summer sample due to low discharges and high water temperatures, which reached up to 23 °C (Sampling site S2). The sample from the beginning of December represents winter sample, since there was a longer period of relatively cold weather. The sample from the end of March is a typical spring sample. Epilithic diatoms in the periphyton communities were sampled from stones (φ = 6-20 cm) at each site, by scraping and brushing off the stone surface (5 cm^2 per stone). Samples for diatom identification and for biomass determination were preserved in 4% formaldehyde, samples for chlorophyll *a* analyses were stored at 4 °C till the next day. For taxonomic identification of diatoms all samples were diluted with distilled water to 50 mL, homogenized with a magnetic stirrer and subsamples were treated with concentrated nitric acid (HNO_3). Permanent slides of diatom frustules were prepared using the high refraction mountant Naphrax®. Diatom taxa were identified and counted using an Olympus CX41 light microscope with an oil-immersion objective at a magnification of

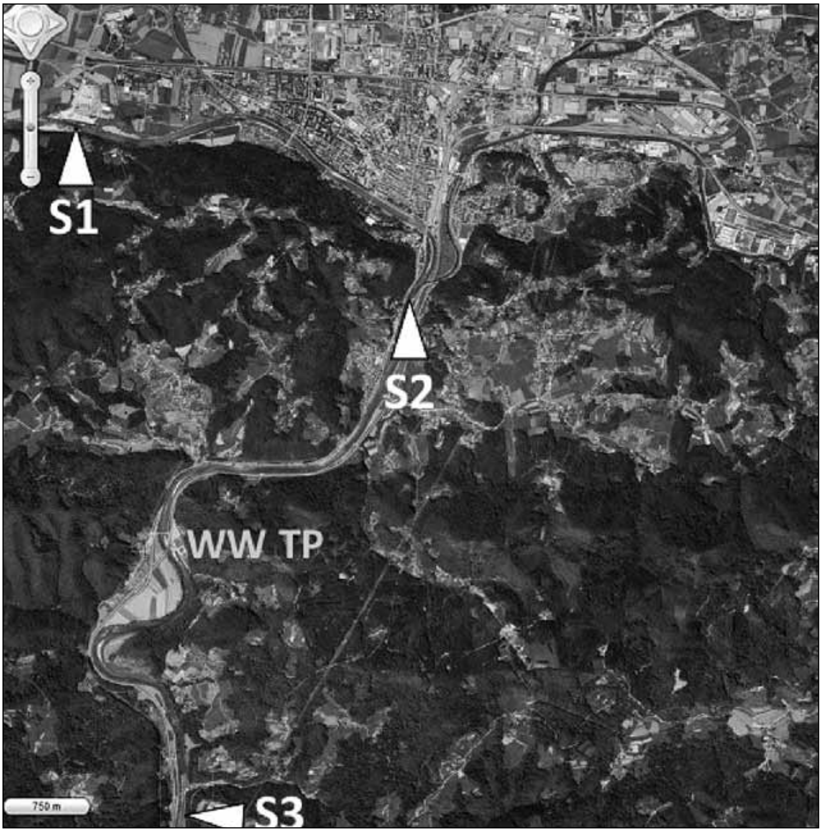

Figure 1: Map showing the location of the sampling sites S1, S2 and S3 along the river Savinja (WW TP = Celje wastewater treatment plant). (Source: Geopedia 2016)

Slika 1: Zemljevid, ki prikazuje lokacijo vzorčnih mest S1, S2 in S3 ob Savinji (WW TP = Celjska centralna čistilna naprava). (vir: Geopedia 2016)

1000×, taxonomy followed Kramer and Lange-Bertalot (1986-1991).

The proportion of diatoms and species composition of cyanobacteria and other algal groups were analysed with the same light microscope at a magnification of 400×. Cyanobacteria and other non-diatom algae were identified using Hindák et al. (1978), Biggs and Kilroy (2000), and Komárek and Anagnostidis (2002). Proportions of diatom taxa among all other algae taxa were obtained by further division of their total share according to the proportions of around 500 counted frustules at a magnification of 1000×.

At each sampling site selected physical and chemical parameters were measured at the same time as the samples were collected. Temperature, pH, O_2 concentration, O_2 saturation, and conductivity were measured using the portable multi-meter PCD 650 (Eutech Instruments, Singapore). Water depth and current velocity above the selected stones were measured as well. The cover of inorganic and organic substrate was estimated according to the AQEM (2002) protocol (see Tab. 1).

Table 1: The structure of organic and inorganic substrates at the sampling sites (S1-S3). Mean coverage (%) of each fraction of the substrate; CPOM - coarse particulate organic matter, FPOM - fine particulate organic matter.
Tabela 1: Deleži različnih tipov organskega in anorganskega substrata na posameznih vzorčnih mestih (S1-S3). Navedeni so povprečni deleži (%) pokrovnosti posameznega tipa substrata; CPOM – večji delci organskih snovi, FPOM – drobni delci organskih snovi.

