

# Longitudinal and seasonal succession of algal periphyton colonization in lowland river (Tundzha River Part of Turkey)

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The aim of this paper is to determine and compare the environmental factors controlling longitudinal colonisation of periphytic algae in agricultural and urbanization effects of a lowland river, the Tundzha River, located in Turkish Tunca. To investigate the effect of the environmental factors on periphyton colonization at the river, 6 stations were selected and samples were collected between April 2012 and March 2013. Canonical correspondence analyses have been applied to clarify relationships between environmental variables and periphytic algae. During the study, the Shannon-Wiener diversity index ( $H'$ ) varied from 1.62 to 3.91. The phytoplankton biovolume was positively related to pH, temperature, salinity, chlorophyll- *a* and nutrients, and was negatively correlated with dissolved oxygen, turbidity, and silicate. Out of the 5 divisions and of 73 identified species of phytoplankton, the diatoms, namely *Fragilaria ulna*, *Cymbella tumida*, *Cocconeis placentula*, *Gomphonema acuminatum* and *Cymbella cystula* were found to be dominant. In addition to these species, the biovolumes of filamentous diatom *Melosira varians*, filamentous blue-green algae *Oscillatoria limosa*, and placoderm desmids *Cosmarium botrytis* were determined to be at high levels during the year. The euglenoid blooms in St.5 showed many times during the study period.

# **Longitudinal and Seasonal Succession of Algal Periphyton Colonization in Lowland River (Tundzha River Part of Turkey)**

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24 **Keywords:** Periphytic algae, species composition, longitudinal changes, Tundzha River

## 25 INTRODUCTION

26 The term 'periphyton' or 'aufwuchs' has been commonly used in literature to include all  
27 organisms (heterotrophs and autotrophs) growing in association with submerged substrata  
28 (Wetzel, 2001). Periphytic algae, a member of the periphyton family, is one of the most  
29 important microorganism communities, which can be found in almost all aquatic systems and  
30 play an important role in the maintenance of ecosystems by supporting fluvial food webs,  
31 removing nutrients from the water column and attenuating the current and stabilizing sediments  
32 (Stevenson, 1996; Masseret et al., 1998; Dodds & Biggs, 2002). Fluvial ecosystems differ from  
33 one another in their quantity, timing and temporal patterns of river flow, and this profoundly  
34 influences their physical, chemical and biological condition (Allan & Castillo, 2007). One of the  
35 components of freshwater bodies is a benthic algal community which is affected by all these  
36 three conditions in one way or another.

The composition of a benthic algal community can be influenced by many proximate factors including light, temperature, currents, substrate, scouring by floods, water chemistry and grazing. Light and nutrients, upon interaction with temperature, influence biomass accrual while disturbance (substrate turnover and transport, high current velocities) and grazing lead to the dislodgement of algae. The importance of any one factor to algal growth depends upon whether some other factor is in even shorter supply, and these environmental conditions vary by location and season (Allan & Castillo, 2007).

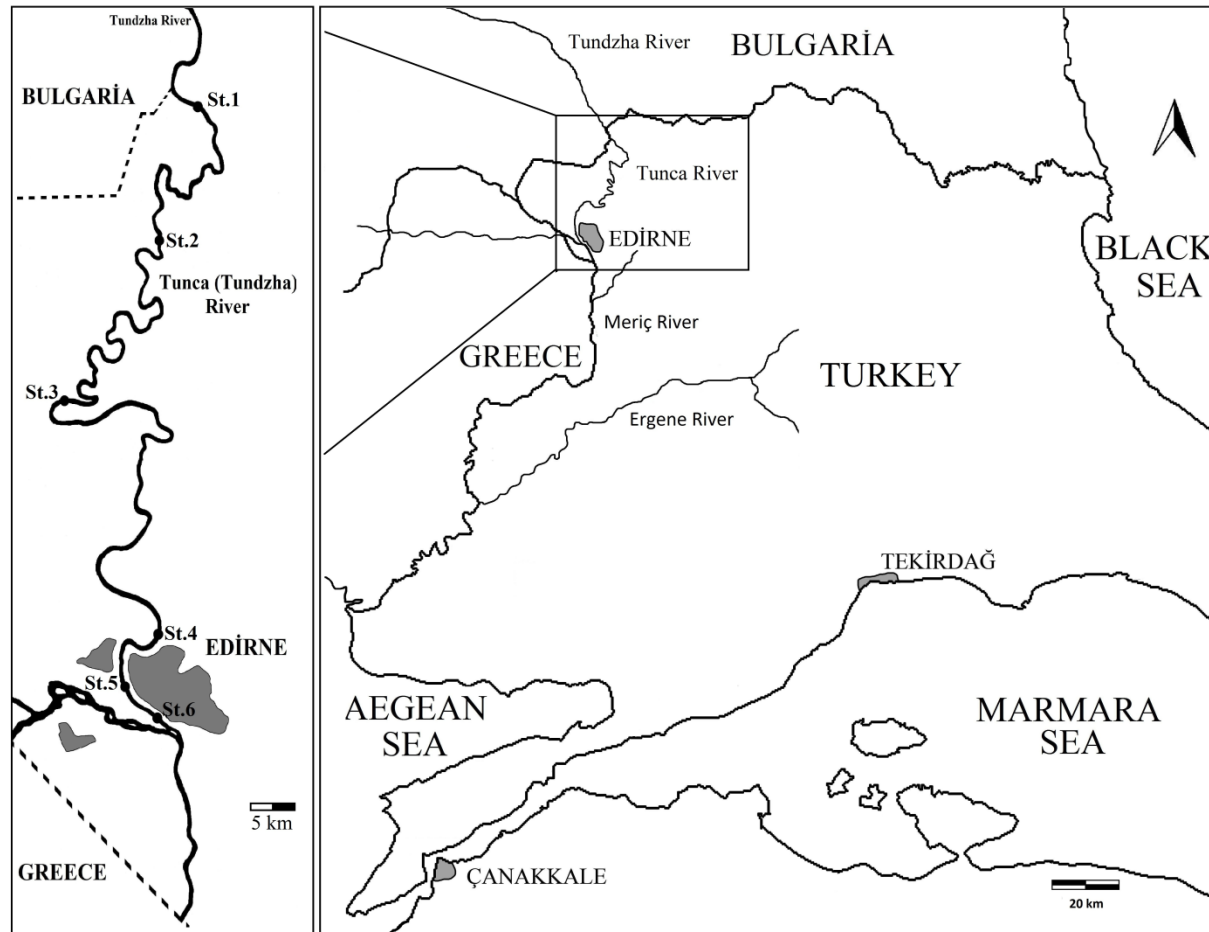
This paper analysed the effects of physical and environmental factors on seasonal variations of periphyton, biovolume, community structure and abundance at the 6 stations chosen from the Tundzha River. The seasonal fluctuations of the periphytic algal communities in the Tundzha River and the effects of agricultural activities and urbanization were analysed statistically.

## EXPERIMENTAL PROCEDURES

### *Study area and sampling*

The Tundzha River runs from west to east before turning south to join the Maritsa River in Edirne, Turkey. The river, whose source is in the Stara Planina Mountains, flows for a length of 350 km in Bulgaria and 56 km in Turkey. The catchment areas within Bulgaria and Turkey are about 7.780 and 155 km<sup>2</sup>, respectively.

The part of Tundzha River located within the present study area is surrounded by human settlements and agricultural areas where paddy, mustard and other crops are cultivated with the application of fertilizer and pesticides throughout the year. The river bank is characterized by scattered bushy and patches of tree formations (Fig. 1).



**Figure 1.** Location of Tundzha River and sampling sites within the Turkish Thrace.

Samplings were performed monthly in 6 stations from April 2012 to March 2013. The stations were selected longitudinally for random sample collections starting at the Bulgarian-Turkish border and continuing to the part of the river where it flows into the Maritsa River. First, three stations were selected outside of the city (rural zone), and then, another three stations were selected around the city (urban zone). During sampling, temperature (Hg thermometer), pH (Lovibond Sensodirect pH200), dissolved oxygen (DO) (Lovibond Sensodirect Oxi200) and conductivity (Lovibond Sensodirect Con200) were measured on-site with related equipment. Silica, nitrite-nitrogen, nitrate-nitrogen, total suspended solids (TSS) and total phosphorus content (TP) (Apha, 2012) of water from sampling stations were measured in the laboratory. Stations were subjectively split into two groups as (1) rural zone (stations 1, 2 and 3) and (2) urban zone (stations 4, 5 and 6). Stations, their specifications and sampled periphyton types are

shown in Table 1. The stations were chosen according to the environmental and habitat characteristics that we believed would best represent the river. The first 3 stations were chosen from outside the city centre, as seen in Figure 1 and Table 1. At this region of the river, there are some villages and settlements, and on both sides of the river, there is intensive agricultural activity; particularly rice, sunflower and wheat. Stations 4, 5 and 6 were identified as urban zones and were chosen from around the city centre. At this region, modifications and the effects of the city on the river, riverside and river bed are abundant.

**Table 1.** Stations and collected algal sampling types.

	St. No	Locality	Bottom	Collected periphyton sampling		
Rural zone	St.1	After the Bulgarian-Turkish Border	Pebbles and sand	Epilithic		
	St.2	Enter the Yolüstü Village	Pebbles and sand	Epipelic	Epilithic	Epiphytic
	St.3	Meander near Değirmenyeni village	Muddy and macrophytes	Epipelic	Epiphytic	
Urban zone	St.4	Enter the Edirne City	Muddy and macrophytes	Epipelic	Epilithic	Epiphytic
	St.5	Middle in the Edirne City	Ooze	Epipelic	Epilithic	
	St.6	Before of wedding Meriç River	Ooze	Epipelic		

For algal periphyton sampling, only three categories of periphyton were considered: (1) that randomly chosen from the littoral (epilithon), (2) samples present as attached to stems of both living and dead macrophytes such as *Typha* sp. and *Phragmites* sp. (epiphyton), and (3) benthic periphyton overlying mud and sand (epipelon). These delineations are generally consistent with those for other wetlands and littoral habitats (Goldsborough & Robinson, 1996; Azim et al., 2005). For epilithon sampling, stones of desired size were scraped with a brush or a knife in a 200 ml water body, and the surface area of the sampled stone was measured. For epiphyton sampling, samples were obtained from stems of macrophytes via a scraping method and washed in 200 ml water, and the diameters and lengths of the sampled stem parts were measured. The epipelic algal community was sampled by means of a glass tube 0.8 cm in diameter and 100 cm in length, from the shore side of the river (littoral zone). The pipe was moved in a circular direction over the surface of the sediment, releasing the thumb to take up the sediment (Margalef, 1949; Round, 1953; Sladeckova, 1962; Aloï, 1990). One 50 ml subsample of periphyton-containing water was used for algal counting according to Utermöhl (1958), using a Olympus CK2 inverted microscope with an objective of a 400-fold increase. For each course,

600-800 cells were counted. A 50 ml subsample of periphyton homogenate was also preserved in Lugol's solution for taxonomic identification of the algal community. To determine the diatoms, water samples were treated with H<sub>2</sub>O<sub>2</sub>, and permanent slides were prepared using Naphrax resin as the embedding medium for diatom frustules. The remaining slurry (100 ml) filtered from the glass-fibre filters (Whatman GF/C) and chlorophyll-*a* were measured using ethanol as a solvent, according to Nush (1980). Species' density was estimated according to Ros (1979) and the results were shown in the number of individuals per unit area (ind cm<sup>-2</sup>). The biovolume estimate followed Hillebrand et al. and Sun & Lui from the multiplication of the densities of each individual by the average biovolume of organisms of the species, according to the size of analysed populations, and the final result was given in mm<sup>3</sup>cm<sup>-2</sup> (Hillebrand, et al. 1999; Sun & Lui, 2003). Morphological details of all sampled algae were studied using a research microscope (Olympus CX21), and identification of taxa was done with the help of available literature (Husted, 1930; Cleve-Euler, 1952; Pestalozzi, 1952, 1982; Prescott, 1973; Patrick & Reimer, 1975; Komárek & Fott, 1983; Krammer & Lange-Bertalot, 1991a, 1991b. 1999), and finally, all species were checked in the Algaebase site (Guiry et al., 2010).

