Development of master curricula in ecological monitoring and aquatic bioassessment for Western Balkans HEIs / ECOBIAS

Task 1.2 REPORT

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Task 1.2 Analysis of Partner Countries Knowledge/Skills/Practice in ecological monitoring and freshwater bioassessment

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As the aim of this task was to investigate and analyze knowledge, skills, and practice in ecological monitoring and bioassessment in Partner Countries, Bosnia and Herzegovina and Montenegro to select priority subject areas for strengthening within the ECOBIAS curricula and LLL trainings, the following report was made.

The EU WFD establishes a legal framework to protect and enhance the ecological status of all waters and protected areas including water-dependent ecosystems, prevent their deterioration, and ensure long-term, sustainable use of water resources. According to the WFDEcological status is an assessment of the quality of the structure and functioning of surface water ecosystems. It shows the influence of pressures (e.g. pollution and habitat degradation) on the identified quality elements. Ecological status is determined for each of the surface water bodies of rivers, lakes, transitional waters, and coastal waters, based on biological quality elements and supported by physico-chemical and hydromorphological quality elements. The overall ecological status classification for a water body is determined, according to the 'one out, all out' principle, by the element with the worst status out of all the biological and supporting quality elements (Table 1).

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Table 1 Surface water bodies, water body category and ecological status of potential

RBMP	River						Lake					
	High	Good	Unknown	Moderate	Poor	Bad	High	Good	Unknown	Moderate	Poor	Bad
2nd	11 767	34 730	5 174	40 854	13 654	5 779	3 957	9 663	1 129	7 904	1 960	790

NUTS0	River						Lake					
	High	Good	Unknown	Moderate	Poor	Bad	High	Good	Unknown	Moderate	Poor	Bad
AT	1 593	2 137	107	3 038	942	248	10	45		6	1	
BE	14	131	14	102	140	126				6	7	5
BG	43	375	78	273	69	35	6	7	6	11	5	2
CY	7	90		73	3	1			3	5		
CZ	4	202	2	560	218	58		9	17	16	20	15
DE	9	598	249	3 257	3 093	1 792	17	174	21	249	192	77
DK	494	1 810	1 918	1 617	844	1 093	50	114	169	189	129	205
EE	4	385	1	205	47	3	4	56		26	3	
ES	591	1 837	81	1 325	402	154	24	132	5	90	32	43
FI	312	914	17	477	153	40	1 122	2 604	80	641	144	26
FR	908	3 886	7	4 199	1 318	388	10	118	31	209	51	16
HR	288	330		290	233	343	11	6		5	3	12
HU	4	71	91	408	279	110	2	12	54	29	15	3
IE	245	1 085	847	597	412	6	293	184	168	112	30	25
IT	369	2 827	1 235	1 934	899	229	10	60	143	124	8	2
LU		3		70	27	10						
LV	1	41		117	37	7	6	51		172	21	9
MT			3						2			
NL		2		80	124	40			3	179	208	61
NO	4 694	7 848	421	4 555	1 585	429	1 160	2 956	421	1 451	369	69
PL	23	1 383	2	2 754	334	90	55	304		553	70	62
PT	52	941	92	543	207	64	1	10		6	6	
RO	1	1 907		976	2	5		94	6	30		
SE	1 846	2 949	1	9 121	947	228	1 071	2 555		3 207	504	85
SI	6	77	3	42	8	1		4		5	3	
SK	55	793		526	126	10						
UK	204	2 108	5	3 715	1 205	269	105	168		583	139	73

1) River basin districts and sub-units as reported in the 2nd RBMPs.
2) For river water bodies, the size value is the length (km). For other water body categories, the size value is the area (km²).
3) 'Unchanged' water bodies are water bodies that have not been redelineated between the 1st and 2nd RBMP.
4) Percentages per row can only be calculated by number of water bodies.

Bioindication and biomonitoring as a young science have a great tradition in using freshwater biota as reliable indicators of the aquatic ecosystem health. Different groups at a different level of organization (individual, population, community, and ecosystem) have been used worldwide by national water authorities in defining the regional specific routine monitoring programs. Until the early nineties of the last century, the routine monitoring of surface waters in the major part of Europe has mainly comprised the chemical and physical parameters. However, some European countries were using biological parameters as a part of their routine monitoring programs for assessing and classifying the water quality of rivers.

Since then, a wide variety of biologically based stream assessment methods, often using benthic macroinvertebrates, have been developed in many European countries. In general, macroinvertebrates algae and fish are commonly used for constructing routine monitoring programs. However, of all the freshwater organisms that have been considered for use in biological monitoring, benthic macroinvertebrates (mainly consisting of aquatic insects, mites, mollusks, crustaceans, and annelids) are most often recommended (Hellawell 1986, Bonada et al). There are many advantages to using macroinvertebrates in water quality assessment: 1) being ubiquitous, they are affected by perturbations in all types of waters and habitats, 2) Large numbers of species offer a spectrum of responses to perturbations, 3) The sedentary nature of many species allows spatial analysis of disturbance effects, 4) Their long life cycles allow effects of regular or intermittent perturbations, variable concentrations, etc., to be examined temporally 5) Qualitative sampling and analysis are well developed, and can be done using simple, inexpensive equipment, 6.) Taxonomy of many groups is well known and identification keys are available, 7) Many methods of data analysis have been developed for macroinvertebrate assemblages 8) Responses of many common species to different types of pollution have been established, 9) Macroinvertebrates are well suited to experimental studies of perturbation, and 10) Biochemical and physiological measures of the response of individual organisms to perturbations are being developed. Beside all these advantages there are some difficulties which have to be considered: 1)Quantitative sampling requires large numbers of samples, which can be costly, 2) Factors other than water quality can affect distribution and abundance of organisms, 3) Seasonal variation may complicate interpretations or comparisons, 4) Propensity of some macroinvertebrates to drift may offset the advantage gained by the sedentary nature of many species, 5) Perhaps too many methods for analysis available, 6) Certain groups are not well known taxonomically, 7) Benthic macroinvertebrates may not be sensitive to some perturbations, such as human pathogens and trace amounts of some pollutants, and 8) Poorly established relationships between specific stressors and most commonly used metrics (Hauer and Lamberti 2007).

Nixon et al. (1996) analyzed all routine monitoring programs in European countries until 1996. and most of these methods indicate are constructed to detect organic pollution in rivers and streams, indicating eutrophication, acidification, and salinization. In addition, most bioassessment approaches are, however, limited to a single impact factor and are only applicable in a restricted geographic range or for a certain stream type. Therefore, there was a strong need for constructing more complex systems which would consider different impact factors, to enable an integrated assessment of streams. This was important due to diversification of anthropogenic impact on aquatic ecosystems, where organic pollution, once the main anthropogenic factor on streams in past decades, was declining in most European countries and other impact factors, such as deterioration of stream morphology, are becoming increasingly important. Changes in land use, direct exploitation of organisms, climate change, and invasion of alien species are the direct drivers of change in nature which have accelerated with an enormous rate during the past 50 years.

A big breakthrough in the bioassessment research of aquatic ecosystems was the multimetric approach. RBP ((Barbour et al., 1999), AQEM/STAR protocol (AQEM, 2002); www.eu-star.at) are some of the largest projects which had an output in multimetric indices. This type of indices have been commonly used in routine monitoring programs for freshwater and brackish water ecosystems in Europe (Hering et al., 2006, Hering et al., 2004)) and the United States ((Barbour et al., 1999, Davis and Simon, 1995, Hughes et al., 1998, Karr and Chu, 1998, Stoddard et al., 2008)). Multimetric indices simplify complex biological data in the form of individual metrics but keeping a sufficient amount of information regarding the ecosystem's health. One of the first approaches in Europe for water bodies monitoring, based on macroinvertebrates has been the Dutch EBEOSWA (PEETERS et al., 1994), which is now implemented into the Dutch national water quality control system. This approach has metrics related to current velocity, saprobity, trophy and substrate types. However, for some regions of Europe, e.g. Greece and Poland, due to regional specificity, there were no any indices adjusted to the regional specificity. Also, there were some attempts to harmonise and intercalibrate assessment and indication methods within Europe, e.g. between Austria and Germany.

