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A comparison of various indices based on benthic macrofauna for the assessment of the ecological status of selected Carpathian streams

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Abstract

The biological indices MMI-PL, BMWP-PL, and %EPT were applied to compare the ecological status of Carpathian flysch stream sectors situated above and below sewage treatment plants. MMI-PL was found to be the most sensitive, whereas BMWP-PL indicated a very good ecological status at all sampling stations despite an increased trophic level of water in polluted sectors. %EPT proved to be an unreliable index because of its strong fluctuations resulting mainly from unstable densities of benthic invertebrate groups not included therein. The analyses of coli titers and water chemistry in the same stream sectors revealed temporary pollution at both types of stations.

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INTRODUCTION

In Poland, the assessment of the ecological status of running waters is performed by Regional Inspectorates for Environmental Protection on the basis of biological indices, such as the Macrophyte Index for Rivers (MIR) (Szoszkiewicz et al. 2010) and the Diatom Index (Picińska-Faltnowicz 2009). Up to now, the biotic index based on benthic macrofauna composition BMWP-PL (Biological Monitoring Working Party) and the diversity index *d* were used (Kownacki & Soszka 2004). BMWP-PL is a modification of the British BMWP score (Armitage et al. 1983) and the index *d* is a modified version of the Margalef index, although the use of these indices has not been obligatory. Recently, the assessment system module MMI (Multimetric index, known in Poland as MMI-PL) has been implemented (Bis et al. 2012). Sometimes the values of %EPT (the sum of all individuals of Ephemeroptera, Plecoptera, and Trichoptera divided by the sum of all collected macroinvertebrates) have also been used for water quality classification (Lenat 1988).

Running waters in the Polish national parks situated in the Carpathians are considered as relatively clean, and therefore they have been used as reference points for various biological indices (Lewin et al. 2013). Most of the streams in the Bieszczady National Park (henceforth: BdNP) are also relatively clean, and their bottoms are inhabited by a variety of macroinvertebrates, including three groups whose composition is well documented: mayflies (Kukuła 1991, 1998; Klonowska-Olejnik 2000), stoneflies (Kukuła 1998, Fiałkowski 2000), and caddisflies (Kukuła 1998, Szczyński 2000). The natural aquatic fauna of the BdNP is highly important, as it contains many endemic, rare, and endangered species (Klonowska 1987, Kukuła & Szczyński 2000, Szczyński 2000). The BdNP is visited by increasing

numbers of tourists, especially from May to September. This results in a gradual eutrophication of streams, mainly those flowing through tourist centers, which has been observed especially in summer, at high water temperatures and low discharge (Kukuła 1998).

Recently, in addition to water chemistry analyses, an ecological assessment based on benthic fauna was taken into account during the preparation of the “Water Protection Plan” for the BdNP (Dumnicka et al. 2011), and data collected for the purpose of that assessment are used in this study.

The aim of the present study was to select the most appropriate and sensitive method for determining the ecological status of running mountain waters of the flysch stream type in the Carpathians (Ecoregion No. 10, according to Blachuta et al. 2010). For this purpose, data on benthic macroinvertebrates collected in stream sectors located above and below sewage treatment plants were used. Chemical water parameters were studied to demonstrate a possible relationship with the ecological status of the selected running waters. Moreover, the levels of fecal *Escherichia coli* in water at all sampling stations were measured to determine the direct pollution impact of tourism.

So far, except for the paper by Lewin et al. (2013), values of the BMWP-PL index were calculated in Poland for lowland rivers (e.g. Czerniawska-Kusza 2005, Koperski & Golub 2006, Korycińska & Królak 2006) and for some submontane running waters (Czerniawska-Kusza 2011, Wyźga et al. 2013), whereas the MMI-PL index is known only for the Polish streams in the Sudety Mountains (Przesmycki & Jusik 2013).

