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Physicochemical monitoring, Biodiversity and Biological monitoring of the Vit River, Bulgaria

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ABSTRACT

The results of the first for Bulgaria ecomonitoring researches, carried out during three seasons (spring, summer and autumn) of 2011 in 4 biotopes of the Vit River valley in the direction middle - lower course trough tests performed to find chemical pollution, are presented; analysis of the biological diversity of bioindicative groups of organisms (bioindicative macroinvertebrate fauna), occupying different trophical levels in the researched freshwater ecosystems. The physicochemical monitoring was made on the basis of indicators of: acidity, insoluble substances, conductivity, biological oxygen demand (BOD₅), chemical oxygen demand (COD), nitrate ions, sulphate ions, copper, lead, etc.

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Introduction

Macroinvertebrates are between the most frequently used bioindicating organisms to evaluate the situation of the freshwater ecosystems. Studies of the biodiversity and the state of freshwater ecosystem of the Vit River are relatively small (Gartsiyanova 2009; Yaneva 1979; Nikolova et al., 2008; Pehlivanov et al. 2009; Stancheva et al., 2007, etc.).

The major negative anthropogenic impacts on the river ecosystem of the river Vit associated with the development of agriculture. The area of study has a large share of agricultural land (79% of national, average 65%) used mainly for growing wheat, corn, sunflower, types of fruits - apples, pears, quinces, cherries, apricots, walnuts, and also large areas of vineyards. Anthropogenic load is mainly due to the use of fertilizers, pesticides and wastewater from livestock. Insignificant share of industrial pollution sources (town of Pleven: Sugar factory, Oil factory, "Plama" fuel factory; village of Yasen: Plant Tobbacco factory; town of Gulyanci: port Somovit on the Danube River, etc.) or of municipal wastewaters.

Vit River is included in the national monitoring program (Water Body Type BGTR7-Large Loess Rivers). River Tuchenitsa is not included (Water Body Type BGTR11 - Small and medium karst rivers). Following the river before the town of Pleven and through it is seen periodically infusing the River Tuchenitsa waste water overflowed channels. This is due to the absence of main collectors of the project for reconstruction of the town of Pleven. Infusion of untreated waste water causes pollution of the river Tuchenitsa, the degree may be demonstrated through sampling and analysis. In recent years, however, missing the data for the Tuchenitsa river. These researches would allow making a characterization of the situation and a prognostication of the changes in the studied freshwater biocenoses under the influence of the anthropogenic effects; to plan possibilities for preservation of the natural water resources

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and to plan possibilities to prevent any transborder pollution and the consequences related to it, etc. **Material and Methods**

The Vit River (189 km) is a one of the biggest tributary of Danube River in central northern Bulgaria (Danube Basin, Bulgaria) (Fig. 1). The source of the Vit River is in Stara Planina



Fig. 1. Vit River Basin

Mountain, below Vezhen Peak at an altitude of 2,030 m, and it empties into the Danube close to town of Gulyantsi. The river has a watershed area of 3,220 km², its main tributaries being Kamenska river, Kalnik and Tuchenitsa river.

During 2011, 24 samples of water and sediments, 28 taxa and 827 specimens of macrozoobenthos in the region between village of Yasen (before town of Pleven) and town of Gulyanci (before the Danube River) from the Vit River, were examined.

Ecomonitoring researches, carried out during three seasons (spring (22.03-22.06), summer (22.06-22.09) and autumn (22.09-22.12)) and in four biotopes: Biotope 1 – before village of Yasen and before town of Pleven; Biotope 2 – after village of Opanec and after infusion of Tuchenitsa river; Biotope 3 – after town of Gulyanci (last part of the Vit river, before Danube river); Biotope 4 – Tuchenitsa river, after town of Pleven, in direction middle – lower course (Fig. 1).

