Macedonian Journal of Ecology and Environment
Vol. 16, issue 1-2
pp. 5-9
Skopje (2014)
ISSN 1857 - 8330
Original scientific paper
Available online at www.mjee.org.mk

Vertical dynamics of some indicator microorganisms in Tunca river at Turkish Thrace

Вертикална динамика на некои индикаторски микроорганизми во реката Тунџа во Турска Тракија

Pinar ALTINOLUK [1*], Belgin CAMUR-ELIPEK [2] & Halide AYDOGDU [3]

[1] Trakya University, Technology Research and Development Centre, 22030, Edirne, Turkey
[2] Trakya University, Faculty of Science, Department of Biology, 22030, Edirne, Turkey
[3] Trakya University, Arda Vocational School, 22100, Edirne, Turkey

Although freshwater resources are the most essential material to sustain the life, they have been under a lot of impacts from human activities. These ecosystems sustain a lot of different groups of microorganisms. But, discharges from domestic and industrial wastewaters, agricultural pesticides and fertilizers to the water bodies may change the bacterial composition. The presence of some indicator bacteria in water ecosystems can point to the quality of a water resource. In this research, the vertical distribution of some bacterial indicators (total coliform, faecal coliform and *Escherichia coli* bacteria) and their dynamics were analyzed in Tunca River located at Edirne Province in Turkey (Turkish Thrace). The water samples were taken from three different depths (surface, middle, and bottom) at seasonal intervals during 2011. Some physicochemical parameters (temperature, dissolved oxygen, pH, and suspended solids) were also measured. It was concluded that the bacterial concentration in Tunca River decreases with increasing of depth. Therefore, it is suggested that the surface water of Tunca River should not be used for irrigation.

Key words: Bacteria, indicator microorganisms, total coliform, faecal coliform, *Escherichia coli*.

Водните ресурси се под голем број различни влијанија од страна на човековите активности, иако се есенцијални воопшто за животот. Водните екосистеми поддржуваат голем број различни групи микроорганизми. Но, испустите од домашна и индустриска канализација, пестицидите и ѓубривата во земјоделството предизвикуваат промени во составот на бактериите во водните тела. Присуството на некои индикаторски бактерии во водните екосистеми можат да ни укажат на квалитетот на водните ресурси. Во оваа студија беше истражувана вертикалната дистрибуција и динамика на некои бактериски индикатори (вкупни колиформни, фекални колиформни и *Escherichia coli*) во реката Тунџа во провинцијата Едрене на Турција (Турска Тракија). Примероци од вода беа земани на три различни длабочини (површина, среден дел и дно) во различни сезони во 2011 година. Анализирани беа и некои физичко-хемиски параметри (температура, растворен кислород, pH, суспендирани честички). Од анализите беше заклучено дека концентрацијата на бактериите опаѓа со зголемување на длабочината. Затоа, се препорачува да не се користат површинските води на реката Тунџа за наводнување.

Клучни зборови: бактерии, индикаторски микроорганизми, вкупни колиформни, фекални колиформни, *Escherichia coli*.

Introduction

Water is an essential material to sustain the life and it is intended the water resources should be safe for human/animal consumption and usage. But, a freshwater ecosystem contains different groups of microorganisms from naturally or discharges from domestic / industrial / agricultural activities. Bacteriological quality of an aquatic ecosystem is determined by hygiene index or indicator microorganisms such as coliform bacteria (Saha et al. 2009; Bushati et al. 2010). In the recent years, total and faecal coliforms, and *Escherichia coli* bacteria have been used as the main organisms of bacterial indicators (Niewolak & Golas 2000; Anderson et al. 2005; Agbogu et al. 2006; Servais et al. 2007; Mishra et al. 2010). The

Submitted: 05.07.2014
Accepted: 10.11.2014

* pinar_altinoluk@yahoo.com.tr; belginelipekcamur@trakya.edu.tr; halideaydogdu@trakya.edu.tr

presence of faecal coliform bacteria in an aquatic environment may indicate that the water body has been contaminated with the faecal material by the wastes from humans or animals. Furthermore, if the ratio of faecal coliform bacteria reaches to high levels in an aquatic ecosystem, there is a great probability that pathogenic organisms are also present in the water resource and there is a risk of a disease based on epidemiological evidence of waterborne diseases.