STUDY AREA

Several running waters in BdNP were investigated within the “Water Protection Plan” (Dumnicka et al. 2011), but only a few of them have been taken into account in the present study. Sampling stations 1 and 2 were located on the Wołosatka stream, stations 3, 4, 5, and 6 on the Wołosaty stream, stations 7 and 8 on the Rzeczyca stream, and stations 9 and 10 on the Terebowiec stream (Fig. 1). The odd-number sampling stations were situated above the sewage treatment plants, whereas the even-number stations – below the plants: for example, station 2 in the village of Wołosate, station 4 below the sewage plant of the “Górski” hotel (the largest hotel in BdNP), located in the village of Ustrzyki Górne – the main

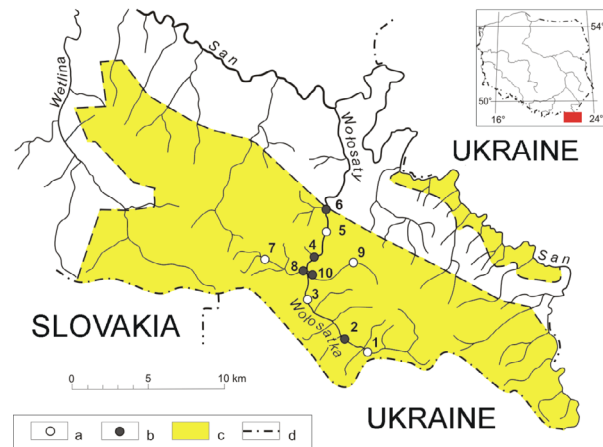


Fig. 1. Location of the sampling stations in the Bieszczady National Park; a – sampling stations above sewage treatment plants, b – stations below sewage treatment plants, c – the BdNP area; d – the state border

tourist center in the region. All sampling stations were situated in BdNP, at altitudes from 630 to 800 m. The streams and their catchments were fully described by Dumnicka & Kukuła (1990), Kukuła (1998), Kukuła & Szczesny (2000), and Szczesny (2000).

MATERIAL AND METHODS

Water samples for chemical analyses were taken 10 times from April 2009 to March 2010. Temperature, pH, and conductivity were measured *in situ* with a portable instrument (Elmetron pH-meter CX-742). Water samples for dissolved oxygen content and BOD analyses were collected into glass bottles and examined in the laboratory by Winkler’s method.

The ammonium content was measured in the laboratory by ion chromatography ICS 1000, and chloride, nitrate and phosphate anions – by chromatography IC DX-320 with an analytic column AS15 4 mm (DIONEX). The detection limit for ammonium and chloride was $1.5 \mu\text{g dm}^{-3}$, whereas for nitrate – $1 \mu\text{g dm}^{-3}$.

Statistical significances were determined by Student’s *t* test, using paired data.

Water for bacteria and benthic samples were collected three times: in May, August, and November 2009. The number of fecal *Escherichia coli* was determined by the fermentation test-tube method

according to Polish standards (PN-77, C-04515/07). The sampling procedure for macroinvertebrates covered all the habitats at each station and corresponded to the Multi Habitat Sampling (MHS) method (Bis et al. 2012), but owing to the small size of some streams and their location in the protected area of the national park, the number of partial samples was reduced to ten. Because of the small number of specimens, they were usually selected from half of the sub-samples (except for Chironomidae). For the purpose of the “Water Protection Plan”, Ephemeroptera, Plecoptera, and Trichoptera were identified by specialists to the species level, and identification of the remaining groups was carried out to the family level according to Rozkošny (Ed.) (1980) and Kolodziejczyk & Koperski (2000).

The BMWP-PL index was calculated according to the instruction provided by Kownacki & Soszka (2004). The MMI-PL assessment module is based on the multimetric system based on six different metrics, which provide information mainly on tolerance to pollution, as well as on taxa richness (Buffagni et al. 2004, Van de Bund 2009 after Bis et al. 2012). MMI-PL values range from 0 (bad ecological status) to 1 (high ecological status). The ecological status of the running waters was determined by classifying them into one of the five quality classes (Table 1). Unlike BMWP-PL and MMI-PL values, %EPT are not attributed to water quality classes in Poland. Some authors using this index in their publications, arbitrarily adopted a three- or five-degree scale for %EPT (e.g. Czerniawska-Kusza & Szoszkiewicz 2007, Lewin et al. 2013). In this paper, we assumed the values of %EPT as indicated in Table 1.