The basic abiotic and biotic characteristics of the water habitat in the region under research were determined (temperature, pH, dissolved oxygen in water mg/l, oxidizability, BOD₅, ionic groups, heavy metals, structure of the river bed, dominant tree vegetation, etc.). Samples of water and sediments were collected according to the Guidance on sampling of rivers and watercourses - ISO 5667-6:1990, introduced as a Bulgarian standard in 2002.

Samples of macrozoobenthos were collected by the EN 27828:1994/ISO 7828:1985; EN ISO 9391:1995/ISO 9391:1993; EN ISO 5667-1:2006/AC:2007; EN ISO 5667-3:2003/AC:2007, etc.

Methods for presentation of biological data (EN ISO 8689-2:2000) are enjambment. Methods have been developed at European level projects AQEM and STAR (AQEM consortium, 2004). All samples were fixed in 4% formaldehyde. They are laboratory sorted by systematic groups and then are kept in 70% ethyl alcohol. Analyses of the biological diversity of bioindicative groups of organisms (bioindicative macro invertebrate fauna - macrozoobenthos) were determined according to quantitative data. The criteria and methods of Cheshmedzhiev et al., 2011; Pielou, 1975; Yaneva and Cheshmedzhiev, 1999; Haase et al., 2006; Rosenberg et al., 1997; Russev, 1993; Furse et al., 2006, etc. have been applied. Modified Irish Biotic Index (MIBI), Ecological Quality Ratios Index for Ecological Quality Assessment (EQR), Index based on presence of Ephemeroptera, Plecoptera, Trichoptera Index (EPT), Rhithron Feeding Type Index (RETI) and Saprobic index of Pantle and Buck (SPB) were used for biomonitoring analysis. For an ecological evaluation of the situation of the analyzed freshwater ecosystems, principal biotic indexes have been fixed: Tn/spe - total number of taxa; Tn/sps - total number of specimens, HB (index of Brillouin, diversity), Dmg (index of Margalef, diversity); H' (index of Shannon, diversity), etc. (Lenat, 1988, 1993; Maguran, 1988; McGarrigle, 1992; Yaneva et al., 1999; Russev, 1993; Furse et al., 2006, etc.).

Results and Discussion

General characterization of the studied biotopes Physicochemical monitoring

Studies have been performed of freshwater ecosystems from the Vit River between the village of Yasen, before town of Pleven and after the village of Gulyanci, before the Danube River. The section under research is 12-15 m. wide and about 0.2-0.5 m. deep; with moderately flowing clear waters, with occasional rapids. The river bed is of sand and stones. The waterside vegetation is represented mainly by *Platanus orientalis*, *Alnus glutinosa* and *Salix* spp. The water of the river in this region of study is weak alkali with acidity from 7.48 pH to 8.46 pH, measured by temperature of the water from 16.4 $^{\circ}$ C – 27.4 $^{\circ}$ C during the three seasons of study. The oxidizability, COD and BOD₅ values were determined for each season (Table 1).

The waters of the Vit River in the region under research for the three seasons are distinguished by a low content of ionic groups (chlorides, sulfates, nitrite nitrogen, nitrate nitrogen, phosphates, cyanides) compared to Limit Admissible Concentration (LAC) for second category of surface flowing waters (in accordance with the BG State Standards – Regulation 7) with the exception of N-NO₂ in Biotope 2 and Biotope 3 compared for IIIrd category of waters (0.069-0.083 mg/l and 0.051-0.124 mg/l, respectively). Conductivity for all samples compared for the first category of surface following waters (Table 1; Fig. 2-5).

There was not any increased content of heavy metals detected (Mn, Fe, Pb, Cd, Cu, Zn) for the three seasons of the period under research (in accordance with the BG State Standards – Regulation 7 and H-4/2012 of the Ministry of Environment and Waters of Bulgaria for LAC for second category of surface flowing waters) with the exception of the zinc in Biotope 3 compared for IIIrd category of waters (12.3 mg/l) (Table 1; Fig. 2-5).