The EU Water Framework Directive (WFD), requires advanced multimeric assessment systems. To determine the ecological status of streams and rivers, aquatic biota, including macrophytes, benthic algae, and phytoplankton, benthic invertebrates and fish were recommended to be used as biological indicators. According to WFD, the detection of the ecological status must have been based on reference conditions, pristine aquatic ecosystems. A major challenge has been, how to obtain a quality score by means of a measure that calculates the distance of the ecosystem towards the reference ecosystem.

The EU funded project AQEM (The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates) had the main objective to develop a framework for a future European stream assessment system based on benthic macroinvertebrates. To realized this goal the following tasks were set: to develop and test an assessment procedure for streams and rivers using benthic macroinvertebrates, according to the EU Water Framework Directive; – to outline a European stream typology; – to adapt the assessment method to regional conditions to allow comparable application in all EU member states; – to define quality targets for the ecological status of streams and rivers; – to combine this new assessment method with the methods presently used in the EU member states; – to test the method to applied water management.

The AQEM assessment system currently covers 29 European stream types. It was designed to classify a sampling site into an Ecological Quality Class ranging from 5 (high) to 1 (bad) based on a macroinvertebrate taxa list, which has been obtained from sampling the site using the multihabitat sampling method (Figure 1); To develop the multimetric index a large number of metrics was tested for each of 29 stream types. Metrics were selected according to the extent of their correlation with the degradation gradient. Only metrics that were able to make a difference between reference sites and one or more stress classes were selected as suitable for the multimetric systems.

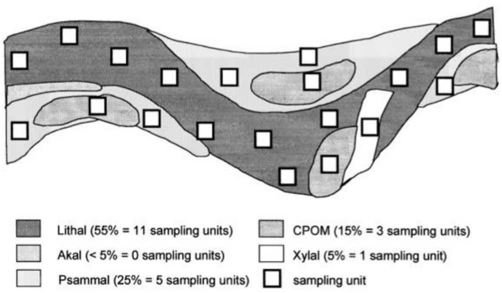

Figure 1 The multihabitat sampling method (Hering et al 2004)

Table 2. Part of the metrics used for the construction of AQEM multimetric system. (Hering et al 2004).

AQEM code	Stream type	Metrics used to assess the ecological quality	Predicted response to increasing perturbation
A01	Mid-sized streams in the Hungarian Plains	organic pollution	
		• Saprobic Index Zelinka & Marvan	increase
		• Number of Ephemeroptera+Plecoptera-taxa	decrease
		• (%) Ephemeroptera+Plecoptera-taxa/total taxa (sp)	decrease
		• (%) Ephemeroptera+Plecoptera individuals / total individuals	decrease
		• Total number of families	decrease
		• Number of sensitive taxa	decrease
		• (%) Littoral+profundal	increase
		• Abundance of Plecoptera	decrease
		• (%) Shredder	decrease
		• Diversity (Margalef)	decrease
A02	Mid-sized calcareous pre-alpine streams	organic pollution	
		• Saprobic Index Zelinka & Marvan	increase
		degradation in stream morphology	
		• Number of EPT-taxa	decrease
		• Total number of taxa	decrease
		• (%) EPT-taxa/total taxa	decrease
		• Number of sensitive taxa	decrease
		• Abundance of Plecoptera	decrease
		• Abundance of Trichoptera	decrease
		• Diversity (Margalef)	decrease
A03	Small non-glaciated crystalline alpine streams	organic pollution	
		• Saprobic Index Zelinka & Marvan	increase
		degradation in stream morphology	
		• Number of EPT-taxa	decrease
		• Total number of taxa	decrease
		• Number of sensitive taxa	decrease
		• Abundance of Plecoptera	decrease
		• Ratio Oligochaeta and Diptera/total-taxa	increase
		• Abundance of Oligochaeta	increase
		• RETI	decrease
		• Diversity (Margalef)	decrease
		• (%) Littoral and Profundal preferences	increase
A04	Mid-sized streams in the Bohemian Massif	organic pollution	
		• Saprobic Index Zelinka & Marvan	increase
		degradation in stream morphology	
		• Number of EPT-taxa	decrease
		• Abundance of all taxa	variable
		• Index of Biocoenotic Region	variable
		• (%)Oligochaeta and Diptera taxa	increase
		• (%) Littoral preferences	increase
		• (%) Gatherers/collectors	increase
		• Total number of taxa	decrease
		• Abundance of Trichoptera	decrease

Continued on p. 13

The general architecture of a multimetric approach (Figure 2), as applied in the AQEM consists of the following steps: 1. The starting point is the taxa list obtained from the sampling site, which is to be assessed. 2. Based on this taxa list some metrics are ca calculated. 3. Generally, the metric results are individually converted into scores by comparing their values with the values of the same metrics in the stream-type specific referencecondition. 4. Thescoresorresultsofthemetricsarecombinedin a simple multimetric index (usually the average of all scores). This procedure enables the user to view both the final assessment result (Ecological Quality Class) and the individual metric results, allowing further interpretation of the data for future management procedures (Hering et al., 2004)

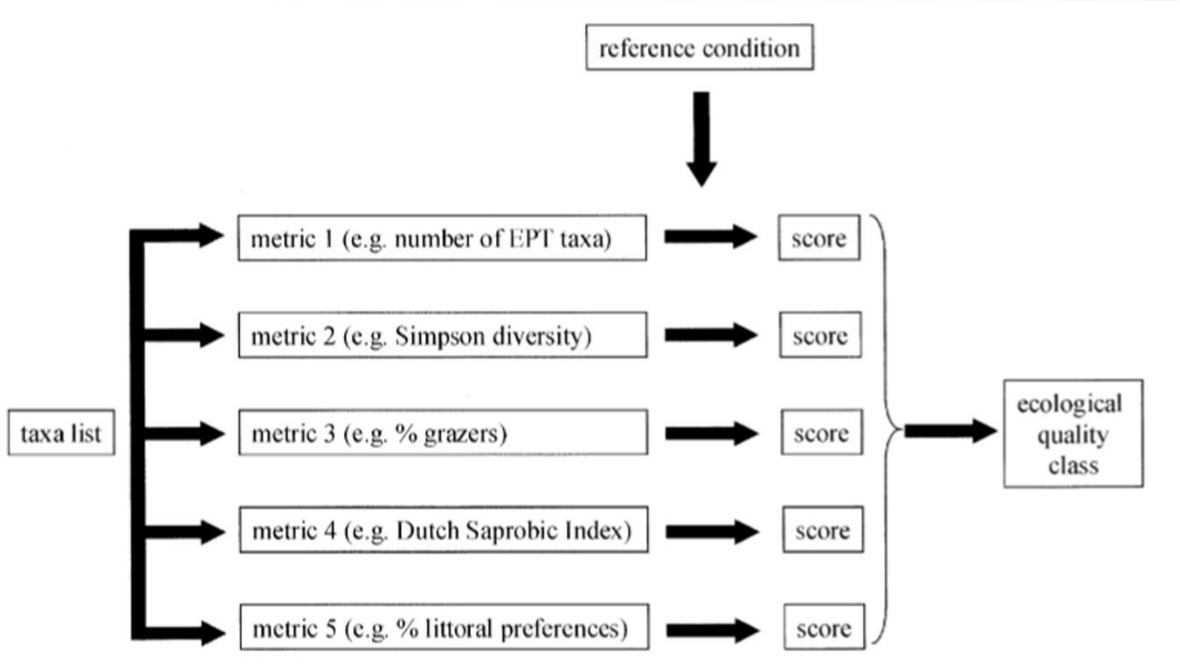

Figure 2 General scheme of a multimetric calculation (Hering et al 2004)

Having all this in mind, modernization of master programs in the Balkan countries and therefore the education of future professionals is an important step in the direction of efficient monitoring and possible restoration of key freshwater bodies in this region according to the WFD. This is particularly important since WFD is sporadically implemented in Bosnia and Herzegovina. Bosnia and Herzegovina and Montenegro can benefit from these experiences made part of running modules in further European countries.

Content

1. Results of questionnaires relate Bosnian knowledge / skills / practice in EMAB

2. Results of questionnaires relate Montenegrin knowledge / Skills / Practice in EMAB

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ANALYSIS OF EXISTING CURRICULA RELATED TO EMAB IN PARTNER COUNTRIES

1. RESULTS OF QUESTIONNAIRES RELATE BOSNIAN KNOWLEDGE / SKILLS / PRACTICE IN EMAB

1.1. ADRIATIC BASIN AGENCY

Adriatic Basin Agency conducts an annual monitoring program where biological components in rivers are sampled once a year, and phytoplankton in some heavily modified water bodies is sampled 3-4 times per year. They monitor phytobenthos and macroinvertebrates, Phytoplankton (including chlorophyll-a) and Zooplankton, also Macrophytes and Ichthyofauna.