RESULTS

At all sampling stations and at all sampling times, such chemical parameters as conductivity, pH, dissolved oxygen and nitrates, were within the range corresponding to water purity class I according to Dz.U. (2011). The value of conductivity ranged between 90 and 260 $\mu\text{S cm}^{-1}$, and the mean value of pH was 7.6. During the study period, fluctuations in the oxygen content were observed from a higher mean value of 11.7 $\text{mg O}_2 \text{ dm}^{-3}$ (SD = 0.5) in early spring and autumn to a considerably lower mean value of 7.9 $\text{mg O}_2 \text{ dm}^{-3}$ (SD = 0.3) in August. There were no significant differences between the stations above and below the sewage treatment plants (Student's *t*-test).

Table 1

Ecological status according to various values (ranges) of macroinvertebrate metrics for the flysch type of streams

Ecological status	BMWP-PL	MMI-PL	%EPT
Very good	>100	>0.89	>40
Good	70-99	0.89-0.69	30-40
Moderate	40-69	0.69-0.46	20-30
Poor	10-39	0.46-0.23	10-20
Bad	<10	<0.23	<10

In water sampled below the sewage treatment plants (stations: 2, 4, 6, 8, 10), especially during the tourist season, elevated values of phosphates, ammonium, BOD, and chloride were recorded (Fig. 2 A, B). The highest concentration of PO_4^{3-} was found in July (0.39 mg dm^{-3}), and the maximum NH_4^+ , BOD, and Cl^- concentrations (2.2 $\text{mg NH}_4^+ \text{ dm}^{-3}$, 8.96 $\text{mg O}_2 \text{ dm}^{-3}$ and 1.9 $\text{mg Cl}^- \text{ dm}^{-3}$) were observed in August at station 2. The maximum values of PO_4^{3-} , NH_4^+ , and BOD found below the sewage treatment plant classified this stream stretch below water purity class II (Dz.U. 2011). An elevated value of Cl^- (compared with values characteristic of flysch rock in this area) was also observed in May at station 4 (2.3 $\text{mg Cl}^- \text{ dm}^{-3}$) (Fig. 2 A, B). The only significant differences ($p < 0.001$) were found between stations 5 and 6 for PO_4^{3-} , NH_4^+ and Cl^- , station 9 and 10 for NH_4^+ and Cl^- (Student's *t*-test).

Escherichia coli titers varied widely during the study period. In the Wolosaty stream, this parameter ranged from 10 to 0.0001 ml^{-1} (Fig. 3 A). A high level of *E. coli* was noted at station 4 below the sewage treatment plant in August (0.001 ml^{-1}), while the level of contamination with *E. coli* (0.0001 ml^{-1}) in the same stream above the sewage plant was even higher. The values of *E. coli* titers in November were 1 or 10 at almost all stations, except for station 9 with the value of 0.01 ml^{-1} (Fig. 3 A).

The benthic fauna collected at particular sampling stations ranged from 500 to 5000 individuals (Fig. 3 B). The numbers of macroinvertebrates were almost always higher at stations below the sewage plants than at stations located above the plants. Particularly abundant specimens were collected in May and August at station 4 and in May at stations 6, 8, and 10. During the same period, the highest number of Chironomidae was found (1120, 2400, 3340, 3600, and 1056 ind., respectively), whereas their number was significantly smaller (318 ± 249 individuals) for the remaining benthic samples.