	S1	S2	S3
filamentous algae	67	53	75
submerged macrophytes	.	.	1
xylal	2	4	3
CPOM	.	2	3
FPOM	5	27	17
megalithal	5	.	40
macrolithal	5	5	20
mesolithal	50	20	20
microlithal	40	60	20
psammal	.	10	.
argyllal	.	5	.

Water samples were analyzed in the laboratory for the concentration of nitrates and soluble reactive phosphorous (SRP). Chlorophyll *a* content was measured spectrophotometrically according to the method described in (Urbanič and Toman, 2003). Concentration of nitrates were measured with the Na-salicylate method (Monteiro et al. 2003), while the soluble reactive phosphorus (SRP) was measured using the $SnCl_2$ method (APHA 1998). Periphyton biomass was also determined as dry weight (at 105 °C) of the biofilm covering the sampled stones scratched from another 5 cm^2 rectangle on each stone. Results of these measurements are given in Table 2.

Table 2: Measured environmental variables on sampling sites S1, S2 and S3 including mean, minimum and maximum values.
Tabela 2: Izmerjene vrednosti okoljskih spremenljivk na merilnih mestih S1, S2 in S3, vključno s povprečnimi, minimalnimi in maksimalnimi vrednostmi.

	S1			S2			S3		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Temperature (°C)	11.8	4.5	21.0	13.2	5.5	23.2	13.1	5.3	22.8
pH	8.2	8.1	8.3	8.2	8.1	8.3	8.3	8.1	8.5
conductivity (µS/cm)	379	375	384	683	630	738	462	458	469
O_2 (mg/L)	12.6	11.7	14.2	13.1	12.3	12.5	13.1	12.0	14.8
O_2 saturation (%)	115	106	130	123	113	142	123	116	138
nitrate (mg/L)	5.2	3.9	6.0	5.1	4.8	5.5	5.8	4.8	6.6
current velocity (m/s)	0.55	0.42	0.63	0.57	0.46	0.77	0.32	0.28	0.39
dry mass of periphyton (mg/cm^2)	5.3	3.3	6.4	4.2	1.7	7.6	7.6	7.1	8.8
Chlorophyll a (mg/m^2)	5.2	4.0	5.9	3.5	2.3	4.2	7.6	6.5	9.1

Data analyses

Relative abundance (percentage values) of the diatom taxa were calculated for each sample. The Shannon-Wiener (S-W) diversity index was used to estimate diatom diversity and the saprobic index (SI) was calculated using saprobic (s_i) and indicator values (G_i) according to Kosi et al. (2006) to determine water quality using the following formula:

$$SI = \frac{\sum_{i=1}^{n}(h_i \times G_i \times s_i)}{\sum_{1=1}^{n}(h_i \times G_i)}$$

(h_i – abundance of the taxon i;
n – number of taxa)

The trophic index was calculated in the same manner as saprobic, however the trophic and indicator values according to Rott (1999) were found in Kosi et al. (2006). The cluster analyses were performed with the program Syn-Tax (Podani 2001) to establish the similarity between diatom communities from different sampling sites/seasons. As a method of linkage, unweighted pair group method with arithmetic mean (UPGMA) was used and the Sørensen index served as a similarity measure for the creation of a dendrogram.

Detrended correspondence analysis (DCA) was applied to the diatom percentage data to explore the patterns of species changes and biological species turnover (the gradient length). The eigenvalue for the first DCA axis was < 0.4 (0.35, while gradient length was 1.70 SD (standard deviation units of species turnover) and indicated linearity (ter Braak and Verdonschot 1995) and therefore redundancy analysis (RDA) was chosen to explore the relationships between diatom assemblages and explanatory variables.

Separate RDAs for smaller groups of all studied environmental and temporal variables were performed to test the significance of their influence on the variation of species composition. Forward selection of explanatory variables was used to provide a ranking of the relative importance of the specific variables and to avoid co-linearity. Unrestricted Monte Carlo test with 499 permutations was used to test the statistical significance of the variables and canonical axes. A series of RDAs were done with subsets of statistically significant variables ($p < 0.05$) and the proportions of variance explained by these variables were calculated. Ordination of the samples according to the species composition was made using DCA and most important environmental parameters were passively projected on the ordination diagram. The whole set of analyses was performed using CANOCO 4.5 (ter Braak and Šmilauer 2002).

Relationships between the diatom diversity and environmental factors were explored with Spearman correlation coefficients in SPSS version 17.

