



Ecological Status Assessment of Mountain and Semi-mountain Streams in the Aegean Watershed: Applicability of Biotic Indices BMWP and ASPT Based on Macroinvertebrates

Biljana Rimcheska & Yanka Vidinova*

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria

Abstract: In the past decades, the majority of the European countries have developed evaluation systems for freshwater ecosystems, including quality assessment through the living organisms as biological quality elements. Based on this practice, the aim of the present study is to provide indicative ecological status estimation of mountain and semi-mountain streams in a poorly studied area of the North Macedonian – Bulgarian cross-border territory. We focused on four indices for benthic macroinvertebrates: Biological Monitoring Working Party (BMWP), Average Score Per Taxon (ASPT), Irish Biotic Index (BI) and EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa richness. In total, 38 sites in both countries were sampled during autumn 2017 and spring 2018, all belonging to the R3 and R5 river type. The water quality was assessed from excellent to poor (I – IV class). The majority of the studied rivers do not achieve a good ecological status, mainly due to hydromorphological stress and habitat degradation (damming/mining/hydropower stations) and/or organic/anthropogenic pollution. Statistical treatment of the dataset indicated the strongest and most significant positive correlation between BMWP and EPT-taxa and negative correlation between BMWP and BI. The correlation between the General Degradation Score and the applied ecological quality indices clearly outlined a negative trend. The results confirmed the sensitivity of the indices BMWP and EPT to seasonal changes of the macroinvertebrate communities in running water ecosystems.

Key words: Water quality, biotic indices, benthos, R3 and R5 river types, Bulgaria, North Republic of Macedonia.

Introduction

According to the terminology of the Water Framework Directive (WFD) (DIRECTIVE 2000/60/EC) and the European Environment Agency – EEA (2018), the ecological status is an assessment of the quality of the structure and functioning of surface water ecosystems. Freshwater macroinvertebrates are important indicators of river health and the structural changes at community level can be

indicative for the ecological status of the aquatic ecosystems (MENEZES et al. 2010). Based on their sensitivity to long-term alterations in the aquatic environment, they are an essential element in river monitoring, unlike the physical and chemical factors which detect the current state of the water quality (ROSENBERG & RESH 1993). Moreover, in Annex 5 of the WFD benthic macroinvertebrate fauna is defined as an obligatory biological quality element in water quality assessment and in determining

*Corresponding author: rimceska@gmail.com

the ecological status of water bodies (EEA, 2018). River management (e.g. damming, construction of small hydropower stations, or river water exploitation mainly for agriculture purposes), point sources of pollution and diffuse loads of agricultural runoff affect macroinvertebrate communities, especially from the small watersheds of semi-mountain and mountain rivers (KUBECKA et al. 1997, NEGISHI et al. 2002, CHEIMONOPOULOU et al. 2011, BENETTI et al. 2012, KAIL et al. 2012, COOPER et al. 2013).

Macroinvertebrates are among the few reliable indicators for the effects of physical and chemical recovery of water bodies (ORMEROD 2004) and confirm some chemical and other biological indicators of river quality (CLEWS & ORMEROD 2009). The application of a unified biotic index is hampered by the specific geographic distribution of organisms and the typological differences between rivers (KORYCIŃSKA & KROLAK 2006). Thus, hydrobiologists use different biotic indexes based on the Biological Monitoring Working Party (BMWP) from which is derived the Average Score Per Taxon (ASPT) (ARMITAGE et al. 1983). BMWP gives information both about the taxonomic diversity of the benthic macroinvertebrates and also on their tolerance to pollution (REICE & WOHLBERG 1993, HAWKES 1997, WETZEL 2001). The implementation of BMWP in different European regions requires its adaptation not just because of the differences in macrozoobenthic communities between the ecoregions, but also because of the different tolerance of the species (OERTEL & NOSEK 2006, KOLAŘIKOVÁ et al. 2014, SKOULIKIDIS et al. 2002, BUSS & SALLES 2006, ZAMORA-MUÑOZ & ALBA-TERCEDOR 1996, KOWNACKI et al. 2004). So far, five versions of BMWP and three versions of ASPT have been adapted and used successfully in Europe (CARDOSO et al. 2005). Recently, the Turkish adapted version of BMWP (TR-BMWP) was developed as well (KAZANCI et al. 2016).

Currently, for the purposes of water quality assessment in Bulgaria, a modification of the Irish Biotic index (CLABBY & BOWMAN 1979) is used – Adapted Biotic Index (ABI) (JANEVA & CHESHMEDJIEV 1999, ORDINANCE H-4/2012). BMWP and ASPT were used less often as the first attempt was a study of semi-mountain rivers in a region affected by mining activities (STOYANOVA 2012). The implementation of the WFD requires the use of the biotic indices BMWP and ASPT, which provide an integrated assessment of the ecological status of aquatic ecosystems. To date, there are no studies aimed at comparing ABI with the BMWP and ASPT indices, which motivated this study.

The aims of the present study were to (i) assess the ecological status of mountain and semi-moun-

tain rivers in the Aegean watershed of Ecoregion 7 via various biotic indices; (ii) assess the similarity between the samples and sampling sites, respectively, based on the ratings given by various indices; (iii) test their applicability in evaluation of different kind of loading in rivers and/or hydromorphological degradation; (iv) test the relationship between the biotic indices based on the obtained scores.