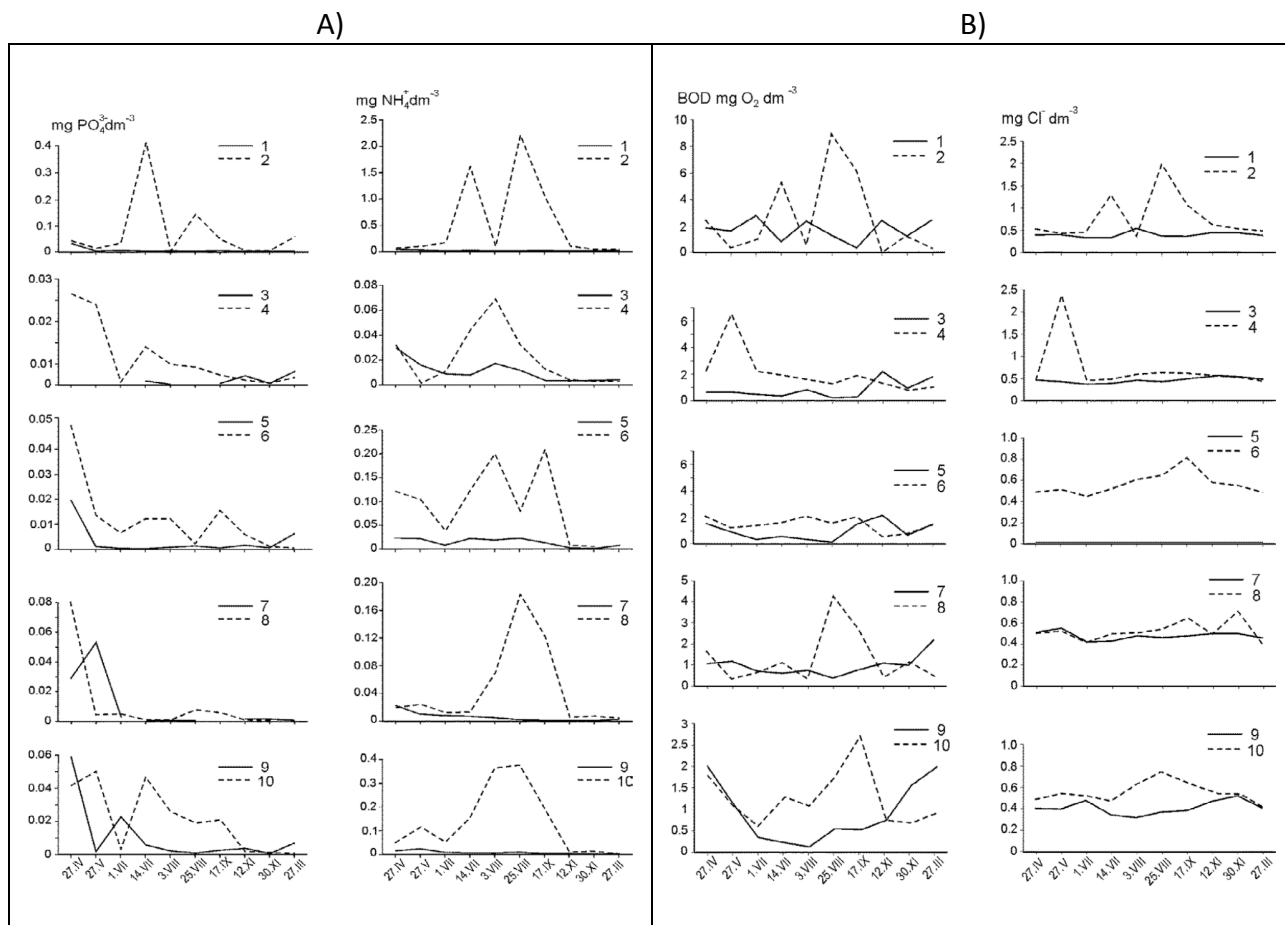


Fig. 2. A) Monthly changes in PO_4^{3-} and NH_4^+ values at the sampling stations; B) Monthly changes in BOD and Cl^- values at the sampling stations. Solid line – for stations above sewage treatment plants (Stations 1, 3, 5, 7 and 9), dashed line – for stations below sewage plants (Stations 2, 4, 6, 8 and 10)