# Statistical analysis

The relationships between periphytic algal groups, chlorophyll-*a*, and water quality parameters were explored by canonical correspondence analysis (CCA) using the XLSTAT-ADA statistical package (Addinsoft, 2015). The relationships between environmental parameters and dominant species were evaluated using Spearman's rank correlation test. Changes in periphyton dynamics were examined using Shannon's diversity index, H', calculated from the biovolume data as:

$$H' = -\sum_{i=1}^s p_i \log_2 p_i$$

Where  $p_i$  is the relative biovolume of species and  $i$  and  $s$  are number of species (Sommer, 1993). Equitability (E) was calculated as  $H'/H'^{\max}$ . A Bray-Curtis analysis was performed to reveal similarities, if any, among stations based on periphytic algal species diversity and abundance (Bray & Curtis, 1957).

## 129 RESULTS

### 130 *Environmental conditions*

131 The values of the physical and chemical parameters of the sampling stations including  
 132 dissolved oxygen (DO), water temperature, conductivity, pH, silica,  $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N, and total  
 133 phosphate in the water from six sampling stations on the Tundzha River are provided in Table 1.  
 134 Basically, among these parameters, water temperature showed obvious seasonal change, as the  
 135 water temperature of the river ranged from 2°C to 28°C during the sampling period. The river  
 136 water was slightly alkaline with pH values that varied between 7.65 and 8.85 (mean 8.4). DO  
 137 was recorded ranging from 2.66 to 8.75  $\text{mg L}^{-1}$  (mean 5.6), representing a congenial environment  
 138 for biota. Conductivity was recorded ranging from 408 to 812  $\mu\text{S cm}^{-1}$  (mean 563).  $\text{NO}_2^-$ -N was  
 139 estimated at an average 0.027  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$ -N, was estimated at an average 6.4  $\text{mg L}^{-1}$ . TP was  
 140 recorded ranging from 142 to 746  $\mu\text{g L}^{-1}$  (mean 357). TSS was recorded ranging from 83 to 480  
 141  $\text{mg L}^{-1}$  and  $\text{SiO}_2$  was recorded ranging from 325 to 1890  $\mu\text{g L}^{-1}$  (mean 965) (Table 2).

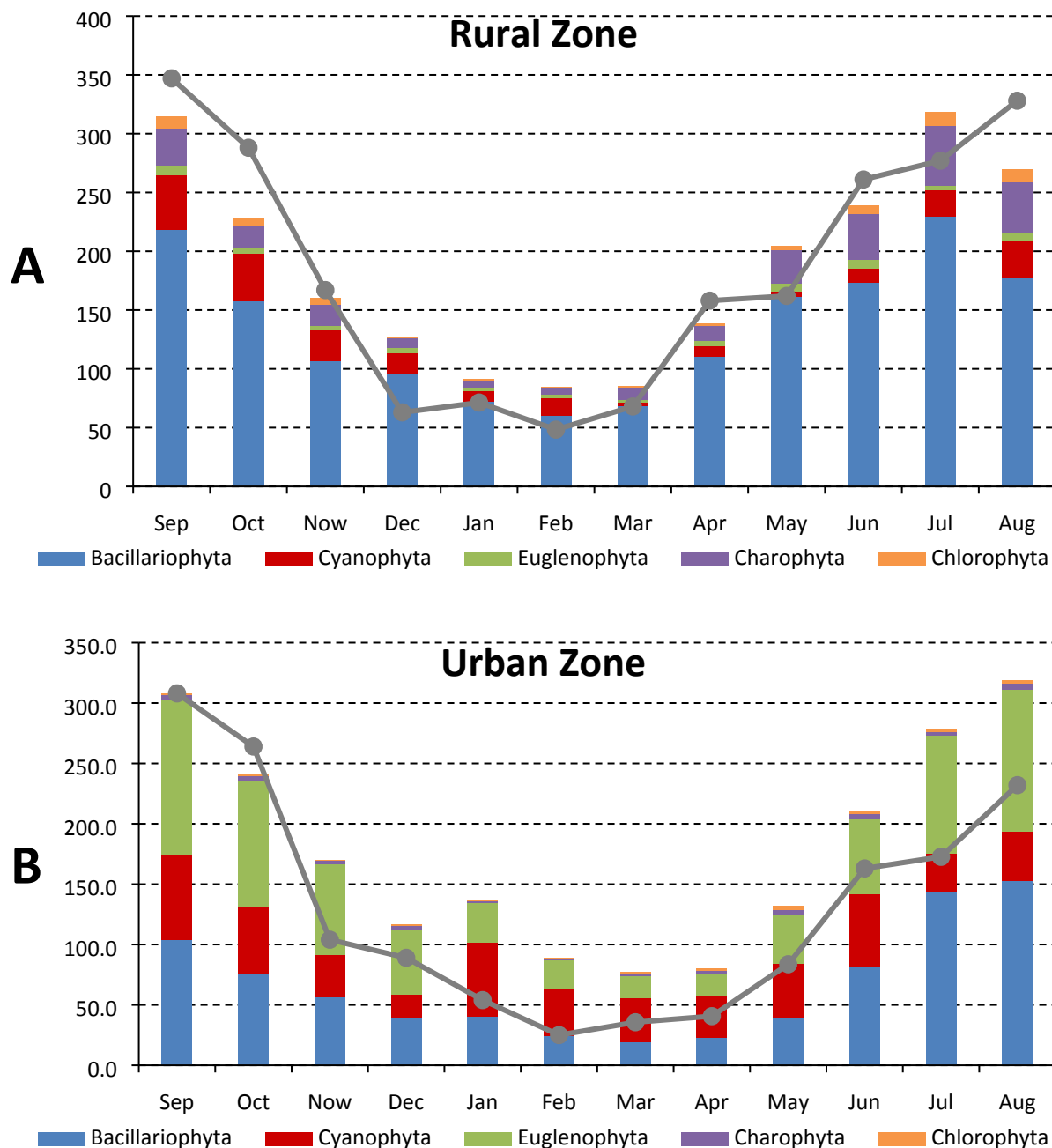
142 **Table 2.** Summary of physical and water quality characteristics of the study parts of the Tundzha  
 143 River (mean values of chemical variables measured at the sampling stations).

	Rural Zone				Urban zone			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Temperature (°C)	15,8	4,3	15,2	24,2	15,8	4,2	16,0	24,6
Diss. Ox. ( $\text{mg L}^{-1}$ )	5,13	6,27	4,61	7,48	4,67	5,82	4,43	6,45
pH	8,91	9,20	8,64	8,09	8,46	8,77	8,49	7,84
Conductivity ( $\mu\text{S cm}^{-1}$ )	519	479	655	566	534	471	666	616
N- $\text{NO}_2^-$ ( $\text{mg L}^{-1}$ )	0,04	0,03	0,02	0,01	0,05	0,05	0,02	0,01
N- $\text{NO}_3^-$ ( $\text{mg L}^{-1}$ )	2,31	4,69	12,30	4,81	5,07	6,25	12,89	7,91
TP ( $\mu\text{g L}^{-1}$ )	45,7	47,8	56,2	64,2	65,7	50,6	54,3	72,1
$\text{SiO}_2$ ( $\mu\text{g L}^{-1}$ )	1550	773	801	1169	1239	628	623	941
Chl- <i>a</i> ( $\mu\text{g L}^{-1}$ )	267,3	60,9	129,3	288,7	225,3	56,0	53,3	189,2
TSS ( $\text{mg L}^{-1}$ )	135	239	207	162	148	332	263	173
Biovolume ( $\text{mm}^3\text{cm}^{-2}$ )	234,4	100,9	142,8	288,8	246,4	114,0	96,6	269,6

145 The seasonal variation of chlorophyll-*a* (chl-*a*) concentration and calculated biovolume  
 146 showed considerable difference between the rural and urban zones ( $p < 0.05$ ). Maximum chl-*a*



147 was recorded in September at the rural zone (St.3) as  $422 \mu\text{g cm}^{-2}$ , and minimum chl-*a* was  
 148 measured in November at the urban zone (St.4) as  $19 \mu\text{g cm}^{-2}$ . Maximum biovolume at the rural  
 149 zone was recorded in July at St.3 as  $318.4 \mu\text{m}^3 \text{cm}^{-3}$  and the minimum biovolume was measured  
 150 in April at St.5 as  $52.4 \mu\text{m}^3 \text{cm}^{-3}$  (Fig. 2).



153 **Figure 2.** Seasonal composition of chlorophyll-*a* and biovolume on the Tundzha River (A-rural  
 154 zone, B-urban zone). X axis showed biovolume ( $\mu\text{m}^3 \text{cm}^{-3}$ ) and chlorophyll-*a* ( $\mu\text{g cm}^{-2}$ ).

Mean chlorophyll-*a* concentration at the rural zone was 160.8, 282.5 and 229.9  $\mu\text{g cm}^{-2}$  at St.1, St.2 and St.3, respectively. The same seasonal data was also obtained for biovolume ( $r=0.71, 0.67, 0.79$ ;  $p<0.01$  at St.1, St.2 and St.3). Similarly, mean chlorophyll-*a* concentration at the urban zone was 154.1, 125.8 and 114.8  $\mu\text{g cm}^{-2}$  at St.4, St.5 and St.6, respectively. Therefore, the similar seasonal data was also obtained for biovolume ( $r=0.61, 0.53, 0.57$ ;  $p<0.01$  at St.4, St.5 and St.6).

Total algal biovolume was positively correlated with chlorophyll-*a*, TSS, and nitrate-nitrogen and negatively correlated with TP at the rural zone ( $r=0.74, r=0.66$ , and  $r=-0.74$ ;  $p<0.01$ , respectively) and the urban zone ( $r=0.58, r=0.61$ , and  $r=-0.52$ ;  $p<0.01$ , respectively).

### *Periphyton composition*

From the total of 73 taxa examined in this study, 51 taxa of Bacillariophyta (69.8%), 8 taxa of Chlorophyta (10.9%), 5 taxa of Cyanophyta (6.9%), 5 taxa of Charophyta (6.9%) and 4 taxa of Euglenophyta (5.5%) were identified (Table 3). The highest number of periphytic algal taxa (69 taxa) was obtained at St. 3 where the river meanders, the water flow is low and aquatic macrophytes are common. The lowest taxa number (41 taxa) was obtained at St.5. The species richness values in all sampling stations are provided in Figure 3.

**Table 3.** Periphytic algal communities of the Tundzha River.

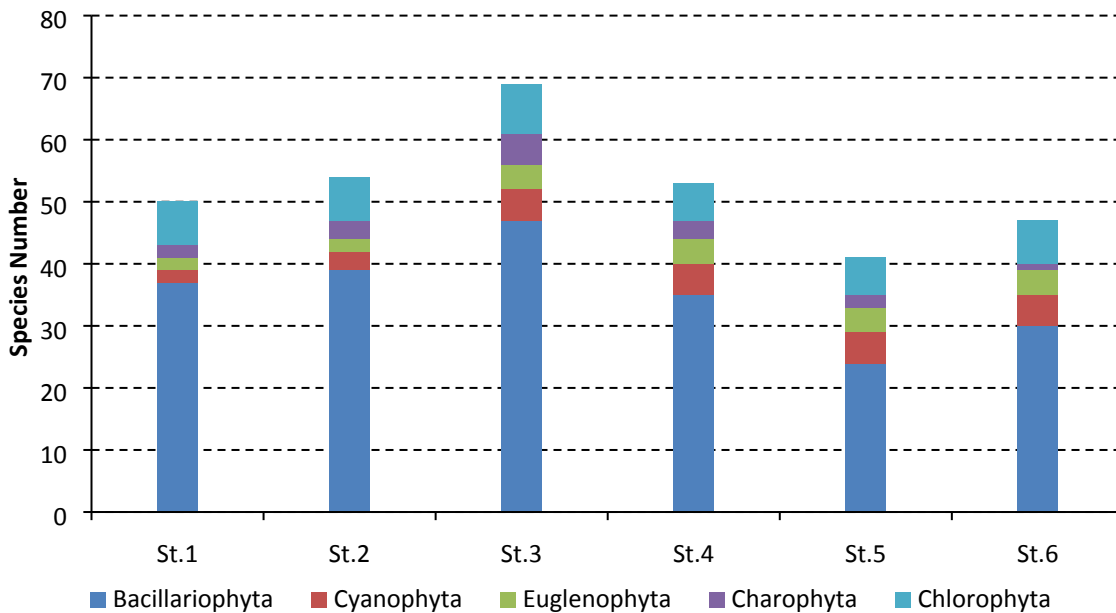
	St.1	St.2	St.3	St.4	St.5	St.6
<b>Empire</b> Prokaryota						
<b>Kingdom</b> Eubacteria						
<b>Subkingdom</b> Negibacteria						
<b>Phylum</b> Cyanobacteria						
<b>Class</b> Cyanophyceae						
<i>Anabaena affinis</i> Lemm.	□	□	■	■	■	■
<i>Leptolyngbya granulifera</i> (Copeland) Anag.	□	■	■	■	■	■
<i>Lyngbya</i> sp.	□	□	■	■	■	■
<i>Oscillatoria limosa</i> Agardh	■	■	■	■	■	■
<i>Oscillatoria tenuis</i> Gomont	■	■	■	■	■	■
<b>Empire</b> Eukaryota						
<b>Kingdom</b> Protozoa						
<b>Phylum</b> Euglenophyta						
<b>Class</b> Euglenophyceae						
<i>Euglena acus</i> Ehren.	■	■	■	■	■	■

