Report

Ecological Status Classification Schemes for the Khrami River basin

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Countries : Armenia, Azerbaijan, Belarus, Georgia, Moldova, Ukraine

Address : Hulla & Co. Human Dynamics KG
13, Knjaz Dondukov Blvd. , fl. 3
Sofia 1000, Bulgaria

Tel. number : +35929359978

Fax number : +35929359970

Contact person Ivan Davidov

Signatures : __________________

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Author of report: Peter Rončák & Romina Álvarez-Troncoso

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EC M & E team __________________ __________________ ______________

[name] [signature] [date]

EC Delegation __________________ __________________ ______________

[name] [signature] [date]

ENPI Bureau __________________ __________________ ______________

[task manager] [name] [signature] [date]

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1 INTRODUCTION

The objective of this ecological classification is to assign rivers (natural water bodies) into 5 classes of ecological status: high, good, moderate, poor, and bad. The high status corresponds to undisturbed conditions showing no or minor deviation from the reference values, whereas in good and moderate status classes the deviation is slight and moderate, respectively. The deviation is measured in relative terms as the Ecological Quality Ratio (EQR = observed value/reference value). The key problems are to set numerical class boundaries for the quality parameters and elements and to combine the information from many parameters and elements.

The scheme used classifies river water bodies based on: (i) macroinvertebrate status as a biological element, (ii) physico-chemical status and (iii) hydromorphological elements. To establish reference conditions, values and class boundary data from previous surveys in the Khrami River basin (2012 – 2015) were used. Where possible, the estimated reference values and class boundaries are compared with those reported from other EU Member countries.

In chapters below, both methodologies to establish the reference conditions values for selected metrics, seasonality of the metrics and their variations within water body type and among types are presented and discussed and also relationships between biological quality elements and physico-chemical parameters were investigated (correlation analysis). These steps went to the development of ecological classification schemes (EQR boundaries for H/G, G/M, M/P and P/B for 5 metrics) for river water types (characteristic for the pilot river basin). Furthermore, those ecological classification schemes for the Khrami River basin were tested based on the data from the JFS 2016 (spring) for 9 sampling locations that covered three river water types (Small mountainous rivers, Alpine meadows rivers and Middle mountainous rivers)..

2 SAMPLING LOCATIONS IN THE PILOT RIVER BASINS

To develop an ecological status classification scheme for the water body group types (see 2.1) the following three main types of sampling locations were selected in the Khrami basin:

- Where reference conditions (or insignificant anthropogenic impact) are expected (representing high quality status);
- Where lower water quality status are expected, with pressure from significant point sand diffuse pollution sources (representing good, moderate, poor and bad status);
- Where the river crosses a state boundary.

2.1 Sampling location with specific reference conditions

The WFD provides 3 options to define reference conditions:

- Use of data from physically similar, quasi-pristine locations.
- Modelling.
- Expert judgement.

In the JFSs it was decided to use a method combining elements of the first and third of these options. Selection of the potential locations to represent reference conditions involved the following main steps:

- An initial scoping study using maps and historical literature for preliminary identification.
- Pressure – impact analysis results.
- Reconnaissance mission by experts (hydrology, hydrobiology, chemistry).
- Sampling for macro-invertebrates, hydro-morphological quality elements and physicochemical parameters.
- Reassessment of the selected locations based on the data from the first JFSs rounds.
- A second reconnaissance mission to confirm (or otherwise) the locations for reference conditions.

The above steps allowed for more or less adequate selection of sampling locations representative of the specific reference conditions.

Because no typology was originally undertaken for the Khrami River basin, the river network was subdivided into three water body groups:

- Alpine meadow rivers;
- Small gravel mountainous rivers;
- Middle gravel mountainous rivers.

For those groups reference condition locations were selected and data from the JFS (2012 – 2015) was analysed to develop an ecological status classification scheme (ESCS). It should be mentioned that Alpine meadow river types were not sampled in the Khrami River pilot basin and data from the Debed River basin (There are similarities regarding the altitude, area, climate, hydrology.) were used during the analysis and ESCS development process.

All together data from the following sampling stations were assessed in the Khrami River basin with expected reference conditions:

- Alpine meadows rivers – The Pambak River at Khnkoyan and the Dzoraget River - upstream of Katnarat;
- Middle gravel mountainous type - the Khrami River – Khramhesi, the Mashavera River – Dmanisi;
- Small gravel mountainous rivers - the Kldeisi River – Bediani.
  (Note: Due to lack of data from the Khrami River basin also one sampling location as representative for the small gravel mountainous type in the Debed River basin – Shnokh –

upstream from Teghut (neighbouring river basin with similarities in habitat conditions was included into the analysis)).

Photographs of three sampling locations expected to be reference conditions are shown in Fig. 1 - 3.

Figure 1 Sampling location Khramhesi on the Khrami River

Figure 2 Sampling location Dmanisi on the Mashavera River

Figure 3 Sampling location Bediani on the Kldeisi River

2.2 Sampling and analysis methods

A modified multi-habitat sampling method was used, in accordance with AQEM/STAR methodology. The replicates were sampled using a 30 x 30 cm mouth size net (0,5 mm mesh size). The replicates were selected according to the percentage ratio of the main bottom substrata (pro-rata multi-habitat approach). Kick-sampling and wash sampling was undertaken, usually, entailing a 50:50 ratio between kick-sampling and wash sampling for the upper part of the river basin and 100 % kick-sampling for the downstream part (alluvial plain). The way of sampling is illustrated in Fig. 4. The total area sampled per site was 1 m^2. Samples were fixed with the ethanol (80%), stored in a cool box and delivered to the laboratory for sorting and identification.