Materials and Methods

Study area

Field studies were conducted within the catchment area of the Struma River watershed (SW Bulgaria/ SE Republic of North Macedonia) and the catchment in the upper course of the Mesta River in Bulgaria; the upper regions of the Kriva Reka and the spring region of the Bregalnitsa River watershed in R. North Macedonia. The examined river sections were defined as R3 and R5 river types – mountain and semi-mountain rivers in Eastern Balkans (Ecoregion 7) in accordance with the recent typology of Bulgaria (CHESHMEDJIEV & MARINOV 2008, WEST AEGEAN RIVER BASIN MANAGEMENT PLAN 2016-2021). Twenty-five sampling sites were selected along the tributaries of the Struma River watershed from the cross-border Bulgarian-Macedonian territory, six sites from the Mesta River watershed (Bulgaria), four sites from the upper course of the Bregalnitsa River watershed and three sites from the upper regions of the Kriva Reka from the Vardar watershed (R. North Macedonia) (Fig. 1). The sampling sites selection was done considering three main criteria: (i) to be

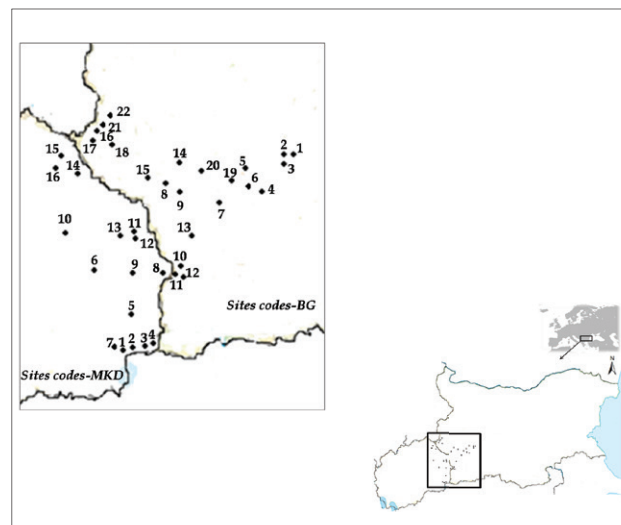


Fig. 1. Sampled sites from the territory of Bulgaria and R. North Macedonia (7th Ecoregion, Eastern Balkans). Legend: Sites №№ 1÷6 (BG) – Mesta River watershed; №№ 7÷22 (BG) and №№ 1÷9 (MKD) – Struma River watershed; №№ 10÷16 (MKD) – Vardar River watershed.

Table 1. List of the rivers and the sampling sites, selected from the study area.

Sitecode	River/Place	Altitude (m)	Latitude	Longitude
1_MKD	Baba River, downstream Koleshinski Waterfall	425	41.3703	22.8078
2_MKD	Lomnitsa River, downstream Smolarski Waterfall	435	41.3709	22.9003
3_MKD	Drazevska River upstream Drazevo Village	389	41.3715	22.9185
4_MKD	Star Dol River upstream Staro Konjarevo Village	464	41.3627	22.9529
5_MKD	River Shtuka, upstream Stuka Village.	379	41.479	22.821
6_MKD	Dvorishka (Prevedenska, Cironka) River, spring region	1138	41.5719	22.8473
7_MKD**	Barlen River, upstream Gabrovo Village	387	41.3764	22.7816
8_MKD	Dvorishka (Cironka) River, downstream Dvorishte Village	901	41.5848	22.9336
9_MKD	Dvorishka (Cironka) River, upstream Dvorishte Village	944	41.5935	22.9004
10_MKD	River Ratevska, downstream Rusinovo Village	833	41.6952	22.8325
11_MKD	River Klepalska Reka, upstream HPS***	1165	41.6666	22.9614
12_MKD	River Ambarska Reka, upstream HPS***	1165	41.6673	22.9622
13_MKD	River Klepalska Reka, downstream HPS***	1122	41.6744	22.9428
14_MKD	River Kriva Reka, upstream Uzem Village	864	42.2152	22.4356
15_MKD	River Luchka Reka, downstream Dobrovitza Village	789	42.2566	22.3492
16_MKD	River Kriva Reka, upstream Kriva Palanka Town	670	42.2122	22.3452
1_BG	Cherna Mesta River, upstream HPS***	1125	42.07875	23.72581
2_BG*	Small brook, tributary to Cherna Mesta River, near site 1_BG	1121	42.07776	23.72564
3_BG	Right tributary to Cherna Mesta River, upstream Cherna Mesta Village	1040	42.06104	23.72682
4_BG	Mesta River, downstream Yakoruda Town	851	41.998757	23.62883
5_BG	Klinoshtica River, upstream Dobarsko Village	1220	41.98504	23.46597
6_BG	Draglishka River, at Dolno Draglishte Village	831	41.93432	23.51248
7_BG	Elovitsa River, at Prevala	1038	41.89799	23.33618
8_BG	Sushichka River, upstream Sushitsa Village	873	41.80629	23.02944
9_BG	Sushichka River, downstream Sushitsa Village	780	41.81839	23.07301
10_BG	Lebnitsa River, after border with N Macedonia	789	41.55756	22.99431
11_BG	Dvorishka (Cironka) River	814	41.56622	22.98840
12_BG	Tributary of Lebnitsa River, downstream Dobri Laki Village	797	41.56890	22.99031
13_BG	Tsaparevska River, upstream Tsaparevo Village	622	41.63227	23.07884
14_BG	Oshtavska River, before its mouth to Struma River	190	41.75985	23.15539
15_BG	Eleshnitsa River, at Chetirtsi Village	457	42.24273	22.87364
16_BG	Sovolyanska Bistritsa River, near Kutugertsi Village	797	42.31128	22.50344
17_BG	Bobeshinska River, before its mouth to Sovolyanska Bistritsa R.	806	42.31732	22.49856
18_BG*	Sovolyanska Bistritsa River, upstream Gurlyano Village	1090	42.24026	22.55357
19_BG**	Bachevska River, at Bachevo v.	949	41.9290	23.4480
20_BG**	Blagoevgradska Bistritsa River, near "Parangalitsa" Reserve	1528	42.0424	23.3643
21_BG**	Lomnishka River, at Lomnitsa Village	824	42.3762	22.5482
22_BG**	Dragovishtitsa River, downstream Dolno Ujno Village	594	42.4043	22.594

Legend: *Sites collected only in autumn 2017; **Sites collected only in spring 2018; ***Hydroelectric power station.

natural or close to natural conditions, (ii) without or with different anthropogenic impact on the freshwater ecosystems, (iii) to be easily accessible.