<i>Euglena polymorpha</i> Dangeard	■	■	■	■	■	■
<i>Phacus acuminatus</i> Stokes	□	□	■	■	■	■
<i>Trachelomonas volvocina</i> (Ehren.) Ehren.	□	□	■	■	■	■
<b>Phylum Bacillariophyta</b>						
<b>Class Fragilariophyceae</b>						
<i>Achnanthes hungarica</i> (Grun.) Grun.	■	■	■	□	■	■
<i>Amphora ovalis</i> (Kütz.) Kütz.	■	■	■	■	■	■
<i>Caloneis amphisbaena</i> (Bory) Cleve	■	■	■	■	■	■
<i>Cocconeis placentula</i> Ehren.	■	■	■	■	□	■
<i>Craticula cuspidata</i> (Kütz.) Craw.&Mann	■	■	■	■	□	□
<i>Cymatopleura elliptica</i> (Bréb.) Smith	■	■	■	□	■	■
<i>Cymatopleura solea</i> (Bréb.) Smith	■	■	■	□	□	□
<i>Cymbella cystula</i> (Ehren.) Kirch.	■	■	■	■	■	■
<i>Cymbella lanceolata</i> (Agardh) Kirchner	□	□	■	□	□	□
<i>Cymbella naviculiformis</i> Heiberg	■	■	■	□	□	■
<i>Cymbella tumida</i> (Bréb.) van Heurck	■	■	■	■	■	■
<i>Diatoma vulgare</i> Bory de Saint-Vincent	■	■	■	■	■	■
<i>Epithemia sores</i> Kützing	■	□	■	□	□	□
<i>Fragilaria acus</i> (Kütz.) Lange-Bertalot	■	■	■	■	■	■
<i>Fragilaria crotonensis</i> Kitton	■	■	■	■	■	■
<i>Fragilaria ulna</i> (Nitz.) Lange-Bertalot	■	■	■	■	■	■
<i>Fragilariforma virescens</i> (R.) Wil.&Round	□	□	■	■	□	■
<i>Gomphonema acuminatum</i> Ehren.	■	■	■	□	□	□
<i>Gomphonema parvulum</i> (Kütz.) Kütz.	■	■	■	■	□	■
<i>Gomphonema truncatum</i> Ehren.	■	■	■	■	■	■
<i>Gyrosigma acuminatum</i> (Kütz.) Rab.	□	□	■	□	□	■
<i>Gyrosigma attenuatum</i> (Kütz.) Rab.	□	□	■	□	□	□
<i>Hantzschia amphioxys</i> (Ehren.) Grunow	□	■	■	■	□	□
<i>Mastologia smithii</i> Thwaites	■	■	□	■	■	□
<i>Navicula capitata</i> Ehren.	■	■	■	■	□	■
<i>Navicula cryptocephala</i> Kützing	■	■	■	■	■	□
<i>Navicula gracilis</i> Ehren.	■	■	■	■	■	■
<i>Navicula radiosa</i> Kützing	□	□	■	□	□	□
<i>Navicula tripunctata</i> (Müll.) Bory	□	□	□	■	■	□
<i>Navicula viridula</i> (Kütz.) Ehren.	■	■	■	■	■	■
<i>Nitzschia acicularis</i> (Kütz.) Smith	■	■	■	■	■	■
<i>Nitzschia angustata</i> (Smith) Grunow	■	■	■	■	□	□
<i>Nitzschia flexa</i> Schumann	□	□	■	■	□	■
<i>Nitzschia gracilis</i> Hantzsch	□	■	■	□	□	■
<i>Nitzschia hungarica</i> Grunow	■	■	■	■	■	■
<i>Nitzschia obtusa</i> Smith	□	□	■	□	□	□
<i>Nitzschia palea</i> (Kütz.) Smith	■	■	■	■	■	■
<i>Nitzschia parvula</i> Smith	■	■	■	□	□	□

<i>Nitzschia sigmoidea</i> (Nitz.) Smith	■	■	■	■	□	■
<i>Nitzschia tryblionella</i> Rab.	□	□	□	■	■	■
<i>Pinnularia acuminata</i> Smith	□	■	□	□	□	□
<i>Pinnularia brebissonii</i> (Kütz.) Rab.	■	■	■	□	□	□
<i>Pinnularia viridis</i> (Nitzsch) Ehren.	■	■	■	■	□	□
<i>Rhoicosphenia curvata</i> (Kütz.) Grunow	■	■	■	■	■	■
<i>Rhopalodia gibba</i> (Ehren.) Müller	□	□	■	□	□	□
<i>Sellaphora pupula</i> (Kütz.) Meres.	□	■	■	■	□	□
<i>Surirella robusta</i> Ehren.	■	□	■	■	■	□
<b>Class Coscinodiscophyceae</b>						
<i>Aulacoseira italica</i> (Ehren.) Sim.	■	■	■	■	□	□
<i>Melosira varians</i> Agardh	■	■	■	■	■	■
<b>Class Mediophyceae</b>						
<i>Cyclotella atomus</i> Hustedt	■	■	■	■	■	■
<i>Cyclotella meneghiniana</i> Kützling	■	■	■	■	□	■
<b>Phylum Charophyta</b>						
<b>Class Conjugatophyceae</b>						
<i>Closterium lunula</i> Ehren. & Ralfs	■	■	■	■	■	□
<i>Closterium parvulum</i> Nageli	□	■	■	■	□	□
<i>Cosmarium botrytis</i> Ralfs	■	■	■	■	■	■
<i>Mougeotia</i> sp.	□	□	■	□	□	□
<i>Spirogyra</i> sp.	□	□	■	□	□	□
<b>Phylum Chlorophyta</b>						
<b>Class Chlorophyceae</b>						
<i>Monoraphidium contortum</i> (Thuret) Fott	■	■	■	■	■	■
<i>Pediastrum boryanum</i> (Turpin) Meneg.	■	■	■	□	□	□
<i>Scenedesmus acuminatus</i> (Lag.) Chodat	■	■	■	■	■	■
<i>Scenedesmus bicaudatus</i> Dedusenko	■	■	■	■	■	■
<i>Scenedesmus quadricauda</i> (Turpin) Bréb.	■	■	■	■	■	■
<i>Scenedesmus sempervirens</i> . Chodat	■	■	■	■	■	■
<i>Schroederia antillarum</i> Komárek	□	□	■	□	□	■
<i>Tetraëdron minimum</i> (Braun) Hansgirg	■	■	■	■	■	■

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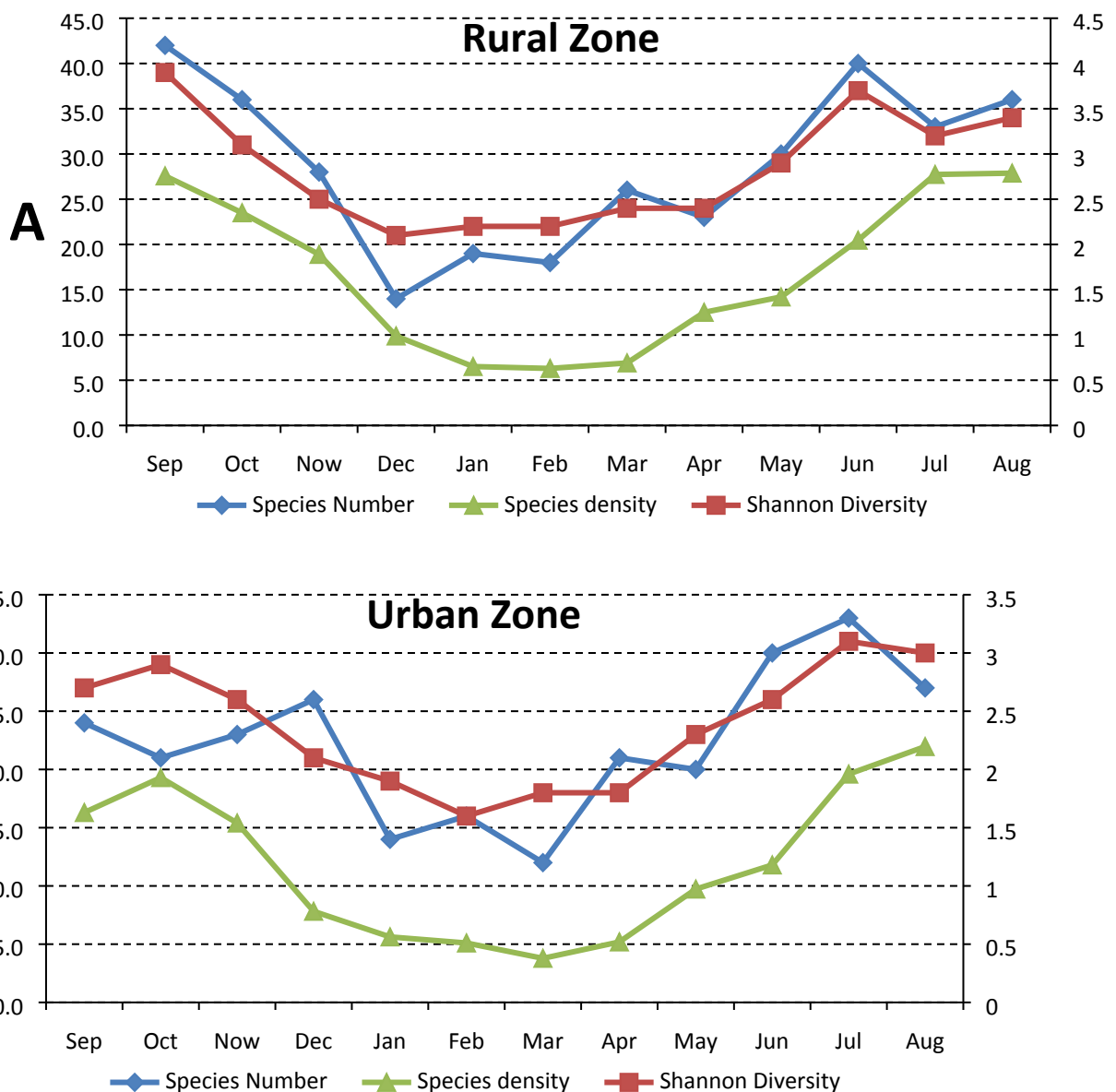
**Figure 3.** Species richness values in all sampling stations.

The monthly total taxonomic richness varied from 12 to 42 among stations. Species diversity at the rural zone showed a regular distribution pattern by increasing in spring and decreasing at mid-autumn (with a monthly average of 29), while it was irregular at the urban zone (monthly average of 22) in all stations during the 12-month study period, with no regular patterns. Species density at the rural zone showed two peaks, in June and September; these peaks were in August and October at the urban zone (Fig. 4a, b) which may be due to the availability of nutrients.

The Shannon index ( $H'$ ) ranged from 2.1 to 3.9 in the rural zone. In general, high values were found during the summer to autumn (except November), and low values were found during winter to early spring months. Maximum values were recorded in summer months (Fig. 4a). Very strong positive correlations were recorded between the Shannon diversity index and the number of species at all stations on this part of the river ( $r=0.71$ ,  $r=0.73$  and  $r=0.89$ ;  $p<0.01$ , St.1, St.2 and St.3, respectively).

The Shannon index ( $H'$ ) calculated for the urban zone ranged from 1.6 to 3.1. In contrast to the rural zone, the highest diversity values were found in autumn, and the lowest values were found in spring months. Positive correlations were recorded between the Shannon diversity index

192 and the number of species on all stations on this part of river ( $r=0.59$ ,  $r=0.39$  and  $r=0.47$ ;  $p<0.01$ ,  
193 St.1, St.2 and St.3, respectively) (Fig. 4b).