Results

Distribution of diatoms and diversity of diatom assemblages

A total of 50 diatom taxa were identified in the samples. Almost all of them that is 45 taxa were found at the sampling site S2, in the regulated channel, downstream the urban area. The highest number of diatom species in a single sample (37) was found in summer samples at second and third site (Tab. 3), and the lowest (30) at the upper sampling site in all seasons. Two species occurred with a high share in all seasons and at all sampling sites, namely *Achnanthes biasolettiana* (18-46%) and *Nitzschia fonticola* (4-14%). The similarity of diatom assemblages is presented in Fig. 2, which indicates that about three quarters of taxa were common to all samples (Tab. 3). The dendrogram (Fig. 2) showed that two clusters are formed according to seasons. The left subgroup uniting the summer samples from all three locations (S1_P, S3_P and S2_P) shows a higher similarity of the samples within seasons than sites. Moreover, the cluster in the middle unites winter samples (S1_Z, S2_Z and S3_Z) from all three sites.

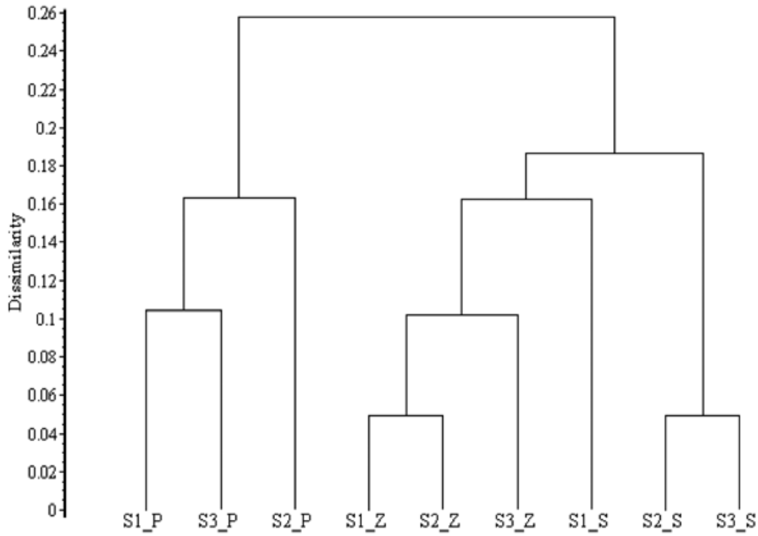

Figure 2: Dendrogram showing the similarity of diatom communities in different sampling sites (S1-S3) in different seasons/months (P = summer, Z = winter, S = spring).

Slika 2: Dendrogram, ki kaže podobnost združb kremenastih alg na različnih vzorčnih mestih (S1-S3) v različnih letnih časih / mesecih (P = poletna, Z = zimska, S = spomladanska).

In the samples from the upper site (S1) 38 diatom taxa were found all together (Tab. 3). The most abundant species (≥ 5%) were: *Achnanthes biasolettiana*, *Nitzschia fonticola, Nitzschia dissipata, Navicula capitatoradiata, Cymbella affinis* and *Navicula menisculus*.

The total number of diatom taxa was the highest at site S2 downstream of the town Celje where 45 diatom species were found. The highest number per sample was in the summer (Tab. 3). The majority of co-dominant species were the same as in site S1: *Achnanthes biasolettiana*, *Nitzschia fonticola, Nitzschia dissipata, Navicula capitatoradiata, Cymbella affinis,* but here additional taxa occurred with high abundance: *Amphora pediculus, Gomphonema angustatum* and *Cymbella minuta*.

The site S3 which is in the regulated channel like site S2 exhibited the most diverse diatom assemblage in December when S-W index reached the value 4.1 (Tab. 3). Ten taxa reached ≥ 5% of the relative abundance. *Achnanthes biasolettiana* occurred with a high share in all seasons. Other common diatom species included: *Amphora pediculus, Nitzschia fonticola, Navicula capitatoradiata, Cymbella affinis, Navicula menisculus, N. reichardtiana, Diatoma vulgaris, Cocconeis placentula.*

Table 3: Number of diatom taxa, Shannon-Wiener (S-W) diversity index, saprobic indices, the percentage (%) of diatoms in phytobenthos and relative abundances (%) of common diatom species on sampling sites (S1-S3) in different seasons/months (S – September/P – summer; D – December/Z – winter; M – March/S - spring). Abundances reaching at least 5% are in bold; +, species present with relative abundance < 1%; . , not detected

Tabela 3: Število taksonov diatomej, Shannon-Wiener (SW) indeks raznolikosti, saprobni indeksi, delež kremenastih alg v fitobentosu in relativne abundance (%) pogostih diatomej na vzorčnih mestih (S1-S3) v različnih letnih časih / mesecih (S – september/P – poletni; D – december/Z – zimski; M – marec/S - spomladanski). Abundance, ki dosegajo vsaj 5% so v krepkem tisku; +,: vrste so prisotne z <1%; . takson/vrsta ni zaznan/a.