Macroinvertebrate sampling

Altogether, 69 samples of bottom macroinvertebrates were collected in October 2017 (autumn) and in April/May 2018 (spring) (Table 1). These samples were collected with standard hydrobiological hand

net (mesh size 500 µm) applying kick & sweep multihabitat procedure (CHESHMEDJIEV et al. 2011). The collection of bottom fauna was according EN ISO 10870:2012.

At the laboratory, samples were elutriated from inorganic substrata, passed through nested 1 mm and 0.5 mm sieves. All macroinvertebrate specimens were sorted by systematic groups and labelled; the taxa were identified to the lowest possible level (Ta-

Table 2. Level of identification of the systematic groups established during the study.

Systematic group	Level of identification	Systematic group	Level of identification
Turbellaria	genera, species	Plecoptera	genera, species
Oligochaeta	family, genera, species	Coleoptera	genera, species
Hirudinea	genera, species	Heteroptera	genera, species
Gastropoda	genera, species	Megaloptera	genera, species
Bivalvia	genera	Trichoptera	genera, species
Crustacea	genera, species	Diptera	family, genera, species
Ephemeroptera	genera, species	Nematoda	presence
Odonata	genera, species		

ble 2). All collected and analysed specimens were preserved in 70% ethanol and stored in the Institute of Biodiversity and Ecosystem Research - Bulgarian Academy of Sciences (IBER-BAS), Sofia, Bulgaria.

Data analysis

For the purposes of the indicative ecological status assessment, the following metrics were used: Biological Monitoring Working Party Score - BMWP (Hungarian version), Average Score per Taxon - ASPT (Hungarian version), Irish Quality Rating System – QRS (Adapted version) (SOUFI et al. 2015) and EPT index (Ephemeroptera, Plecoptera and Trichoptera taxa richness). BMWP, ASPT and EPT indices were calculated using the ASTERICS software package (AQEM Consortium 2002). Class boundaries for the biotic indices were used as in RIMCHESKA & VIDINOVA (2019).

Hydromorphological degradation

The degree of hydromorphological degradation was assessed *in situ* at each study site. We assessed the departure from naturalness as a result of human pressures on river hydromorphology and determined if there was organic matter inflow present in the river based on *River bank degradation*, *Organic pollution and Inorganic debris present*, where we calculated the *General degradation score* (GDS) as an average value. The assessment was performed on a 500 m long reach encompassing the 100 m sampled reach and extending upstream to account for drift. In terms of degradation, quantitative scoring was given using a scale of 1 (no degradation) to 5 (maximum degradation) (Table 3).

Analytical methods

The data set was processed using Statistica 7 and Primer 6. A cluster analyse (Euclidean distance, complete linkage), based on the scores values of all applied indices (preliminary normalised) for each collected sample, was performed to assess the similarity level between the studied sites. Further, after

checking of the available data for normality (Kolmogorov-Smirnov Test), multiple linear regression was applied to test the relationship between the biotic indices.

Canonical Correspondence Analysis (CCA) in the CANOCO package version 5.0 (TER BRAAK & ŠMILAUER 2012) was used to test the relationship between major macroinvertebrate taxonomic groups and hydromorphological degradation. A Monte Carlo test using 999 permutations ($p < 0.05$) was performed to test the significance of the correlations between taxon occurrence and hydromorphological degradation scores. To evaluate the effect of this negative impact on the semi-mountain and mountain rivers we compared the GDS and its impact with the indices scores (BMWP, BI, EPT, ASPT).

Multiple linear regression was used to test the presence of trends in metric values against the GDS in Statistica, 13.0 (TIBCO Software Inc. 2017). Prior to the analysis the metrics were normalised as they would be when used for monitoring purposes, representing the WFD Ecological Quality Ratio (EQR):

$$\text{Metric EQR} = \frac{(\text{metric value at site} - \text{lowest metric value in dataset})}{(\text{highest metric value in dataset} - \text{lowest metric value in dataset})}$$

Results

During this survey, 272 taxa belonging to 15 systematic groups were established (Table 2). The taxonomic composition covers to a great extent the common inhabitants of river types R3 and R5 in Ecoregion 7. The values of the selected biotic indices (BMWP, ASPT, EPT and BI) suggested the water quality of the studied sites ranged from high (I class) to poor (IV class) (Fig. 2). The calculated scores for sites 4, 5, 6, 9 and 12 (from Bulgaria) and 1, 8, 12, 13 (from North Macedonia) in autumn and for sites 1, 3, 5, 6, 7, 12, 13, 15, 17 (_BG) and 2, 5, 8, 9, 10, 11, 12 and 16 (_MKD) in spring, indicated matching water quality class for the indices BMWP and BI (Fig. 2, a, b).

Table 3. The degree of hydromorphological degradation at each study site. Scoring on a scale of 1 – 5 with one representing no degradation and five representing maximum degradation (Site abbreviations featured in Table 1).