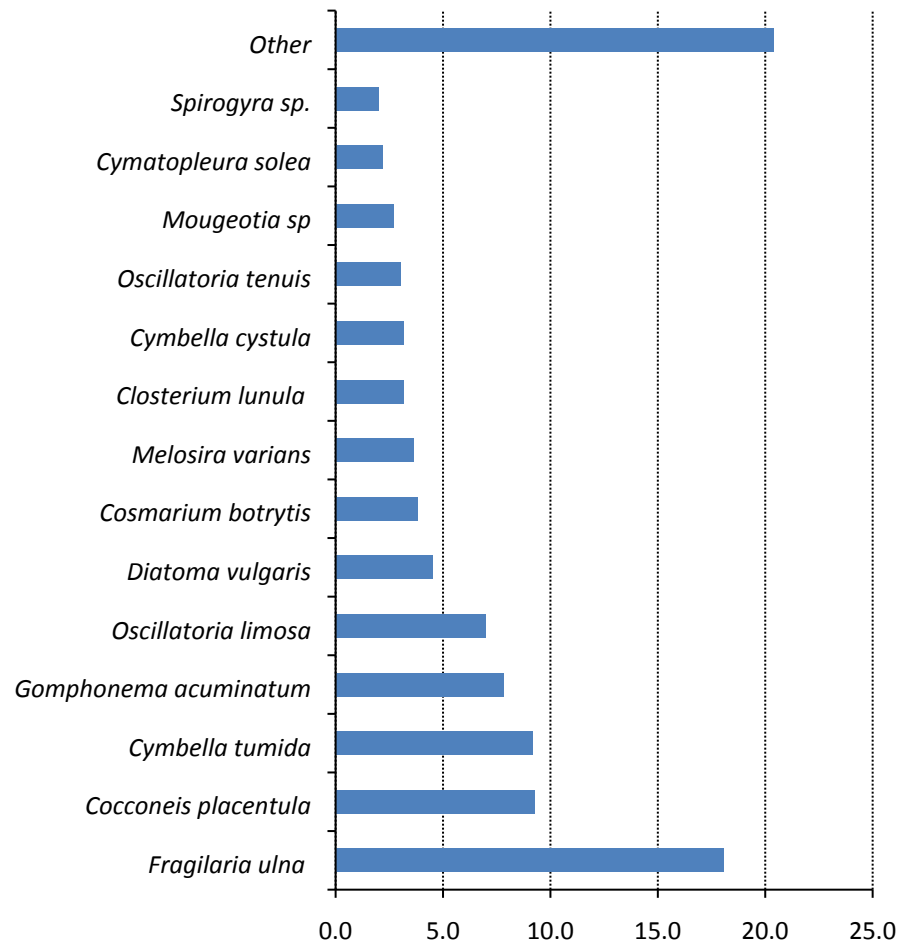
195

196 **Figure 4.** Seasonal composition of species density, species number and Shannon diversity in the  
197 Tundzha River (A-rural zone and B-urban zone). First X axis is given species density (%) and  
198 species number; second X axis is given Shannon index ( $H'$ ).

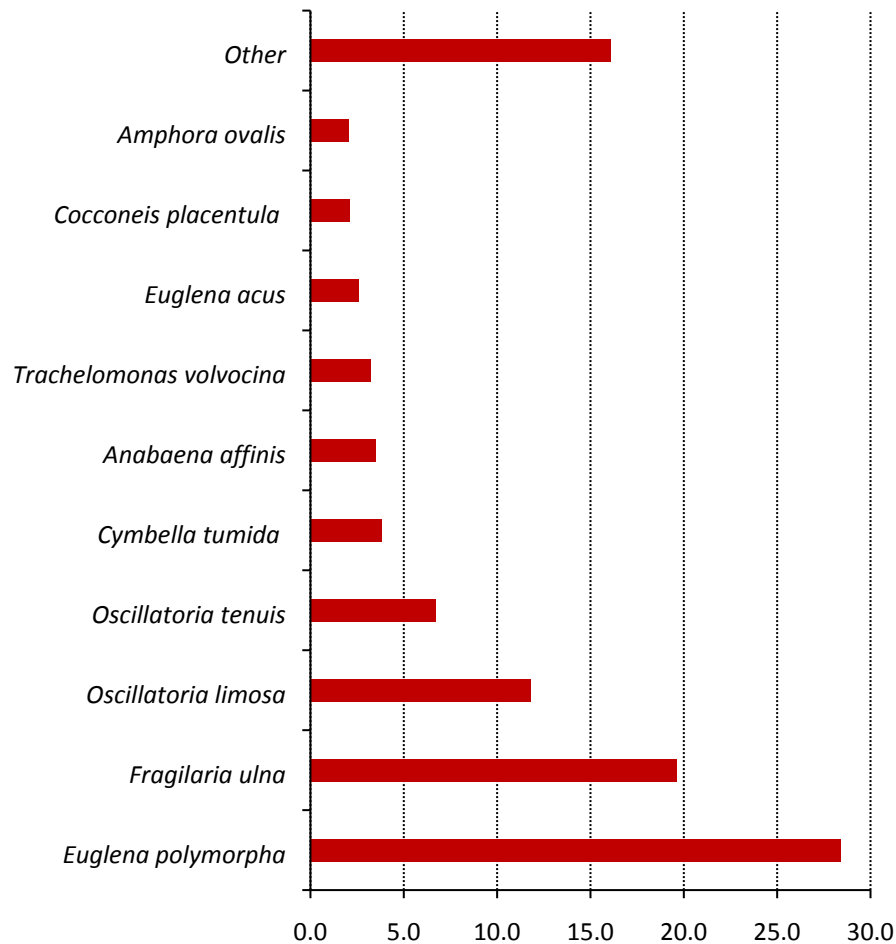
199 A total of 71 taxa were identified in the first 3 stations, which were regarded as the rural  
200 zone. The predominant species on this section of the river were mostly attached diatoms,  
201 including *Fragilaria ulna*, *Cymbella tumida*, *Cocconeis placentula*, *Gomphonema acuminatum*

and *Cymbella cystula*. In addition to these species, the biovolume of filamentous diatom *Melosira varians*, filamentous blue-green algae *Oscillatoria limosa*, and placoderm desmids *Cosmarium botrytis* were determined to be at high levels during the year. *Diatoma vulgare*, *Amphora ovalis* and chlorococcal green algae *Scenedesmus quadricauda* were the second-most dominant (subdominant) organisms, and the filamentous multicellular green algae *Mougeotia* sp. and *Spirogyra* sp. were found with high biomass values at St.3, which is characterized by low water current and dense macrophytes, during the whole sampling period. Although green algae *Monoraphidium contortum*, *Tetraëdron minimum* and diatoms *Achnanthes hungarica* and *Nitzschia palea* were found in high numbers at all stations, they never became biovolume dominant nor subdominant in periphyton because of their small size. The list of species in the rural zone with relative biovolume values above 2% are shown in Figure 5a.

A total of 59 periphyton taxa were identified in stations of the urban zone (St.4 to 6), and the predominant species on this section of river at all stations were mostly euglenoids *Euglena polymorpha*, *Euglena acus* and *Trachelomonas volvocina*. In addition, filamentous blue-green algae *Oscillatoria limosa*, *Oscillatoria tenuis* and *Anabaena affinis*, loosely attached diatom taxa *Fragilaria ulna* and attached diatom *Cymbella tumida*. In addition, flagellate euglenoid *Phacus acuminatus*, filamentous cyanophyte *Leptolyngbya granulifera* and adnate diatom species *Navicula tripunctata* were the second-most dominant taxa of this zone. Although green algae *Tetraëdron minimum* and diatom *Nitzschia palea* were found in high numbers at all stations, they never became biovolume dominant nor subdominant in periphyton because of their small size. The list of species in the urban zone with relative biovolume values above 2% are shown in Figure 5b.

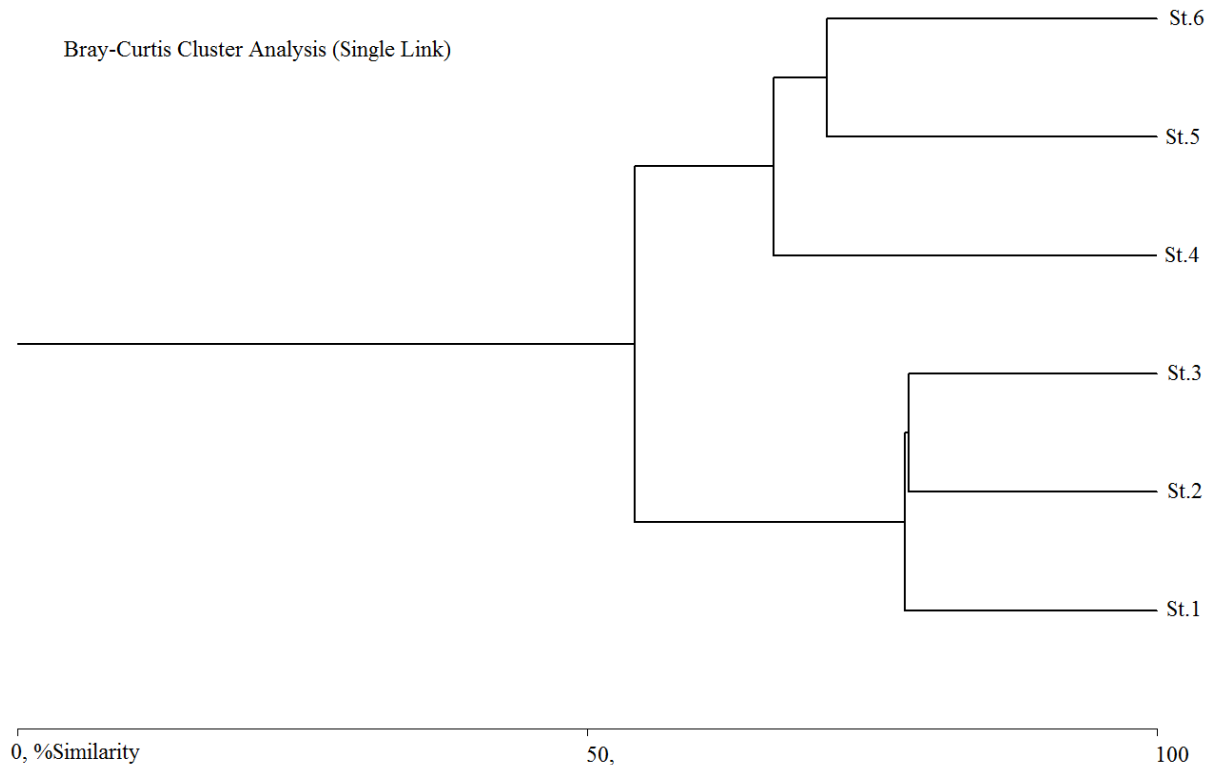






**Figure 5.** Highest relative biovolume in Tundzha River (A-rural zone, B-urban zone).

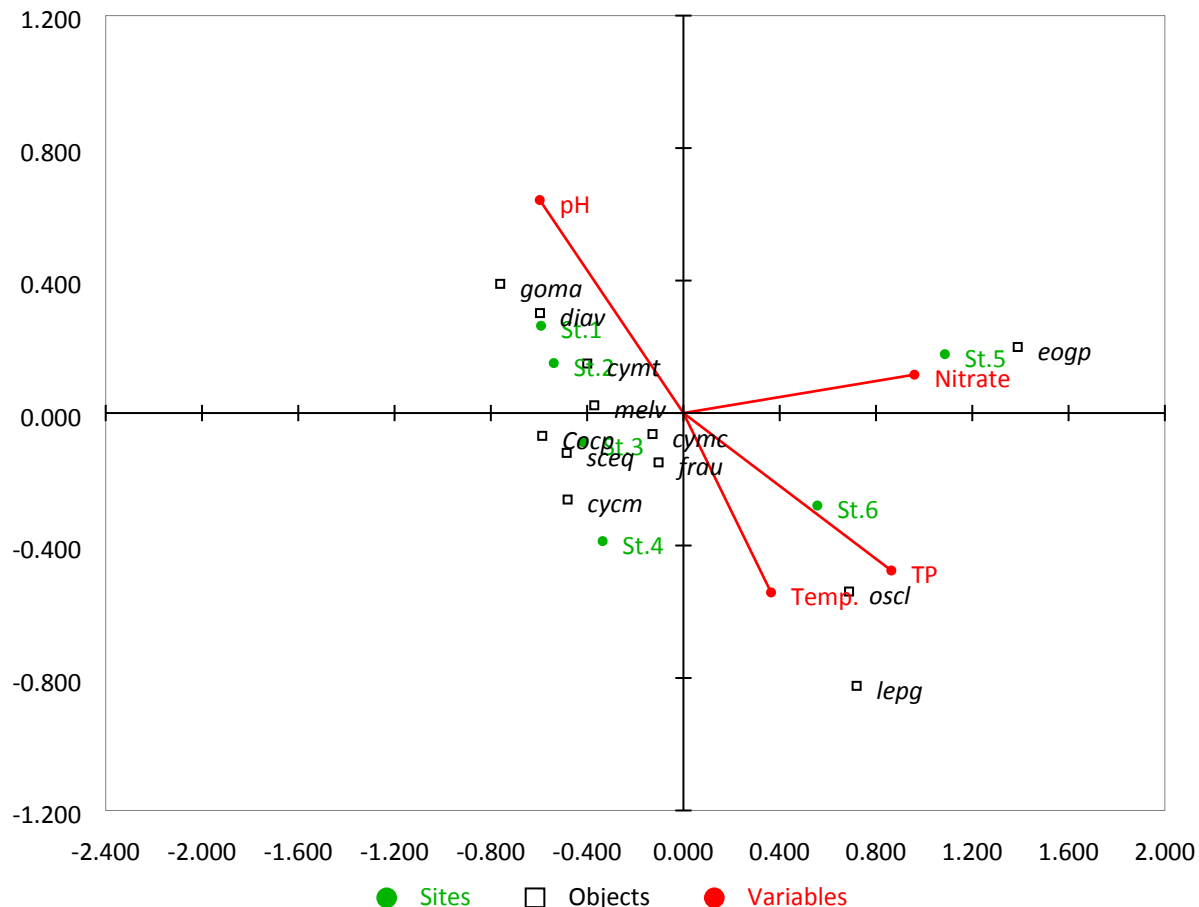
When floristic compositions of all 6 stations were used to obtain a similarity index through a cluster analysis, St.2, St.3 and St.1 are similar to each other, and St.5, St.6 and St.4 are similar to each other, respectively, for the dynamics (distribution, both in terms of species and the number of individuals) of periphyton in the river (Figure 6). The Bray-Curtis similarity index compares the stations both according to species findings and frequency of the specimens. The contaminants in the river were not measured between the two zones in terms of nutrients, but according to Bray-Curtis similarity indexes, there were obvious differences for both regions in terms of species composition and abundance.