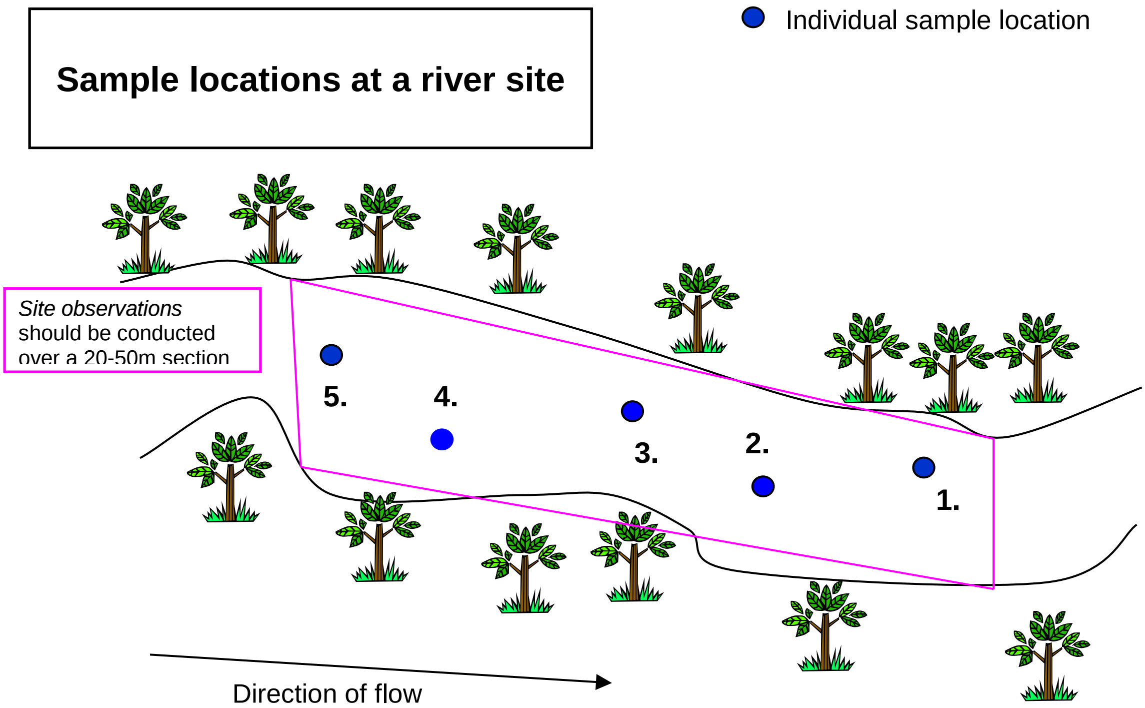

Figure 4 Scheme of the placement of replicate samples within a single river riffle stretch in relation to direction of flow.

Water samples were collected and analysed by staff from National Environmental Agency of Georgia.

2.3 Macroinvertebrates community

The available macroinvertebrate data from the Khrami River basin consisted of samples from 3 reference locations (Alpine meadows, small and middle gravel mountainous river group types) and samples from 7 monitoring locations impacted by human activities. Samples were collected seasonally during 2012 - 2016: spring (April-May), summer (only August 2012 and 2013) and autumn (October-November). The reference locations data has consisted 30 samples and monitoring data of 69 samples, respectively. The most abundant macroinvertebrates families were as follows:

- Diptera: *Chironomidae, Tipulidae and Simuliidae;*
- Trichoptera: *Rhyncophilidae, Hydropsychidae, Goeridae and Hydroptilidae;*
- Ephemeroptera: *Heptageniidae and Baetidae*;
- Plecoptera: *Perlidae and Leuctridae;*
- Gastropoda: *Acroloxidae and Lymnaeidae* .

For illustration, the overall number of families per order (2012 - 2016) is presented for the Khrami River basin in Fig. 5.

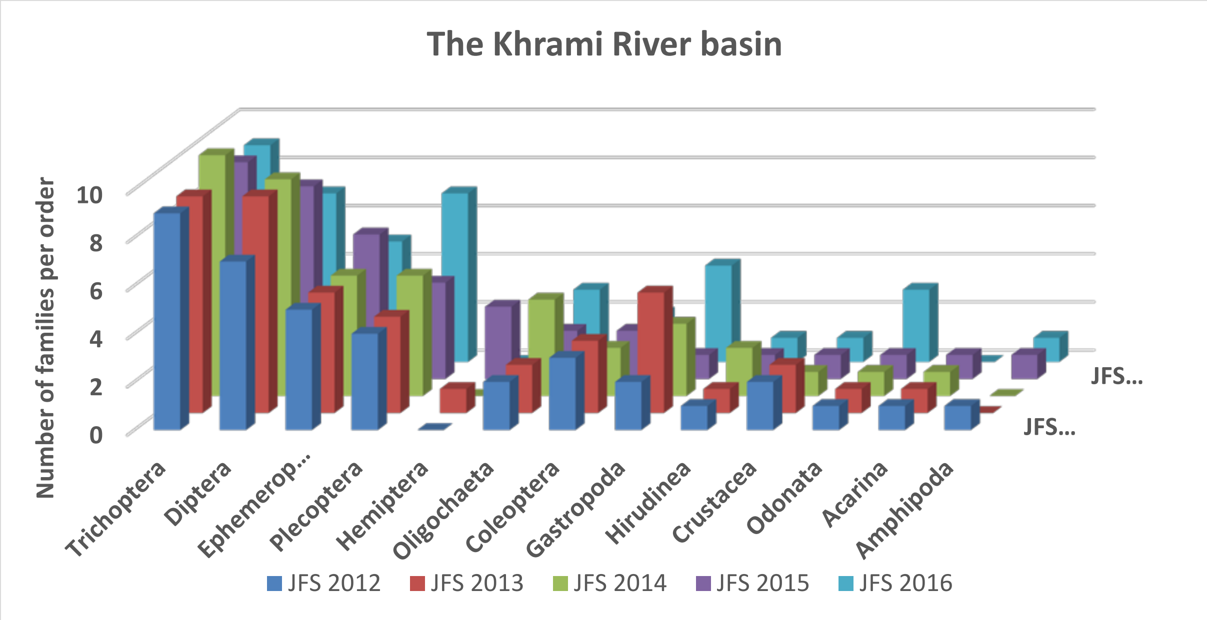

Figure 5 Number of macroinvertebrate taxa in taxonomic groups for the Khrami River basin (JFS 2012 - 2016)

2.4 Environmental data

All sampling locations were surveyed for general physico-chemical parameters and other specific pollutants (heavy metals) as it is presented in Tab. 1.

Table 1 Physico-chemical parameters and other specific pollutants used in the assessment of the ecological status in the Khrami River basin

Quality elements	Indicative parameters
thermal conditions	*water temperature*
oxygenation conditions	*dissolved oxygen, oxygen saturation* *BOD5* *COD* *total suspended solids*
nutrient conditions	*NO3* *NH4* *PO4 (orthophosphates)*
salinity	*Conductivity* *Cl* *SO4* *total dissolved solids (total mineralization)*
acidification status	*pH*
Other specific pollutants - heavy metals	*As, Cd, Cu, Ni, Pb, Zn*

Standardised methods were used for the subsequent analysis of the physico-chemical quality parameters.
Hydro-morphological degradation of the streams was evaluated based on the Hydromorphological Site Protocol and Hydromorphology Assessment Protocol and quantified by the hydromorphology quality score (HMQS).

3 METRICS AND REFRENCE CONDITIONS VALUES

The normative definitions of ecological status classes given in Annex V of the WFD, include five characteristics of river benthic invertebrate assemblages:

(i) Composition and
(ii) Abundance of taxa,
(iii) Ratio of disturbance sensitive taxa to insensitive taxa,
(iv) Level of diversity, and
(v) Occurrence of major taxonomic groups.