This study was performed in Tunca River to determine the vertical distribution and the dynamics of indicator microorganisms (total coliform, faecal coliform and *Escherichia coli*). Although, there are some studies which have been performed on this topic in the world, the studies in Turkey are very rare (Ribeiro & Kjerfve 2002; Lokoska 2006; Mestres et al. 2009; Pote et al. 2009; Cardak & Altug 2010; Koloren et al. 2011). Also, some physicochemical parameters like temperature, dissolved oxygen, pH, and suspended solids were measured to determine the environmental conditions to evaluate the bacterial distribution.

Tunca River (Bulgarian: Tundzha or Tundja) is born in Bulgaria and enters to European part of Turkey Turkey at Edirne Province (Turkish Thrace). The area of the River Basin is around 8000 km^2 and its length is approximately 390 km. The River is the biggest tributary of Meric River (Bulgarian: Maritsa; Greek: Evros) which is the border river between Turkey and Greece. Although a lot of limnological studies have been carried out in Tunca River (Uzunov 1980; Russev et al. 1984; Janeva & Russev 1985; Uzunov & Kapustina 1993; Kavaz, 1997; Oterler 2003; Kirgiz et al. 2005; Camur-Elipek et al. 2006; Georgieva et al. 2010; Vassilev et al. 2010) there have been no study concerning the vertical dynamics of some indicator microorganisms.

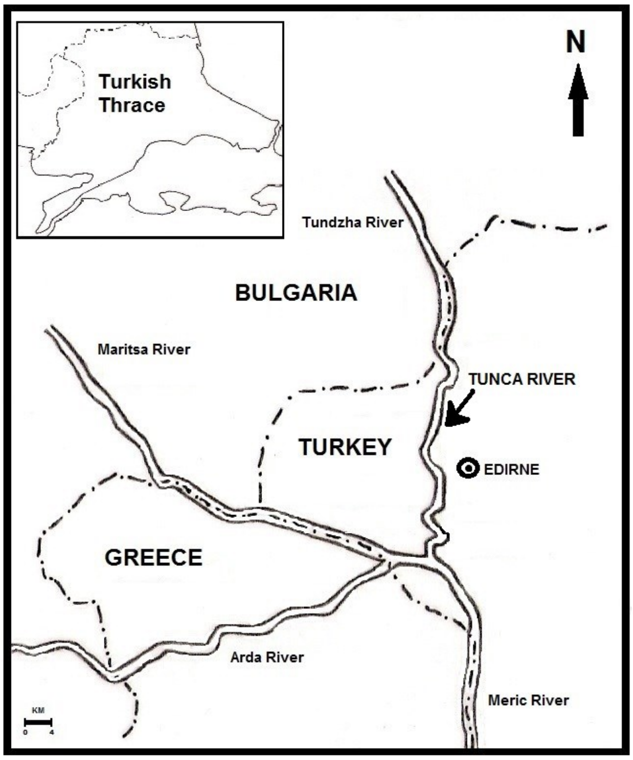

Figure 1. Location of Tunca River in Turkish Thrace and the study area (arrow)

Materials and methods

In the present study, the water samples were taken from different depths in Tunca River that belongs to Turkish segment located in Turkish Thrace (Fig. 1). In order to characterize the distribution of bacterial community along the depth profile, the sampling was carried out up to 2 meters in depth at seasonal intervals during 2011. The surface (0.5m), middle and bottom samples (2m) of the water column was taken by using Ruttner water sampler. The samples were kept in 100 mL of sterile sampling bottles. After the transportation of the samples to the laboratory, bacterial material was analysed immediately within few hours under cold chain.

The counts of the bacteria belonging to total coliform, faecal coliform and *E. coli* were determined by using the multiple-tube fermentation technique (through the presumptive-confirmed phases or completed test) (Hurst et al. 2002). Standard tables for three tubes were used to interpret the results to give the MPN (Most Probable Number) of the bacteria (Hurst et al. 2002). LST Broth (35±0.5 ºC at 24 hours) was used as culture media for the all bacteria. Also, EC Broth (44.5±0.2 ºC) was used for faecal coliforms and *E. coli* bacteria, and the TW Broth was also used for *E. coli* bacteria (44.5±0.2 ºC) as indicator culture media and incubation conditions (Hurst et al. 2002).

Bray-Curtis index was used in order to determine the similarity ratios of bacteria during the seasons and different depths of the water columns.