Site	Riverbank alternation	Organic pollution	Inorganic debris present	General degradation score
1_MKD	1	1	1	1
1_MKD	1	1	1	1
2_MKD	1	1	1	1
2_MKD	1	1	1	1
3_MKD	1	1	1	1
3_MKD	1	1	1	1
4_MKD	1	1	1	1
5_MKD	1	1	1	1
6_MKD	1	1	1	1
7_MKD	1	1	1	1
8_MKD	1	4	5	3.33
9_MKD	1	2	2	1.67
10_MKD	1	3	2	2
11_MKD	1	1	1	1
12_MKD	3	1	1	1.7
13_MKD	1	1	1	1
14_MKD	1	4	1	2
15_MKD	1	2	1	1.33
16_MKD	1	3	2	2
1_BG	1	1	1	1
2_BG	1	1	1	1
3_BG	1	1	1	1
4_BG	1	5	4	3.33
5_BG	1	1	1	1
6_BG	1	4	1	2
7_BG	4	1	1	2
8_BG	1	1	1	1
9_BG	1	1	1	1
10_BG	1	1	1	1
11_BG	1	1	1	1
12_BG	1	4	3	2.67
13_BG	1	1	1	1
14_BG	1	2	1	1.33
15_BG	1	1	1	1
16_BG	1	1	1	1
17_BG	1	1	1	1
18_BG	1	1	1	1
19_BG	1	1	1	1
20_BG	1	1	1	1
21_BG	1	2	2	1.67
22_BG	1	2	3	2

More pronounced seasonal dynamics of the values was observed for the indices BMWP and EPT, while those of BI and ASPT varied in smaller ranges. Indices scores for sites 1, 2, 5, 9, 11, 12 (_MKD) and 1, 10, 16 (_BG) indicated excellent/good water quality. At the sites with the lowest riverbed water level 13 and 17 (_BG) we established moderate (in autumn) and excellent water quality (in spring). As the most polluted stood out 11 sites: 4, 5, 6, 19, 22 (_BG) and 4, 8, 10, 13, 14, 15 (_MKD) (Fig. 2).

All the detected dependencies between the studied biotic indices were positive. Although no significant correlations were reported, the strongest correlation was evident between BMWP and EPT (0.80, $p < 0.05$). With respect to the correlations between BI and other indices, the most pronounced was the one with ASPT (0.50, $p < 0.05$), while the weakest one was with BMWP (0.42, $p < 0.05$) (Fig. 3). This trend was also confirmed by the results of the linear multiple regression analysis. The assessment of the correspondence between the studied indices as dependent variables showed high and significant correlation between BMWP and EPT-Taxa and it was the highest value (0.80, $p < 0.05$). The results showed negative correlation between independent variables BMWP and BI, -0.02 and -0.04 ($p < 0.05$), respectively (Table 4).

The cluster analysis based on indices scores values separated the samples in two well-distinguished groups (A and B) (Fig. 3). Considering the differences in benthic groups' composition, the studied sites with the lowest water quality assessment were grouped in cluster A. Moreover, most of the clustered sites contained tolerant taxa, mainly of the subclass Oligochaeta and some species of the order Trichoptera. According to the dominant presence of some taxa tolerant to organic pollution (aquatic worms, crustaceans, etc.), the most polluted sites 4_BG, 5_BG, 6_BG and 8_MKD were clustered together. As one of the most hydro-morphologically disturbed sites, this group also included 13_MKD and 20_BG (Table 3). The lowest biological indices scores were calculated at these sampling sites. It is evident that, the lowest water quality was registered in autumn at sites 6_BG (BMWP-62, BI-3.5, ASPT-5.6, EPT-Taxa-11) and 13_MKD (BMWP-46, BI-3, ASPT-5.1, EPT-Taxa-6). This separation explained the highest linkage distances within this group and subgroup B₂ where the highest water quality was obtained (Fig. 2).

The second group (B) was divided into two subgroups (B₁ and B₂), each comprising of sites/samples which indices score values were more (B₁) or less (B₂) dynamic over time in terms of season and/or anthropogenic loading. The samples (resp.