**Figure 6.** Results of clustering analyses, based on differences in the floristic composition of phytoplankton in the Tundzha River (Bray-Curtis distance).

### Canonical analyses

The CCA ordination demonstrated the importance of the different environmental factors in determining dominant periphytic algal species. At St.6, *Leptolyngbya granulifera* and *Oscillatoria limosa* were placed close to temperature and total phosphate; in St.5, *Euglena polymorpha* was close to the nitrate vector and in St.1 and 2 *Cymbella tumida*, *Diatoma vulgaris*, *Gomphonema acuminatum* and *Melosira varians* were placed near the pH vector in the ordination diagram of CCA (Fig. 7).



245

246 **Figure 7.** Biplot of first and second CCA axis for periphyton in the Tundzha River.  
 247 Environmental variables: Cocp, *Cocconeis placentula*; Cymc, *Cymbella cystula*; Cymt, *Cymbella*  
 248 *tumida*; Diav, *Diatoma vulgaris*; Frau, *Fragilaria ulna*; Goma, *Gomphonema acuminatum*;  
 249 Melv, *Melosira varians*; Lepg, *Leptolyngbya granulifera*; Oscl, *Oscillatoria limosa*; Eogp,  
 250 *Euglena polymorpha*; Sceq, *Scenedesmus quadricauda*; Temp, Temperature; DO, Dissolved  
 251 Oxygen; TP, Total Phosphorus.

## 252 DISCUSSION

253 Species composition and distribution of periphytic algal assemblages in a fluvial  
 254 ecosystem are known to be affected and regulated by single or combined effects of physico-  
 255 chemical and environmental conditions (Vis, et al., 1998; Cardinale et al., 2000). The results of  
 256 the present study revealed significant differences in distributional patterns of periphytic algae in

relation to water quality. Water temperature, TSS and nutrients were found to have an effect on longitudinal distributions of periphytic algal populations throughout the study period.

When the rural and urban zones of the river that have a low conductivity value were compared according to water chemistry condition, a decrease in DO, SiO<sub>2</sub>, Chl-*a* and biovolume was determined. However, an increase was detected in nitrite, nitrate, total phosphorus, pH and TSS values.

If we compare previous studies with the current physicochemical status of the river which also covers our study area, there are studies providing data about physicochemical characteristics (Öterler, 2014; Altınoluk, 2014). According to these data, in particular, DO quantity has decreased, but pH has increased, over time. There is no significant change in the NO<sub>2</sub>-N values, but NO<sub>3</sub>-N values were determined to be higher, especially in spring and summer seasons. However, in a study by Borisova et al. (2013) on the Bulgaria section of the river, mean conductivity was 626 µS cm<sup>-1</sup>, which is higher than our study (563 µS cm<sup>-1</sup>). The pH values in the river were in a narrower band than our data (7.82-8.68), and DO values were in a wider band when compared to our data (3.36-11.22 mg L<sup>-1</sup>) (Borisova et al., 2013).

The establishment of periphytic algal communities are influenced by chemical changes in water, along with urbanization process (Baker et al., 2009; Wu et al., 2009; Dunck et al., 2013). This study revealed differences in the distribution and structure of epiphytic algae among the sections of the Tundzha River under different impacts.

Among the rural zones which were chosen from agricultural estates, especially St.3, the highest bio volume and species richness in terms of epiphytic algae were found, as they are located in a region which is rich in macrophytes, and the river is enlarged and meanders and the current velocity is low. These results may be assigned to several factors, such as changes in nutrient input and light availability, which may also have a strong impact on algal communities (David et al., 2006).

Similar to previous studies (Whitford & Schumacher, 1963; Hauer & Lamberti, 2007; Sabater & Sabater, 2000), biovolume and chlorophyll-*a* content in both study zones showed an increase starting from mid-spring until autumn including September, but the taxa represented with an increase differed from those reported in the former studies. According to Allan (2007), algal communities in streams are often dominated by diatoms in spring, blue-green algae in summer and green algae later (Hauer & Lamberti, 2007), but these trends did not always apply to

the Tundzha River at the study sites. The community structure and longitudinal distribution of periphyton in the Tundzha River showed a distinct difference between the two zones. The diatoms were recorded as the dominant group with 72.1%, and desmids were the subdominant group in the rural zone, whereas diatoms and euglenoids accounted for 37.3% and 36.8% of the periphyton in the urban zone, respectively. Diatoms continued to be the dominant group in the rural zone throughout the year, but in the urban zone, they were only dominant from June to August, with blue-greens dominant from January to May. Euglenoids, on the other hand, showed an increase in their biovolumes with increasing temperature values and became the dominant group in the river in autumn months including December. In aquatic zones, detrimental agriculture practices, effluent treatment plants, urban discharge and other wastes play an important role in the increase in nutrients, especially nitrogen and phosphorus (Epa, 2006). In our present study, the rural zone can be classified, considering its nitrogen concentrations, as mesotrophic, and the urban zone as eutrophic to hypereutrophic. As a result of urbanization related to human activity, particularly at St.4 and its region, there is an increase in the nutrient concentration; accordingly, in particular, the euglenoids are almost established as monoculture at times. Presence of flagellated and motile euglenoids in periphyton, particularly at St.5, with an increasing pattern from June to November, shows the negative effects of organic pollution originating from the city on the river.

The euglenoids were determined to be dominant organisms in the urban zone in the river. As a result of statistical analyses, euglenoids are determined to have a positive correlation with nitrate. Besides, TSS values are determined to be higher when compared to those of the rural zone. It is possible that the flocculation of the river in this region is abundant not only in benthic algae, but also in plankton. Öterler et al. (2014) noted that *E. polymorpha* is the dominant species among plankton. It is possible that because of the planktonically excessive increase of euglenoids in the urban zone, and as a result of the occurring flocculation, pressure is placed on the composition of the species in the benthic region.

Five taxa (*Fragilaria ulna*, *Cocconeis placentula*, *Cymbella tumida*, *Oscillatoria limosa* and *Oscillatoria tenuis*) were found as dominant species in both the rural and urban zones of the Tundzha River. Similar results were found by Cardinale et al. (2006) and Stelzer and Lamberti (2001). *Fragilaria* sp. was the pioneer species (35.6 mm<sup>3</sup> cm<sup>-2</sup> in average) during the study period. The other diatoms, *Cocconeis* sp. and *Cymbella* sp. reached high biomass values in the

rural zone, whereas euglenoids and filamentous cyanophytes were found in high biomass values in the urban zone occasionally. The excessive growth of some filamentous cyanophytes and euglenoids has long been associated with eutrophication, and in this study, filamentous blue-green algae were positively correlated with TP and temperature, and euglenoids were positively correlated with nitrate. Seasonal variation in epiphytic algal diversity and number of species showed a highly different pattern between the two zones. In the rural zone, the highest diversity values were recorded in early summer, and the lowest values were recorded in early winter; whereas in the urban zone, the highest and lowest diversity values were recorded in mid-summer and late winter, respectively. The obtained data showed that the highest periphytic diversity ( $H'$ ) values were recorded when periphyton was dominated by several epiphytic algal species, and lowest values were recorded when periphyton was dominated by few species in both zones. The periphyton community was dominated by species which are tolerant to eutrophic conditions in the urban zone, whereas mesotrophic taxa dominated in the periphyton community in the rural zone.

While we were planning our study, we classified 6 stations, chosen from the river, as rural or urban zones. But the results of physicochemical analyses did not find a statistically significant difference between the two zones. However, modifications are seen in the sediment structure as a result of the modifications of the riverside and river bed in the urban zone. As a result of this, the difference between algae composition at the regions named as rural and urban zones can be explained by the deficiency of macrophytes on which epiphytic algae can hold on to, and that the river basin structure is relatively covered with ooze, correspondingly degrading the substrate on which benthic algae can hold on to.

In conclusion, our research findings recorded 73 taxa of periphytic algal flora in the Tundzha River, which play significant roles in primary production and nutrient cycling. Despite their significance, many aspects of the geographical distribution, biodiversity and ecology of periphytic algae are poorly understood (Hasler, 2008). The contaminants in the river were not measured between the two zones in terms of nutrients, but there are obvious differences between the regions in terms of species composition and abundances. This study opens the door for further research on periphytic algae and the ecological role governed by periphytic algae in an ecosystem like the Tundzha River, where the pressure of agricultural activities and urbanization changes the area continuously.

350

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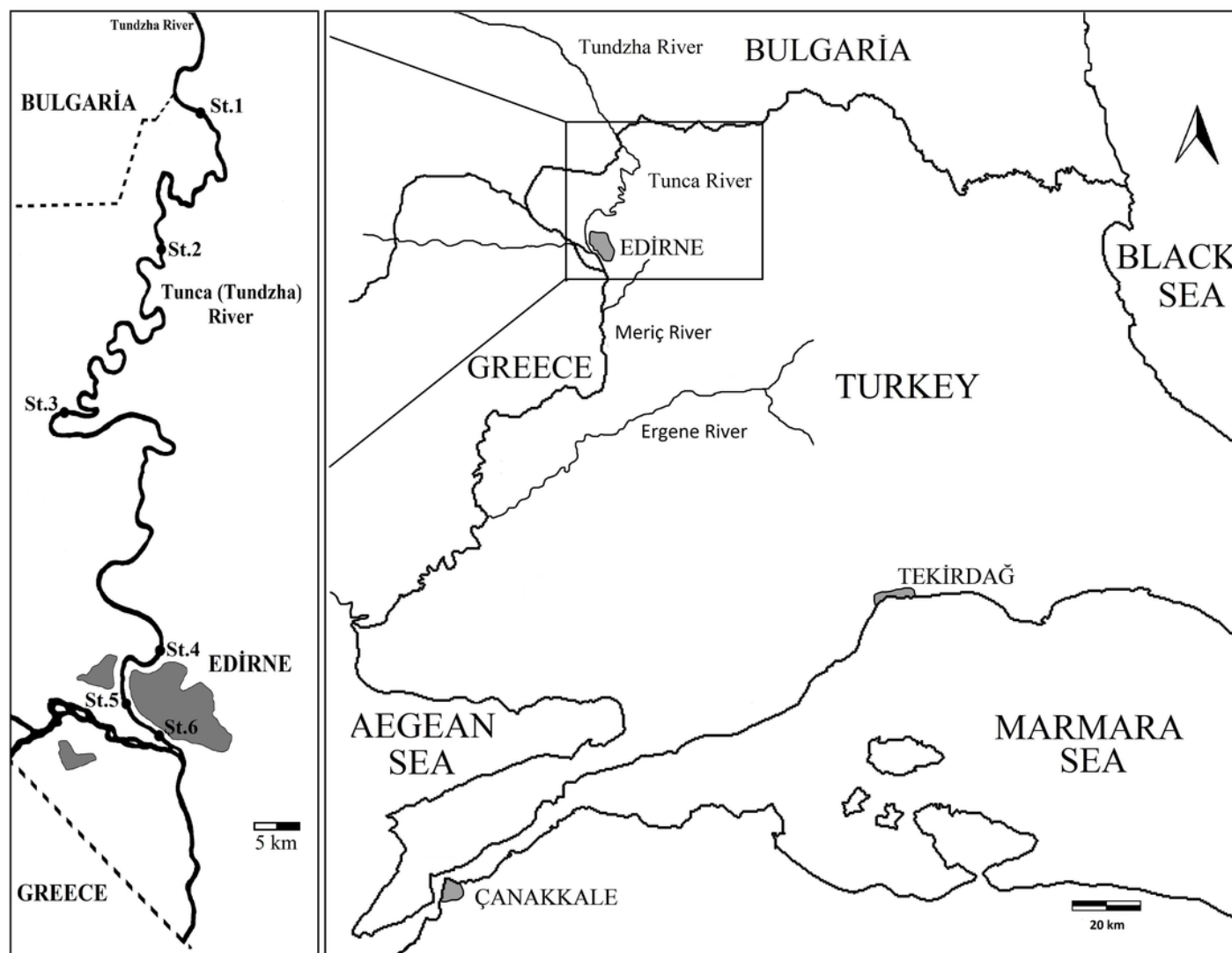
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1

Location of Tundzha River and sampling sites within the Turkish Thrace.