The number of collected macroinvertebrate taxa was 41, whereas their number ranged from 18 to 28 at particular stations and sampling times. The highest number of taxa was usually found in November. The number of taxa was similar at stations situated above and below the sewage treatment plants, but sensitive families with a higher score according to the BMWP-PL index were found more often at unpolluted stations. The most sensitive families were represented frequently by caddisflies, especially Glossosomatidae (score 10) and Odontoceridae (10). The others, e.g. Leptoceridae (10), Taeniopterygidae (9), Goeridae (9) and Lepidostomatidae (9), were observed only at a few sampling stations, mostly in November. Species of high nature value, such as *Annitella chomiacensis* Dzierżewicz 1908, *Hydropsyche tabacarii* Botosaneanu 1960, and *Rhythrogena wolosatkae* Klonowska 1987 (Ephemeroptera) were collected only at a few unpolluted stations. Oligoneuriidae (8)

and Heptageniidae from the genera *Epeorus* and *Rhythrogena* (8) were the only mayflies found. Other taxonomic groups were represented by Taeniopterygidae (9), Perlidae (Plecoptera) and Athericidae (Diptera) (8).

Values of the BMWP-PL index calculated for the sampling stations both above and below sewage treatment plants, were always over 100, which classified both stream sectors of all streams as having a very good ecological status (Table 2). The highest values of this index were found at all sampling stations in November. Values of MMI-PL at almost all sampling stations were over 0.89, which also corresponds to the highest ecological status. Only in two cases, i.e. at sampling stations 6 and 8 (both situated below sewage plants), the MMI-PL values were 0.82 and 0.87 in May, which classified the respective streams sectors as having a good ecological status. Out of the six key metrics used for

Table 2

Values of BMWP-PL, MMI-PL, and %EPT calculated for the 10 sampling stations. Stations 1, 3, 5, 7, and 9 were situated above sewage treatment plants. Stations 2, 4, 6, 8, and 10 were situated below sewage treatment plants

Stream	Station	MMI-PL V	BMWP-PL V	%EPT V	MMI-PL VIII	BMWP-PL VIII	%EPT VIII	MMI-PL XI	BMWP-PL XI	%EPT XI
Wołosatka	1	0.94	124	45	1	125	71	1	178	53
Wołosatka	2	0.98	152	28	0.99	134	40	1	153	67
Wołosate	3	0.92	143	18	1	127	39	1	167	72
Wołosate	4	0.92	131	22	0.92	134	22	1	167	61
Wołosate	5	0.97	156	20	1	151	32	1	187	70
Wołosate	6	0.82	147	5	1	118	42	1	170	70
Rzeczyca	7	0.94	115	33	0.99	127	62	1	138	59
Rzeczyca	8	0.87	133	59	1	132	10	0.93	155	47
Terebowiec	9	1	134	72	1	139	65	0.99	142	55
Terebowiec	10	0.92	107	34	1	117	60	1	153	84

