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Ecological characteristics of the main river catchments in Vrachanska Planina Mountains

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Abstract. Assessment of the ecological status of river ecosystems of the major watersheds in the Vrachanska Planina Mts. (Leva River, Cherna River and some tributaries) is made. The assessment is carried out by determining the composition and structure of phytobenthos, benthic macroinvertebrate communities and fish. The following indexes are calculated: diatom pollution index IPS, macrozoobenthic Biotic Index and Fish Based Index (BFI), adopted for assessing the ecological status as required by WFD (Directive 60/2000). Additionally, various physical and hydrochemical analyzes are performed. Based on our results the majority of the mountainous zones of the studied rivers is "good" or "high" ecological status. Deteriorated ecological conditions is observed downstream some villages: for Leva River below the village of Zgorigrad and for Cherna River nearby the village of Dolno Ozirovo. This is probably owing to contamination with organic matter from the human settlements in the area. An accident pollution (with a predominantly protein character) was found in the Cherna River near the Lupovaka area.

Key words: macrozoobenthos, fish, diatoms, ecological status, biotic indices, Bulgaria.

Introduction

The anthropogenic impact on the river ecosystems on the territory of the Vrachanski Balkan Nature Park has not been studied until 2013. We assume that there is a high probability of contamination in the major river catchments (Leva River and Cherna River), owing to the several villages without sewage systems, an abandoned pit mine "Mir", a dairy establishment and livestock farming. In addition, the typology of the studied rivers (semi-mountainous and mountainous types) has a number of specific features, such as a strong karst influence and presence of specific bottom substrata (bedrock, calcareous, rocks, etc.). The objective of this work is to present data for the actual ecological state of the river ecosystems assessed using selected biological and water chemistry indicators, in accordance with the Water legislation in Bulgaria (Regulation N-4) as well as to identify biological reference conditions in very calcareous rivers in Bulgaria.

Material and Methods

Study sites

The study was carried out in July 2013 at 12 sampling sites. They are located along the Leva River catchment (sites 1 – 3, 9 and 10) and Cherna River catchment (sites 4 – 8,

11, and 12) as follows: 1. Dyasna River - upstream the mine "Mir"; 2. Leva River - upstream the village of Zgorigrad; 3. Leva River - downstream the village of Zgorigrad; 4. Gluharska River – upstream; 5. Gluharska river – downstream; 6. Cherna River - downstream the village of Lyutadzhik; 7. Cherna River - upstream the village of Gorno Ozirovo; 8. Cherna River near the village of Dolno Ozirovo; 9. Dyasna River - downstream mine "Mir"; 10. Dyasna River - upstream of the inflow into Leva River; 11. Cherna River - near "Tarsov val" area; 12. Cherna River - near "Lupovaka" area (Table 1).

Table 1. Geographic coordinates (North latitude, East longitude) and altitude of the river sites.

Station	**1 St.**	**2 St.**	**3 St.**	**4 St.**
North latitude	43^0 09' 30.5"	43^0 10' 10.3"	43^0 11' 25.9"	43^0 10' 27.0"
East longitude	23^0 29' 14.9"	23^0 30' 21.6"	23^0 31' 48.9"	23^0 27' 15.3"
altitude (a.s.l.)	801 m	564 m	422 m	608 m
Station	**5 St.**	**6 St.**	**7 St.**	**8 St.**
North latitude	43^0 11' 35.4"	43^0 11' 51.6"	43^0 13' 07.5"	43^0 14' 18.2"
East longitude	23^0 25' 30.0"	23^0 24' 38.6"	23^0 23' 35.0"	23^0 21' 33.9"
altitude (a.s.l.)	412 m	379 m	328 m	278 m
Station	**9 St.**	**10 St.**	**11 St.**	**12 St.**
North latitude	43^0 09' 31.6"	43^0 09' 56.3"	43^0 09' 56.5"	43^0 10' 45.9"
East longitude	23^0 29' 18.8"	23^0 29' 50.6"	23^0 25' 26.5"	230 24' 51.9"
altitude (a.s.l.)	777 m	615 m	575 m	490 m

River typology according to the Water Framework Directive

All studied rivers belong to the mountainous and semi-mountainous river types (R2 and R4) in Ecoregion 12 (Pontic province), according to the national typology of Bulgaria, using system B of the Water Framework Directive (Cheshmedjiev et al 2010). At the same time, a number of special cases in the typology are presented within the study area as follows:

- Rocky gorges and various rock formations
- Bedrock substrata at low altitude
- Areas with moderate to slow velocity alternating with waterfalls
- Karst deposits in bottom substrata
- Typical seasonal variations in water level (semi-dry conditions during summer and autumn)

Water chemistry

We investigated the lower zones of the main rivers (sites 3 and 8) and the high mountainous area of Cherna River (sites 11, 12). The following parameters have been measured *in situ*: dissolved oxygen (oximeter WTW Oxi 330i); pH (pH meter WTW pH330i) and conductivity (WTW Cond 330i). In the laboratory we measured $N\text{-}NH_4$ (ISO 7150-1:2002); $N\text{-}NO_2$ (EN 26777:1997); $N\text{-}NO_3$ (ISO 7890-3:1998); PO_3^{4-} and total phosphorous (EN ISO 6878:2005); total nitrogen (EN ISO 11905-1:2001); BOD_5 (EN 1899-2:2004).

Phytobenthos

Epilithic diatoms were collected from the upper surface of stones at sites 3, 8 and 11 according to the European Standard EN 13946:2003 (European Commitee for Standardization 2003). The samples were fixed *in situ* with 4% formaldehyde. In the laboratory, pretreatment of the samples was done following A.5.2 method (EN 13946/2003) with cold sulfuric acid (H_2S0_4) and potassium permanganate ($KMnO_4$). The cleaned material was mounted on permanent slides with Naphrax©. Light microscopy was performed following the European Standard EN 14407:2004 (European Committee for Standardization 2004) on Amplival Carl Zeiss, with 100x oil-immersion objective. Diatoms were identified mainly according to Krammer & Lange-Bertalot (1986-1991) and Lange-Bertalot (2001). Four hundred valves per slide were counted. Diatom pollution index IPS (Coste in CEMAGREF 1982, 1984) was calculated using Omnidia ver.5.3. (Leconite *et al.* 2003). The classification system for karst springs (R15 river type) instead of typical classes for mountain or semi-mountain conditions (R2 or R4 river types) have been used to assess properly ecological status of phytobenthos (benthic diatoms) in these explicit calcareous conditions.

Macrozoobenthos

Macrozoobenthic samples were collected from sites 1-11 using a hand-held net with mesh size of 500 μm (EN ISO 10870:2012). The multihabitat approach was applied (adapted from Cheshmedjiev *et al.* 2011). At each station 10 sub-samples from different substrates were collected, according to EN ISO 16150:2012. Ecological status was determined using the following metrics (after Cheshmedjiev & Varadinova 2013): Biotic index (adopted from Yaneva & Cheshmedjiev 1999), total taxa number (TTN index); number of species from Ephemeroptera, Plecoptera, Trichoptera orders (EPT index). The ecological assessment was carried out according to the criteria for R-4 river type (sites 3, 6, 7, 8) and R-2 river types (the remaining sites) under Regulation N-4 (for TTN index and biotic index BI) and following Cheshmedjiev & Varadinova (2013) for EPT index.

Ichthyofauna

Fish samples were collected from the downstream parts of the two studied catchments - sites 3 and 8. Scientific device for electrofishing (Hans Grassl IG 200-2) according to standard EN 14011:2003 was used. The ecological status was determined based on the BFI index v.1.8/26.3.2010 (Mihov 2010). The scope of the BFI index is presented in accordance with Regulation N-4 for R-4 river type.

Results

Water chemistry

Most of the measured values for the physical and the chemical parameters for sites 3, 8, 11 correspond to „good" or „high" ecological status (Table 2). In Cherna River near Lupovaka area (site 12) was found deterioration of the environmental situation based on the dissolved oxygen, ammonium nitrogen, phosphates, total phosphorus, total nitrogen, BOD_5. Moreover, we found serious pollution by organic substances (for instance proteins). The samples were collected on 15.XI.2013, after torrential rains, when on the water surface

was observed foaming, and the river water became white in colour.