The Agency does not conduct sampling and monitoring ourselves since it doesn't have the appropriate laboratory. They provide public procurement through public procurement procedures defining all the data to be collected and processed using the Water Framework Directive, legislation and (where appropriate) the following standards:

Table 3 Methods of water quality sampling, and biological quality elements methods used.

Parameter	Method
Water samples	BAS EN ISO 5667-1:2008, BAS EN ISO 5667-1/Cor1:2008, Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques BAS EN ISO 5667-3:2014 Water quality - Sampling - Part 3: Preservation and handling of water samples
Chlorophyll-a	BAS ISO 10260 E:2002 Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration

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Benthic macroinvertebrates	BAS EN 27828:2003 Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates BAS EN 28265:2003. Design and use of quantitative samplers for benthic macroinvertebrates on the stony substrate in shallow freshwaters BAS EN 28265:2003. Design and use of quantitative samplers for benthic macroinvertebrates on the stony substrate in shallow freshwaters BAS EN 16150:2013 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 9391:2003 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 8689-1:2003 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 8689-2:2003 Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates
Benthic diatoms	BAS EN 13946:2015 Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes BAS EN 14407:2006 Guidance standard for the identification, enumeration, and interpretation of benthic diatom samples from running waters
Aquatic macrophytes	BAS EN 14184:2015 Guidance for the surveying of aquatic macrophytes in running waters.

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Ichthyofauna	BAS EN 14011:2004 Sampling of fish with electricity; Standard Methods For the Examination of Water Wastewater (1995)
Parameter	**Method**
Water samples	BAS EN ISO 5667-1:2008, BAS EN ISO 5667-1/Cor1:2008, Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques BAS EN ISO 5667-3:2014 Water quality - Sampling - Part 3: Preservation and handling of water samples
Chlorophyll-a	BAS ISO 10260 E:2002 Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration

Monitoring shall be carried out regularly unless the monitoring carried out earlier has shown that the water body concerned has been in good condition and that there is no indication from the impact assessment of human activities that this impact has changed. (In such cases, monitoring is carried out during every third river basin management plan.)

Watershed of the Adriatic Sea in Federation of Bosnia and Herzegovina – 3 river basins Neretva with part of Trebišnjica, part of Cetina river basin and a small part of Krka river basin. Approximately 25-35 stations per year.

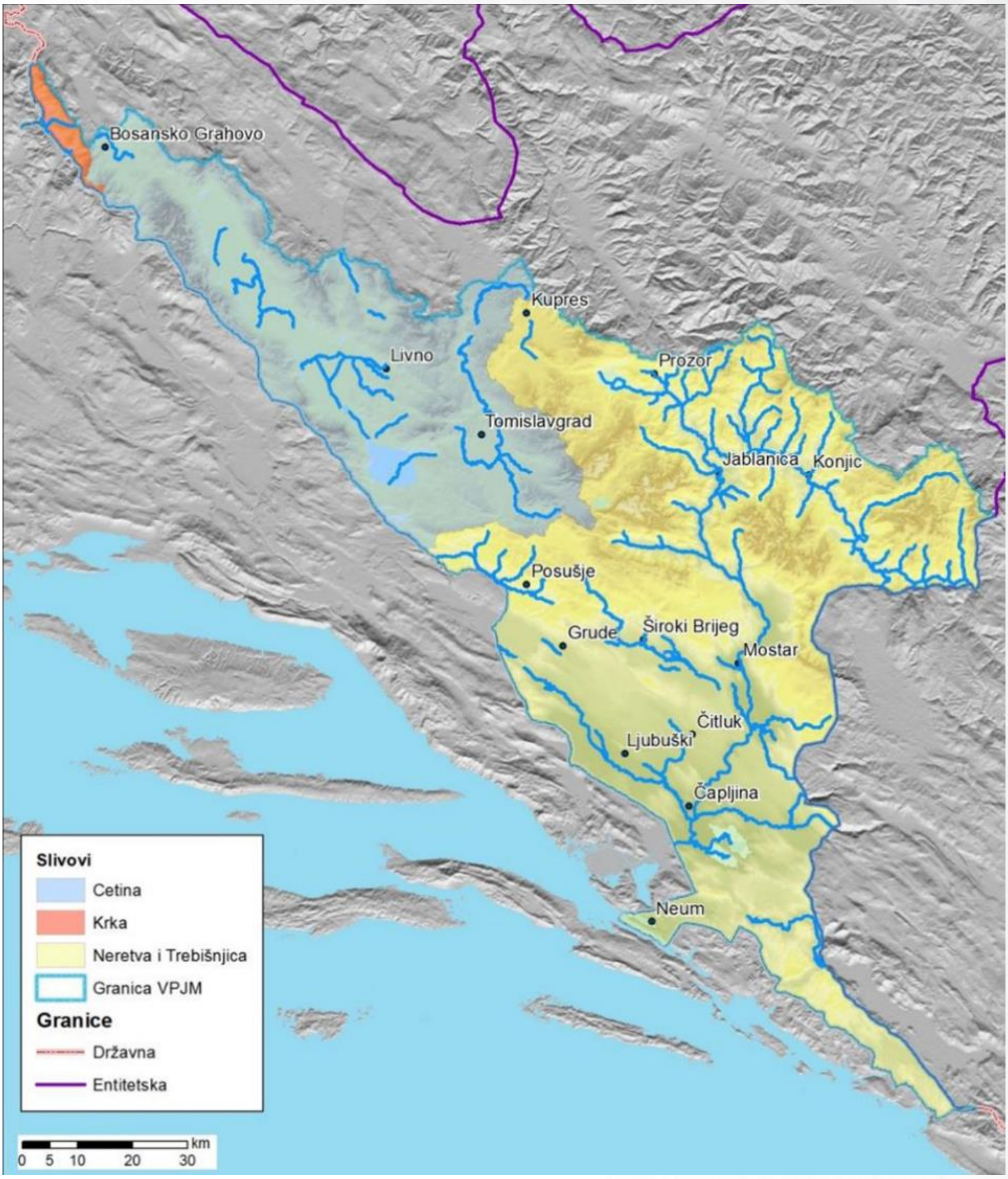

Figure 3 The map of the sampling points explored

1.2. THE UNIVERSITY OF SARAJEVO

The University of Sarajevo conducts monitoring programme annually.

The biological quality elements that this institution monitors are benthic diatoms, aquatic macrophytes, benthic macroinvertebrates, ichthyofauna.

Table 4 The standard and calibrated methods for collecting and processing the data used.

Parameter	Method
Benthic diatoms	BAS EN 13946:2014, Water quality – Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes
Aquatic macrophytes	BAS EN 14184:2015, Water quality Guidance standard for the surveying of aquatic macrophytes in running waters
Benthic macroinvertebrates	BAS EN 16150: 2013 Water quality – Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers; BAS EN 14184: 2015 Water quality - Guidance for the surveying of aquatic macrophytes in running waters
Ichthyofauna	BAS EN 14011:2003, Water quality – Sampling of fish with electricity

The spatial distribution of sample point investigation has been carried out in the entire Federation of Bosnia and Herzegovina.

1.3. THE UNIVERSITY OF EAST SARAJEVO

The University of East Sarajevo has so far had no activities in the field of Environmental monitoring and bioassessment methods.

1.4. JU VODE SRPSKE

Based on the Law on the waters, JU Vode Srpske conducts qualitative monitoring of surface water bodies for bioassessment their ecological and chemical status. The monitoring program is prepared on an annual basis; a sampling frequency depends on monitoring type being carried out (surveillance or operational monitoring) and by the character of the examined quality elements. For biological quality elements and for basic physicochemical parameters that are supportive for a given ecological status sampling is performed mostly two to four times a year, seasonally (or twelve times a year, in case of priority substances for chemical status assessment). Within the routine monitoring, ichthyofauna research is planned once during the river basin management plan, in other words, in six-year cycles.