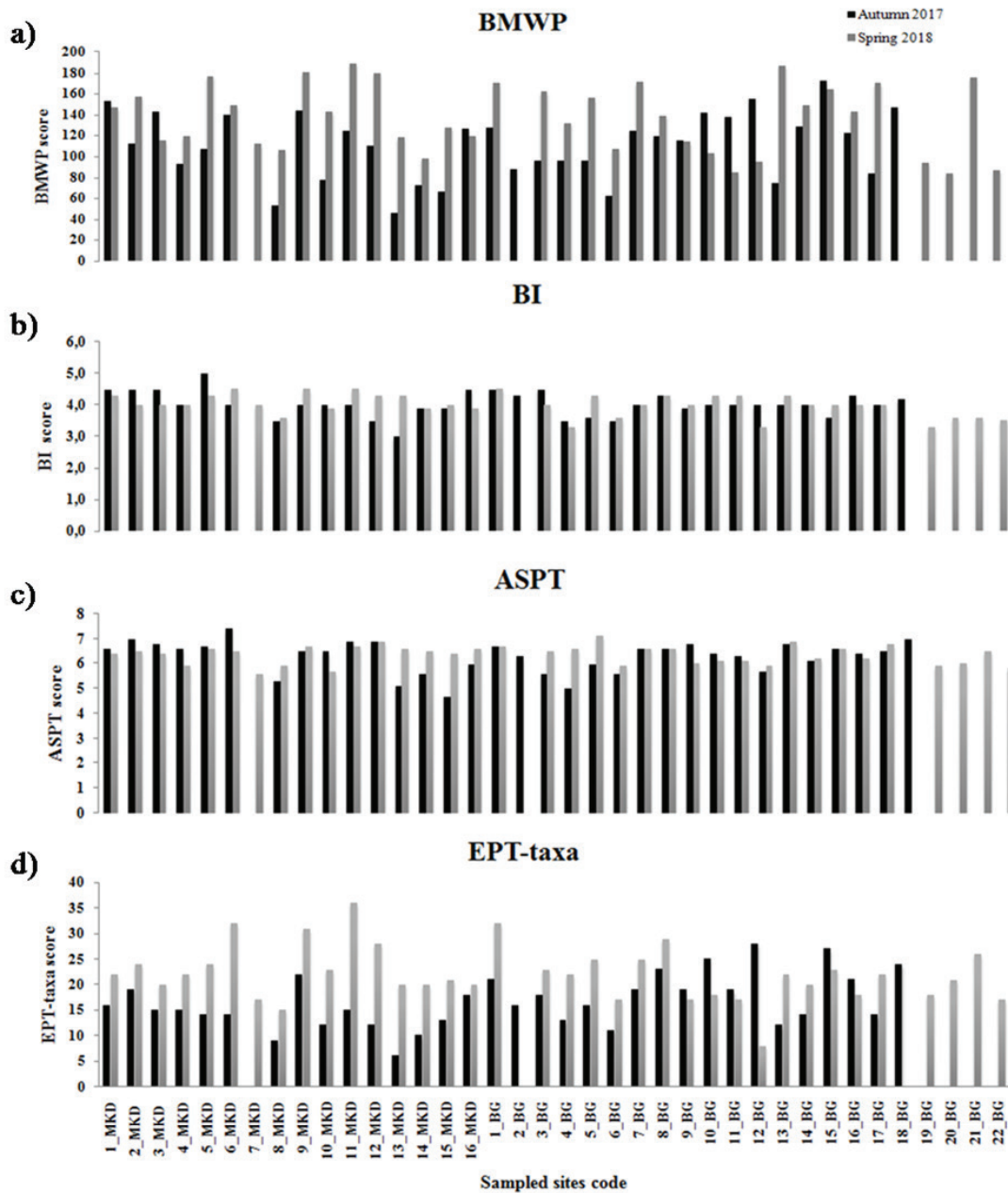


Fig. 2. Dynamics of the values of the applied indices per sampling sites and seasons.

sites) clustered in subgroup B₁ included more tolerant taxa of Trichoptera and Crustacea, compared to the subgroup B₂, where most of the separated sites contained the highest presence of macroinvertebrates characteristic for good water quality in mountain and semi-mountain streams. Besides this, we found that the sites with the highest BMWP scores (11S_MKD, 13S_BG, 9S_MKD, 1S_BG, 17S_BG and 1A_MKD) were clustered in subgroup B₂.

In situ observations of the sampling sites indicated disturbed naturalness owing to human pressures (riverbank degradation, organic pollution, inorganic debris present) on river hydromorphology. Among all the studied sites, 12 were more heavily

Table 4. Multiple regression correlation analyses between dependent and independent variables of the studied biotic indices. *Correlation significant at level $p < 0.05$.

		Independent variable		
		ASPT	EPT-Taxa	BI
Dependent variable	BMWP	0,288*	0,687*	-0,02
		ASPT	EPT-Taxa	BMWP
	BI	0,397*	0,295	-0,04
		BMWP	EPT-Taxa	BI
	ASPT	0,570*	-0,19	0,336*
		ASPT	BMWP	BI
EPT-Taxa	-0,11	0,798*	0,147	

