

Biological Monitoring of Surface Water Bodies of the Adriatic Sea Basin in Bosnia and Herzegovina-An Example

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Abstract

According to the application of the European Water Framework Directive, bioassessment of surface water bodies is one of the most important approaches to provide the deterioration of ecosystems and achieve environmental sustainability. Hydromorphological alternation, nutrient enrichment driven by human activities are the main factors for the ecological compromise of freshwater ecosystems. During the 2022 we done research and bio assessment in 40 water bodies in the Adriatic Sea Basin, and it is included monitoring of

- i. physico- chemical,
- ii. chemical,
- iii. hydro-morphological, and
- iv. biological factors.

Mostly of our investigated water bodies were in category of good or high ecological status or maximum or good ecological potential. Despite the satisfactory conditions we have been noting new water challenges (e.g. eutrophication, water blooms, salinization, invasive species, biodiversity declining) lately. Bioassessment in surface water bodies could be used in a risk assessment framework to identify levels of pollution or contamination, and guide environmental decision making.

Introduction

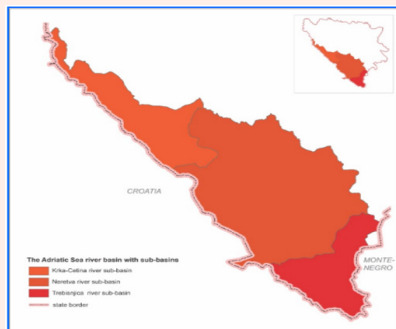
Biological assessment is an essential element of water monitoring programs that helps guide decisions for managing the ecological integrity of environmental resources (1-20). Classical biomonitoring programs have focused primarily on measures linked to various biodiversity metrics, e.g., species richness, beta diversity (4) and indicator species (20-24). By using the results of the above-mentioned measurements, the ecological status or ecological potential is assessed as a measure of the global state of aquatic ecosystems. The mentioned terms are included in the Water Framework Directive - WFD (Directive 2000/60/EC) of the European Parliament and the Council, which established a framework for the action of the European Community in the field of water policy. The Water Framework Directive is the most important part of the EU legislation on water. The assessment of the water body status is based on the ecological and chemical status classification. The ecological status/potential is an assessment of the quality of the structure and functioning of surface water ecosystems, which is reflected through biological elements (abundance and diversity of aquatic organisms), hydro morphological (flow dynamics, depth, etc.) and *physical-chemical* conditions (saturation, temperature, pH). etc.), which are necessary for the survival of communities in aquatic ecosystems. If the status of the biological and physical and chemical element is assessed as good, and of the hydro morphological as moderate, total ecological status will be estimate as moderate. The ecological status is evaluated based on the worst rated component. According to WFD, biological elements used to assess water status/potential include communities of algae, macrophytes, aquatic invertebrates and fish. Based on the quantitative and qualitative composition of communities, the saprobiological condition of investigated water body and the degree of water pollution are determined. Saprobity is determined based on indicator organisms, which by their presence and activity point to different water quality, purity or pollution, and includes the analysis of qualitative and quantitative features of their communities (17). Organisms community in freshwater ecosystems are sensitive indicators of water status, because they integrate and maintain the influence of various environmental factors, various types of contamination, degradation, fragmentation of habitats and etc. (18). This paper presents the water quality, as well as the ecological status/potential of surface water bodies of the Adriatic Sea basin in Bosnia and Herzegovina. The aim is to determine existing disturbances, assess the current state based on the established composition and structure of communities and compare them with reference values, determine water quality categories, and present future challenges.

Materials and Methods

The area of the Adriatic Sea basin in Bosnia and Herzegovina encompasses a karst area where significant surface waters have been formed: Neretva, Trebisnjica, Krka and Cetina (Figure 1). Territorially speaking, these river basins encompass parts of three countries. The larger, north-eastern part belongs to Bosnia and Herzegovina, the smaller, south-western part to the territory of the Republic of Croatia, and the smallest south-east part is in Montenegro. The total surface area of the Adriatic Sea basin district

in Bosnia and Herzegovina is 8.782 km², of which the surface of Neretva and Trebišnjica is 6.041 km², Cetina 2.655 km², and Krka 84.8 km². Significant watercourse are: the Neretva river and the Krka river with the Cetina (Figure 1).