If the normative definitions are to be taken strictly, all of these features of macroinvertebrate community structure should be measured for the purpose of ecological classification. In practice, all these community descriptors can be interpreted in many ways, and a large number of metrics (parameters, variables or indices) commonly used in biological assessment can be used to describe them.

3.1 Selection of metrics

In the case of the Khrami River basin, metrics were calculated with AQEM (Asterics) software and these were used in the selection of candidate metrics. A good metric should have a small within-type variation in reference condition, hence allowing for detection of human impact. This requirement was examined by calculating coefficient of variation for reference sites within a type and by comparing the values between reference conditions and monitoring sites (t-tests). The metric values were also correlated (Pearson correlation) with variables indicative of human influence.
The following metrics were selected:
BMWP Score, BBI, IBE, EPT Taxa and Margalef's Diversity Index.

The Biological Monitoring Working Party Score (BMWP) *is a procedure for measuring water quality using macroinvertebrates as biological indicators. The method is based on the principle that different aquatic invertebrates have different tolerances to pollutants. In the case of BMWP, this is based on the sensitivity/tolerance to organic pollution (i.e. nutrient enrichment that can affect the availability of dissolved oxygen). The division of invertebrates into taxonomic groups is undertaken predominantly at family level (81 families), but with one order (oligochaeta). In the case of BMWP, the presence of mayflies or stoneflies, for instance indicate the cleanest river and are given a* ***tolerance score of 10****. The lowest scoring invertebrates are* ***worms (Oligochaeta) which score 1.*** *The number of different macroinvertebrates is also an important factor, because the better quality water is assumed to contain fewer pollutants that would exclude "sensitive" species resulting in a higher diversity.*

BBI The Belgian Biotic Index is based upon the relative sensitivity of specific indicator species to pollution and the diversity of species present. The index value varies from 0 (extremely bad quality) to 10 (extremely good quality). Initially, the invertebrates present are divided into 7 main faunal groups (at order, family and/or genus level), then the number of systematic units is counted. The division of taxa into systematic units is undertaken predominantly at family and genus level, and results are compared with a pre-determined 5-class look-up table. Overall, the index is based on a combination of taxa diversity (1415 taxa) and presence or absence of specific indicator groups.

IBE: *The IBE method derives from the Trent Biotic Index (Woodiwiss 1964). It is based on two evaluation parameters: taxonomic richness and the presence of pollution-sensitive taxa. This method offers synthetic information about the general conditions of river ecosystems, underlining potential alterations.*

***The ETP Taxa (ETP)** displays the richness within the insects groups (Ephemeroptera, Trichoptera and Plecoptera), which are considered to be sensitive to pollution and will increase with increasing water quality. The ETP Index is equal to the total number of families represented within these three orders of in the sample.*

***Margalef's Diversity Index** is a simple-to-calculate measure of taxanomic (species) richness, best employed on large sample sizes. It is a combination of the number of taxa (species) counted and the total number of individuals sampled.*

3.2 Establishment of the type-specific reference conditions values

Type-specific reference conditions values can be established either on the basis of data from reference sites, or, if data are insufficient, using modelling, expert judgement or their combination and also the reference conditions values from neighbouring countries or catchment areas. In the case of the Khrami River basin, reference conditions values were established from the JFS results (2012 – 2015) for the Alpine meadows, small gravel and middle gravel mountainous river types. For the large river type modelling and/or expert judgement will be applied later to establish reference conditions values for the macroinvertebrates taxa (no locations were found without human impact on the given water body type).

3.2.1 Effect of sampling season

Generally, macroinvertebrate fauna data has differed between spring and autumn. Therefore, an analysis of data from the reference sites was undertaken for the metrics described above. Student´s t-tests were used to test the null hypothesis that "the means of spring and autumn data sets for the selected metrics in the Khrami River basin are equal". As it is presented in Table 2, the results from the analysis have unambiguously refused the „0" hypothesis. The results of selected metrics have shown that there is significant difference between spring and autumn data for the river water group types in the Khrami River basin (p values were much higher than 0,05), except EPT Taxa for Alpine meadow types. However, to confirm the effect of the sampling season on the macroinvertebrate fauna the large data sets to cover different years regarding the climate and precipitation situations should be used. Furthermore, identification methods may also affect the results.

Table 2 Student´s t-test for the spring and autumn data sets of the selected metrics for the Khrami River basin (2012 – 2015)

	BMWP Score	BBI	IBE	EPT Taxa	Margalef's Diversity Index
Alpine meadows river type p values (T2)	0,32	0,05	0,46	**0,05**	0,44
Small mountainous river type p values (T2)	0,33	0,28	0,23	0,3	0,28
Middle mountainous river type p values (T2)	0,09	0,34	0,22	0,39	0,18

3.2.2 Variation of selected metrics

Selected metrics should have low within-type variation (less than 30%). In the case of the Khrami River basin, all five metrics had coefficients of variation (among reference locations) equal to or less than 30 %. The within–type variations are fairly small for sampling seasons (2012 -2015) in reference locations.

Table 3 Coefficients of variation of the selected metrics for different sampling seasons for the Small gravel mountainous water body type of both reference conditions locations and impacted locations in the Khrami River basin (2012 -2014)

	Spring	Summer	Autumn
BMWP Score			
cv – Ref – Con locations	0,3	0,13	0,17
cv – impacted locations	**0,4**	**0,49**	**0,46**
BBI			
cv – Ref – Con locations	0,1	0,05	0,07
cv – impacted locations	**0,3**	**0,29**	**0,34**
IBE			
cv – Ref – Con locations	0,1	0,08	0,11
cv – impacted locations	**0,3**	**0,52**	**0,32**
EPT Taxa			
cv – Ref – Con locations	0,3	0,26	0,18
cv – impacted locations	**0,4**	**0,52**	**0,49**
Diversity Margalef			
cv – Ref – Con locations	0,3	0,24	0,16
cv – impacted locations	**0,4**	**0,38**	**0,26**

Another important aspect of the selected metrics is that their values should be significantly different between reference condition locations and impacted locations. The variation of the metrics values in the monitoring locations (impacted sites) should be higher to leave a scope for values of impacted locations to deviate substantially from those in reference locations in a given water body type (see Table 3).To evaluate such difference Student´s t-tests were applied to data sets from the Khrami River basin. The analysis shows that all selected metrics differed (Student´s t-tests, $p \leq 0.05$) between reference and impacted locations in spring and autumn seasons (2012 – 2015) (see Table 3).