 Table 1. Basic abiotic indices of the studied freshwater

 ecosystems from the Vit River

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Abiotic indices	Min - max	Mean \pm SD	SEMean	C.V.					
Biotope 1									
Temperature (°C)	16.2-26	21.1±6.929	4.9	32.842					
pH	7.48-7.93	7.705±0.318	0.225	4.129					
Conductivity	247-501	374±179.61	127	48.023					
μS/cm									
COD mg/l	6-17	11.5±7.778	5.5	67.636					
BOD ₅ mg/l	4.2-5	21.55±8.132	0.4	12.298					
Biotope 2									
Temperature (°C)	15.8-27.3	8.005±0.388	5.75	37.734					
pH	7.73-8.28	12.2±5.09	0.275	4.858					
Conductivity	389-517	453±90.51	64	19.98					
μS/cm									
COD mg/l	8.6-15.8	12.2±5.09	3.6	41.731					
BOD ₅ mg/l	2-5.2	3.6±2.263	1.6	62.854					
Biotope 3									
Temperature (°C)	16.4-	21.9±7.	5.5	3					
	27.4	778		5.517					
pH	7.94-8.46	8.2±0.367	0.26	4.484					
Conductivity	408-577	492±119.5	84.5	24.264					
µS/cm									
COD mg/l	5-8.3	6.65±2.334	1.65	39.09					
BOD ₅ mg/l	1.6-2.9	2.25±0.919	0.65	40.855					



Fig. 2 Content of Zinc (mg/l)