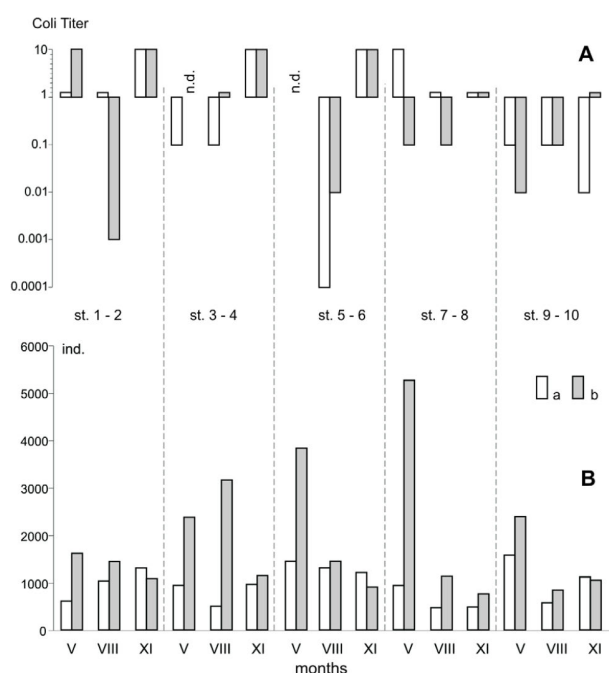


Fig. 3. Changes in *E. coli* titers (A) and the number of macroinvertebrates (B) in May, August, and November; a – stations above sewage treatment plants (Stations 1, 3, 5, 7 and 9), b – stations below sewage treatment plants (Stations 2, 4, 6, 8 and 10)

MMI-PL calculations, $\text{Log}_{10}(\sum \text{EPTD} + 1)$, 1-GOLD, and the Shannon-Wiener index were much lower for those two stations compared to other stations; whereas ASPT, the total number of families (S), and the number of EPT families were similar. The lowest values of BMWP-PL and MMI-PL metrics were found in May (Table 2).

The value of %EPT for all samples ranged from 5% to 73% (Table 2). Of the total 30 samples, in 18 samples %EPT values were higher than 40% (mostly at stations located above the sewage treatment plant), in 5 samples the values ranged from 30% to 40%, and in 7 samples they were lower than 30%. In two cases, the values of %EPT were very low: 5% at station 6 on the Wołosaty stream and 10% at station 8 on the Rzeczyca stream (Table 2).

DISCUSSION

Running waters of the upper San River drainage basin represent the bicarbonate-calcium-magnesium type (Kukula & Szczesny 2000, Siwek & Rzonca 2009) with high concentrations of sulphates and low concentrations of nitrates (Szczesny 2000, Rzonca & Siwek 2009). Most values of physical and chemical parameters determined in our study (Dumnicka et al. 2011) were characteristic of water purity class I (Dz. U. 2011). In shallow, turbulent mountain streams, the oxygenation level rarely drops below values indicating water purity class I (Kukula & Stachowicz-Kawalec 1996) or sometimes remains at a high level as in the High Tatra stream where sewage was not purified (Bombowna 1977). Tourist impact from Wołosate resulted in elevated concentrations of phosphates and ammonium, as well as high values of BOD in the Wołosatka stream below the sewage treatment plant, mostly in summer (May, July, August). Similar but weaker effects were observed in three other streams: Wołosaty, Terebowiec, and Rzeczyca. Nitrate concentrations below sewage treatment plants were not elevated, probably because of the *in situ* nitrate assimilation by algae, mainly diatoms, which were very abundant at such places

(Kukuła & Szczęsny 2000). Moreover, the concentration of chlorides was slightly elevated above the background level at stations below the sewage plants, which indicates its anthropogenic origin. The deterioration in water quality below the sewage plants at the end of the tourist season had been observed earlier in the Wołosaty stream (Kukuła 1998, Kukuła & Kukuła 1996), as well as in the Wołosatka and Terebowiec streams (Kukuła & Szczęsny 2000). This indicates that these sewage treatment plants could not completely purify the water at the end of the 20th century.

Although the efficiency of several sewage treatment plants in chemical cleaning was acceptable, *E. coli* titer results indicated that stream water contamination was temporarily high, usually at stations below the sewage treatment plants. According to Kukuła & Kukuła (1996), the *E. coli* titer in the Wołosaty stream below “Hotel Górski” (station 4 in this study) in October 1995 was 0.00001 ml⁻¹. This value was much higher than the one found in our study, but in 1995 the sewage plant operated less efficiently. High levels of *E. coli* measured in August at two stations in Wołosate indicate that the stream may have been contaminated directly by tourists. Bacteria of this group are characterized by a very short life span in water, so their presence in water indicates a relatively recent introduction of feces into the streams (Stadnicki 2005, Bis et al. 2006). A similar situation was observed permanently in the Terebowiec stream, which may indicate a sustainable inflow of sewage from sparse houses. According to the most recent standards (Dz. U. 2011), this parameter is not included in the ecological status assessment of running waters. However, in our opinion the measurement of *E. coli* value in national park waters is necessary, since they are used for drinking purposes by tourists.