Figure 1: The Adriatic Sea basin in Bosnia and Herzegovina



The Adriatic Sea basin has relatively few rivers with surface runoff. It is dominated by subterranean rivers and sub-surface flows. The rivers of the Adriatic Sea basin usually originate from karst springs, and they are most often the waters of some entire watercourses that have sunk somewhere in a higher part of the topography (19). The valley of the Neretva river is mostly of a canyon type, and its river beds have frequent rapids, cascades and waterfalls. In the broad valleys and fields, the waterfalls are small. The geological structure and petrographic composition of the terrain of the Adriatic basin, which is located in the Dinaric region, evolved during the Paleozoic and Cenozoic era and belongs to a developed karst. It is mainly made of water-permeable rocks, karst limestone and, to a lesser extent, of the water-permeable rock mass of dolomites.

Sampling and bioassessment

In order to assess the ecological state and quality of water, the physico-chemical elements of water and biological elements were analyzed in the framework of these studies: communities of phytobenthos, phytoplankton, macrozoobenthos and macrophytes. Measurements and sampling were carried out in June and July 2022 at 40 locations that include running and standing water surfaces in the Adriatic Sea basin.

Physical and chemical conditions of water

Measurements of physical and chemical conditions of water included measurements of: water temperature (°C), electrical conductivity (µS/cm), dissolved oxygen (mg/L), oxygen saturation (%) and water pH. The mentioned conditions were measured in situ using an ecological probe (WTW Multi-Parameter Instruments). Chemical analyzes of nutrient were done at the Institute of Public Health of the Federation of Bosnia and Herzegovina, and include the analysis of nitrites, nitrates, phosphates, silicates, total nitrogen, total phosphorus, chlorophyll a, etc. Based on above mentioned factors, trophic indices were calculated. Trophic status indices (TSI) of stagnant waters according to Carlson (1977) are determined using TSI values of chlorophyll a concentration (CHL), total phosphorus concentration TSI (TP) and Secchi TSI transparency values (SD). The values of the index result represent the next state of the trophic state, i.e. values < 40 oligotrophic; 40-50 mesotrophic; 50-70 eutrophic; >70 hypertrophic.

Biological elements of water

Phytobenthos: Phytobenthos samples were taken depending on the bottom substrate at research stations according to prescribed protocols (European standard EN 13 946, 2003). Species were determined using a Zeiss light microscope (Axio Images) and scientific literature and keys. The indicator values of species were defined according to

Wegl (1983), and for the saprobiological assessment of phytobenthos communities, the saprobicity index according to (19) and (20) was used.

Macroinvertebrate: Macrozoobenthos samples were taken using a Surber net and tweezers. Laboratory isolation, determination and analysis of macrozoobenthos communities was performed using an Olympus SZX10 stereoscope. Scientific literature and keys were used for determination. To assess the ecological status and organic pollution of aquatic ecosystems, the following are used: Pantle-Buck compatibility index (S), extended biotic index (EBI), BMWP index (Biological Monitoring Working Party) and Shannon-Weaver diversity index. The saprobity index (19) is based on the fact that any pollution of watercourses affects the composition and structure of macrozoobenthos communities, whereby changes are manifested primarily in the reduced abundance or disappearance of species sensitive to pollution (especially shorebirds, water lilies, and tulars), and the increased number of tolerant species. The overall value was determined according to (17) and the database: freshwater ecology.com.

Phytoplankton: Phytoplankton sampling were taken with a boat using a net for phytoplankton with a diameter of 20µm. Sampling was taken in the upper layer of the water column (depth 0.5m) for qualitative and quantitative analysis. The analysis of the samples was carried out with an Opt on microscope. Species are identified using relevant keys and literature. Taxon nomenclature is aligned with the Algae-base database (<http://www.algaebase.org/>). Quantitative analysis, i.e. cell counting, was done according to the EN 15 204 (2004) standard (Water quality - Manual for routine analysis of the abundance and composition of phytoplankton using inverted microscopy - Utermöhl's technique). The sample was processed by the method of sedimentation and counting (22). Cells were counted after sedimentation for at least 24 hours. The results of the analysis are expressed as the number of cells per liter of water. The degree of compatibility was determined based on the relative number of indicator species according to the (19) method.

Macrophytes: Macrophyte sampling methodology was based on the European norm EN 14184:2014 (Comité Européen de Normalisation, 2014), following national sampling protocols. A five-level scale of macrophyte taxa according to the IBMR index is used to assess the coverage in the field. Vascular plants and mosses are assigned to a species, and macroalgae to a genus. The taxon nomenclature is aligned with Flora Europaea base. To assess the trophic state based on the macrophyte biological element, the Macrophyte Biological Index for rivers, IBMR, was calculated. The technical process of creating the IBMR is based on in situ observation of macrophytes to determine each taxon and calculation of percent cover using only a transformed scale of 1 to 5 (from the only species present to high abundance and cover). The calculation of IBMR also relies on defining the specific trophic point (indicator value) Csi and the ecological amplitude coefficient Ei. Trophic-level IBMR is biased by the values assigned to each taxon so that scores represent trophic affinity (1: hypereutrophic, 20: highly holotrophic). The index also corresponds to habitat structure (coastal type, habitat type, substrate).