The biomonitoring study of the hydrobiontic macro invertebrate fauna (macrozoobenthos) in the research region during the three seasons of the research period show the presence of the 28 bioindicating taxa from 12 orders. With the biggest number of taxa are distinguished the Biotope 1 in the summer period of the study (27 taxa), followed by the biotopes 2 and 3 in the three seasons (on 21 and 20 taxa, respectively). With the lowest number of taxa are remarkabled three seasons (on 13 taxa, respectively) in Biotope 4 (Table 2, 3, 4). Gammarus pulex (L.), Ephemerella ignita (Poda), Chaoborus sp., Chironomus plumosus L. were presented in all of the studied biotopes and seasons. Ephemera vulgata (L.) and Rhyacophila nubile Zett. were presented only in Biotope 1, as well as the species Limnophilus flavicornis Fabr. but only in the autumn period. Tipula sp. are presented only in Biotope 3. Nematoda sp., Herpobdella stagnalis Blanchard, Gomphus vulgatissimus L., Lymnaea stagnalis (L.), Planorbis corneus (L.) and Sphaerium corneum (L.) absent only in Biotope 1. Aeschna grandis L. and Gyrinus sp. absent only in Biotope 4. With the biggest number of specimens bioindicating macrozoobenthos were distinguished in Biotope 1 (309 specimens), followed by this in Biotope 2 (111 specimens) and Biotope 4 (107). With the lowest number of specimens are distinguished Biotope 3 (82 specimens). The determined bioindicating taxa were from five sensitivity groups (A, B, C, D, E). The dominant were these from group B (less sensitive forms) - on 11-8 taxa and group C (relative tolerant forms) - on 4 - 3 taxa for biotopes 1 and 2, respectively; from group D (tolerant forms) and group E (most tolerant forms) - in biotopes 3 and 4 (on 8 taxa and on 3 or 4 taxa, respectively). Only in spring group E was presented with 5 taxa in Biotope 3. More weakly were presented sensitive group A (the most sensitive forms) - only in biotopes 1 and 2, presented only on one species during the three seasons. As a whole, the number of taxa from the group B was the highest (total 11 taxa) than these from the group D (total 8 taxa) and groups C and E (total 4 taxa), but they were presented with lower number of specimens than this from group C (176 and 182 taxa, respectively for groups C and B), followed by this in group E (148 specimens). Group C was detected with the most specimens in Biotope 1 during the spring, summer and autumn (on 61, 42 and 61 specimens); the lowest is their number in biotopes 3 and 4 in the spring, summer and autumn period of the study (on 6, 8, 6; on 3, 6, 7 specimens, respectively for the two biotopes). Group B were presented with the highest number of specimens in Biotope 1. followed by these in Biotope 2, but with less higher numbers than these from group C in the some biotopes. Group E was presented with significantly higher number in Biotope 4 and Biotope 3 during the three seasons (on 27, 28, 24 specimens and 8, 14, 20 specimens, respectively) in comparison with these from Biotope 1 (on 3 specimens, respectively) and Biotope 2 (on 4, 6, 8 specimens, respectively). Groups D and A are with the lowest number of specimens (28 and 10 specimens, respectively). The most sensitive group (A) are absented for biotopes 3 and 4. The number of specimens in Biotope 1 is the highest during the autumn (total 111 specimens) and less higher during the spring and summer period of this study (on total 102 and 92 specimens, respectively). The number of specimens was the lowest in Biotope 3 in the spring (22 specimens from the total 82 specimens). In Biotope 2 the spring and autumn period had an equal quantity of specimens (on 42 specimens, respectively), which is higher than this in summer (27 specimens). Only in Biotope 4 the number of specimens is the highest during the summer period (38 specimens from the total of 107 specimens). The highest number of specimens in Biotope 3 is distinguished during the summer (38 specimens) (Table 2). In connection with the results of this survey, for the examined samples of the four biotopes from the Vit River, bioindicating taxa macrozoobenthos belonging to eight saprobic groups were determined $(0-\beta, 0-\alpha)$ 0-p, β , β - α , α , p, χ -p). Saprobic group β are presented with the highest number of taxa and specimens, fixed for Biotope 1 (on 10 taxa and on 43, 70 and 53 specimens during the seasons spring, summer and autumn, respectively). The number of taxa for the exception is from saprobic group p during the summer period of the study, determined for Biotope 4 (19 taxa with total 23 specimens). Saprobic group $0-\beta$ are absent in samples from biotopes 3 and 4. This is also fixed according saprobic group α during the three seasons in Biotope 1, as well as for saprobic group during the summer and autumn in Biotope 1. Saprobic group $0-\alpha$ are absent in samples from summer and autumn period in Biotope 4. After them with the lowest number of taxa and specimens are presented saprobic group 0-p (on 1-2 taxa and on 1-2 specimens, respectively) (Table 3).

Sensetive	N0.taxa (N0.sps.)								
groups	Α	В	С	D	Е				
Biotope 1									
Spring	1(2)	11(34)	4(61)	1(1)	2(3)				
Summer	1(2)	11(45)	3(42)	0	2(3)				
Autumn	1(2)	11(45)	3(61)	0	2(3)				
Biotope 2									
Spring	1(1)	9(17)	3(18)	6(7)	2(4)				
Summer	1(1)	8(12)	3(6)	7(19)	2(6)				
Autumn	1(2)	8(11)	3(19)	7(14)	2(8)				
Biotope 3									
Spring	0	5(5)	3(6)	8(23)	3(8)				
Summer	0	2(2)	3(8)	8(31)	4(14)				
Autumn	0	2(2)	3(6)	8(40)	4(20)				
Biotope 4									
Spring	0	1(1)	1(3)	8(30)	3(27)				
Summer	0	1(1)	1(6)	8(37)	3(28)				
Autumn	0	1(1)	1(7)	8(38)	3(24)				

 Table 2. Number of bioindicative taxa, specimens and sensitive groups

Note: No.taxa - Number of taxa; No.sps. - Number of specimens.