sampling site		S1			S2			S3	
Nr. of taxa per site		38			45			44	
month	S	D	M	S	D	M	S	D	M
season	P	Z	S	P	Z	S	P	Z	S
Nr. of taxa per sample	30	30	30	37	31	31	37	33	30
S-W diversity index	3.6	3.0	2.5	3.3	3.5	3.9	3.9	4.1	3.0
Saprobic index (all algae)	1.72	1.74	1.69	1.67	1.82	1.92	1.78	1.87	1.78
Saprobic index (diatoms)	1.66	1.73	1.64	1.60	1.82	1.92	1.77	1.88	1.79
Trophic index (diatoms)	2.4	2.0	1.7	2.1	2.1	2.1	2.5	2.7	2.2
% of diatoms in phytobenthos	75	77	81	80	82	89	77	87	79
% of diatoms taxa									
Achnanthes biasolettiana	**26**	**46**	**57**	**40**	**29**	**18**	**19**	**19**	**37**
Nitzschia fonticola	**9**	**14**	**13**	4	**11**	**14**	**6**	**10**	**9**
Amphora pediculus	+	+	**5**	+	+	**9**	**8**	**10**	**26**
Nitzschia dissipata	1	**12**	2	+	**19**	**7**	1	**6**	2
Navicula capitatoradiata	**17**	+	+	**12**	.	.	**20**	+	.
Cymbella affinis	**11**	4	+	**14**	1	.	**10**	+	.
Navicula menisculus	**8**	+	+	**5**	2	2	**6**	3	2
Gomphonema angustatum	+	+	2	1	**5**	**13**	+	.	2
Cymbella minuta	+	2	4	+	3	**6**	1	4	2
Gomphonema olivaceum	+	3	1	+	3	4	+	4	1
Diatoma vulgaris	1	2	2	3	2	1	1	**6**	+
Navicula reinchardtiana	.	3	1	.	**5**	2	.	**8**	+
Cymbella silesiaca	3	+	4	1	+	3	+	2	2
Navicula lanceolata	.	2	+	+	4	3	+	**5**	2
Cocconeis placentula	4	+	+	3	+	+	**6**	+	+
Fragillaria capucina vaucheria	2	2	+	1	3	2	1	2	+
Rhoicosphaenia abbreviata	2	+	+	2	+	+	2	3	3
Nitzschia palea	3	+	2	2	+	+	3	+	+
Cymbella sinuata	+	+	2	1	2	4	1	.	2
Navicula gregaria	.	1	+	.	+	3	.	**5**	2
Gomphonema parvulum	2	+	.	3	1	+	2	+	+
Surirella brebissonii	+	+	+	.	2	2	+	4	+
Cocconeis pediculus	2	+	+	+	+	+	2	1	1
Navicula tripunctata	3	+	+	+	+	.	2	+	.
Gomphonema minuta	1	+	+	+	2	+	+	+	.
Navicula veneta	1	+	+	+	+	+	1	+	+
Fragillaria ulna	+	1	+	+	+	.	+	2	.
Navicula atomus	.	.	+	.	.	2	.	.	2
Achnantes minutissima	.	+	.	.	1	+	.	+	1
Melosira varians	+	+	.	+	+	+	+	2	+
Cyclotella meneghiniana	.	.	.	+	+	.	2	+	.

More evident than differences between the sites were similarities between the samples from the same seasons (Tab. 3). Certain diatoms occurred with relatively higher abundance in the summer like: *Navicula capitatoradiata, Cymbella affinis, Navicula menisculus, Cocconeis placentula.* On the other hand, diatom species like *Nitzschia dissipata* and *Navicula reichardtiana,* were more numerous in the winter samples. Two taxa, namely *Amphora pediculus* and *Gomphonema angustatum* reached the highest share in spring at all three sites and were characteristic for spring samples.

The lowest number of diatom taxa was detected at the upper site (S1) where 30 diatom species were found in each sample. The lowest S-W diversity index value (2.5) was calculated for the spring sample from the upper site, which was also the most species-poor, due to the dominance of the species *Achnanthes biasolettiana* (57%). Beside the mentioned species only *Nitzschia fonticola* exceeded the share of 5% (Tab. 3).

Water quality

The average SI value for all sampling sites was 1.76 which indicates a 2nd quality class or β-mesosaprobic level with moderate organic loading. Samples from sites S2 and S3 were classified into 2nd class. Samples from the upper site S1 were classified into 1-2nd class which is the oligo- to β-mesosaprobic class (Tab. 3).

Influence of environmental parameters on diatom species composition

Two parameters statistically significantly explained the species composition of diatom communities: O_2 saturation of the water and saprobic index values calculated on the base of all algae. Saturation of water with O_2 explained 35% and saprobic index explained 33% of the total variance of the diatom community.