3.2.3 Metrics redundancy

When metrics are selected, it is also necessary to test on the redundancy among them. As it can be seen from the results presented in Table 4, there is a weak correlation among them, except correlations between BMWP Score and EPT Taxa (r=0,84) and between EPT Taxa and Margalef's Diversity Index (r=0,713).

Table 4 Pearson correlations between selected metrics ($p < 0.05$). N = 21 (spring and autumn seasons from years 2012 – 2014) for the both small and middle gravel mountainous river types in the Khrami River basin (Correlations ≥ 0.7 or ≤ -0.7 are bolded)

	BBI	IBE	EPT	Margalef's Diversity Index
BMWP Score	0,65	0,22	**0,84**	0,49
BBI		0,48	0,69	0,37
IBE			0,17	-0,055
EPT				**0,73**

Note: in this analysis simple correlation coefficient "r" was used that indicates the relationship and dependence between variables (the close to 1 (or -1) the stronger dependence, the closer to 0 the weaker dependence).

4 CLASS BOUNDARIES AND CLASSIFICATION

Data for 5 selected metrics from three reference conditions locations in the Khrami River basin were used to calculate the reference conditions values distribution (percentiles).
In most cases the reference locations have, unavoidably, some degree of human influence, and thus do not fully represent true reference conditions. This is often taken into account by setting the High/Good –boundary (see Figure 6) to some percentile (e.g. 25th %, 50th %) of the distribution of metric values among the reference locations within a type.

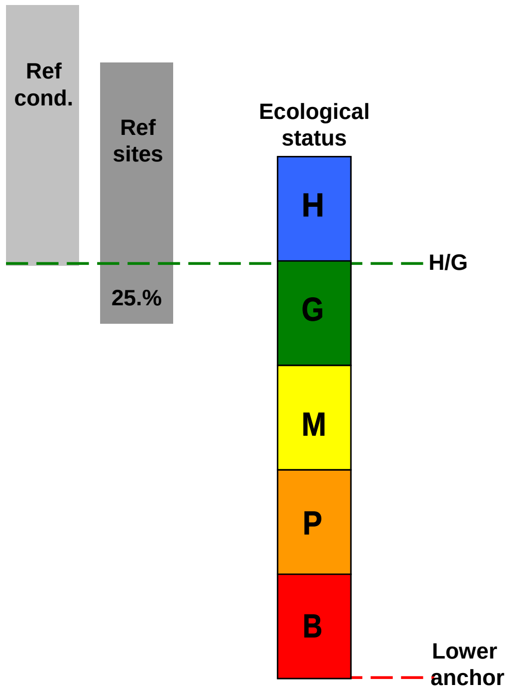

Figure 6 A schematic presentation of the class boundary setting for biological parameters (H = High, G = Good, M = Moderate, P = Poor and B = Bad ecological status.)

A crucial point in boundary setting is the selection the lower anchor (LA) values for metrics. This can be the lowest (i.e. worst) value observed in the data or the theoretical minimum (i.e. worst) value of the metric. The remaining class boundaries Good/Moderate (G/M), Moderate/Poor (M/P) and Poor/Bad (P/B) were established, e.g. by dividing the range between the LA and G/M boundary evenly into three bands (but there are other, inevitably equally arbitrary options).
In the case of the Khrami River basin, H/G boundary was set to the 25th percentile of the type-specific reference value distribution and to illustrate the effect of the H/G-boundary value, also the 50th percentile (median) was used for comparison for the middle mountainous river type (for small mountainous river type the H/G boundary was set to the 25th percentile). The LA was set to the approximate theoretical minimum value (i.e. the lowest attainable value of the metric and quality classes were evenly spaced within the range LA – G/M class boundary. The results are presented in Tables 5, 6, 7 and 8.

Table 5 Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Margalef's Diversity Index for the Khrami River basin (based on JFS 2012 – 2015) for middle mountainous river water type H/G – 25th % tile

	Altitude 500 – 1200 m a.s.l.																			
	Spring										***Autumn***									
	BMWP Score	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef´s Diversity Index***	***EQR***	***BMWP Score***	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef´s Diversity Index***	***EQR***
H/G**	130	**0,9**	9,4	**0,94**	9,8	**0,9**	16	**0,88**	4,6	**0,87**	137	**0,87**	9,0	**0,92**	10,3	**0,93**	14	**0,82**	3,7	**0,92**
G/M***	92	**0,6**	6	**0,6**	6,5	**0,6**	11	**0,6**	3,1	**0,6**	92	**0,6**	5,8	**0,6**	6,6	**0,6**	10	**0,6**	2,4	**0,6**
M/P***	52	**0,4**	4	**0,4**	4,3	**0,4**	7	**0,4**	2,1	**0,4**	62	**0,4**	3,9	**0,4**	4,4	**0,4**	7	**0,4**	1,6	**0,4**
P/B***	26	**0,2**	2	**0,2**	2,2	**0,2**	4	**0,2**	1.1	**0,2**	31	**0,2**	1,9	**0,2**	2,2	**0,2**	3	**0,2**	0,8	**0,2**
Lower anchor	0		0		0		0		0		0		0		0		0		0	

*** H/G – 25th % tile; *** other boundaries were distributed equally*

Table 6 Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Margalef's Diversity Index for the Khrami River basin (based on JFS 2012 – 2015) for middle mountainous river water type with H/G – 50th % tile

	Altitude 500 – 1200 m a.s.l.																			
	Spring										***Autumn***									
	BMWP Score	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef´s Diversity Index***	***EQR***	***BMWP Score***	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef´s Diversity Index***	***EQR***
H/G**	113	**0,85**	*9,0*	**0,9**	*9,0*	**0,83**	*15*	**0,79**	*3,7*	**0,7**	*127*	**0,8**	*9,0*	**0,92**	*9,0*	**0,81**	*13*	**0,76**	*3,4*	**0,85**
G/M***	79	**0,6**	6	**0,6**	6	**0,6**	11	**0,6**	3,0	**0,6**	93	**0,6**	5,8	**0,6**	7	**0,6**	10	**0,6**	2,4	**0,6**
M/P***	53	**0,4**	4	**0,4**	4	**0,4**	8	**0,4**	2,0	**0,4**	63	**0,4**	3,9	**0,4**	4	**0,4**	7	**0,4**	1,6	**0,4**
P/B***	26	**0,2**	2	**0,2**	2	**0,2**	4	**0,2**	1,0	**0,2**	31	**0,2**	1,9	**0,2**	2	**0,2**	3	**0,2**	0,8	**0,2**
Lower anchor	0		0		0		0		0		0		0		0		0		0	

*** H/G – 50th % tile; *** other boundaries were distributed equally*

Table 7 Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Margalef's Diversity Index for the Khrami River basin (based on JFS 2013 – 2015) for small mountainous river water type with H/G – 25th % tile