Results and Discussion

The running waters

The results of the conducted research, along with the assessment of the ecological status/potential, are presented in summary Tables 1 and 2. A great diversity as well as abundance of phytobenthos taxa were recorded. The number of phytobenthos taxa ranged from 16 to 40. Saprobic index values ranged from 1.5 (Rakitnica) to 1.77 (Doljanka). Most of the analyzed stations were in I.-II. category of water, that is, in the category of oligo to betamesosaprobic waters or in category II.

Table 1: Total results of the investigated rivers for the year 2022.

Location	Saprobity Index				EBI		BMWP		Shannon-Weaver Index			IBMR		Ecological Status/Potential		
	F	M	K	K	V	K	V	K	M	F	k	v	k	Physical-chemical indicators	Biological indicators	Total status
Neretva-Zito mislici	1,7	1,7	1,72	I-II	8	II	87	II	1,532	2,853	II	11,21	mesotrophic	MEP	MEP	MEP
Neretva Glavaticevo	1,61	1,3	1,45	I-II	7	III	60	III	2,391	2,908	II	11,98	mesotrophic-oligotrophic	HES	HES	HES
Neretva Dracevo	1,76	1,77	1,76	II	6	III	38	IV	2,978	2,807	II	11,33	mesotrophic	MEP	MEP	MEP
Neretva Bacevici	1,63	1,77	1,7	I-II	9	II	96	II	2,509	2,403	II	10,57	mesotrophic	MEP	MEP	MEP
Neretva 9 Konjic	1,67	1,81	1,74	I-II	7	III	64	III	2,322	2,995	II	11,09	mesotrophic	MEP	MEP	MEP
Neretva Rastani	1,64	1,73	1,68	I-II	8	II	63	III	2,705	2,491	II	11,35	mesotrophic	MEP	MEP	MEP
Listica	1,64	1,74	1,69	I-II	10	I	97	II	1,957	2504	II	11,91	mesotrophic	MEP	MEP	MEP
Tresanica	1,62	1,81	1,71	I-II	7	III	47	III	1,388	2,731	II	7,77	eutrophic	MEP	MEP	MEP
Tresanica	1,68	2,04	1,86	I-II	6	III	23	IV	0,580	2,574	II	12,11	oligotrophic	MEP	GEP	GEP
Bijela	1,73	1,78	1,75	I-II	8	II	54	III	2,21	2,657	II	12,14	oligotrophic	HES	GES	GES
Doljanka	1,77		1,77	I-II						2,456	II	10,89	oligotrophic	HES	GES	GES
Gobelovska	1,53	1,55	1,54	I-II	10	I	112	I	2,144	2,998	II	13,6	oligotrophic	HES	HES	HES
Neretvica	1,57	1,65	1,61	I-II	10	I	108	I	2,054	2,908	II	11	oligotrophic	HES	GES	GES
Rakitnica	1,5	1,52	1,51	I-II	10	I	104	II	3,16	2,600	II	13,5	oligotrophic	HES	HES	HES
Rama 3	1,67	1,72	1,69	I-II						2,801	II			MEP	MEP	MEP
Gracanica	1,8	1,78	1,79	I-II						2,809	II	12,16	oligotrophic	HES	GES	GES
Rika	1,49	1,53	1,51	I	8	II	73	II	3,186	2,994	II	12,28	oligotrophic	HES	HES	HES
Trebizat mouth	1,73	1,69	1,71	I-II	5	IV	22	IV	0,665	2,907	II	10,5	oligotrophic	GES	GES	GES
Mlade Trebizat	1,65	1,76	1,705	I-II	8	II	48	III	1,822	2,601	II	11,66	oligotrophic	MEP	MEP	MEP
Milac	1,55	1,56	1,55	I-II	8	II	56	III	1,127	2,400	II	12,43	oligotrophic	HES	HES	HES

Table 2: Total results of researched lakes and sea for the year 2022.