Diatoms presented on the left side of Fig. 3B were found in the summer samples from all three sites (Fig. 3A) and prefer water with higher O_2 saturation, while diatoms which are found on the right side, prefer water with higher content of dissolved organic matter, that also results in lower O_2 saturation.

Figure 3 A

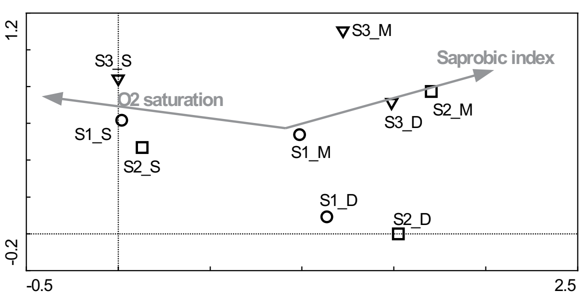

Figure 3B

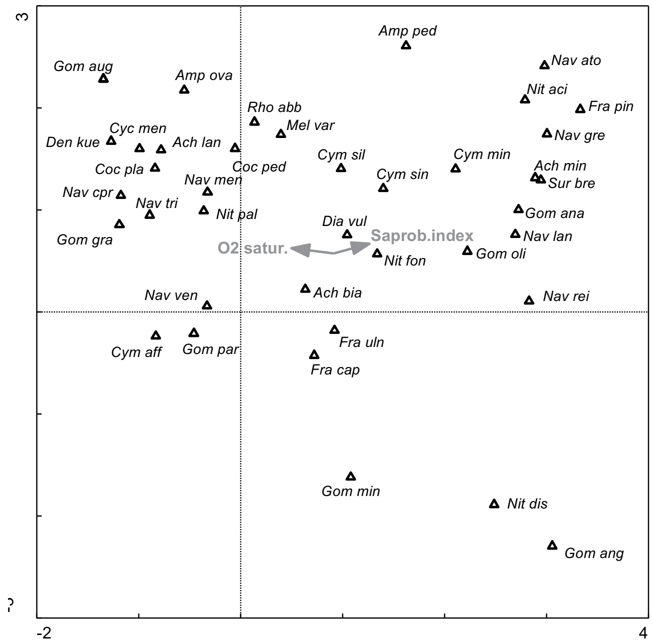

Figure 3: Detrended correspondence analysis (DCA) ordination diagram with passively projected variables of diatom assemblages from various seasons and sites of the Savinja River. Only significant ($p < 0.05$) variables are included. **A** – distribution of the samples and environmental gradients, where sites are represented with numbers (S1, S2 and S3) and seasons/months with letters (S = September, D = December, M = March; **B** – distribution of diatom species present in at least three samples are shown.

Slika 3: Ordinacijski diagram diatomejskih združb z različnih vzorčnih mest v Savinji in iz različnih sezon, narejen na osnovi korespondenčne analize z odstranjenim trendom (DCA) s pasivno projiciranimi spremenljivkami. Vključene so samo statistično značilne ($p < 0.05$) spremenljivke. **A** - razporeditev vzorcev in okoljski gradienti, kjer so vzorčna mesta predstavljena s številkami (S1, S2 in S3) in sezone/meseci s črkami (S = september, D = december, M = marec); **B** - razporeditev vrst diatomej prisotnih v vsaj treh vzorcih:

Legenda / Legend: Ach bia - *Achnanthes biasolettiana,* Ach lan - *Achnanthes lanceolata*, Ach min - *Achnanthes minutissima,* Amp ova - *Amphora ovalis*, Amp ped - *Amphora pediculus,* Coc ped - *Cocconeis pediculus,* Coc pla - *Cocconeis placentula,* Cyc men - *Cyclotella meneghiniana*, Cym aff - *Cymbella affinis,* Cym min - *Cymbella minuta,* Cym sil - *Cymbella silesiaca,* Cym sin - *Cymbella sinuata,* Den kue - *Denticula kuetzingii*, Dia vul - *Diatoma vulgaris,* Fra cap - *Fragilaria capucina,* Fra uln - *Fragilaria ulna,* Fra pin - *Fragillaria pinnata*, Gom ang - *Gomphonema angustatum,* Gom ang - *Gomphonema angustum*, Gom aug - *Gomphonema augur*, Gom gra - *Gomphonema gracile*, Gom min - *Gomphonema minuta*, Gom oli - *Gomphonema olivaceum,* Gom par - *Gomphonema parvulum,* Mel var - *Melosira varians,* Nav ato - *Navicula atomus,* Nav cpr - *Navicula capitatoradiata*, Nav rei - *Navicula reinchardtiana*, Nav ven - *Navicula veneta*, Nav gre - *Navicula gregaria,* Nav lan - *Navicula lanceolata,* Nav men - *Navicula menisculus,* Nav tri - *Navicula tripunctata,* Nit aci - *Nitzschia acicularis*, Nit dis - *Nitzschia dissipata,* Nit fon - *Nitzschia fonticola,* Nit pal - *Nitzschia palea,* Rho abb - *Rhoicosphaenia abbreviata*, Sur bre - *Surirella brebissonii*.