Furthermore, some physicochemical features of the water were also analysed in the same time with bacteriological sampling. Temperature was measured by ordinary thermometer as ^{0}C, pH was measured by pH-meter, dissolved oxygen was measured by oxygen-meter as $mg \cdot L^{-1}$. Also, suspended solids in the water samples were measured in $mg \cdot L^{-1}$ in the laboratory.

Spearman's correlation index was used in order to determine the correlation between the bacterial parameters and measured environmental parameters in Tunca River.

Results and discussion

The counts of the bacteria in water columns of Tunca River are shown in Tab. 1. According to the results, the highest numbers of total coliforms were found at surface water in Autumn and Spring ($1.1x10^5$ MPN/100 mL); while the lowest numbers were found at the bottom in Spring ($2.1x10^3$ MPN/100 mL) (Fig. 2). The maximum number of faecal coliform and *E. coli* bacteria were found at surface water during Autumn and Spring ($1.1x10^5$ MPN/100 mL); and the minimum at bottom in the Summer ($1.5x10^3$ MPN/100 mL) (Fig. 2).

According to Bray-Curtis similarity index, Autumn and Spring showed greatest similarity (77% similarity ratio) for counts of total coliforms. Winter and Summer were the most similar according seasons to the counts of faecal coliforms and *E. coli* bacteria (similarity ratios of 83% and 72%, respectively). Also, Autumn was the most different from Winter for the counts of total coliforms (similarity was only 14%); Autumn was found to be the most different from Summer for the counts of faecal coliforms and *E.coli* bacteria (similarities of only 13% and 8%, respectively). As it also shown in Tab. 1, the bacte-

Table 1. Seasonal changes of the numbers of indicator bacteria in Tunca River

Period	Depth	Total coliform (MPN/100mL)	Faecal coliform (MPN/100mL)	*E. coli* (MPN/100mL)
Autumn	**Surface**	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$
	Middle	$4.6x10^4$	$4.6x10^4$	$4.6x10^4$
	Bottom	$2.4x10^4$	$2.4x10^4$	$2.4x10^4$
Winter	**Surface**	$7.5x10^3$	$7.5x10^3$	$7.5x10^3$
	Middle	$4.3x10^3$	$4.3x10^3$	$4.3x10^3$
	Bottom	$2.3x10^3$	$2.3x10^3$	$2.3x10^3$
Spring	**Surface**	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$
	Middle	$2.3x10^3$	$2.3x10^3$	$2.3x10^3$
	Bottom	$2.1x10^3$	$2.1x10^3$	$2.1x10^3$
Summer	**Surface**	$1.5x10^4$	$9.3x10^3$	$4.3x10^3$
	Middle	$9.3x10^3$	$2.3x10^3$	$2.3x10^3$
	Bottom	$2.3x10^3$	$1.5x10^3$	$1.5x10^3$

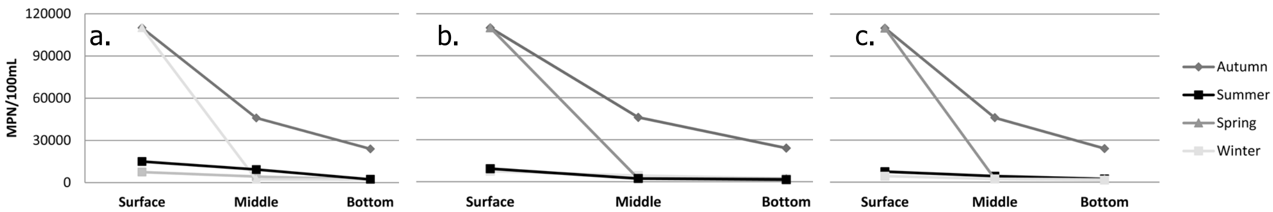

Figure 2. Vertical variation of the numbers of bacteria (a. Total coliforms; b. Faecal coliforms; c. *E. coli*) in Tunca River. Units on vertical axis identical for all three graphs

rial counts of Autumn, Winter and Spring were found at equal ratios. This situation can be explained by the input of bacteria from faecal sources. But, the high counts of total coliforms from the other bacteria in Summer show that the total coliform group includes other bacteria, no only faecal bacteria. Consequently, in this study, it was found that the bacterial load in Autumn was higher than the Winter. This might be due to wash out of the land surface by the rain water. Minimal bacterial numbers in winter might be due to cold climatic conditions, which is not been supportive for bacterial duplication in a greater extent.