 Table 3. Number of bioindicative taxa, specimens and saprobic groups

	Saprobic groups							
N0.taxa	0-β	0-α	0-	β	β-α	α	р	χ-р
(No.sp.)	-		р	-	-		-	
Biotope 1								
Spring	4(22)	1(7)	1(2)	10(43)	1(1)	0	1(1)	1(25)
Summer	3(26)	1(15)	1(2)	10(70)	0	0	1(1)	1(18)
Autumn	3(16)	1(9)	1(2)	10(53)	0	0	1(1)	1(31)
Biotope 2								
Spring	2(5)	1(1)	1(1)	11(17)	2(3)	2(2)	1(3)	1(15)
Summer	2(4)	1(2)	1(1)	10(13)	2(5)	3(12)	1(5)	1(2)
Autumn	2(4)	1(1)	1(1)	10(16)	2(3)	3(8)	1(7)	1(15)
Biotope 3								
Spring	0	1(1)	2(2)	6(6)	4(13)	3(9)	1(6)	1(4)
Summer	0	1(1)	2(3)	3(4)	4(13)	3(11)	1(9)	1(5)
Autumn	0	1(1)	2(3)	4(5)	4(18)	3(15)	1(12)	1(3)
Biotope 4								
Spring	0	1	1(1)	1(1)	4(16)	3(10)	1(18)	1(3)
Summer	0	0	1(1)	1(1)	4(17)	3(13)	19(23)	1(6)
Autumn	0	0	1(1)	1(1)	4(19)	3(14)	1(17)	1(7)

The waters of the Vit River in the region under research for the three seasons are distinguished as a whole by a low content of ionic groups and heavy metals for second category of surface flowing waters. The results dominantly read indicate β mesosaprobic in biotopes 1, 2 and 3, α -mesosaprobic in Biotope 4. The results of the present study showed the highest biotic indices during the tree seasons in Biotope 1 (biotic index on 4, respectively) and the lowest – in Biotope 4 (2, respectively). EPT Index (Fig. 6) is a significant indicator for determination of the ecological status on the freshwater ecosystems (Furse et al., 2006; Cheshmedzhiev et al., 2011). Values of EPT above 10 confirm very good ecological status; values from 10-6 evidence for good ecological status; values from 5-2 – for middle ecological status and values below 2 – for bad ecological status.

The results according made ecological estimation by EPT evidence for good ecological status during the three seasons in Biotope 1 (10, 9, 9). In biotopes 2, 3 and 4 is reviewed middle ecological status. Biotope 4 during the three seasons is showed lowest values of EPT (on 2, respectively) and with ecological status critical gravity to bad ecological status, respectively. According done ecological estimation by RETI Index, biotopes 1 and 2 are distinguished with good ecological status (0.667, 0.692, 0.692, 0.636, 0.6, 0.6, respectively), Biotope 3 – with middle (0.445, 0.335, 0.335, respectively) and Biotope 4 – with bad ecological status (on 0.25, respectively) (Fig. 7).



Note: EPT–Ephemeroptera/Plecopera/Trichotera Index; Sp1-Spring/Biotope1; Su1-Summer/Biotope1; Au1-Autumn/Biotope1; Sp2-Spring/Biotope2; Su2-Summer/Biotope2; Au2-Autumn/Biotope2; Sp3-Spring/Biotope3; Su3-Summer/Biotope3; Au3-Autumn/Biotope3; Sp4-Spring/Biotope4; Su4-Summer/Biotope4; Au4-Autumn/Biotope4.

Fig. 6. Ecological appraisal and values of EPT Index

The EQR index, used to assess changes in ecological condition, determined by dividing the numerical value of the measured parameter and the reference value gives rise to highly complex environmental assessment during the three seasons in Biotope 1 and during the spring in Biotope 2 (BI = 4 and EQR = 0.8, respectively). These indicators are the lowest during the three seasons in Biotope 4 (BI = 2 and EQR = 0.4, respectively).