Table 2. Ecological status of the river sites as based on the physical and chemical parameters of the water. Legend: * – high status; ** – good status; *** – moderate or lower status (outside the scope of the Regulation N-4); bold – extremely high concentrations

Site	O_2	pH	cond.	$N\text{-}NH_4$	$N\text{-}NO_2$	$N\text{-}NO_3$	PO_4^{3-}	T P	T N	BOD
3	8,20 *	8,38 **	524 *	0,023 *	0,007 *	1,47 **	0,023 **	0,04 **	1,61 ***	<1,25 **
8	10,2 *	8,65 --	367 *	0,012 *	<0,002 *	0,008 *	0,036 **	0,05 **	0,27 *	<1,25 **
11	11,8 *	8,29 **	378 *	0,008 *	<0,002 *	1,10 ***	<0,006 *	<0,007 *	1,12 **	<1,25 **
12	6,60 **	7,23 **	512 *	**0,88 *****	0,029 **	0,50 **	**0,31 *****	**1,24 *****	**1,80 *****	**5,50 *****

Phytobenthos

According to the diatom index IPS the sites were characterised as follows:

Site 3 – in "bad" ecological status (IPS=4,9). The dominant taxa were polysaprobes, tolerant to organic pollution (*Nitzschia palea* (Kützing) W.Smith, *Achnanthidium saprophilum* (H.Kobayasi & S.Mayama) Round & L.Bukhtiyarova, *Eolimna minima* (Grunow) Lange-Bertalot & W.Schiller).

Site 8 – in "good" ecological status (IPS=14,2). The dominant taxa were β-mesosaprobes (*Navicula reichardtiana* Lange-Bertalot, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Diatoma vulgaris* Bory de Saint-Vincent).

Site 11 – in "high" ecological status (IPS=19,6). The dominant taxa were oligosaprobes to β-mesosaprobes (*Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi, *Achnanthidium subatomus* (Hustedt) Lange-Bertalot).

Macrozoobenthos

The bottom invertebrates recorded from the mountainous sites of the catchment of Leva River were mainly xenosaprobic or oligosaprobic species (sites 1, 2). The most abundant species was *Gammarus fossarum* Koch 1835 (Amphipoda). In the semi-mountainous sector (site 3) more ubiquitous and β-mesosaprobic species were found. The most abundant taxon was *Hydropsyche* sp. (Trichoptera).

In the catchment of Cherna River the most abundant species was *Oligoneuriella rhenana* (Imhoff 1852), Ephemeroptera. This species is typical for the semi-mountainous river type in Bulgaria (R-4 type). During our study this species was found exactly at this river sector (sites 6-8). Very high abundance had *Hydropsyche* sp. (Trichoptera), which was found at almost all sites. Xeno- or oligosaprobic taxa such as: *Leuctra hirsuta* Bogoescu, Tabacaru, 1960, *Perla pallida* Guérin-Méneville, 1838, *Dinocras cephalotes* (Curtis, 1827), *D. megacephala* (Klapalek, 1907) (Plecoptera); *Epeorus* sp. (Ephemeroptera) and *G. fossarum* were found only from the mountainous sector (sites 4, 5). Overall this mixture of typical mountain reophilic indicators (Perlidae, *Epeorus* sp.), semi-mountain indicators (*O. rhenana*, plentiful *Hydropsyche* sp.) and typical karst elements (*Niphargus bureshi* Fage 1926, plentiful *G. fossarum*) define biological reference conditions for these highly karst rivers (calcareous conditions).

Generally, the ecological status of studied rivers corresponded to „good" or „high" status (Table 3). The Biotic index had the highest values in the mountainous sectors from both catchments (BI= 4 or 4,5). Downstream of the river currents, the values of the Biotic index decreased. In Cherna River catchment this decrease was within the limits of „good" ecological status (BI= 3,5) for semi-mountainous area (sites 6- 8). In the lower part of Leva River (site 3) the Biotic index had minimal value (BI= 3) and indicated „moderate" ecological status.

Indexes TTN and EPT showed a similar dynamics for both watersheds (Table 2). The highest values have been recorded in the mountainous areas of the rivers. The ecological status at these sampling sites was defined as „good" (at sites 1, 2, 6- 8 and site 5 for TTN index), and some case as „high" (at site 4 for TTN index; at sites 4 and 5 for EPT index). Only in Leva River downstream the village of Zgorigrad (site 3) deteriorated conditions were identified and the corresponding ecological status was defined as „moderate" as based on the macroinvertebrate assemblages.