Correlation between environmental parameters and diversity of diatoms

Diatom species richness was positively correlated ($p < 0.01$) with saturation of water with O_2 and marginally ($p < 0.1$) with month of sampling, temperature, submerged macrophytes (Tab. 4).

Shannon-Wiener diversity index was positively correlated ($p < 0.01$) with the trophic index, with saprobic index calculated on the basis of diatoms and other algae ($p < 0.05$), and marginally significant with the saprobic index calculated on the basis of diatoms (Tab. 4).

Table 4: Summary of correlation analysis between diatom species richness (Nr. of taxa) and Shannon-Wiener diversity index (H') and some of the environmental parameters; $p < 0.01$, * $p < 0.05$, + $p < 0.1$.
Tabela 4: Povzetek analize korelacij med vrstno pestrostjo diatomej (število taksonov), Shannon-Wienerjevim indeksom (H') in izbranimi okoljskimi parametri; ** $p < 0.01$, * $p < 0.05$, + $p < 0.1$.

	S-W diversity index	Nr. of taxa
month of sampling	-0.388	-0.639+
Temperature (°C)	0.169	0.630+
O_2 saturation (%)	0.391	0.811**
Saprobic index (based on diatoms)	0.614+	-0.159
Saprobic index (based on all algae)	0.678*	-0.132
Trophic index (based on diatoms)	0.827**	0.389
submerged macrophytes	0.344	0.625+

Discussion

Distribution and diversity of diatoms

Diatoms are the most abundant primary producers in the periphyton community, especially in streams with a stony substrate, with the highest share in spring and autumn periods. In our samples diatoms represented very high share ranging from 75 to 89% of the primary producers (Tab. 3). The highest number of the diatom species was found in September in two sites downstream the town Celje.

The similarity of the species composition of the epilithic diatom communities (Fig. 2) reflected rather the season than environmental factors. The left group uniting the summer samples represents a specific group of diatom assemblages. However, this group still has three quarters of species in common with other samples. The highest similarity within the samples was found among the winter samples collected in the beginning of December. On the other hand the samples collected in the end of March showed the highest heterogeneity in species composition (Fig. 2). Diatom assemblage from the upper site was more similar to winter samples that to other two samples from the same season, which had almost identical species composition having 95% species in common. Possible reason was greater exposure to disturbance in second and third location in spring what also reflected in higher share of pioneer species *Amphora pediculus* (see Rimet and Bouchez 2012). Three quarters of species were the same in all epilithic diatom assemblages that is much higher that is reported for river Kamiška Bistrica by Toman et al. (2014), where only about a third of taxa were common to all samples. A lower rate of similarity is a consequence of stronger gradient in natural factors such as water temperature, as well as the variety of human impacts on the river ecosystem (Toman et al. 2014).

For instance, species *Achnanthes biasolettiana,* was present in all samples (Tab. 3) and was also the most abundant in almost all (except one) communities. According to Hoffman et al. (2011) *Achnanthes biasolettiana* prefers Calcium-rich, oligotrophic to mesotrophic running waters on limestone bedrock of the alpine and pre-alpine regions where they often reach high abundance. This is also the case in our research area and in accordance with our results (Tab. 3). The species *Achnanthes biasolettiana* has the ability to firmly attach to the substratum in changeable water flows (Virtanen et al. 2011) and is capable of quick re-colonization that could be the reasons for its constant presence and dominance in the epilithic community.

Certain diatoms occurred with relatively higher abundance in the summer like: *Navicula capitatoradiata, Cymbella affinis, Navicula menisculus, Cocconeis placentula. Species Navicula capitatoradiata* even reached the highest abundance in the summer sample from the lowest site (S3), the second in the S1 and third in S2 (Tab. 3). Characteristic species for summer assemblages was also *Cymbella affinis* which was the second or third most abundant diatom.

The most abundant winter diatoms with relative abundance ≥ 5% in the winter period, beside the common *Achnanthes biasolettiana* and *Nitzschia fonticola,*were: *Nitzschia dissipata* and *Navicula reichardtiana.* Two of them - *Achnanthes biasolettiana* and *Nitzschia dissipata* were reported as most abundant in winter sample by Toman et al. (2014).

Taxa characteristic for spring samples were *Gomphonema angustatum* and pioneer species *Amphora pediculus.* The latter reached 26% share in S3 indicating the hydrological disturbance of the site due to high water levels.