# **Table 1** (on next page)

Stations and collected algal sampling types.

	St. No	Locality	Bottom	Collected periphyton sampling		
Rural zone	St.1	After the Bulgarian-Turkish Border	Pebbles and sand		Epilotic	
	St.2	Enter the Yolüstü Village	Pebbles and sand	Epipelic	Epilotic	Epiphytic
	St.3	Meander near Değirmenyeni village	Muddy and macrophytes	Epipelic		Epiphytic
Urban zone	St.4	Enter the Edirne City	Muddy and macrophytes	Epipelic	Epilotic	Epiphytic
	St.5	Middle in the Edirne City	Ooze	Epipelic	Epilotic	
	St.6	Before of wedding Meriç River	Ooze	Epipelic		

1

## Table 2 (on next page)

Summary of physical and water quality characteristics of the study parts of the Tundzha River (mean values of chemical variables measured at the sampling stations).

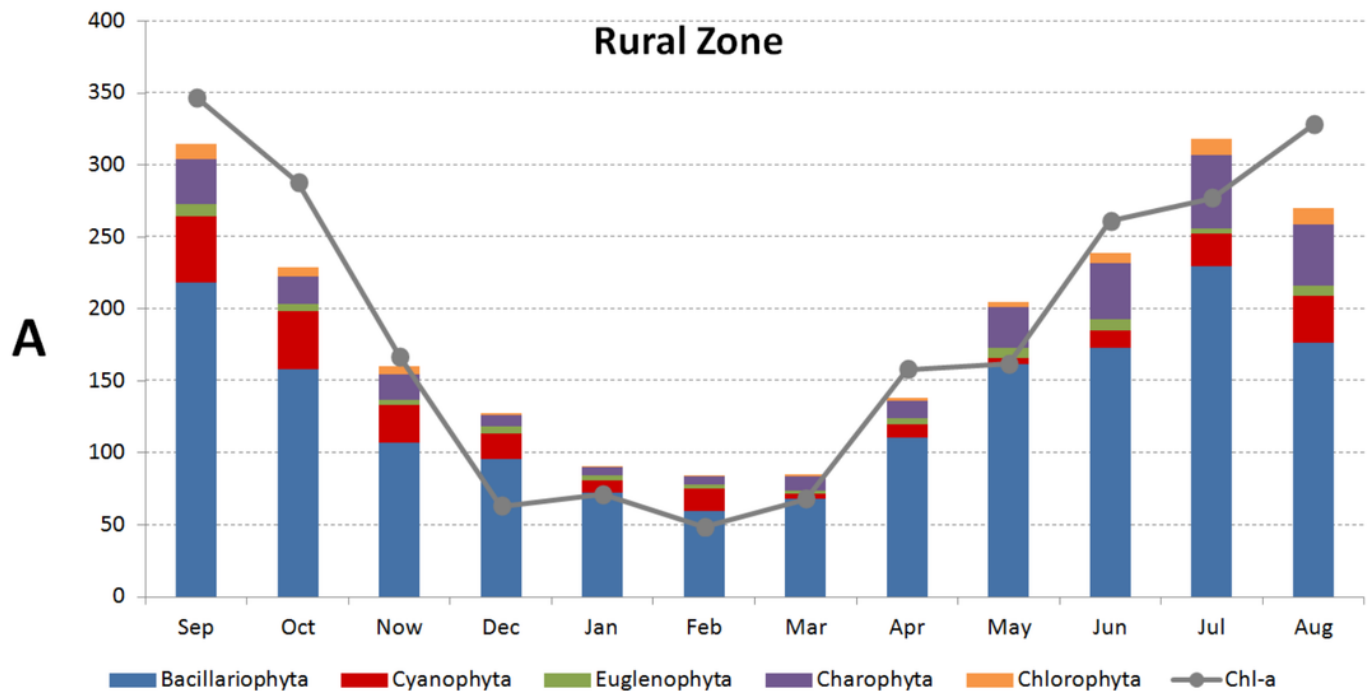
	Rural Zone				Urban zone			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Temperature (°C)	15,8	4,3	15,2	24,2	15,8	4,2	16,0	24,6
Diss. Ox. (mg L <sup>-1</sup> )	5,13	6,27	4,61	7,48	4,67	5,82	4,43	6,45
pH	8,91	9,20	8,64	8,09	8,46	8,77	8,49	7,84
Conductivity (µS cm <sup>-1</sup> )	519	479	655	566	534	471	666	616
N-NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0,04	0,03	0,02	0,01	0,05	0,05	0,02	0,01
N-NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	2,31	4,69	12,30	4,81	5,07	6,25	12,89	7,91
TP (µg L <sup>-1</sup> )	45,7	47,8	56,2	64,2	65,7	50,6	54,3	72,1
SiO <sub>2</sub> (µg L <sup>-1</sup> )	1550	773	801	1169	1239	628	623	941
Chl- <i>a</i> (µg L <sup>-1</sup> )	267,3	60,9	129,3	288,7	225,3	56,0	53,3	189,2
TSS (mg L <sup>-1</sup> )	135	239	207	162	148	332	263	173
Biovolume (mm <sup>3</sup> cm <sup>-2</sup> )	234,4	100,9	142,8	288,8	246,4	114,0	96,6	269,6

1



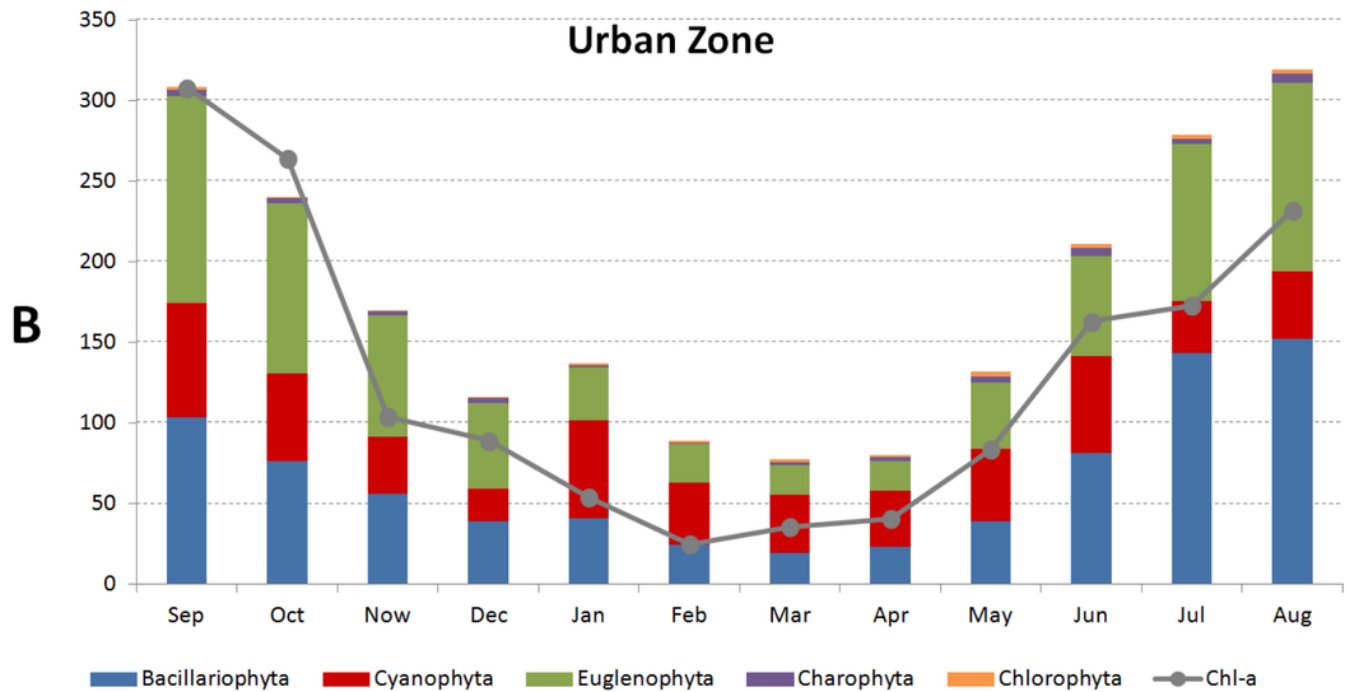
2

Seasonal composition of chlorophyll- *a* and biovolume on the Tundzha River (A-rural zone, B-urban zone). X axis showed biovolume ( $\mu\text{m}^3 \text{ cm}^{-3}$ ) and chlorophyll- *a* ( $\mu\text{g cm}^{-2}$ ).



3

Seasonal composition of chlorophyll- *a* and biovolume on the Tundzha River (A-rural zone, B-urban zone). X axis showed biovolume ( $\mu\text{m}^3 \text{ cm}^{-3}$ ) and chlorophyll- *a* ( $\mu\text{g cm}^{-2}$ ).



# **Table 3**(on next page)

Periphytic algal communities of the Tundzha River.