The results concerning the vertical distribution of bacteria according to the seasons are presented in Fig. 3. According to the data, the bacterial abundance of Tunca River decreases in the following order: surface water > middle water > bottom water.

While it was found that bacterial groups were at the highest concentration in surface water, the lowest number of bacteria was recorded at the middle (Fig. 4). In a previous study which was performed by Riberio and Kjerfve (2002), faecal coliforms were examined at the surface and bottom depth, and it was found that the counts of bacteria at surface samplings were higher. Similar results were reported by Mestres et al. (2009) and they have reported that faecal coliform and *E. coli* bacteria reach maximum ratios at surface (especially at 0.5 m depth) compared to the bottom (Mestres et. al. 2009). In another study which was performed by Koloren et al (2011), it was found that the total coliform bacteria had similar counts at surface and bottom water columns, faecal coliform counts were higher at the surface than the bottom. In the study which was performed by Cardak and Altug (2010) it was reported that the deep discharges in the water cause the highest bacteria counts in the bottom. Also, Lokoska (2006) has found that the bottom has higher total coliform bacteria than the surface. In another study which was performed by Pote et al. (2009), it was reported that the counts of bacteria change by depths depending on the environmental conditions like temperature, sunlight, biological activities and presence of bacteriovorous organisms. These environmental factors are the primary cause for the bacterial differences between bottom and surface. The low water level in Tunca River has significant impact on vertical bacterial distribution, especially due to the increased sunlight and biological activi-

Table 2. Some physicochemical features of Tunca River (WT: Water temperature; DO: Dissolved oxygen; SS: Solid suspended).

Seasons / Parameters	WT (^{0}C)	pH	DO (mg/L)	SS (mg/L)
Autumn	14	8.4	3.6	850
Winter	3	8.7	7.0	400
Spring	22	7.9	7.6	430
Summer	24	7.8	6.1	310

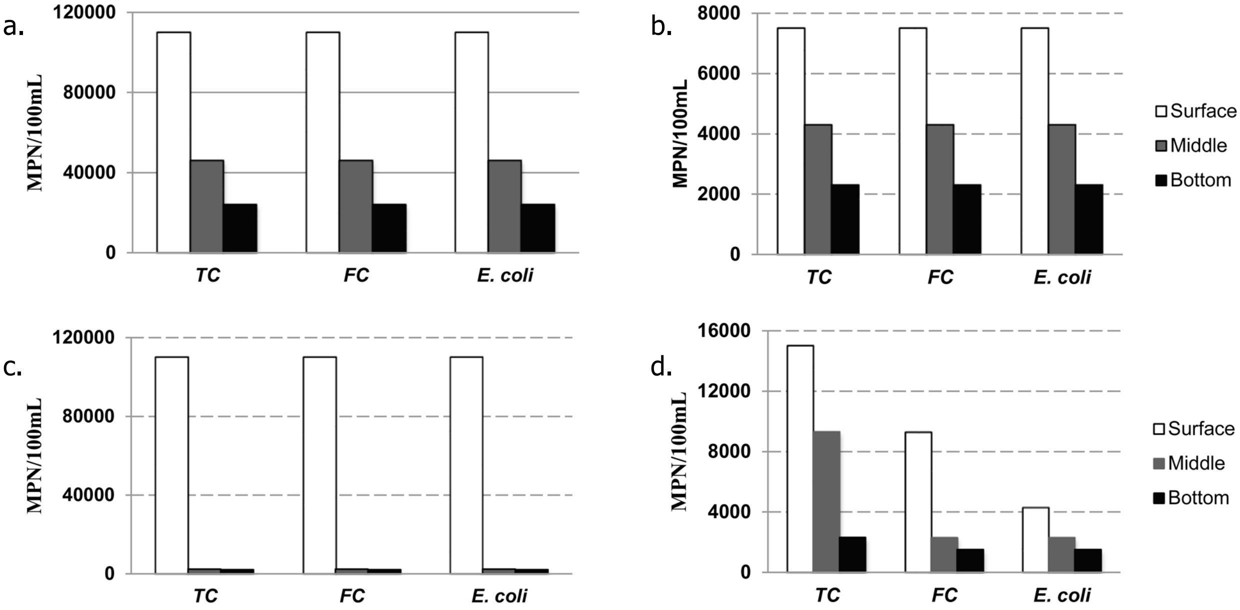

Figure 3. Numbers of indicator bacteria in Tunca River according to the seasons (a. Autumn; b. Winter; c. Spring; d. Summer). Note the different vertical axis values