	Altitude 500 – 1200 m a.s.l.									
	All data									
	BMWP Score	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef´s Diversity Index***	***EQR***
H/G**	108	**0,88**	8	**0,88**	8,9	**0,98**	11	**0,73**	3,3	**0,77**
G/M***	74	**0,6**	5,4	**0,6**	5,4	**0,6**	9	**0,6**	2,58	**0,6**
M/P***	49	**0,4**	3,6	**0,4**	3,6	**0,4**	6	**0,4**	1,72	**0,4**
P/B***	25	**0,2**	1,8	**0,2**	1,8	**0,2**	3	**0,2**	0,86	**0,2**
Lower anchor	0		0		0		0		0	

Table 8 Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Margalef's Diversity Index for the Khrami for Alpine meadow river water type with H/G – 25th % ile (based on JFS 2012 – 2015 data from the Debed River basin)

	Altitude 500 – 1200 m a.s.l.									
	All data									
	BMWP Score	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef's Diversity Index***	***EQR***
H/G**	102	**0,96**	8	**0,88**	7,7	**0,86**	9	**0,9**	3,5	**0,82**
G/M***	64	**0,6**	5,4	**0,6**	4,6	**0,6**	6	**0,6**	2,1	**0,6**
M/P***	42	**0,4**	3,6	**0,4**	3,0	**0,4**	4	**0,4**	1,4	**0,4**
P/B***	22	**0,2**	1,8	**0,2**	1,5	**0,2**	2	**0,2**	0,7	**0,2**
Lower anchor	0		0		0		0		0	

*** H/G – 25th %ile; *** other boundaries were distributed equally*

4.1 Classification schemes for the macroinvertebrates in the Khrami River basin

Based on the previous analysis of the macroinvertebrates data from JFSs in the Khrami River basin (2012 – 2015), classifications schemes were developed for the Alpine meadows, Small gravel mountainous and Middle gravel mountainous river types. Combined data sets (spring and autumn) were used in this process due to fact that the differences in the boundary values for the ecological status classes between spring and autumn are small (see Table 5 and 6). Classification schemes for three types are presented in Tables 9, 10 and 11.

Table 9 Classification scheme for the middle gravel mountainous river type in the Khrami River basin

	Middle gravel mountainous type				
Class	I	II	III	IV	V
EQR	>0,83	>0,6	>0,4	>0,2	≤0,2
BMWP Score	**>120**	**>90**	**>62**	**>31**	**≤31**
EQR	>0,9	>0,6	>0,4	>0,2	≤0,2
BBI	**>9**	**>6**	**>4**	**>2**	**≤2**
EQR	>0,9	>0,6	>0,4	>0,2	≤0,2
IBE	**>10**	**>6,6**	**>4,4**	**>2,2**	**≤2,2**
EQR	>0,88	>0,6	>0,4	>0,2	≤0,2
EPT	**>16**	**>11**	**>7**	**>4**	**≤4**
EQR	>0,78	>0,6	>0,4	>0,2	≤0,2
Margalef's Diversity Index	**>3,68**	**>3,06**	**>2,04**	**>1,02**	**≤1,02**
Multimetrics Index EQR	**>0,86**	**>0,6**	**>0,4**	**>0,2**	**≤0,2**

Table 10 Classification scheme for the small gravel mountainous river type in the Khrami River basin

	Small gravel mountainous type				
Class	I	II	III	IV	V
EQR	>0,88	>0,6	>0,4	>0,2	≤0,2
BMWP Score	**>108**	**>74**	**>49**	**>25**	**≤25**
EQR	>0,88	>0,6	>0,4	>0,2	≤0,2
BBI	**>8**	**>5,4**	**>3,6**	**>1,8**	**≤1,8**
EQR	>0,98	>0,6	>0,4	>0,2	≤0,2
IBE	**>8,9**	**>5,4**	**>3,6**	**>1,8**	**≤1,8**
EQR	>0,73	>0,6	>0,4	>0,2	≤0,2
EPT	**>11**	**>9**	**>6**	**>3**	**≤3**
EQR	>0,77	>0,6	>0,4	>0,2	≤0,2
Margalef's Diversity Index	**>3,3**	**>2,58**	**>1,72**	**>0,86**	**≤0,86**
Multimetrics Index EQR	**>0,85**	**>0,6**	**>0,4**	**>0,2**	**≤0,2**

Table 11 Classification scheme for the Alpine meadows river type in the Khrami River basin (based on data from the Debed River basin)

	Alpine meadows type				
Class	I	II	III	IV	V
EQR	>0,96	>0,6	>0,4	>0,2	≤0,2
BMWP Score	**>102**	**>64**	**>44**	**>22**	**≤22**
EQR	>0,88	>0,6	>0,4	>0,2	≤0,2
BBI	**>8**	**>5,4**	**>3,6**	**>1,8**	**≤1,8**
EQR	>0,86	>0,6	>0,4	>0,2	≤0,2
IBE	**>7,7**	**>4,6**	**>3,0**	**>1,5**	**≤1,5**
EQR	>0,9	>0,6	>0,4	>0,2	≤0,2
EPT	**>9**	**>6**	**>4**	**>2**	**≤2**
EQR	>0,82	>0,6	>0,4	>0,2	≤0,2
Margalef's Diversity Index	**>3,5**	**>2,1**	**>1,4**	**>0,7**	**≤0,7**
Multimetris Index EQR	**>0,88**	**>0,6**	**>0,4**	**>0,2**	**≤0,2**

Note: Multimetric Index EQR values were calculated as averages EQRs values for the selected metrics.

4.2 Comparison of the class boundaries with other regions

Classification schemes established for the Khrami River basin (BMWP Score and EPT Taxa) were compared with the class boundaries established and used in Slovakia for similar types of water bodies (see Table 12 and 13). There are only small differences for both Middle and Small gravel mountainous river types (BMWP Score and for EPT Taxa), except EPT – Taxa for Middle mountainous river types).

Table 12 Comparison of the class boundaries for the BMWP Score and EPT Taxa between the Khrami classification scheme and the Slovak Republic classification scheme (Carpathian middle gravel mountainous river type)

	Middle gravel mountainous type				
	I	II	III	IV	V
BMWP - Khrami	>130	>93	>62	>31	≤31
BMWP - SR	>139	>105	>71	>37	≤37
EPT Taxa - Khrami	>16	>11	>7	>4	≤4
EPT Taxa - SR	>24	>18	>12	>6	≤6

Table 13 Comparison of the class boundaries for the BMWP Score and EPT Taxa between the Khrami classification scheme and the Slovak Republic classification scheme (Carpathian small gravel mountainous river type)

	Small gravel mountainous type				
	I	II	III	IV	V
BMWP - Khrami	>108	>74	>49	>25	≤25
BMWP - SR	>98	>75	>51	>27	≤27
EPT Taxa - Khrami	>11	>9	>6	>3	≤3
EPT Taxa - SR	>15	>11	>8	>4	≤4

4.3 Class boundaries for physico-chemical parameters

Note: The values of the physic-chemical quality elements must be taken into account when assigning water bodies to the high and good ecological status classes.