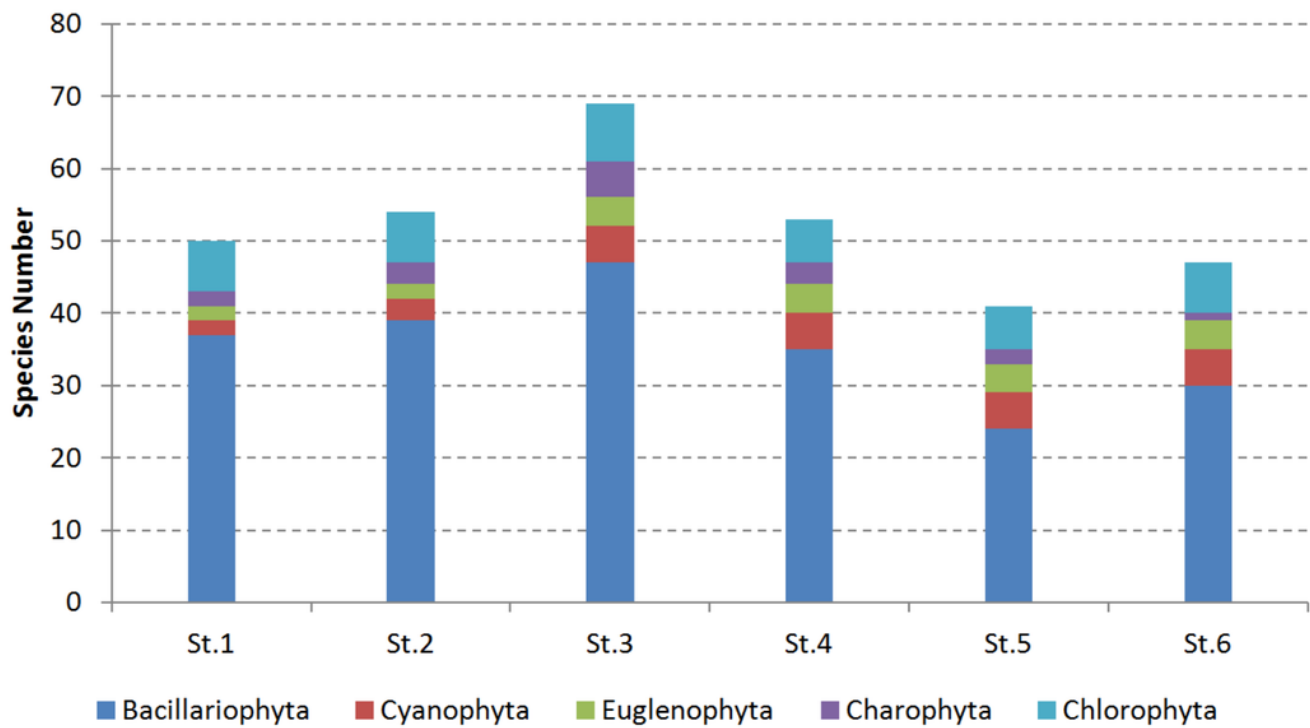
	St.1	St.2	St.3	St.4	St.5	St.6
<b>Empire</b> Prokaryota						
<b>Kingdom</b> Eubacteria						
<b>Subkingdom</b> Negibacteria						
<b>Phylum</b> Cyanobacteria						
<b>Class</b> Cyanophyceae						
<i>Anabaena affinis</i> Lemm.	□	□	■	■	■	■
<i>Leptolyngbya granulifera</i> (Copeland) Anag.	□	■	■	■	■	■
<i>Lyngbya</i> sp.	□	□	■	■	■	■
<i>Oscillatoria limosa</i> Agardh	■	■	■	■	■	■
<i>Oscillatoria tenuis</i> Gomont	■	■	■	■	■	■
<b>Empire</b> Eukaryota						
<b>Kingdom</b> Protozoa						
<b>Phylum</b> Euglenophyta						
<b>Class</b> Euglenophyceae						
<i>Euglena acus</i> Ehren.	■	■	■	■	■	■
<i>Euglena polymorpha</i> Dangeard	■	■	■	■	■	■
<i>Phacus acuminatus</i> Stokes	□	□	■	■	■	■
<i>Trachelomonas volvocina</i> (Ehren.) Ehren.	□	□	■	■	■	■
<b>Phylum</b> Bacillariophyta						
<b>Class</b> Fragilariophyceae						
<i>Achnanthes hungarica</i> (Grun.) Grun.	■	■	■	□	■	■
<i>Amphora ovalis</i> (Kütz.) Kütz.	■	■	■	■	■	■
<i>Caloneis amphisbaena</i> (Bory) Cleve	■	■	■	■	■	■
<i>Cocconeis placentula</i> Ehren.	■	■	■	■	□	■
<i>Craticula cuspidata</i> (Kütz.) Craw.&Mann	■	■	■	■	□	□
<i>Cymatopleura elliptica</i> (Bréb.) Smith	■	■	■	□	■	■
<i>Cymatopleura solea</i> (Bréb.) Smith	■	■	■	□	□	□
<i>Cymbella cystula</i> (Ehren.) Kirch.	■	■	■	■	■	■
<i>Cymbella lanceolata</i> (Agardh) Kirchner	□	□	■	□	□	□
<i>Cymbella naviculiformis</i> Heiberg	■	■	■	□	□	■
<i>Cymbella tumida</i> (Bréb.) van Heurck	■	■	■	■	■	■
<i>Diatoma vulgaris</i> Bory de Saint-Vincent	■	■	■	■	■	■
<i>Epithemia sores</i> Kützing	■	□	■	□	□	□
<i>Fragilaria acus</i> (Kütz.) Lange-Bertalot	■	■	■	■	■	■
<i>Fragilaria crotonensis</i> Kitton	■	■	■	■	■	■
<i>Fragilaria ulna</i> (Nitz.) Lange-Bertalot	■	■	■	■	■	■
<i>Fragilariforma virescens</i> (R.) Wil.&Round	□	□	■	■	□	■
<i>Gomphonema acuminatum</i> Ehren.	■	■	■	□	□	□
<i>Gomphonema parvulum</i> (Kütz.) Kütz.	■	■	■	■	□	■
<i>Gomphonema truncatum</i> Ehren.	■	■	■	■	■	■
<i>Gyrosigma acuminatum</i> (Kütz.) Rab.	□	□	■	□	□	■
<i>Gyrosigma attenuatum</i> (Kütz.) Rab.	□	□	■	□	□	□

<i>Hantzschia amphioxys</i> (Ehren.) Grunow	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Mastologia smithii</i> Thwaites	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<i>Navicula capitata</i> Ehren.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Navicula cryptocephala</i> Kützling	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<i>Navicula gracilis</i> Ehren.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Navicula radiosa</i> Kützling	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Navicula tripunctata</i> (Müll.) Bory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<i>Navicula viridula</i> (Kütz.) Ehren.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia acicularis</i> (Kütz.) Smith	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia angustata</i> (Smith) Grunow	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Nitzschia flexa</i> Schumann	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia gracilis</i> Hantzsch	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia hungarica</i> Grunow	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia obtusa</i> Smith	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Nitzschia palea</i> (Kütz.) Smith	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia parvula</i> Smith	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Nitzschia sigmoidea</i> (Nitz.) Smith	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Nitzschia tryblionella</i> Rab.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Pinnularia acuminata</i> Smith	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Pinnularia brebissonii</i> (Kütz.) Rab.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Pinnularia viridis</i> (Nitzsch) Ehren.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Rhoicosphenia curvata</i> (Kütz.) Grunow	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Rhopalodia gibba</i> (Ehren.) Müller	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Sellaphora pupula</i> (Kütz.) Meres.	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Surirella robusta</i> Ehren.	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<b>Class Coscinodiscophyceae</b>						
<i>Aulacoseira italica</i> (Ehren.) Sim.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Melosira varians</i> Agardh	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<b>Class Mediophyceae</b>						
<i>Cyclotella atomus</i> Hustedt	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Cyclotella meneghiniana</i> Kützling	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
<b>Phylum Charophyta</b>						
<b>Class Conjugatophyceae</b>						
<i>Closterium lunula</i> Ehren. & Ralfs	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<i>Closterium parvulum</i> Nageli	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Cosmarium botrytis</i> Ralfs	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Mougeotia</i> sp.	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Spirogyra</i> sp.	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Phylum Chlorophyta</b>						
<b>Class Chlorophyceae</b>						
<i>Monoraphidium contortum</i> (Thuret) Fott	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<i>Pediastrum boryanum</i> (Turpin) Meneg.	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<i>Scenedesmus acuminatus</i> (Lag.) Chodat	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

<i>Scenedesmus bicaudatus</i> Dedusenko	■	■	■	■	■	■
<i>Scenedesmus quadricauda</i> (Turpin) Bréb.	■	■	■	■	■	■
<i>Scenedesmus sempervirens</i> . Chodat	■	■	■	■	■	■
<i>Schroederia antillarum</i> Komárek	□	□	■	□	□	■
<i>Tetraëdron minimum</i> (Braun) Hansgirg	■	■	■	■	■	■

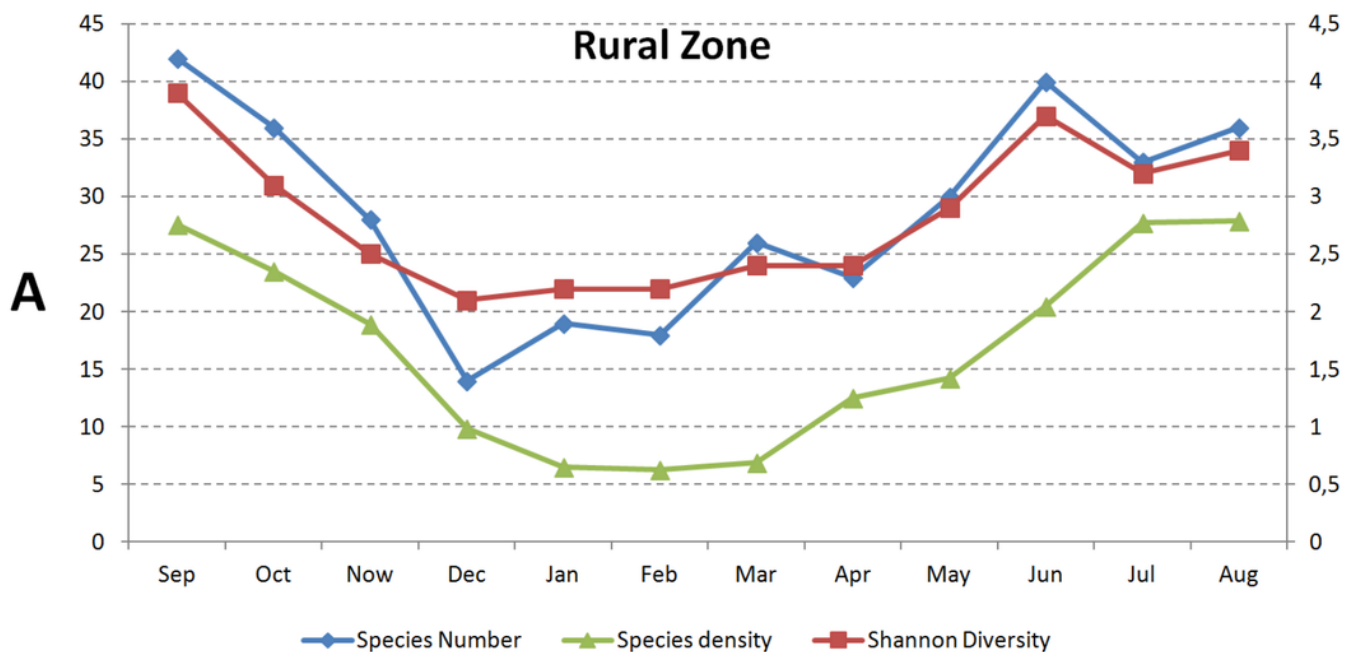
# 4

Species richness values in all sampling stations.



# 5

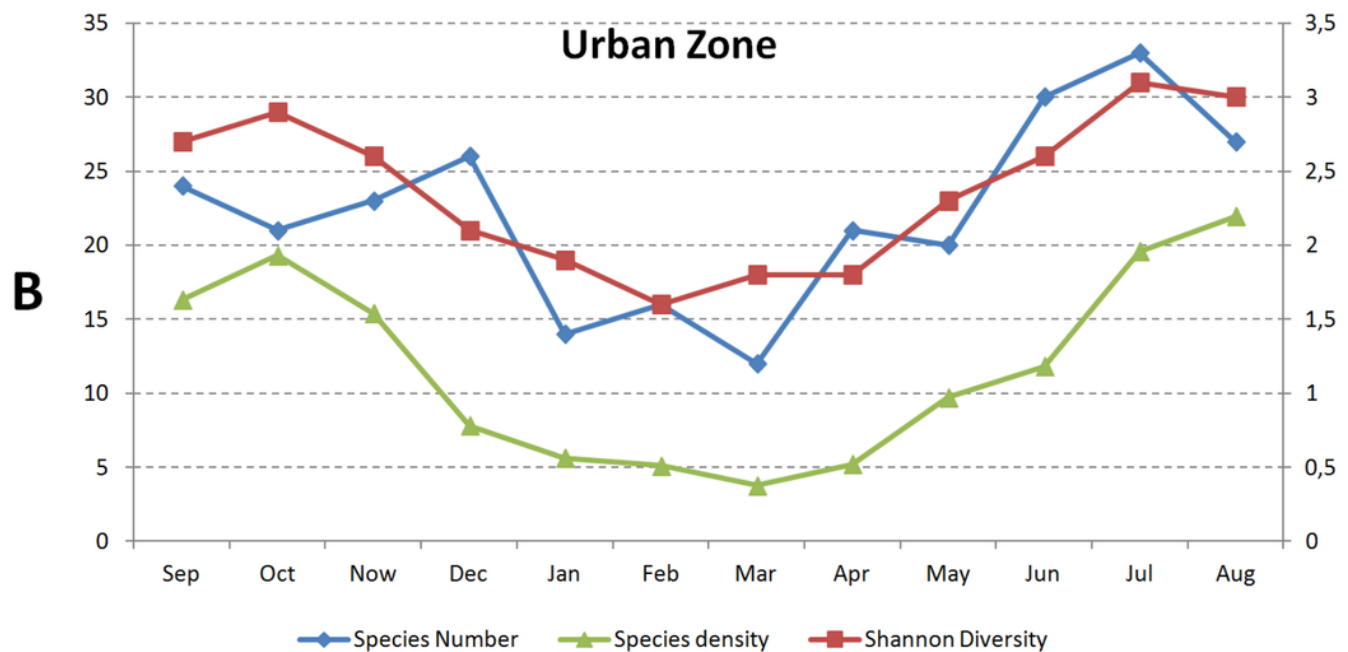
Seasonal composition of species density, species number and Shannon diversity in the Tundzha River (A-rural zone and B-urban zone). First X axis is given species density (%) and species number; second X axis is given Shannon index ( $H'$ ).