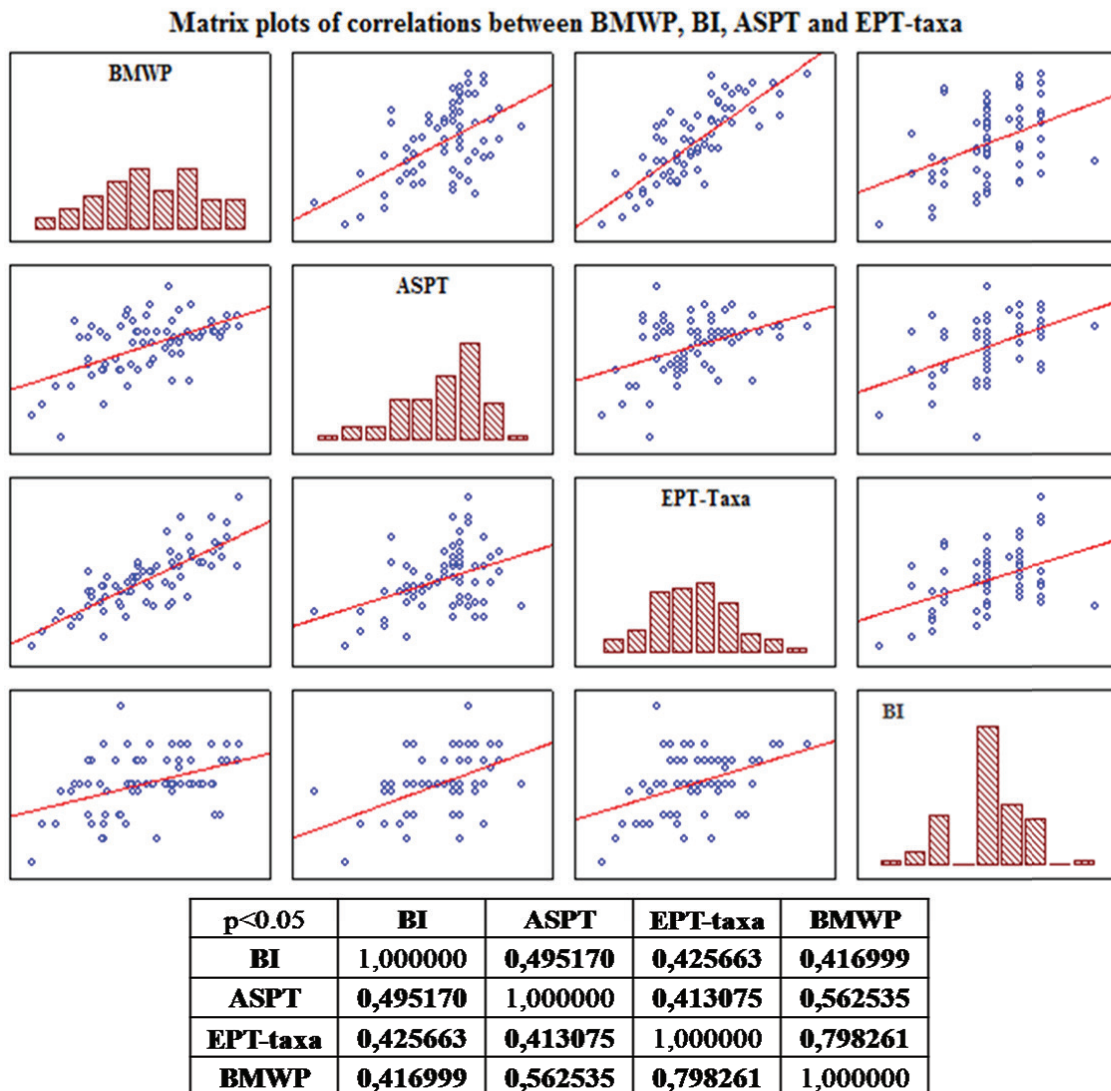


Fig. 3. Matrix plots and correlation values between the studied indices.

influenced with only three of them having the GDS value exceeding 2, namely – 8_MKD, 4_BG and 12_BG (Fig. 5).

The correlation between the GDS and the ecological quality indices (BMWP, BI, EPT, ASPT) clearly outlined a negative trend. Although all regressions were not statistically significant, it was visible that all normalised metrics decreased in value with increasing habitat degradation (Fig. 6).

In the CCA analysis, the four hydromorphological degradation features (including the GDS) explained 24.6% of the total variation of macroinvertebrate communities. The eigen values of the first two axes were 0.09 and 0.02 (Fig. 7). A Monte Carlo permutation test showed that the ordination was statistically significant ($F = 2.7$, $p = 0.004$). The y axis of ordination indicated an obvious separation between sensitive and tolerant taxa.

Discussion

The macroinvertebrate communities from mountain and semi-mountain (R3 and R5 river types) rivers from the Aegean watershed are characterised by specific conditions (CHESHMEDJIEV & MARINOV 2008). During our study, the obtained scores of selected biotic indices (BMWP, ASPT, EPT and BI) assessed the water quality of the studied sites from high (I class) to poor (IV class) (Fig. 2). Compared to the specific situation of each site, the results obtained can be considered to reflect adequately the anthropogenic load at the time of the study (mainly as result of organic/domestic wastewater pollution, mining activities, small hydropower stations and damming or agriculture effluents (Table 3, Fig. 2). All these caused domination of more tolerant macroinvertebrate

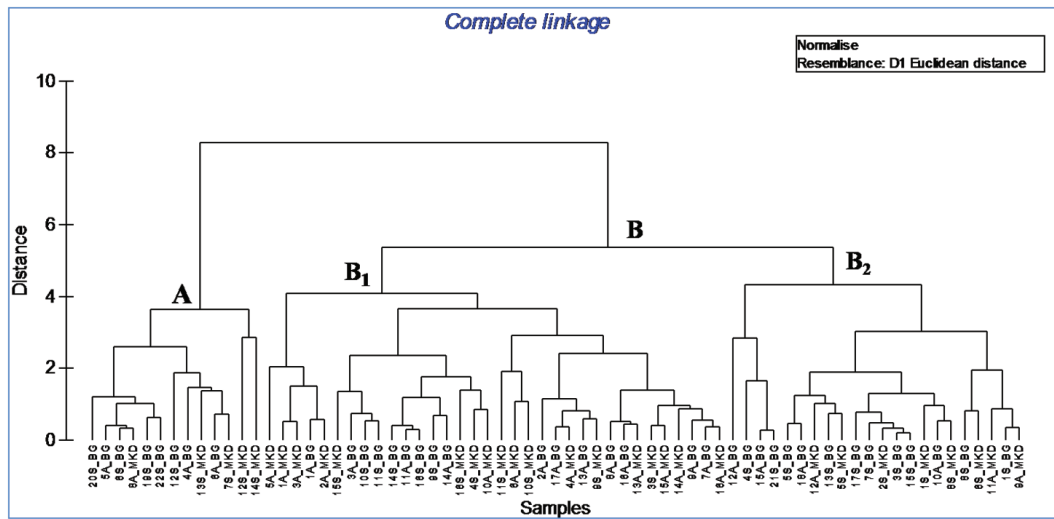


Fig. 4. Dendrogram of the similarities of studied sites based on the values of the applied indices (Complete linkage, Euclidean Distances).

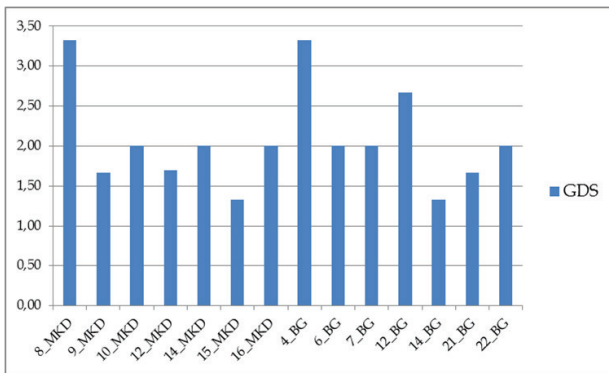


Fig. 5. Values of the General Degradation Score (GDS) measured for the hydromorphologically influenced/impacted sites

taxa (representatives of the groups Oligochaeta, Hirudinea, Trichoptera, etc.).