ties. Also, other physicochemical features (temperature, dissolved oxygen, pH, and suspended solids) have an effect on the distribution of bacteria (Elmaci et al. 2008). The environmental parameters were investigated at the sampling locality and the results are shown in Tab. 2. Water temperature ranged between 3 and 24 ^{0}C (Winter and Summer) while dissolved oxygen values ranged from minimum of 3.6 mg·L^{-1} in Autumn and maximum of 7.6 mg·L^{-1} in Spring. pH values varied between 7.8 and 8.7 in Winter and Summer. Suspended solids fluctuated between 310 mg·L^{-1} in Summer and 850 mg·L^{-1} in Autumn. According to Spearman's index of correlation between the bacteriological counts and the environmental data, it was found any considerable correlation.

According to the Water Pollution Control Regulation (Anonymous 2004), water quality of inland waters is classified into four groups (first quality class: high quality clear waters; second quality class: moderate quality waters; third quality class: polluted waters; and fourth quality class: highly polluted waters. The first, second, third and fourth class water quality amounts for total coliform bacteria (MPN/100ml) is 100, 20000, 100000, >100000 respectively, and these classification for faecal coliform bacteria (MPN/100ml) is 10, 200, 2000, >2000, respectively. According to the amounts of total coliform bacteria, the surface water in Tunca River was found to be in the third class quality level while the middle and bottom depths were found at second class quality levels. In terms of the number of faecal coliform bacteria the water of Tunca river belong to in the fourth quality class at all depths.

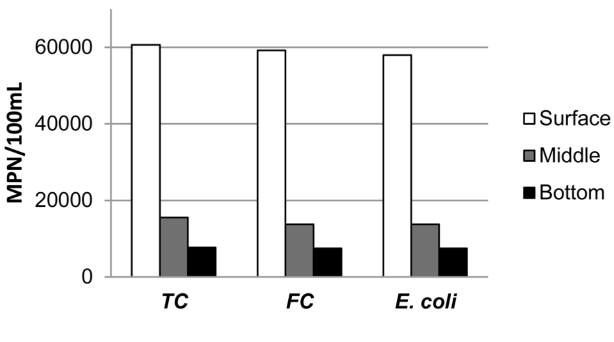

Figure 4. Average values of the number of bacteria in Tunca River

In summary, the bacterial concentration in Tunca River decreased with depth and depends on many environmental factors that also change with depth. Therefore, it is suggested that the surface water should not be used for irrigation.

References

Agbogu, V.N., Umoh, V.J., Okuofu, C.A., Smith, S.I., Ameh, J.B. (2006). Study of the bacteriological and physico-chemical ındicators of pollution of surface waters in Zaria, Nigeria. *African Journal of Biotechnology,* **5**(9): 732-737.

Anderson, K.L., Whitlock, J.E., Harwood, W.J. (2005). Persistence and differential survival of fecal ındicator bacteria in subtropical waters and sediments. *Applied Environmental Microbiology,* **71** (6): 3041-3048.

Anonymous (2004). *Water Pollution Control Regulation*. Official Gazette No: 25687 of 13 December 2004, Ankara.

Bushati, N., Neziri, A., Hysko, M. (2010). Investigation on physico-chemical and microbiological of the parameters of Lake Shkodra. *BALWOIS* 2010-Ohrid, Republic of Macedonia, 25-29 May 2010.

Camur-Elipek, B., And Arslan, N., Kirgiz, T., Oterler, B. (2006). Benthic macrofauna ın Tunca River (Turkey) and their relationships with environmental variables. *Acta Hydrochimica Hydrobiologica,* **34** (4): 360-366.

Cardak, M., Altug, G. (2010). Distribution of members of the family *Enterobacteriaceae* in the Istanbul Strait. J*. Black Sea/Mediterranean Environment,* **16** (3): 295-310.

Elmaci, A., Teksoy, A., Topac, F.O., Ozengin, N. Baskaya, H.S. (2008). Monitoring of seasonal variation of microbial quality in Lake Ulubat. Uludag University *Journal of The Faculty of Engineering and Architecture,* **13** (1): 93-103.

Georgieva, N., Yaneva, Z., Dospatliev, L. (2010). Analyses of natural water quality in Stara Zagora region according to the parameters sulfates and chlorides. *Trakia Journal of Sciences,* **8** (2): 517-524.