The most diverse genus was *Navicula* with 13 species found, which was in accordance with findings of Soltanpour-Gargari et al. (2011) and Toman et al. (2014).

The lowest value of the diversity index (2.50) was calculated from the spring sample at the first site (S1) due to the dominance of *Achnanthes biasolettiana* (57%), which has a pioneer character.

The greatest diversity index value (4.1) and the highest periphyton biomass were in the S3 winter sample, where nitrate concentration was high (6.6 mg/L). Moreover, the highest trophic index was calculated for this sample. Measurements showed the highest concentration of dissolved oxygen and pH, as well as coverage with filamentous algae at the site S3. We can assume from this that diversity of epilithic diatom community increases with increasing amount of nutrients and dissolved organic matter (DOM), meaning that the studied part of the Savinja River is in considerably good ecological status. This sample contained 33 diatom species and had the highest number of taxa (8) with relative abundance of at least 5%, i.e., *Achnanthes biasolettiana, Nitzschia fonticola, Amphora pediculus, Nitzschia dissipata, Diatoma vulgaris, Navicula reichardtiana, Navicula lanceolata, Navicula gregaria.* Significant correlations between Shannon-Wiener diversity index and trophic as well as saprobic index (Tab. 4) were calculated, meaning that the higher the concentration of nutrients and dissolved organic matter, the higher the diverstity of diatoms.

Water quality

Water quality was evaluated using the saprobic index. Upper site belongs to oligo-β-mesosaprobic status, characteristic of moderate organic loading.

Samples from the sites downstream of the town Celje were classified into 2nd class which is the β-mesosaprobic class (Tab. 3) and indicates low organic loading. The exception was the summer sample from the second location (S2), which was classified to 1st-2nd class and displayed slightly better condition. The most abundant taxon (except in S3_P) was *Achnanthes biasolettiana* which is characteristic for β-mesosaprobic state (Hoffman et al. 2011). Our results are similar to those obtained by Koren (2009), but on the base of trophic index we found out that there was less nutrient loading on the site S2 (downstream the town) than seven years ago. However, the trophic index reached the highest values in the lowest site (S3), which can be explained with suboptimal efficiency of tertiary purification processes and nutrient removal in the Celje wastewater treatment plant (WWTP).

Influence of environmental parameters on diatom species composition

Influences of environmental factors on the diatom community were tested using RDA. The significant variables explained almost 36% of diatom species composition (Tab. 5), which is lower than results published by Passy (2007), where the share reached 60%. A possible reason for mentioned differences could be much smaller size of research area. Soininen (2007) reports that the relative importance of environmental and spatial factors varies with study scale and distance effects are negligible over small scales.

The highest share of variability of the studied epilithic diatom community was explained by O_2

saturation and saprobic index calculated on the base of all algae.

Diatoms presented on the left side of Fig. 3B seemed to prefer water with higher O_2 saturation and were found in the summer samples from all three sites (Fig. 3A). At the time of summer sampling the water temperature was relatively high (21-23 °C), which contributed to the high saturation values. Diatoms distributed on the right side of the diagram (Fig. 3B) are found in the downstream sites S2 and S3 (Fig. 3A) and prefer water with high content of dissolved organic matter that also results in lower O_2 saturation.

Parameters that influence the structure of epilithic communities often have synergistic effects making the influence of a single parameter on the species composition, diversity and other community characteristics hard to define. Furthermore, there is also the influence of biotic interactions (grazing, competition) which are very difficult to quantify. The mentioned facts could be possible reasons for the differing conclusions on the importance of various factors in structuring epilithic diatom communities (Soininen 2007, Lange et al. 2011, Beltrami et al. 2012).

Correlation between environmental parameters and diversity of diatoms

Species diversity of certain community is determined by the diversity of habitats, amount and diversity of nutrients, water temperature, flow regime and stability of the ecosystem, which depends mainly on hydrological disturbances and pollution (Moss 2010, Zelnik 2015). Velghe et al. (2012) calculated negative correlation between diatom species richness and amount of phosphorous. In our case S-W diversity index (diatoms) was unexpectedly positively correlated ($p < 0.05$) with the saprobic index calculated on the basis of all algae, moreover highly significant ($p <0.01$) correlation was calculated with trophic index (Tab. 4). These findings indicate low content of organic matter and nutrients, too low even to enable the thriving of species-rich epilithic diatom community, which needs higher amounts of nutrients and/or organic matter to support high number of species. Diatom species richness was positively correlated with O_2 saturation, which is expected to decrease with increasing content of DOM. Due to turbulent flow and fully insolated channel, water contains sufficient amount of oxygen even in reaches with higher content of organic matter.