The elements of biological quality that are monitored by the Water Resources of Srpska are: Phytoplankton (chlorophylle a), Phytobenthos, Macroinvertebrates and Ichthyofauna.

Sampling and quality assessment methods for physical, chemical and biological parameters are defined by Regulation on classification and categorization of watercourses of the Republic of Srpska, 42/01. By that Regulation, for biological parameters evaluation, only the saprobic index, S, Pantle, Buck (1955) is standardized. Lists of sampling and determination methods are given in tables.

Table 5 Methods of water quality sampling and biological quality elements methods used

Parameter	Method
Water samples	BAS EN ISO 5667-1:2008, BAS EN ISO 5667-1/Cor1:2008, Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques BAS EN ISO 5667-3:2014 Water quality - Sampling - Part 3: Preservation and handling of water samples BAS ISO 5667/6:2017 Water quality - Sampling - Part 6: Guidance on sampling of rivers and streams
Chlorophyll-a	BAS ISO 10260 E:2002 Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration
Benthic macroinvertebrates	BAS EN ISO 10870:2014 Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters BAS EN ISO 8689-1:2003 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers

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	BAS EN ISO 8689-2:2003 Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates
Benthic diatoms	BAS EN 13946:2015 Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes BAS EN 14407:2006 Guidance standard for the identification, enumeration, and interpretation of benthic diatom samples from running waters

Table 6 List of data processing methods (*methods for which the laboratory is not accredited; with bold letters is marked an index which is standardized by the Regulation on classification and categorization of watercourses of Republic of Srpska, 42/01).

Parameter	Method
Saprobic index, S, pantle, Buck (1955)*	MSZ 12 756:1988
Shannon Weaver diversity index *	(Shannon & Weaver, 1949)
IPS – Indice de Polluosensibilité *	Coste in CEMAGREF, 1982
EPI-D – Eutrophication/Pollution Index*	Dell'Uomo, 2004
CEE*	
TDI – Trophic Diatom Index *	Kelly & Whitton, 1995
Trent Biotic index*	Woodwiss, 1964

Biological monitoring working party (BMWP index*)	Armitage et al., 1983
Average score per takson (ASPT index*)	Armitage et al., 1983.
Zelinka, Marvan saprobic index*	Zelinka, Marvan, 1961
Belgian biotic index (BB index*)	Flanders, 1990.-2010
Chandlar-ov index, CH*	Chandlar, 1970
EPT i % EPT in relation to the total number of individuals in sample*	
Margalef diversity index *	Margalef, 1958.

Results of chlorophyll-A concentration assay

Tests performed on the measurement profiles included in all three monitoring programs for 2017. A total of 3 series of tests were performed at NM1, 4 for OM and 10 series of tests for TNMN profiles from January to December 2017 (Annex 4, Tables 1 to 17).

According to ISO 10 260: 2002, variant B, collection of algae and other suspended matter from water is done by filtration. Extraction of the pigment from the suspended material retained on the filter is carried out with hot ethanol.

Spectrophotometric determination of chlorophyll concentration is performed in the extract. The chlorophyll concentration and the feopigment concentration are estimated based on the difference in absorbance at 665 nm before and after acidification of the extract.

The presence of chlorophyll is directly related to the number or mass of algal cells, since it accounts for 1-2% of the dry mass of planktonic algae. For this reason, the concentration of chlorophyll is taken as an indirect indicator of the amount of algal biomass and the intensity of primary production.

The quality of water on the tested TNMN profiles was in the largest number of measurements 767 (77%) was in the 1st class, in 15 (17.2%) measurements were in the 2nd class, 2 (2.3%) were in the 3rd class of water quality - Bosnia (Modrica), Bosnia (Usora) Sava (Raca) in Class IV (Bosnia, Modica) in July. The measured concentrations ranged from 0.51 mg / m3 to a maximum of 75.0 mg / m3.

In addition to profiles in the TNMN program that have been testing chlorophyll concentrations for more than ten years, in 2017, these analyzes included profiles in Operational and National Monitoring Monitoring within three and four cycles, respectively, from April to October.

The results for Chlorophyll Concentration for Operational Monitoring, as expected, show that out of a total of 68 measurements, 51 (75%) in grade I, 10 (14.7%) in grade II, and 7 measurements (10.3%) in grade III. Trebisnjica (Gorioca prag), Bosnia (Rudanka), Spreca (Stanic River). The range of concentrations measured ranged from 0.37 mg / m3 to a maximum of 20.13 mg / m3.

The results for the concentration of chlorophyll for National Monitoring, as expected, show that of the total 72 measurements, 60 (83.3%) in grade I, 6 (8.33%) in grade II and 5 (6.94%) in grade III and one measurement in the worst, V class watercourse - 59.0 mg / m3 for Dragočaj (Kuljani). The range of concentrations measured ranged from 0.30 mg / m3 to a maximum of 59.0 mg / m3.

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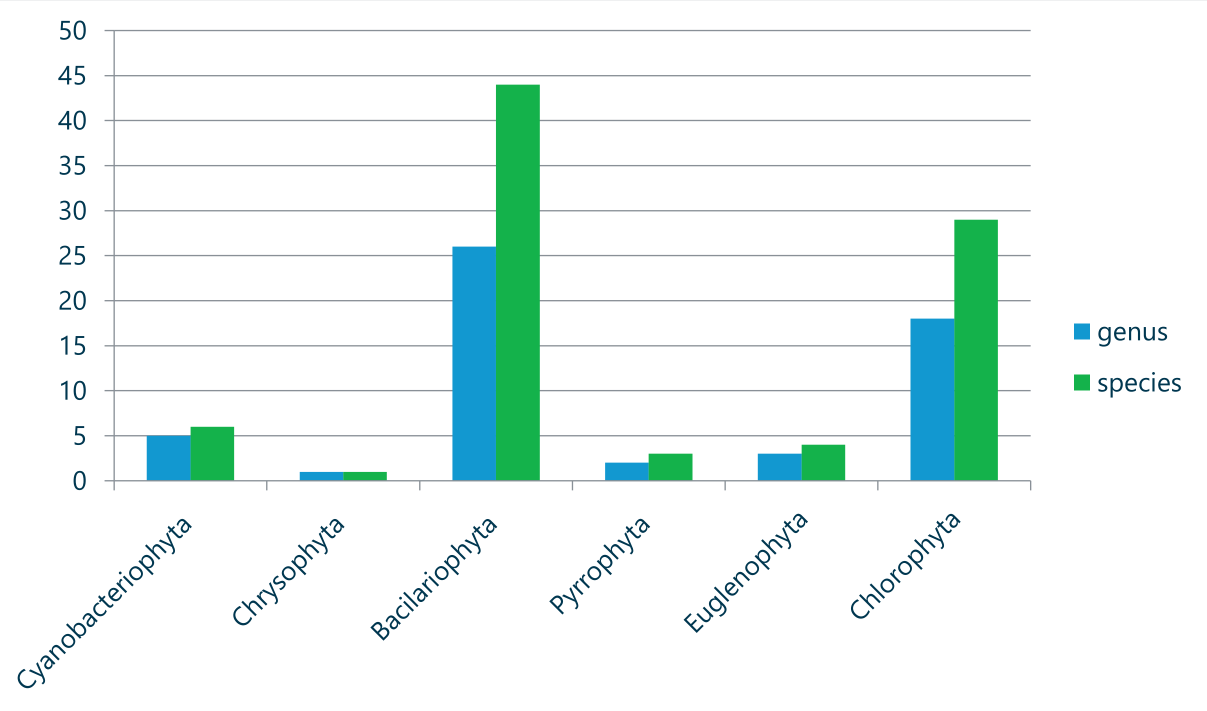

Figure 4 Phytoplankton test results

Table 7 Phytobenthos test results (qualitative analysis on generic level).