The comparison between the estimates of the potentially affected and unaffected areas showed that the scores of the BMWP and BI indexes reflected the potential for interference captured at each site (Table 3, Fig. 5). SOUFI et al. (2006) suggest that BI appears to be more sensitive where poorer species richness is observed and in the cases of higher number of species, taxa do not influence stronger on its score. This statement was valid for sampling site 4_BG (spring), where we recorded lower BI score (3,3 - III class) and higher water quality based on BMWP (132 - II class). For the Lomnishka River (21_BG), the BMWP score indicated class I category (score 176), while BI suggested II class (score 3.6), but the collected macroinvertebrates taxa belonged to the higher scores of the BMWP score system (ARMITAGE et al. 1993). This score difference between the two indices was probably a result of the rule for the

evaluation of the BI index: “if at the sampled site the number of Oligochaeta is more than 20 specimens, that site cannot be scored as class I” (JANEVA & CHESHMEDJIEV 1999) as noted also by SOUFI et al. (2006).

Although the BMWP index is expected to respond negatively with increase of organic pollution, its low values may also reflect low habitat heterogeneity (ARMITAGE et al. 1983, FERREIRA et al. 2004). In line with this statement, we recorded the lowest classes scores of the BI, EPT-taxa and ASPT indices for site 13_MKD, where the lowest indices scores were likely owing to low habitat heterogeneity caused by physical/ mechanical barriers (HPS). ASPT is more dependent on seasonality (ZAMORA-MUÑOZ et al. 1995). It should be emphasised that in most cases the BMWP was more sensitive than BI.

Furthermore, the scores of the used indices revealed the sensitivity to organic pollution and habitat loss at selected sites. In this study the class boundaries of EPT and ASPT (they present the species richness of the studied sites and not their pollution tolerance) were always higher than BMWP and BI by one or two classes, indicating the higher sensitivity of BMWP and BI indices (CLABBY, BOWMAN 1979, ARMITAGE et al. 1983, ZAMORA-MUÑOZ et al. 1995, BODE et al. 1997, SOUFI et al. 2006).

Considering the differences in the values of the applied indices, the studied sites with the lowest water quality assessment clustered together. Most of the clustered sites contained tolerant taxa, mainly of Oligochaeta and Trichoptera. Herein, according to dominant presence of macroinvertebrate groups more tolerant to organic pollution – representative taxa from Oligochaeta and Crustacea cluster the

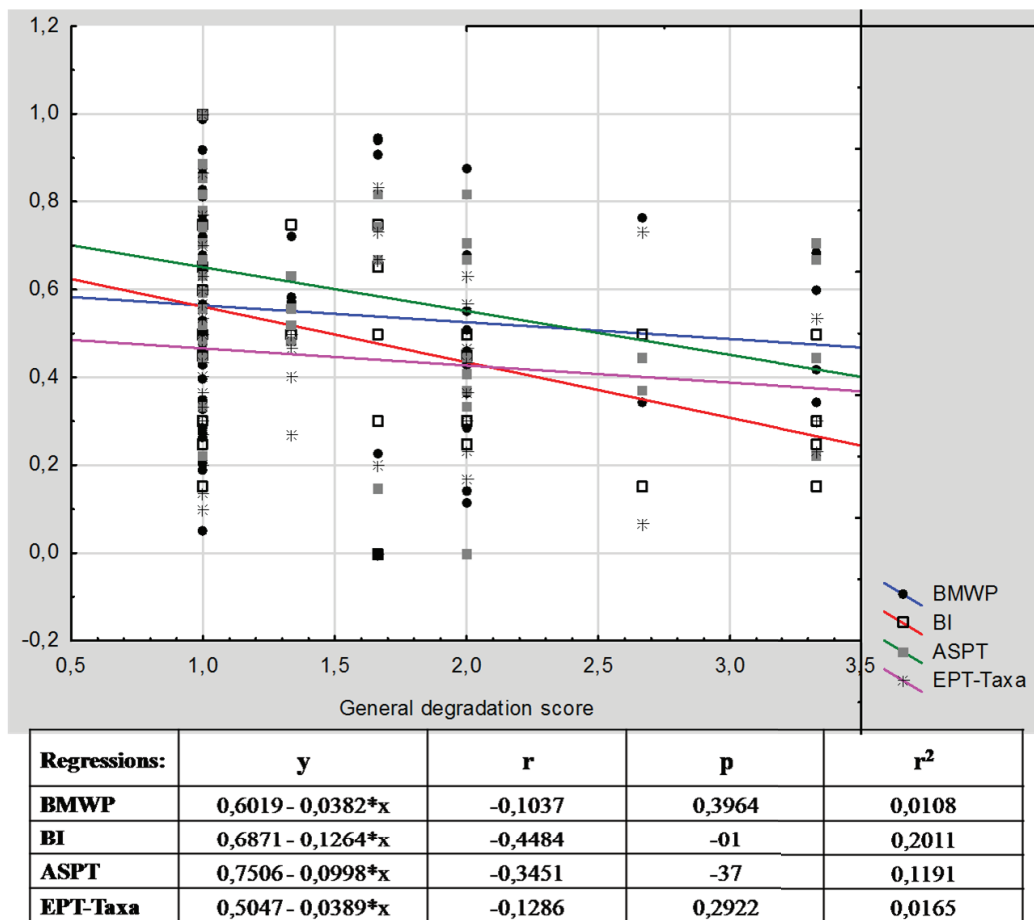


Fig. 6. Multiple linear regression of normalized metric EQRs against general degradation scores.