Hurst, C.J., Crawford, R.L., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D. (2002). *Manual of Environmental Microbiology*. In: Toranzos, G.A., McFeters, G.A., Borrego, J.J. Detection of microorganisms in environmental freshwaters and drinking waters. ASM Press, Washington, D.C. p.205-210.

Janeva, I.J., Russev, B.K. (1985). Trends in changes of the hydrobiological and saprobiological state of the Tundzha River. II. May-November 1981, *Hydrobiology* (Sofia)*,* **26**: 15-36. (in Bulgarian with English summary).

Kavaz, E. (1997). *Benthic macroinvertebrate fauna of Tunca River*. Trakya University, Institute of Natural Sciences, Master Thesis. 38p.

Kirgiz, T., Camur, B., Arslan, N. (2005). Preliminary study of Enchytraeidae (Oligochatea) in Tunca River. *Proc. Estonion Acad. Sci. Biol. Ecol.,* **54** (4): 310-314.

Koloren, Z., Tas, B., Kaya, D. (2011). Microbiological pollution of Gaga Lake in Ordu (Turkey). *The Black Sea Journal of Sciences,* **1** (3): 74-85.

Lokoska, L. (2006). *Microbiological water quality of Lake Prespa and ıts tributaries*. BALWOIS Conference on Water Observation and Information System for Decision Support, Ohrid.

Mestres, M.; Sierra, J.P.; Pascual, A.; Mösso, C.; Gonzalez del Rio, J.; Rodilla, M. (2009). Bacterial pollution in Cullera Bay (Spanish Mediterranean Coast). *Journal of Coastal Research,* **56**: 861-865.

Mishra, A., Bhatt, V.D., Sevak, N., Shah, P., Patel, K., Patel, C. (2010). Comparative study of physico-chemical and microbial parameters on lotic and ground-waters in selected outlying areas of central Gujarat. *J. Chem. Pharm. Res.,* **2** (4): 174-177.

Niewolak, S., Golas, I. (2000). Estimation of the degree of pollution and sanitary and bacteriological state of underground and surface waters in Omulewski aquifer area after liquidation of large cattle and pig farms. *Polish Journal of Environmental Studies,* **9** (6): 487-503.

Oterler, B. (2003). *An investigation on Tundza River phytoplancton and their relationships with water quality*. Trakya University, Institute of Natural Sciences, Master Thesis. 68p.

Pote, J., Goldscheider, N., Haller, L., Zopfi, J., Khajehnouri, F., Wildi, W. (2009). Origin and spatial-temporal distribution of faecal bacteria in a Bay of Lake Geneva, Switzerland. *Environmental Monitoring and Assessment,* **154** (1-4): 337-348.

Ribeiro, C.H., Kjerfve, B. (2002). Anthropogenic influence on the water quality in Guanabara Bay, Rio de Janeiro, Brazil. *Regional Environmental Change,* **3** (1-3): 13-19.

Russev, B. K., Nikolova, M. I., Dimitrova, M.A. (1984). Hydrobiological and saprobiological alterations in The Tundzha River. *Hydrobiology* (Sofia), **22**: 59-73. (in Bulgarian with English summary).

Saha, M., L., Khan, M., R., Ali, M., Hoque, S. (2009). Bacterial load and chemical pollution level of the River Buriganga. *Bangladesh J. Bot.,* **38** (1): 87-91.

Servais, P.; Billen, G.; Goncalves, A.; Armisen, T. (2007). Modelling microbiological water quality in the Seine River drainage network past, present and future situations. *Hydrology and Earth System Sciences,* **11**: 1581-1592.

Uzunov, J. I. (1980). Water Oligochets (Oligochaeta, Limicola) from some Bulgarian rivers. Frequency and Domination. *Hydrobiology* (Sofia), **12**: 79-89. (in Bulgarian with English summary).

Uzunov, Y., Kapustina, L. (1993). Current review on Oligochaeta from macrozoobenthic communities of the Bulgarian rivers. *Lauterbornia,* **13**: 73-83.

Vassilev, M., Apostolou, A., Velkov, B., İvanova, P., Panayotova, M., Dobrev, D., Pehlivanov, L. (2010). Status of Gobiid Ichtyofauna (Gobiidae) in Bulgaria: taxonomical, conservative, ecological and social aspects. *Natura Montenegrina*, **10** (2): 115-124.