International Monitoring (TNMN)	Operational monitoring	National Monitoring
Achnanthes, Cocconeis, Diatoma, Denticula, Epithemia, Fragillaria, Frustulia, Hantzschia, Gomphonema, Gyrosigma, Cyclotella, Cymatopleura, Cymbella, Nitzschia, Stauroneis, Synedra, Surirella	*Achnanthes, Cocconeis, Diatoma, Denticula, Epithemia, Gomphonema, Gyrosigma, Cyclotella, Cymatopleura, Cymbella, Nitzschia, Synedra, Surirella*	*Achnanthes, Cocconeis, Diatoma, Denticula, Epithemia, Gomphonema, Gyrosigma, Cyclotella, Cymatopleura, Cymbella, Nitzschia, Synedra, Surirella*
Notes: As dominant and subdominant taxa in the examined profiles. The calculated values for the saprobability indices were within the limits of the class II of the watercourse	Notes: The highest number of taxa is on average in the group of watercourse class II indicators	Notes: For the purpose of determining the watercourse quality based on the composition of phytobenthos (silicate algae), the data were further processed by OMNIDIA software

Tabela 8 Calculated values for the saprobility index S (Pantle-Buck, 1955) based on phytoplankton composition, profiles included in International Monitoring, TNMN, 2017 Research.

Serial number	Watercourse	Profile	Profile tag	Series I		Series II		Series III		Series IV	
				S	Quality class	S	Quality class	S	Quality class	S	Quality class
1	Una	Kozarska Dubica	U01	2.00	II	1.94	II	1.99	II	1.94	II
2	Vrbas	Razboj	V01	2.02	II	2.17	II	1.97	II	2.01	II
3	Sava	Rača	S01	2.03	II	2.08	II	2.02	II	1.96	II
4	Bosna	Usora	B03	2.04	II	2.19	II	2.18	II	2.1	II
5	Drina	Foča	D05	1.83	II	1.84	II	1.68	II	1.91	II
6	Sava	Gradiška	S04	2.00	II	1,57*	II	1,87*	II	2,03*	II
7	Bosna	Modriča	B01	2.10	II	2.14	II	2.21	II	2.17	II
8	Drina	Pavlovića most	D01	1.9	II	2.01	II	1.88	II	1.95	II
9	Una	Novi Grad nizvodno	U04	1.9	II	2.05	-	2.01	II	1.9	II

Monitoring is carried out on the watercourses of the Republic of Srpska (Regional River Basin District of the Sava River and the Regional River Basin District of the Trebišnjica River).

Table 9 Results of calculated values for the saprobility index S, (Pantle-Buck, 1955) based on phytoplankton, Operational Monitoring, 2017 surveys.

Serial number	Watercourse	Profile	Profile tag	Series I		Series II	
				S	Quality class	S	Quality class
1	Sana	Novi Grad	U13	2.04	II	2,09	II
2	Sana	Prijedor	U14	2,03	II	2,11	II
3	Sana	Ribnik	U15	1.86		2,00*	II
4	Vrba	Delibašino selo	V02	1,89	II	1.91	II
5	Vrbas	Novoselije	V03	1.30	I	1,89	II
6	Crna Rijeka	Bjelajci	V14	1.75	II	2.12	II
7	Ugar	Ugar	U13	1,90	II	1,93	II
8	Bosna	Rudanka	B02	1.99	II	2,19	II
9	Spreča	Stanića rijeka	B11	2,15	II	2,28	II
10	Usora	Matuzići	B12	2,14	II	-	-
11	Ukrina	Lužani	Uk01	2,05	II	2.07	II
12	Lim	Rudo	D16	1,82	II	1,96	II
13	Ćehotina	Brioni	D18	1,99	II	2.02	II
14	Trebišnjica	Dražin do	T03	1,88	II	2.00	II
15	Trebišnjica	Gorica prag	T02	1,89	II	1.81	II
16	Mušnica	Srđevići	T11	1,93	II	1.99	II
17	Neretva	Ulog	N01	1.81	II	1,81	II

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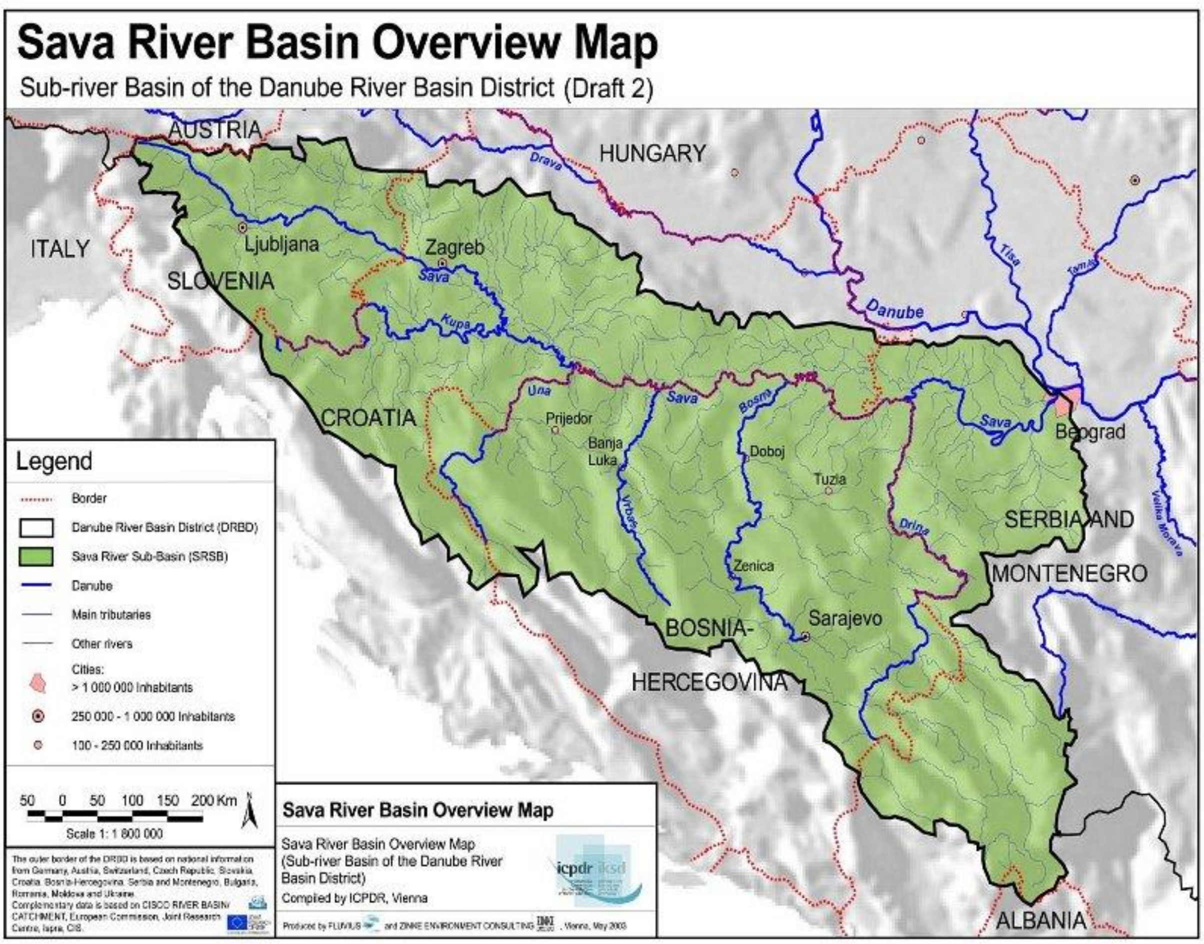