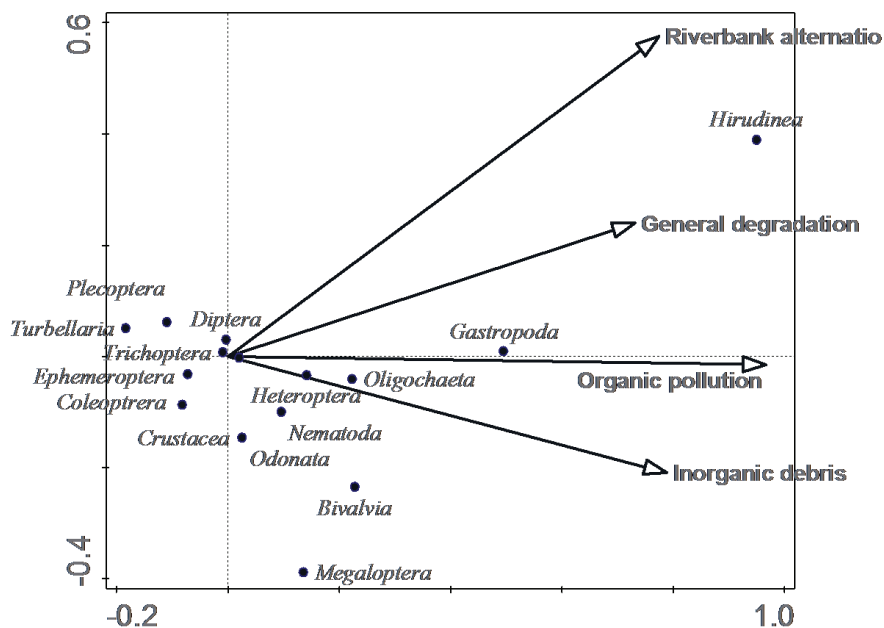


Fig. 7. CCA ordination of macroinvertebrate groups and hydromorphological degradation features. Macroinvertebrate groups are marked with black circles and hydromorphological degradation features with arrows. Arrow lengths on the ordination show the relative importance of the explanatory variables (hydromorphological degradation features), and their direction - relative to each other and to the groups - indicates positive or negative correlations.

most polluted sites 4_BG, 5_BG, 6_BG, 10_MKD and 15_MKD. Furthermore, the lowest biological indices scores for water quality assessment were calculated at these sampled sites as well. From fig. 2 it is evident that the lowest water quality was measured during autumn at sample sites 6_BG (BMWP-62, BI-3.5, ASPT-5.6) and 15_MKD (BMWP-66, BI -3.9, ASPT-4.7). However, this separation explains the highest linkage distances within this group and subgroup B2 where the highest indices scores were obtained.

From the Fig. 4 it can be noticed a good example, where sample sites 5_BG (5A_BG-Group A and 5S_BG-Subgroup B₁) and 15_MKD (Subgroup B₁) correspond to and complements with the typical transition from moderate (as lowest) at autumn period, to high/good water quality the next spring. For two of the sites (5_BG and 15_MKD) we recorded a transition from moderate (as lowest) in autumn to high/good water quality the following spring, likely owing to increased water level during the late winter period. In addition, there was evident change of dominant group Trichoptera with Ephemeroptera (site 5_BG) and fewer Crustacea replaced by more specimens of Ephemeroptera at site 15_BG.

The water quality changed along a longitudinal gradient: lower scores were obtained at sample sites after the local mines (14_MKD), small hydropower stations (3_BG, 13_MKD) or settlements (4_BG, 6_BG, 12_BG, 17_BG). Site 20_BG was affected directly by the construction of a cascade of few small hydropower stations and indirectly by a significant disturbance of the upstream sector of the Blagoevgradska Bistritsa River. This site was initially selected as one of the non-affected upstream (referent) sites but gained scores of BMWP (III class), BI and ASPT (II class), although EPT amounted to 21 taxa (I class).

Our CCA results demonstrated that the macroinvertebrate groups at the right side of the ordination (i.e. Hirudinea and Gastropoda) seemed the most tolerant to habitat degradation as it was concluded also by WARD et al. (1999), CUPŞA et al. (2009), CHEIMONOPOULOU et al. (2011), KAZANCI et al. (2015). Furthermore, on the left side of the ordination, sensitive invertebrate groups such as Turbellaria and EPT groups (Ephemeroptera, Plecoptera and Trichoptera) positioned themselves on opposite sides of all degradation features, proving once more their role as valuable bioindicator groups (HODKINSON & JACKSON 2005, AKAMAGWUNA et al. 2019).

Concerning the selection of a relevant referent sites, we based our evaluation on the scores gained during spring, because of the higher diversity of

benthic macroinvertebrates, as well as the highest water levels follow after winter. On the other hand, in summer/or early autumn most of the insect taxa normally are absent from the freshwater ecosystems, due to peculiarities in their live cycles (ROSENBERG & RESH 1993). According to the requirements of Annex 5 of WFD (DIRECTIVE 2000/60/EC) and EEA (2018) for assessment of water quality based on benthic macroinvertebrate fauna, the calculated indices scores in this study identified three relevant referent/or near natural sites from Bulgaria – 1, 13, 17 (_BG) and six from North Macedonia – 1, 2, 5, 9, 11, 12 (_MKD) (Figs. 2, 4; Table 3).

Conclusions

Based on our results, we can conclude that BMWP is more sensitive than BI. Thus, we highly recommend its wider application in Bulgaria in future water quality assessments based on aquatic macroinvertebrates, although currently it is not obligatory and regulated according to the Bulgarian legislation yet. Based on the results of the case study on semi-mountain and mountain rivers, we could emphasize that basic disturbances, such as hydro-morphological degradation (damming and/or small hydropower stations), regulated discharge and intensive water use (for local agricultural purposes) influence mainly the dynamic of the presented family groups and further affect the scores/classes of the analysed indices. If additional information from the study area are not observed and take into account, than the lower BMWP and/or BI scores may lead to misunderstanding and misinterpretation (organic with hydro-morphological degradation) of the current pollution source.

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