Based on the pressure and impact analysis organic pollution from untreated waste waters, nutrients from agricultural activities, and heavy metals from mining in the Khrami River basin are stress factors. Therefore, BOD5, COD, O2, NH4, NO3, PO4 and 6 heavy metals were included in the JFS programme and also to the classification system development (correlation analysis).Table 13 Calculated background concentrations for selected heavy metals in the Khrami River basin (based on the results from JFSs 2012 – 2015).

4.3.1 Background concentrations

It is important to know the background (natural) concentrations of heavy metals in the river in order to assess the contribution of human activities into the total heavy metals load detected. Background concentrations were calculated using a statistical method based on theoretical log-normal distribution defined by two parameters (mean value, μ, and standard deviation, σ). Data sets of heavy metals available for the upstream part of the Khrami River basin, from the 2012 – 2014 JFs were used in the calculations:

1. Six sampling locations were selected as location with natural, or minimal anthropogenic influence.

2. The data sets from these sampling locations were used to calculate statistical parameters.
3. One value, close to “0” was added to the original data sets (the detection limit divided by 100).
4. All values in the data sets were re-calculated as log-values.
5. Both the mean value and standard deviation are calculated from the log-values data sets to create theoretical log-normal distribution functions.
6. The percentile in the range from 1 to 99% tile was calculated.
7. An estimate of the background concentration values was made from the log-normal probability curve, as a percentile.

Following the above procedure the background concentrations of heavy metals (As, Cu and Zn) in the Khrami River were estimated (see Table 14). For illustration, the background concentration of Zn is shown in Fig. 7.

Table 14 Calculated background concentrations for selected heavy metals in the Khrami River basin (based on the results from 2012 – 2014 and 2016 JFSs)

	Cu µg/l	Zn µg/l
Background concentration	1,1	5,9

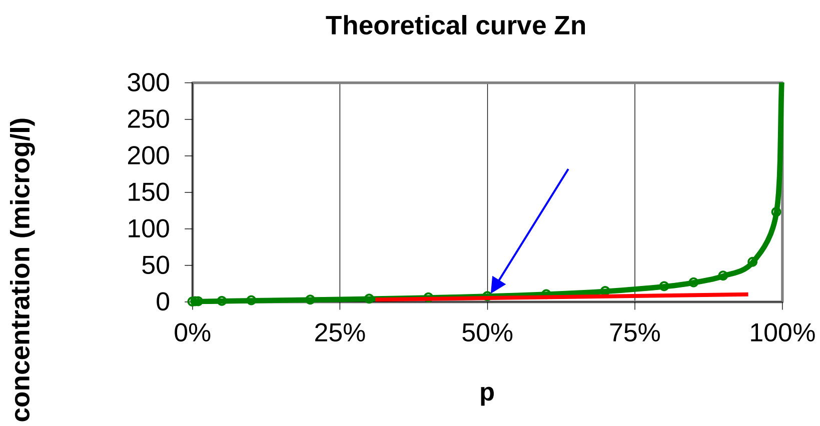

Figure 7 Background concentration of Zn for the Khrami River basin (based on data from JFSs)

4.3.2 Relationship with stressors

To find critical threshold or class boundary values for variables indicative of human induced stress (water quality should support biotic elements (Annex V)) they can be correlated with the estimated biological EQRs or biological status. In this case, Pearson correlations were used to analyse the relation between BOD_5 and other selected water quality parameters, and also between the BMWP Score and selected water quality parameters. Results from the analysis have shown that there is only a weak relationship between BOD_5 and other general physico-chemical parameters (COD, NH_4, NO_3 and PO_4) (see Table 15) and also between BMWP Score (see Table 16). However, the direction of relationships between BMWP Score and tested water quality parameters are in the expected directions (negative for BOD_5, COD and nutrients and positive for O_2).

Table 15 Pearson correlations between selected BOD5 and selected physico-chemical parameters ($p < 0.05$). N = 42 (2012 – 2015) for the Khrami River basin

	O_2	COD	N-NH_4	N-NO_3	P-PO_4
BOD 5	-0,057	0,63	0,32	-0,079	0,32

Table 16Pearson correlations between BMWP Score and selected physico-chemical parameters and heavy metals ($p < 0.05$) spring and autumn seasons from 2012 – 2015 (Cu concentrations were not used for 2015) for the Khrami River basin

	BOD_5	O_2	COD	$N\text{-}NH_4$	Cu
BMWP – RC	-0,28	0,43	-0,24	-0,26	-0,15
BMWP - All	-0,28	0,14	-0,28	-0,28	-0,28

Due to weak correlations it was not possible to indicate the association between the pressure variables and metrics. Therefore, it was decided to use data from national monitoring, JFSs. Finally, expert judgement was used in setting the EQR boundaries of the selected pressure variables. The boundaries for high and good ecological status are summarized in Tables 17, 18 and 19).

Table 17 Classification scheme for general physico-chemical parameters for Alpine meadows and Small gravel mountainous river types in the Khrami River basin

Parameter		unit	I	II	III
Temperature	90%-tile	°C	<18	<21	≥21
Conductivity		µS/cm			
pH		-	(7,0; 8,5)	(6,0; 7,0> or <8,5; 9)	≤ 6,0 or ≥ 9,0
Dissolved oxygen	Min	mg/l	>8,0	>6,0	≤6,0
BOD5	Mean	mg/l	<2,0	<4,0	≥4,0
COD-Cr	Mean	mg/l	<6,0	<15,0	≥15,0
N-NH4	Mean	mg/l	<0,15	<0,5	≥0,5
N-NO3	Mean	mg/l	<1,5	<3,0	≥3,0
P-PO4	Mean	mg/l	<0,03	<0,08	≥0,08

Table 18 Classification scheme for general physico-chemical parameters for Middle gravel mountainous and braided river types in the Khrami River basin

Parameter		unit	I	II	III
Temperature	Mean	°C	<20	<23	≥23
Conductivity		µS/cm			
pH		-	(7,0; 8,5)	(6,0; 7,0> or <8,5; 9)	≤ 6,0 or ≥ 9,0
Dissolved oxygen	Min	mg/l	>7,0	>6,0	≤6,0
BOD5	Mean	mg/l	<3,0	<5,0	≥5,0
COD-Cr	Mean	mg/l	<7,0	<15,0	≥15,0
N-NH4	Mean	mg/l	<0,2	<0,5	≥0,5
N-NO3	Mean	mg/l	<2,0	<3,0	≥3,0
P-PO4	Mean	mg/l	<0,04	<0,08	≥0,08

Table 19 Classification scheme for other specific pollutants relevant for the Khrami River basin

Parameter		unit	I	II	III
Cu	Mean	µg/l	<1,1	<5,0	≥5,0
Zn	Mean	µg/l	<5,9	<30,0	≥30,0

Mean annual concentrations (in case of oxygen concentration minimum value) of the physico-chemical parameters (general conditions) are recommended to be used to classify ecological status.