Table 3. Ecological status of the river sites as based on different biological parameters of the macrozoobenthos after Regulation N-4. Legend: * – high status; ** – good status; *** – moderate status;

Index / site	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
Biotic index	4 *	3,5 **	3 ***	4,5 *	4 *	3,5 **	3,5 **	3,5 **	4 *	4 *	4,5 *
TTN index	13 **	12 **	9 ***	17 *	12 **	11 **	13 **	14 **	no	no	no
EPT index	8 **	6 **	4 ***	12 *	12 *	7 **	8 **	8 **	no	no	no

Fish

The fish fauna of the Vrachanska Mts. is composed of species that are typical for semi-mountainous Bulgarian rivers. A total of eight species have been found in the area. In Leva River (site 3) the greatest abundance and biomass had the Romanian barbell *Barbus petenyi* Heckel 1852 (77 ind., 475 gr). In addition, the fish index value indicated „moderate" ecological status (BFI = 0,54). For Cherna River (site 8) the greatest abundance had *B. petenyi* (35 ind.) and Golden spined loach *Sabanejewia balcanica* (Karaman 1922) (46 ind.), while the European chub *Squalius cephalus* (Linnaeus 1758) had the highest biomass (257 gr). Species composition and age structure of the ichthyofauna was well balanced. The value of BFI index indicated „good" ecological condition (BFI = 0,74).

Discussion

The high increase in the nutrients concentrations and deterioration of the oxygen regime on Cherna River in the mountainous area demonstrated that in the territory of the Nature Park Vrachanski Balkan there were sources of serious organic pollution that extends beyond the standards for water quality. This situation was most likely temporary and may be due to past pollution of karst groundwater, which stands out after heavy rains.

All applied biological quality elements (phytobenthos, benthic macroinvertebrates and ichthyofauna) showed that in urban areas there was deterioration in environmental conditions (mainly downstream village of Zgorigrad). However, it was assumed that no

permanent degradation of aquatic ecosystems due to drought, hydromorphological pressure or other anthropogenic changes in the catchment area of the two major watersheds. Moreover - mountain river sections were in natural conditions, anthropogenic uninfluenced and with high biological integrity.

All rivers in the Nature Park Vrachanski Balkan should be classified as a specific case of mountain river type (R2 - calcareous) or semi-mountain river type (R2 - calcareous) under seasonal semi-drying conditions. We recommend very careful application of the type-specific classification systems in this case. For example, the classification system for karst springs (R15 river type) should be used in the phytobenthic assessment, while usual classification system for mountain and semi-mountain conditions (R2 and R4 river types) are usable for a proper ecological status assessment based on analyses of benthic macroinvertebrate communities.

We suggest the application and compliance of the "*Program for the study of ecological status and chemical status of surface waters*", already developed for the needs of Directorate of the Nature Park Vrachanski Balkan and implementation of measures for prevention of any further water pollution.

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Екологична характеристика на основните речни водосбори във Врачанска планина

СВЕТОСЛАВ ЧЕШМЕДЖИЕВ, ЛЮБОМИР КЕНДЕРОВ, ТИХОМИР СТЕФАНОВ, ПЛАМЕН ИВАНОВ, ВЕСЕЛА ЕВТИМОВА, ЯНКА ВИДИНОВА, ВИОЛЕТА ТЮФЕКЧИЕВА, ТЕОДОРА ТЕОФИЛОВА

(Резюме)

Извършена е оценка на екологичното състояние на речните екосистеми от основните водосборите във Врачански Балкан (река Лева, река Черна и някои притоци). Оценката е осъществена чрез определяне на състава и структурата на фитобентоса, бентосните макробезгръбначни съобщества и рибната фауна. Изчислени са следните индекси: диатомеен индекс на замърсяването IPS, макрозообентосен Биотичен индекс и Базиран на риби индекс (BFI), приети за оценка на екологичното състояние според изискванията на Рамковата директива за водите (Директива 60/2000). Допълнително са извършени различни физични и хидрохимични анализи. Въз основа на нашите резултати, по-голямата част от планинските зони на изследваните реки са в „добро“ или „отлично“ екологично състояние. Влошаване в екологичните условия се наблюдава след някои села: за Лева река под село Згориград и за река Черна при село Долно Озирово. Това може би се дължи на замърсяване с органични вещества от населените места в областта. Регистрирано е и инцидентно замърсяване (с предимно протеинов характер) в река Черна в близост до местността Луповака.