The ecological status of all the studied stream sectors, as indicated by the values of the BMWP-PL and MMI-PL metrics, was very good, which explains why Šporka et al. (2009) treated the running waters of the Carpathian national parks as reference sites. In shallow, well-oxygenated streams in the Bieszczady Mountains, the fauna diversity was high, and many invertebrate families characteristic of clean waters (score 10-7 in BMWP-PL calculations) were present there, both at stations considered clean and at those slightly polluted. High diversity of benthic fauna at slightly polluted but well-oxygenated sampling stations on the Carpathian rivers studied previously,

e.g. Kryniczanka (Szczęsny 1974) and Dunajec (Kownacki et al. 2002), was observed. The only differences between clean and polluted stations was the percentage contribution of sensitive taxa and the total density of macroinvertebrates.

Very high MMI-PL values were obtained, despite the fact that the number of samples collected was half the amount recommended by Bis et al. (2012), which made it possible to reduce the intrusion into the water bodies of the protected areas. The results of MMI-PL, which includes some biological and ecological metrics, showed a decline in the ecological status at only two stream stretches. This indicates that the MMI-PL index is more suitable for following the changes in natural invertebrate communities in streams than BMWP-PL, which was confirmed by Przesmycki & Jusik (2013) for streams in the Sudety Mountains. In the case of this index, the final results depend on both qualitative and quantitative data. To obtain a correct assessment of the ecological status by MMI-PL, the macroinvertebrates present in subsamples must be removed very accurately. This refers especially to small specimens of Chironomidae and Oligochaeta. In this study, a careful removal of Chironomidae specimens resulted in a low percentage of EPT (<30%) recorded at seven sites, mainly below the sewage treatment plants. Low values of this index were also found by Lewin et al. (2013) for some streams situated within protected areas of the Carpathians.

Bis et al. (2012) suggested that the optimal sampling time for the assessment of the ecological status is between April and June. It seems to be appropriate, because in our study the effect of pollution was observed in the results obtained for samples collected in May. Nevertheless, the results of chemical analyses revealed a decline in the water quality during the peak of the tourist season in August. In assessing the ecological status of running waters, MMI-PL seems to be more sensitive than BMWP-PL, whereas %EPT is less suitable for this purpose, since it changes considerably depending on the number of other macroinvertebrate taxa.

Although the ecological status of the streams was very good, the study revealed that the numbers of sensitive and endangered species, such as *Rhithrogena wolosatkae* and some Trichoptera species, e.g. *Hydropsyche tabacarii* and *Annitella chomiensis* were reduced (Klonowska-Olejnik 2000, Szczęsny 2000). Habitat alterations at the stations below the sewage treatment plants, such as a heavily silted up bottom resulting from algae cover, may have a negative

impact on benthic macrofauna. Therefore, not only mechanical and chemical, but also biological sewage purification should be implemented in protected areas to eliminate the excess of biogens from running waters.

CONCLUSIONS

1. The MMI-PL index was the most appropriate for the assessment of the ecological status of Carpathian flysch streams (Ecoregion No. 10), because it is based on various key metrics.
2. In the running waters of BdNP, which are well-oxygenated and have a low biogens content, a small inflow of sewage did not induce a decline in the number of taxa (at the family level). Consequently, the values of the BMWP-PL index were high at all times at all sampling stations.
3. Nevertheless, even a minor pollution of the streams poses a threat to the rare benthic species of Ephemeroptera and Trichoptera characteristic of this region.
4. *E. coli* titer investigations in the running waters of BdNP, not covered by Dz.U. (2011), are recommended, since tourists frequently drink directly from streams.

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