Conclusion

Our results revealed considerable changes in diatom species composition during the year which exceeded the changes between the sites despite all of the human influences in urban landscape, which is not in accordance with the findings of (e.g. Passy 2007), who observed minor changes in diatom community composition between seasons. However, saprobic index as well as trophic index calculated for single samples showed the differences between the sampling sites. Saprobic index was lower in the site S1 which was classified into oligo- to β-mesosaprobic class, whereas S2 and S3 were classified into β-mesosaprobic class. Trophic index reached the highest values in the site S3, which can be explained with suboptimal efficiency of nutrient removal in the WWTP.

The highest share of variability of the epilithic diatom community was explained by oxygen saturation (35%) and saprobic index (33% of TVE), which are greatly influenced by human impacts. Both above mentioned parameters were also positively correlated with diversity of diatom communities, meaning that the increasing amount of organic matter and nutrients, respectively, increase the diversity of diatom community. Since the Water Framework Directive has been accepted by the European Commission and member states, the official monitoring system is more focused on the evaluation of ecosystem status than water quality status. Community of benthic diatoms is an essential element of the mentioned monitoring, as the diatoms respond to the amount of nutrients and dissolved organic matter. These characteristics define them as good indicators of an ecological status and should be used further in monitoring. We also confirm the importance of sampling date for the monitoring.

Povzetek

Spremljanje ekološkega stanja vodnih ekosistemov je bistvenega pomena za oceno človeškega vpliva na vodno okolje in vrednotenje učinkovitosti upravljanja z vodami. Slabšanje kakovosti vode je posledica industrijskih, komunalnih in kmetijskih virov, ki proizvajajo širok spekter polutantov. Ker so alge zelo dober pokazatelj sprememb v kakovosti vode, smo jih uporabili za določanje stanja izbranega odseka reke Savinje na območju Celja in njegove okolice.

Na približno 10 km dolgem odseku smo izbrali tri vzorčna mesta. Med mesecem septembrom 2011 in marcem 2012 smo izvedli tri vzorčenja, in sicer poletno, zimsko in spomladansko. Perifiton smo vzorčili po metodi pobiranja in strganja kamnov s skalpelom in ščetko. Ob vsakem vzorčenju smo spremljali tudi hidrološke, kemijske in fizikalne parametre. S Shannon-Wienerjevim indeksom smo ocenili diverziteto perifitonske združbe, s saprobnim indeksom organsko obremenjenost vodnega okolja, s trofičnim indeksom po Rottu (1999) pa smo spremljali obremenjenost s hranili. Glede na vrednosti saprobnega indeksa smo vzorčna mesta uvrstili v kakovostne razrede.

Naši rezultati so pokazali, da se je perifitonska združba spreminjala sezonsko ter med vzorčnimi mesti, hkrati pa so se spreminjali tudi abiotski dejavniki. V vseh vzorcih so prevladovale kremenaste alge, po deležu pa so jim sledile zelene alge in cianobakterije. Ugotovili smo, da je izbran odsek reke Savinje malo ali zmerno organsko obremenjen. Na podlagi vrednosti trofičnega indeksa smo ugotovili, da je s hranili najbolj obremenjeno vzorčno mesto S3, kar pripisujemo vplivu iztoka iz centralne čistilne naprave Celje in suboptimalni učinkovitosti terciarnega čiščenja. S klastrsko analizo narejeno na podlagi Sørensenovega indeksa podobnosti, kjer upoštevamo samo prisotnost vrst, ne pa številčnosti smo potrdili našo hipotezo, da bodo razlike v vrstni sestavi in diverziteti perifitonske združbe večje med sezonami kot pa med posameznimi vzorčnimi mesti. Rezultati naše raziskave so bili zelo podobni rezultatom, ki jih je dobil Koren (2009), le na podlagi trofičnega indeksa smo ugotovili, da se je stanje obremenjenosti s hranili na mestu S2 izboljšalo. Z nizom redundančnih analiz (RDA) smo ugotovili, da ima na taksonomsko sestavo združbe diatomej statistično značilen vpliv nasičenost s kisikom in saprobni indeks izračunan s pomočjo vseh alg. Pri analizi korelacij med diverziteto in ostalimi parametri, smo izračunali pozitivno korelacijo med Shanon-Wienerjevim indeksom ter trofičnim in saprobnim indeksom, medtem ko je bilo število taksonov v statistično značilni povezavi z nasičenostjo s kisikom. Iz dobljenih rezultatov sklepamo, da na perifitonsko združbo v reki Savinji na preučevanem odseku mesto Celje kljub vsemu nima velikega vpliva in da odsek ni bil tako onesnažen kot smo pričakovali. Potrdimo lahko tudi pomembnost datuma vzorčenja v monitoringu, ki ga moramo upoštevati za ustrezno vrednotenje ekološkega stanja in primerjavo z drugimi območji in leti.

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