Figure 5 Map of the Regional River Basin District of the Sava Rive

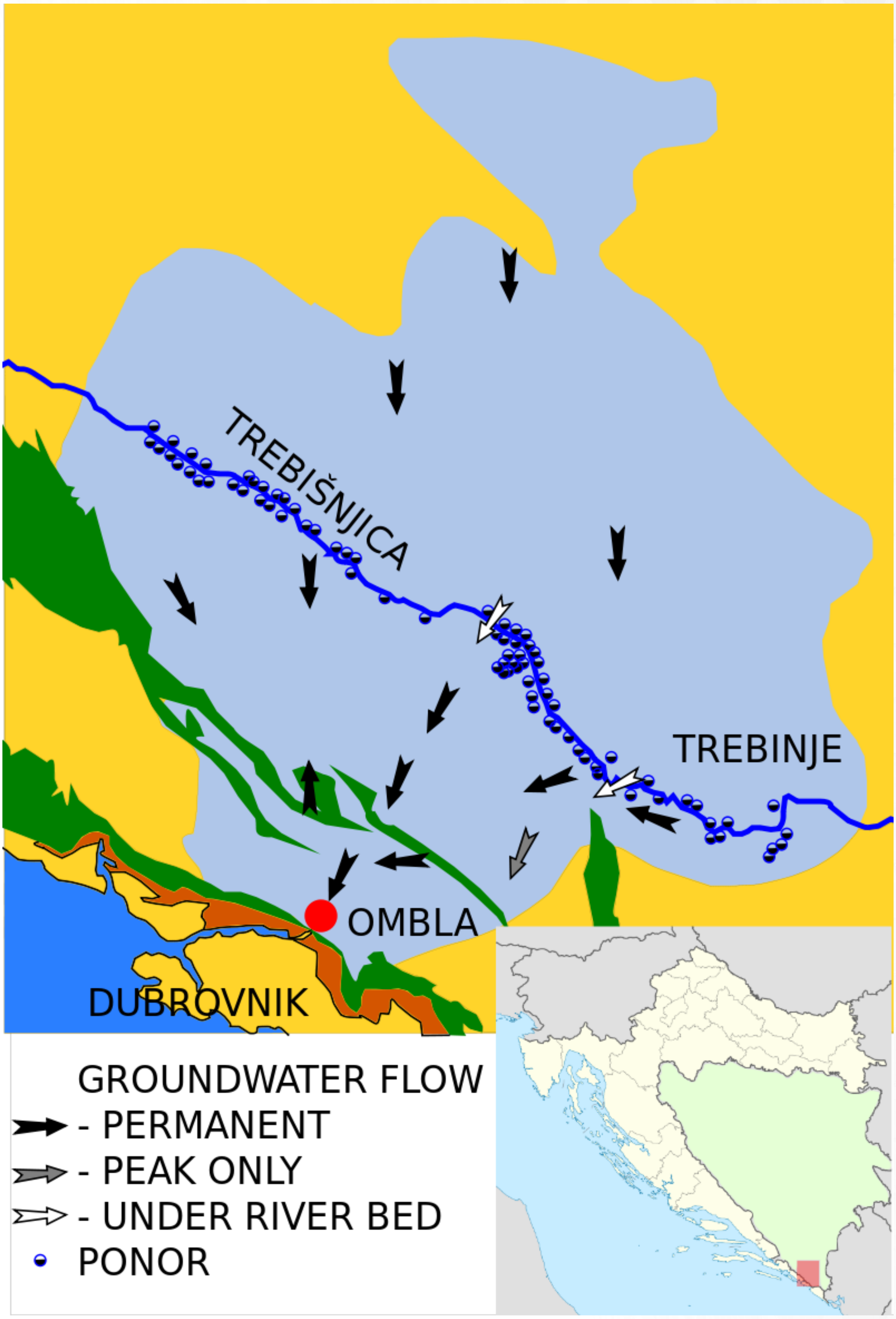

Figure 6 Map of the Regional River Basin District of the Trebišnjica River.

1.5. UNIVERSITY OF MOSTAR

The University of Mostar conducts monitoring programme three times per year at least.

They monitor physical and chemical elements of water quality and biological elements (composition and abundance of aquatic macrophytes, benthic diatoms, benthic macroinvertebrate fauna and ichtyofauna) in rivers. Also, they monitor biological elements (composition and abundance of phytoplankton, zooplankton, other aquatic flora (macrophytes), benthic invertebrate fauna and fish fauna) in lakes.

Table 10 The standard and calibrated methods for data collecting and processing.

Parameter	Method
Water samples	BAS EN ISO 5667-1:2008, BAS EN ISO 5667-1/Cor1:2008, Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques BAS EN ISO 5667-3:2014 Water quality - Sampling - Part 3: Preservation and handling of water samples
Chlorophyll-a	BAS ISO 10260 E:2002 Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration
Benthic diatoms	BASEN 13946:2004 Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers
Phytoplankton	Planktonic mesh size 20 µm is used for sampling phytoplankton. Quantitative analysis; cell counting was performed following EN 15 204, relative

	abundance is estimated according to Pantle-Buck. The water quality category is given according to Liebmann (1962).
Benthic macroinvertebrates	BAS EN ISO 8689-1:2003 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 8689-2:2003 Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates
Aquatic macrophytes	BAS EN 14184:2015 Guidance for the surveying of aquatic macrophytes in running waters.
Zooplankton	Planktonic mesh size 40 µm is used for sampling the zooplankton community. Tolerance for pollution is given according to Patrick and Palavage 2012.
Ichtyofauna	BAS EN 14011:2004 Water quality - Sampling of fish using electricity. The processing of samples covers taxonomic identification, counting, measurement of biologic parameters (length, weight etc.), and examination of fish for external anomalies.

Table 11 List of data processing methods

Parameter	Method
Saprobic index, S, pantle, Buck (1955)	MSZ 12 756:1988
Shannon Weaver diversity index	Shannon & Weaver, 1949
The Macrophyte Biological Index for Rivers	IBMR, Haury et al., 2006
Extended Biotic Index	*EBI;* Woodiwiss, 1978
Biological monitoring working party (BMWP index)	Armitage et al., 1983
EFI index (European Fish Index)	Fame Consortium, 2004
EFI+ index (European Fish Index +)	EFI+ Consortium, 2009
Zelinka, Marvan saprobic index	Zelinka and Marvan, 1961
Margalef diversity index	Margalef, 1958
Pollution tolerance score	Patrick and Palavage 2012.

Three river basins were monitored, namely: the Neretva basin and parts of the basins of the Trebišnjica and Cetina rivers. All of these sites are in Herzegovina and comprise 41 sites and are part of regular biological monitoring.

1.6. UNIVERSITY OF TUZLA

Monitoring is conducted occasionally within the framework of project activities, not as part of periodic operational monitoring. Project activities are based on weekly, monthly or seasonal sampling dynamics. Depending on the type of project and the need for biomonitoring, the process of biomonitoring is considered essential for environmental monitoring. Biomonitoring programs are actively integrated into all projects, and they are mostly seasonal in nature unless it is a target group of organisms where there is monthly monitoring for a shorter period of time (2-3 months).

Phytobenthos, macrophytes, fish and macrozoobenthos are the most usually studied biological quality elements within the project activities. Phytoplankton has been less frequently studied as a target group. The most frequently used condition indicator is macroinvertebrates and fish community of the target area.

Table 12 Standard and calibrated methods for data collecting and processing.

Parameter	Method
Benthic diatoms	BAS EN 13946:2015 Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes BAS EN 14407:2015 Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes
Benthic Macroinvertebrates	BAS EN ISO 8689-1:2003 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 8689-2:2003 Biological classification of rivers – Part 2:

	Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates
Aquatic macrophytes	BAS EN 14184:2015 Guidance for the surveying of aquatic macrophytes in running waters
Fish	BAS EN 14011:2004 Sampling of fish with electricity

Index testing in bioassessment is often one of the objectives in the research. In Bosnia and Herzegovina, phytobenthos methods have not yet been calibrated. Macrophytes have been investigated in the form of phytocoenology and bioindication according to Ellenberg and Pignati values, and less frequently using trophic indices, since that system is not established in Bosnia and Herzegovina for macrophytes in monitoring programme.

Standard sampling methods are used, which include river area transect, zoning and mapping of the environment, the influence of abiotic factors, etc. For target groups, macroinvertebrate sampling is performed using the standard Surber net. Sampling is performed in different zones for comparative analysis.

Most often, scientific investigation have been carried out on different waters belonging to the river basin Sava in North-Eastern Bosnia. These are the following waters: Lake Modrac, Lake Snježnica, Hazna, Vidara and Paučko jezero (Lake Paučko); rivers Gostelja, Spreča, Oskova, and Turija. Part of research was conducted on rivers Una, Vrbas, Bosna and Sava. The focus of scientific research is the most often water bodies in North-Eastern Bosnia belonging to the Sava River Basin (Lake Modrac, rivers Spreča, Turija, Tinja, Oskova, Gostelja), many pit lakes, lakes Hazna, Vidara, Snježnica, Paučko). In addition to the scientific projects, larger rivers such as the Una, Vrbas, Bosna, Sava were also selected as research sites in the recent period. Since bees and honey are the best indicators of environmental pollution, we are monitoring the areas we consider to be at risk of pollution. Our monitoring covers different localities in Bosnia and Herzegovina.

Regarding the geographical distribution of the area of activity, in most cases it is related to the Tuzla Canton, and the focus is on protected landscapes, and places that are restricted due to individual target populations. It is not exclusive that we go outside the Tuzla Canton and enter projects at the national level in order to collect new data from biomonitoring projects, but such cases are much rarer and most often in collaboration with colleagues from across BiH.