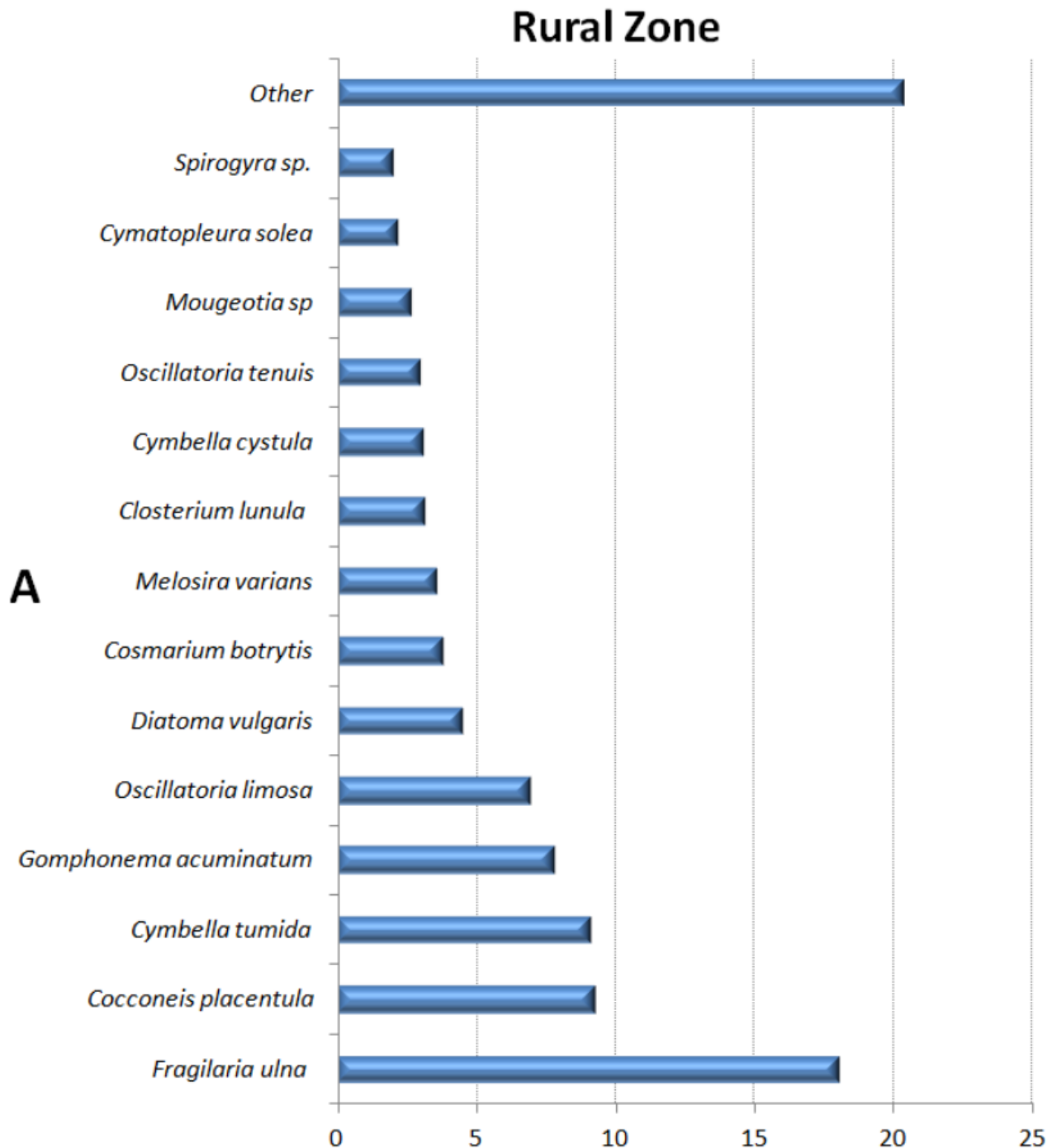
# 6

Seasonal composition of species density, species number and Shannon diversity in the Tundzha River (A-rural zone and B-urban zone). First X axis is given species density (%) and species number; second X axis is given Shannon index ( $H'$ ).



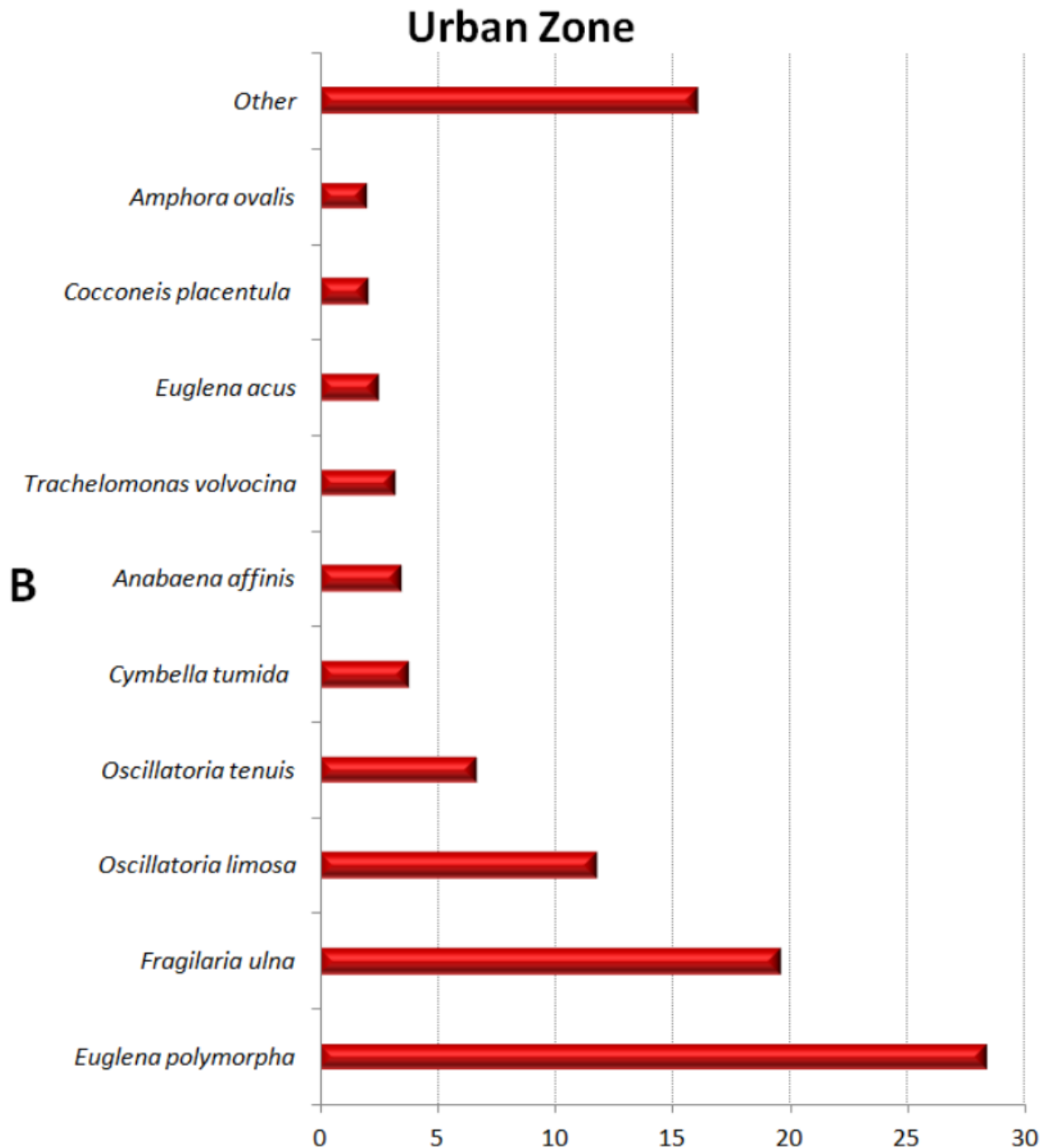
7

Highest relative biovolume in Tundzha River (A-rural zone, B-urban zone).



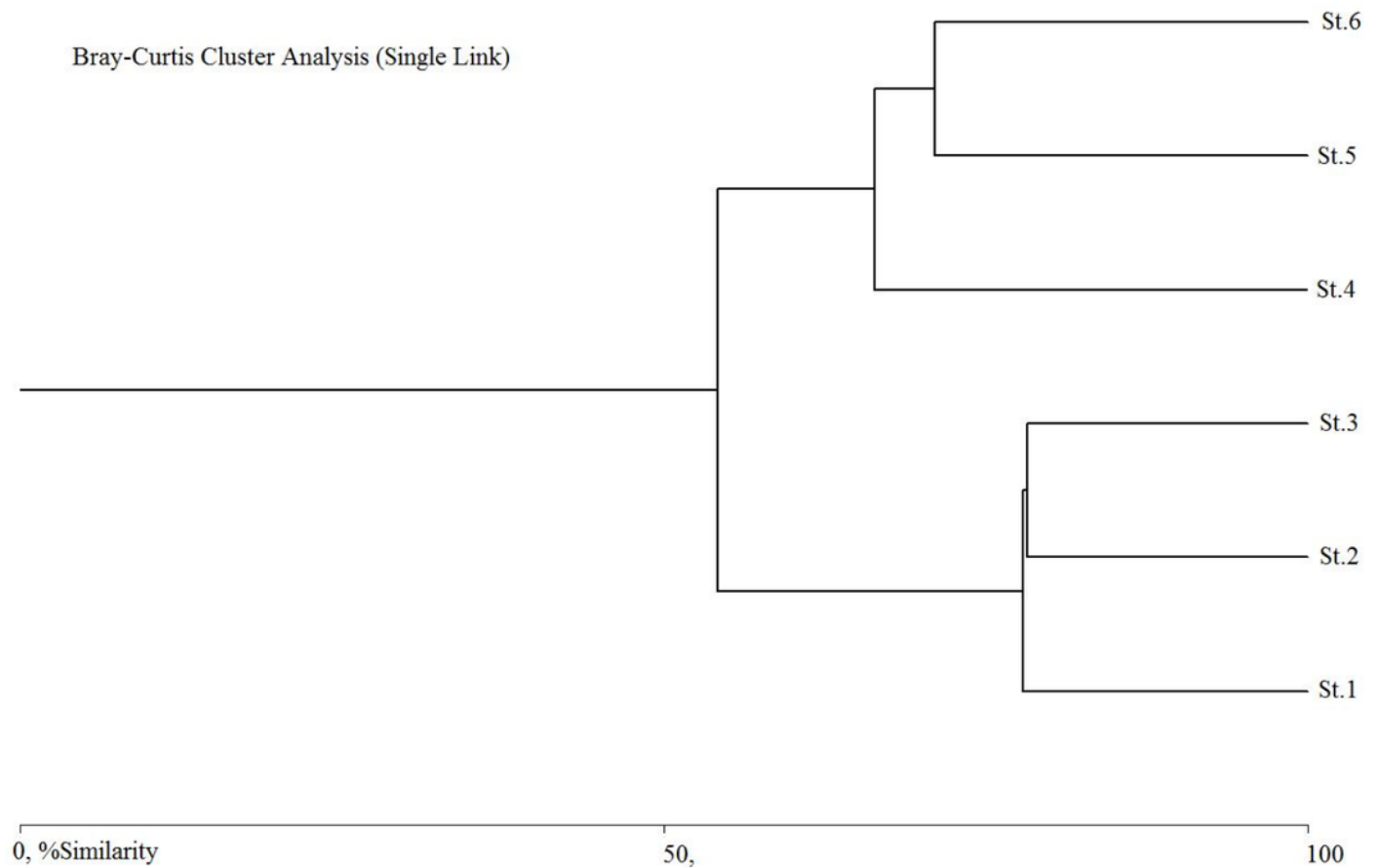
8

Highest relative biovolume in Tundzha River (A-rural zone, B-urban zone).



# 9

Results of clustering analyses, based on differences in the floristic composition of phytoplankton in the Tundzha River (Bray-Curtis distance).



# 10

Biplot of first and second CCA axis for periphyton in the Tundzha River. Environmental variables: Cosp, *Cocconeis placentula*; Cymc, *Cymbella cystula*; Cymt, *Cymbella tumida*; Diav, *Diatoma vulgaris*; Frau, *Fragilaria ulna*[i]; Goma, Gomphone