The determined indices for variety of Brillouin (HB), Margalef (Dmg) and Shannon (H') (Table 4) show highest values for biotopes 1, 2 and 3. Values of HB and H' higher than 2 evidences for β -mesosaprobic and values above 1- for α mesosaprobic. Values above 3 and below 1 correspond on oligosaprobic and polisaprobic status, respectively. Values of Dmg higher than 8 shows good ecological status in freshwater ecosystems (Russev, 1993). HB and H' are above 2 in biotopes 1, 2 and 3 and below 3. These values confirmed β -mesosaprobic conditions during the three seasons. HB and H' are below 2 only during the three seasons in Biotope 4 and these values confirmed a-mesosaprobic conditions. The mean reasons are serious anthropogeneus impact from industry and farm activity near the region after town of Pleven and Biotop 4. The river ecosystem as a whole has better condition during the spring and autumn periods of the study (Table 4).

After Yaneva (1979) to this period, scientific examinations for ecological appraisal for condition of the freshwater ecosystem of the Vit Rivet, based on the bioindicative macrozoobenthos communities are no accomplished. The refered studies of other authors are on the biodiversity of fish and fish communities (Nikolova *et al.*, 2008; Pehlivanov *et al.*, 2009), on epilithic diatom flora (*Stancheva et al.*, 2007), etc.



Note: RETI–Rhithron Feeding Type Index; Sp1-Spring/Biotope1; Su1-Summer/Biotope1; Au1-Autumn/Biotope1; Sp2-Spring/Biotope2; Su2-Summer/Biotope2; Au2-Autumn/Biotope2; Sp3-Spring/Biotope3; Su3-Summer/Biotope3; Au3-Autumn/Biotope3; Sp4-Spring/Biotope4; Su4-Summer/Biotope4; Au4-Autumn/Biotope4.

Fig. 7. Ecological appraisal and values of RETI Index Table 4. Basic biotic indicators and values of

descriptive statistics

Biotopes	Biotic indicators								
and seasons	Tn/tx	Tn/sps	HB	Dmg	H'	BI (EQR)			
Biotope 1									
Spring	19	101	2.10	3.90	2.35	4(0.8)			
Summer	27	92	2.11	3.54	2.36	4(0.8)			
Autumn	17	112	2.06	3.39	2.28	4(0.8)			
Biotope 2									
Spring	21	47	2.09	5.19	2.57	4(0.8)			
Summer	21	44	2.34	5.29	2.87	3-4 (0.7)			
Autumn	21	54	2.18	5.01	2.62	3-4 (0.7)			
			Biotope 3			• • •			
Spring	20	49	2.29	4.88	2.76	3(0.6)			
Summer	17	55	2.20	3.99	2.58	2-3 (0.5)			
Autumn	17	69	2.26	3.78	2.59	2-3 (0.5)			
Biotope 4									
Spring	13	61	1.91	2.92	2.19	2(0.4)			
Summer	13	72	1.94	2.81	2.19	2(0.4)			
Autumn	13	70	1.98	2.82	2.26	2(0.4)			
Mean± SD	18.25± 4.159	68.83± 22.16	2.16± 0.19	3.96± 0.93	2.468± 0.227	3.08± 0.85			
Mean SE	1.20	6.4	0.05	0.26	0.66	0.25			
C.V.	22.788	32.205	8.896	23.43 4	9.194	27.514			
Min-Max (Med)	13-27 (18)	44-112 (65)	1.91- 2.6(2.1 45)	2.81- 5.29 (3.84)	2.19- 2.87 (2.46)	2-4 (0.4-0.8)			

Note: Tn/taxa – Total number of taxa; Tn/sps – Total number of specimens; HB - index of Brillouin, diversity; Dmg - index of Margalef, diversity; H² – index of Shannon, diversity; BI(EQR)-Biotic index(Ecological Quality Ratios for Ecological Quality Assessment).

The present studies were made in applying the new approach to integrated environmental assessment in four biotopes of the river, giving rise to changes in the definition of ecological status and trophic structure of river communities.

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