1.7. SAVA RIVER WATER AGENCY

Sava River Water Agency conducts monitoring following the current legislation in the field of water. The each biological quality element is monitored twice per year.

Table 13 Standard and calibrated methods for data collecting (All biological quality parameters are accredited by the BiH Accreditation Institute).

Parameter	Methods
Water samples	BAS EN ISO 5667-1:2008 Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques - Amendment AC BAS EN ISO 5667-1/Cor1:2008 Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques - Amendment AC BAS EN ISO 5667-3:2014 Water quality - Sampling - Part 3: Preservation and handling of water samples
Phytoplankton	Standard methods 10200-B2a

	APHA-AWWA-WEF 2012
Aquatic macrophytes	BAS EN 15460:2009 Water quality - Guidance standard for the surveying of macrophytes in lakes BAS EN 14184:2015 Water quality - Guidance for the surveying of aquatic macrophytes in running waters
Benthic macroinvertebrates	BAS EN ISO 8689-1:2003 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 8689-2:2003 Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates BAS EN 16150:2013 Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers BAS EN ISO 10870:2014 Water quality - Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters
Bentic diatoms	BAS EN 13946:2015 Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes

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Table 14 List of data processing methods

Parameter	Methods
Phytoplankton	BAS EN 15204:2008 Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)
Macroinvertebrate benthos	Standard methods 10500C (1i2) APHA-AWWA-WEF 2012 Standard methods 10400-A APHA-AWWA-WEF 2012 Standard methods 10400-D(3e(1) APHA-AWWA-WEF 2012 Standard methods 10400-D (3e(2) APHA-AWWA-WEF 2012
Bentic diatoms	BAS EN 14407:2015 Water quality - Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes

The area under the monitoring programme comprises the entire Sava River Basin belonging to the Federation of BiH.

1.8. INTERNATIONAL UNIVERSITY OF TRAVNIK

The University of Travnik has so far had no activities in the field of Environmental monitoring and bioassessment methods.

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1.9. UNIVERSITY OF BANJA LUKA

The University of Banja Luka conducts continuous monitoring programme once a year, since 2016. Besides, this institution conducts monitoring activities occasionally within different scientific projects, expert studies and research work.

Biological quality elements that this institution monitors are microorganisms, phytoplankton, benthic diatom, periphyton, benthic macroinvertebrates, aquatic macrophytes and ichthyofauna.

Table 15 Standard and calibrated methods for data collecting.

Parameter	Methods
Phytoplankton, Benthic diatom, Periphyton, Benthic macroinvertebrates, Aquatic macrophytes	Conducted following APHA-AWWA-WEF, 1999 and Official Gazette of Republic of Srpska, No. 42/01)
Ichthyofauna	Conducted following the standard for sampling fish with electricity for water quality analysis (EN 14011: 2003) and Official Gazette of Republic of Srpska, No. 42/01; net sampling (in cooperation with commercial fisherman and in accordance with the standard for sampling fish with multi-mesh gillnets, EN 14757:2005 and Official Gazette of Republic of Srpska, No. 42/01)

Table 16 List of data processing methods

Parameter	Methods
All investigated groups	Qualitative and quantitative composition
Saprobic index	According to (Pantle-Buck, 1955) for phytoplankton, periphyton, phytobenthos, macrozoobenthos and ichthyofauna
Chlorophyll-a	Standard spectrophotometric method, (APHA-AWWA-WEF, 1999)

Continuous monitoring of microorganisms and ichthyofauna includes surface freshwaters of the Republic of Srpska (watershed of Sava and Trebišnjica rivers) with 39 localities. Occasional monitoring of microorganisms, phytoplankton, phytobenthos, macrozoobenthos, aquatic macrophytes and ichthyofauna includes different types of stagnant and running freshwaters of B&H (accumulations, mountain lakes, rivers, brooks, springs, wetlands...). Also, we occasionally perform monitoring of drinking water quality.

3. RESULTS OF QUESTIONNAIRES RELATE MONTENEGRIN KNOWLEDGE / SKILLS / PRACTICE IN EMAB

The legal prerequisite for the protection of surface and underground waters in Montenegro is stated in the Law on Waters (Offical Gazzette 02/17). This Law transposes the WFD and as such provides the obligations concerning monitoring and reporting. The Law identifies the Institute for Hydrometeorology and Seismology of Montenegro as the institution in charge of monitoring the qualitative and quantitative parameters, following the endorsed annual program. Thus, this Institute conducts the monitoring of relevant biological parameters following the WFD (stated in the table below). The information from this monitoring is reported to the Agency for the Protection of Nature and the Environment and is a part of their Annual Reports on the State of the Environment.

The Agency for the Protection of Nature and the Environment conducts monitoring of certain biological components of surface waters. This follows the national requirements of the nature protection policy (Law on the Nature Protection, Law on the Environment) and based on national and other relevant nature protection related indicators (i.e. not WFD-related, and thus does not follow WFD requirements). The results are part of the annual national Reports on the State of the Environment.

Also, a team of researchers from the Faculty for Biology of the University of Montenegro have capacities for biological monitoring and are occasionally engaged to conduct monitoring about particular needs. They are currently monitoring part of River Tara to assess the impacts of the highway construction.

Apart from biological monitoring, Centre for Ecotoxicological Research and the Municipality of Bijelo Polje conduct the monitoring of physical and chemical parameters, which are reported on the national and local levels (i.e. not towards WFD).

2.1. UNIVERSITY OF DONJA GORICA

The University of Donja Gorica does not carry out any monitoring programs. Since the University does not carry out any monitoring programs they engaged the Institute for Hydrometeorology and Seismology of Montenegro, Agency for the Protection of Nature and the Environment of Montenegro and the University of Montenegro.

2.2. INSTITUTE FOR HYDROMETEOROLOGY AND SEISMOLOGY OF MONTENEGRO

The institution conducted monitoring programme annually – in the season between April and October, in two batches. Biological elements planned to conduct refers to Benthic diatoms, Phytoplankton, Benthic macroinvertebrates. Macrophytes will be conducted in the near future.

Table 17 Standard and calibrated methods for data collecting and processing

Parameter	Methods
Benthic diatoms	For sampling: MEST EN 15708:2014; MEST EN 13946:2016; MEST EN 14407:2016. Indices: IBD, IPS, IDG, EPI-D, TID, SID, CEE; light microscope Nikon; Lange-Bertalot (program Omnidia calculates the index)
Phytoplankton	Fluorospectrometer method – bbe FluoroProbe
Chlorophyll-a	Standard spectrophotometric method, APHA-AWWA-WEF, 1999 BAS ISO 10260 E:2002
Benthic macroinvertebrates	For sampling: MEST EN 16772:2017;

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	Indices – UBS, OSI, BMWP, EPTS; binocular microscope (program Asterix calculates indices), eu taxa – electronic identification keys

The monitoring network covers 13 water courses with 36 monitoring stations, 3 lakes with 11 monitoring stations. They are both in Danube and Adriatic basins (i.e. cover all of Montenegro).