However, in the case of the EPIRB project only two sampling rounds in given year can be used to classify the ecological status. Due to this fact, it was decided to evaluate data (long-term data at least 3 years) from the national monitoring programme (Kazreti sampling site on the Mashavera River) for selected parameters and calculate the mean and standard deviation values for each month. The data from the JFSs sampling round were compared with the given month whether they fit with the long term values (see Fig. 8 and 9). This approach was used to illustrate the necessity to use the annual means values of physico-chemical parameters to support the ecological status classification of surface (running) water.

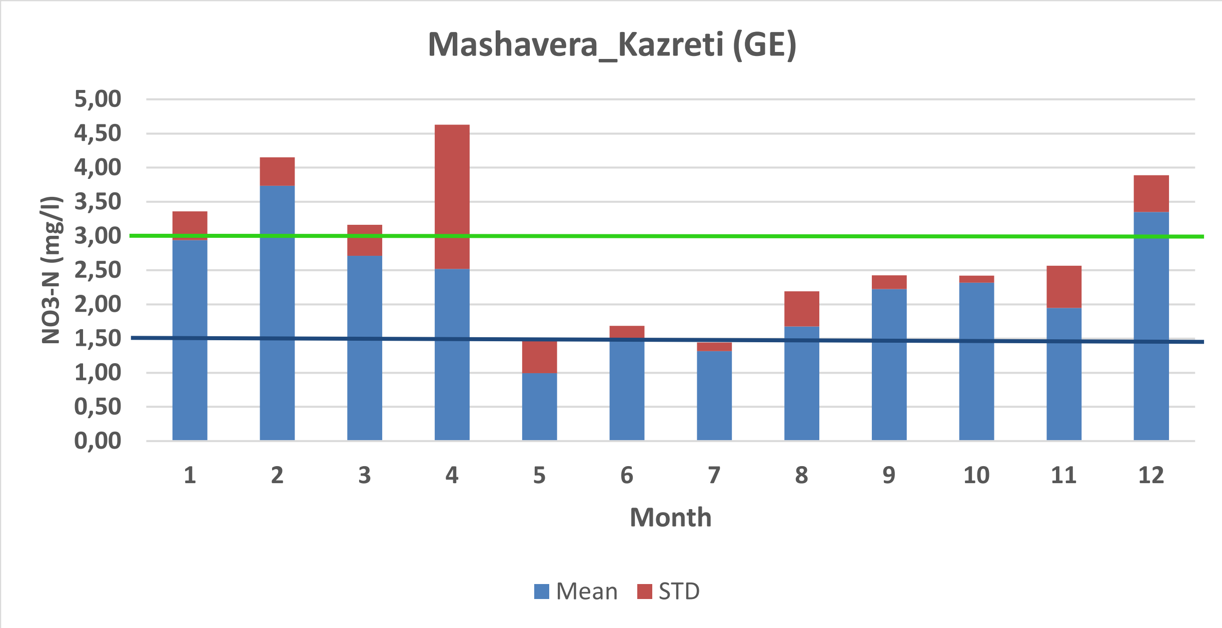

Figure 8 Long-term monthly mean concentrations and standard deviations of NO_3-N (blue line is High status and green line Good status limit values).

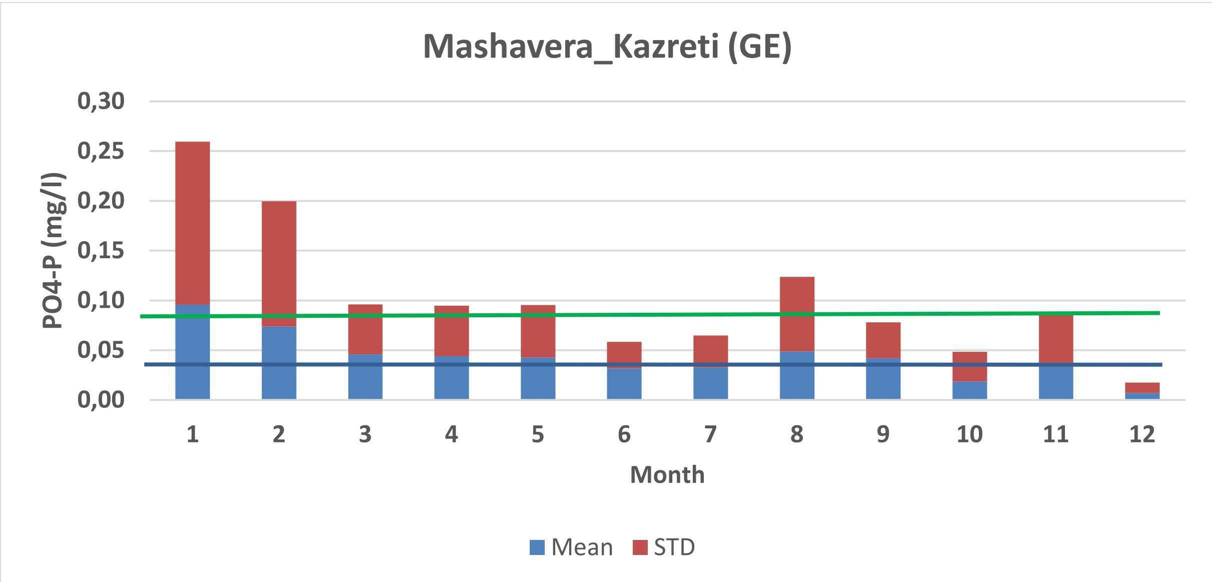

Figure 9 Long-term monthly mean concentrations and standard deviations of PO_4-P (blue line is High status and green line Good status limit values).

4.4 Class boundaries for hydromorphological quality elements

Hydromorphological quality elements (HMQE) must be taken into account when assigning water bodies to the high ecological status class. For the other status classes, the HMQE are required to have conditions which are consistent with the biological values set for the biological class for each water body type.

The HMQE assessment system for the classification of the ecological status in EPIRB project pilot river basins is presented in Tab. 20. This preliminary Hydromorphological Quality Score system was used in Slovakia to classify the water bodies (SHMI, 2004).

Table 20 Preliminary boundaries of the hydromorphological quality classes (SHMI, 2004)

Hydromorphological quality class		Limit values	Colour
1	High	1,0 – 1,7	
2	Good	1,8 – 2,5	
3	Moderate	2,6 – 3,4	
4	Poor	3,5 – 4,2	
5	Bad	4,3 – 5,0	

4.5 Relationship between biological, hydromorphological and physico-chemical quality elements

There are three quality elements that are used in the assessment of the ecological status of the water bodies: biological, physico-chemical and hydromorphological quality elements (Fig. 10). To classify ecological status, the WFD stipulates that the lower of the values for the biological and physico-chemical monitoring results for the relevant quality elements should be used (Annex V, 1.4.2. (i)). This is consistent with the WFD "one out all out" approach.

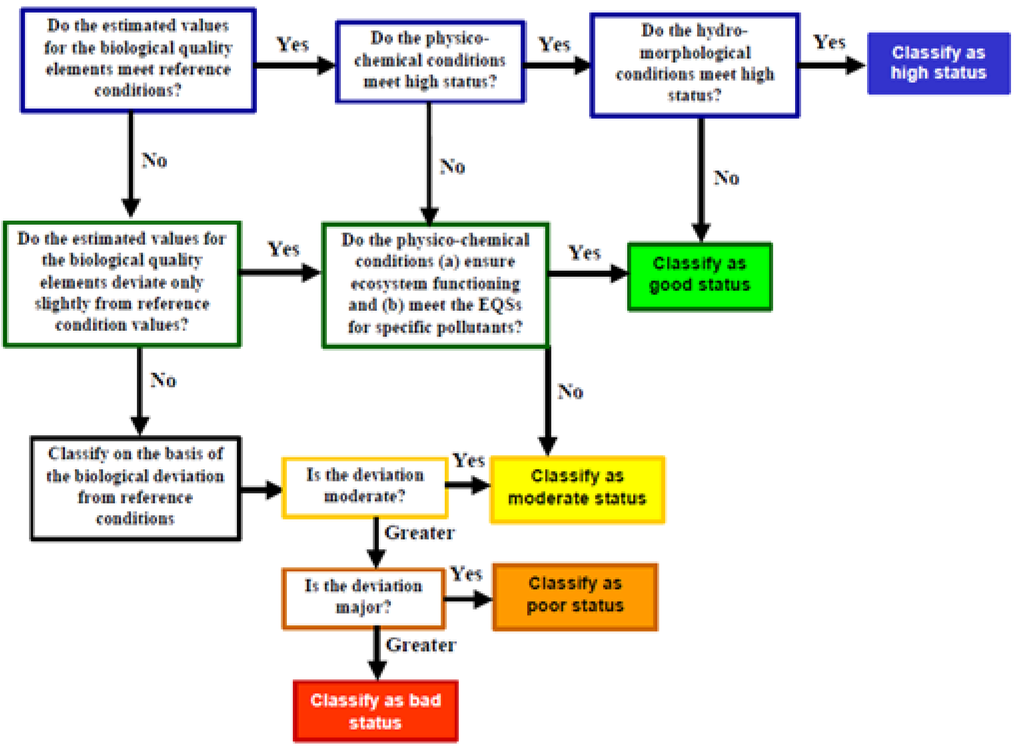

Figure 10 Assessment of the ecological status (reproduced from WFD CIS Guidance documents 10 and 5)

5 CLASSIFICATION EXERCISE

The developed ecological status classifications systems for the Khrami River basin were used to classify the sampling locations based on the data from the spring sampling round JFS 2016. After calculation of EQRs for each metric and Multimetrics Index was calculated as mean value from individual metrics. Subsequently, the assessment scheme as illustrated on Fig. 10 was applied and results of classification are presented in Tab. 21. As it is visible from the ecological status classification of the assessed locations, the results reflects the natural conditions and human activities pressures as well.
Note: Heavy metals (Cu and Zn) were excluded from the assessment

Table 21 Ecological status classification of the sampling locations in the Khrami River basin (spring JFS 2016)

		Class									
River	**Sampling location**	**BMWP**	**BBI**	**IBE**	**EPT**	***Margalef's Diversity Index***	***Multimetris Index EQR***	**Physico-chemical parameters**	**HM Quality Score**	**Overall ecological status**	**Impact**
Khrami	**Kramhesi**	137	10	11	16	3,54					**Location with RC**
Khrami/Kldeisi	**Bediani**	114	9	9	14	2,83					**Location with RC**
Khrami	**Nakhiduri**	105	9	9	11	3,57					Settlements and agriculture
Mashavera	**Dmanisi**	158	10	11,6	18	4,09					**Location with RC**
Mashavera	**Didi Dmanisi**	100	8	8,6	10	2,91					**Agriculture and municipalities**
Mashavera	**Kazreti**	52	6	6	5	2		**N/A**	**N/A**		**Mining industry**
Mashavera	**Kianeti**	23	3	5	3	1,56		**N/A**	**N/A**		**Mining industry**
Mashavera	**Khidiskuri**	41	5	6,6	4	2,14		**N/A**	**N/A**		**Mining industry and waste water from Bolnisi and dredging**
Debed	**Sadakhlo**	47	4	4,4	3	1,77		**N/A**	**N/A**		**Mining industry in Armenia and municipalities**

N/A – not assessed

6 CONCLUSIONS AND RECOMMENDATIONS

During the EPIRB Project 2012-2016 many data have been collected in the field and that information was used to develop this first approach of Ecological Status Classification System for the Khrami River basin. The system used methodologies applied in the EU Member State during the implementation of the WFD. Reference condition values were established for 5 metrics (BMWP Score, BBI, IBE, EPT-Taxa and Margalef's Diversity Index) to assess the river benthic community based on the normative definitions of the WFD.

The results of the assessment have shown certain seasonality and also very weak correlations between biotic indices and physico-chemical parameters. This fact can be caused by insufficient data sets and also hydrological and hydraulics conditions (natural conditions) in the pilot river basin and also due to uncertainties of data from the laboratory analysis (high variations of heavy metals).

On the other hand, classification system reacted on the human induced alterations in the pilot river basin and classified sampling locations into several classes from high to bad.

This ESCS includes macroinvertebrates for the biological evaluation but it is necessary to include more biological elements as macrophytes and phytobethos in the future.

This is a first approach for rivers but also for other water categories it is necessary to develop a system for classification: lakes, artificial waters and heavily modified water bodies.

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