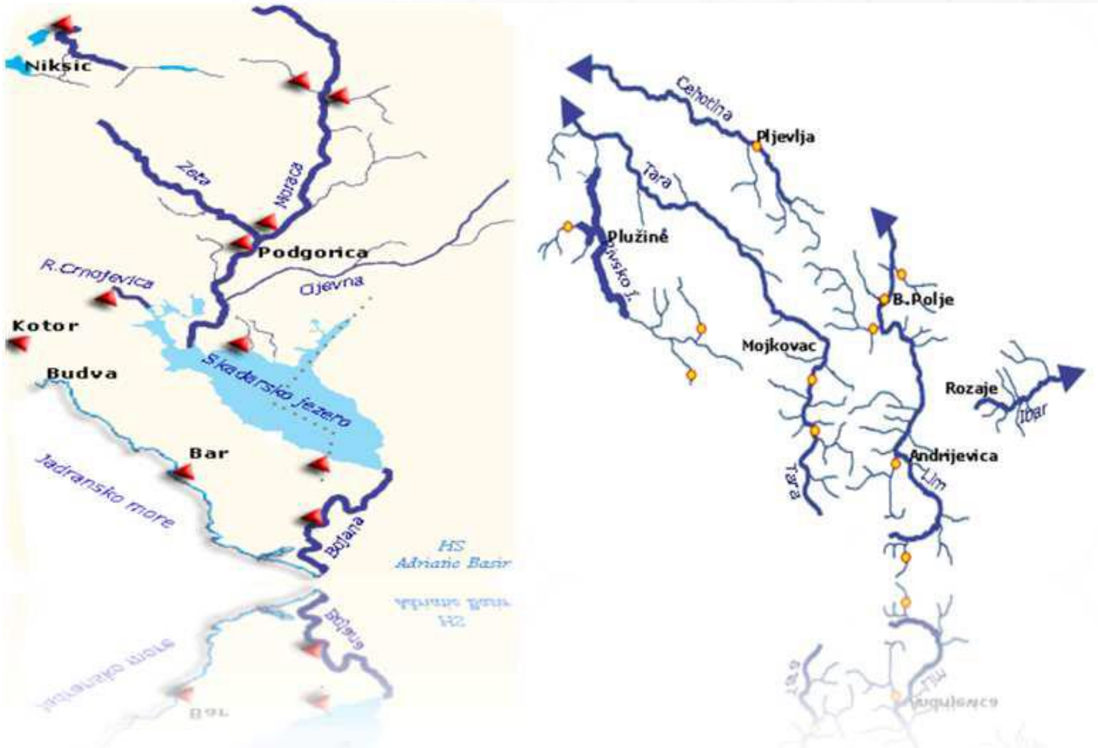

Figure 7 The network of automated hydrological stations in Montenegro.

2.3. AGENCY FOR THE PROTECTION OF NATURE AND THE ENVIRONMENT OF MONTENEGRO

Agency for the Protection of Nature and the Environment of Montenegro conducts monitoring programme annually, encompassing the season between March and October.

Biological quality elements monitored are:

- Aquatic macroinvertebrates (Turbellaria, Hirudinea, Cladocera, Copepoda, Ephemeroptera, Odonata, Placoptera, Trichoptera, Diptera)
- Molluscs (Malacofauna)
- Decapoda
- Ichthyofauna

Standard and calibrated methods for data collecting and processing used are nets for collecting plankton, bottom nets for benthic organisms and fish nets for ichthyofaunal. Fixation of collected organisms was done in the field using formalin. Material determination was done in the laboratory using identification keys.

Spatial distribution of investigated sample relates to four national parks: Skadar Lake, Lovćen, Durmitor and Biogradska Gora.

Figure 8 National Park Skadar lake. https://www.earthtrekkers.com/photographing-lake-skadar-pavlova-strana/.

Figure 9 National Park Lovćen. https://sr.wikipedia.org/wiki/media/datoteka:Lovcen-008-p1010045.jpg.

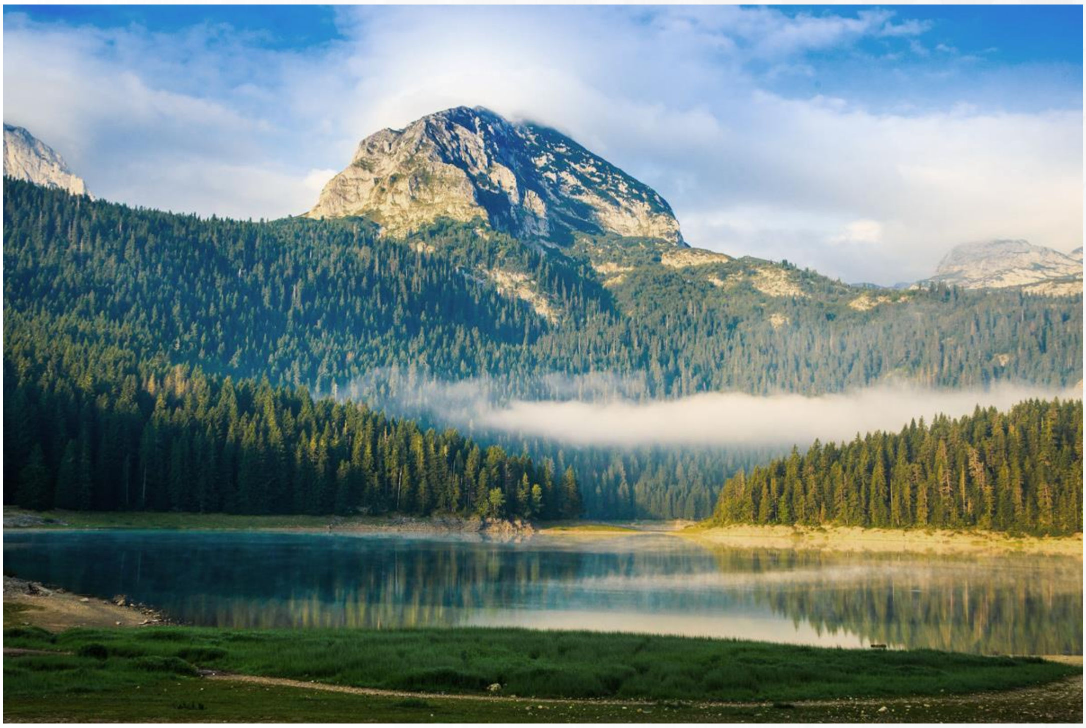

Figure 10 National Park Durmitor. https://vsar.edu.rs/2017/12/07/obelezavanje-dana-planina-12-decembra-u-vsar/durmitor/

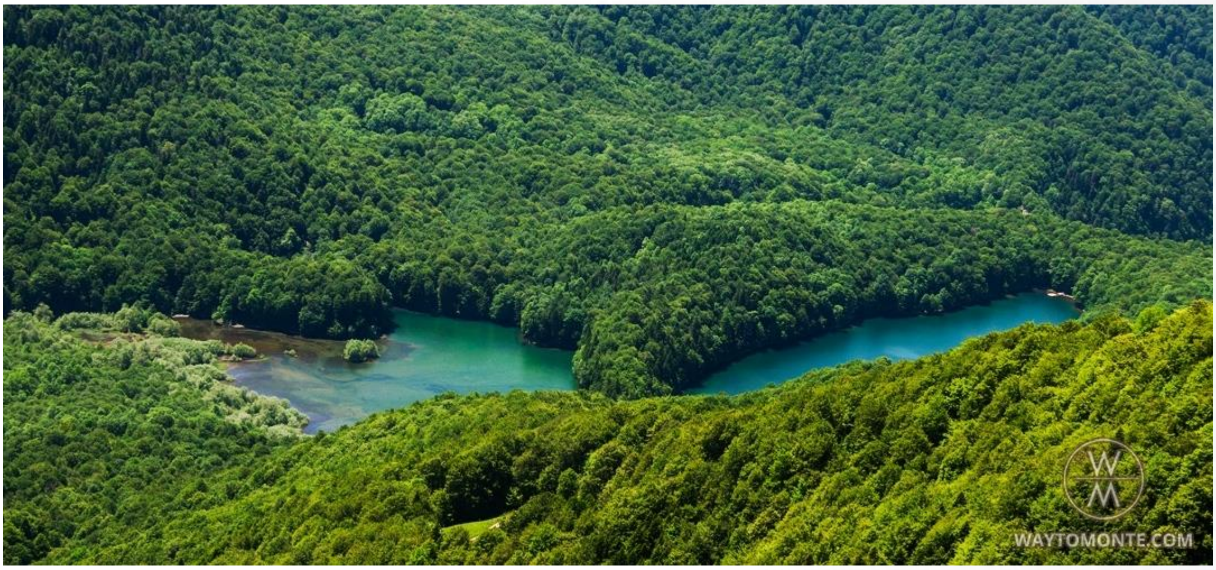

Figure 11 National Park Biogradska Gora. https://waytomonte.com/rs/p-887-national-park-biogradska-gora.

2.4. UNIVERSITY OF MONTENEGRO

Faculty for Biology of the University of Montenegro conducts monitoring programme monthly.

The focus of their research is the benthic macroinvertebrates.

Qualitative and quantitative analysis following the WFD. Lotic and lentic sites are sampled separately.

The spatial distribution of the investigated sample applies only to Tara River, at three localities between Trebaljevo and Matesevo (to monitor the impacts of the highway construction).

Figure 12 Map of the Tara River

Figure 13 Map of the Tara river canyon (detailed).

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