	F	K	K	IBMR	TSI	Trophy
Rama lake (Rumboci entrance) - August	178	I-II	DEP		38,9	mesotrophic
Rama lake (Rumboci entrance) - December	162	I-II	DEP		38,6	mesotrophic
Rama lake - (middle) -August	2	II	DEP		46,3	mesotrophic
Ramsko lake - (middle) - December	178	I-II	DEP		40,8	mesotrophic
Rama lake output/hydropower dam - August	175	I-II	DEP	10,03-MESOTROPHIC	45,3	mesotrophic
Rama lake output/hydropower dam - December	172	1-11	DEP		38,5	mesotrophic
Jablanica lake 1 (shallow part) - August	178	1-11	DEP	4,81-EUTROPHIC	38,9	mesotrophic
Jablanica lake (incidental) - August	178	1-11	DEP	8,4-EUTROFIC	39,1 mesotrophic	mesotrophic
Jablanica lake 2 - (middle) - August	168	I-II	DEP		39,15 mesotrophic	mesotrophic
Jablanica lake 3 (deeper part Hudutsko) - August	177	1-11	DEP		37,47 mesotrophic	mesotrophic
Jablanica lake 3 (deeper part Hudutsko) - December	173	1-11	DEP		40,6	mesotrophic

Busko lake 1 - December	177	1-11	DEP		47,9	mesotrophic
Busko lake 2 - December	181	II	DEP		43,12 mesotrophic	mesotrophic
Busko lake 3 - December	168	1-11	DEP		44,7	mesotrophic
Lipa lake - August	182	I-II	DEP		40,04 mesotrophic	mesotrophic
Lipa lake - December	163	I-II	DEP	5,7 EUTROFIC	39,26 mesotrophic	mesotrophic
GKB Trebisnjica - August	175	I-II	DEP	7,57 EUTROFIC	39,2	mesotrophic
GKB Trebisnjica - December	166	I-II	DEP		41,6	mesotrophic
Deran lake	174	1-11	DEP	8,08 EUTROFIC	44,5	mesotrophic
Ecological status/trophy						

The communities of macroinvertebrates at the investigated stations also show a significant diversity and abundance of taxa. The number of taxa ranged from 15 to 17. The saprobity index at the investigated stations ranged from 1.3 (Neretva Glavicevo) to 2.04 (Tresanica). According to the saprobity index values, most of the water bodies included in this research belong to the category of oligo to betamesosaprobe waters, or betamesosaprobic waters, that is, waters I. to II. or II. quality categories. An exception is the Neretva Glavicevo station, which belongs to the category of oligosaprobic waters (category I). Other indices used for macroscopic invertebrates, which are generally less sensitive and less reliable (EBI, TBI, BMWP and Shannon-Weaver index) relatively followed the obtained values of the saprobity index, except in cases of less diversity of taxa at the station (EBI, TBI, BMWP) or distinct dominance of one taxon (Shannon-Weaver index). Saprobity indices calculated for phytobenthos and macroinvertebrate mostly show uniform values.

According to the legal regulations, i.e. the Decision on the characterization of surface and underground waters, reference conditions and parameters for water condition assessment and water monitoring in Bosnia and Herzegovina, and in accordance with the physical-chemical indicators and the values of the compatibility index, a high ecological status has been established for the stations Neretva-Glavicevo, Gobelovska rijeka, Rakitnica, Rika and Milač, while for all the other analyzed stations a good status was established. Maximum ecological potential was determined for modified water bodies, while good ecological potential was determined for Tresanica. Also, the qualitative-quantitative composition of macrophytes was analyzed and the trophic index IBMR, which indicates the trophic status, was calculated for each station. The obtained trophic status represents the deviation of the structure and function of the ecosystem from the natural or reference state. Trophic status ranged from 7.77 (Tresanica) to 13.5 (Rakitnica). Most of the analyzed stations were in the category of mesotrophic condition.

Stagnant water

A great diversity and abundancy of phytoplankton taxa were recorded on the investigated locations, especially in terms of cyanobacterial, diatoms and dinoflagellats. Most of the stations were in the oligo to beta-mesosaprobic category (Table 2). A good ecological potential was recorded for all researched locations (Table 2). In the majority of analyzed lakes, primarily Busko and Jablanica lakes, during sampling and examination of the sample, an increased biomass was recorded, and it was hinted that a more intense blooming of cyanobacteria and algae would follow. By calculating the TSI value, the above mentioned was confirmed (Table 2).

Regarding on the macrophyte vegetation, for some analyzed stations, especially deeper than 50 meters, the trophic state could not be determined due to the absence of macrophytes. For most of the stations (Deransko lake, GKB-Vrutak, Jablaničko lake) the eutrophic state has been established. Comparing the conducted research with earlier research conducted in these areas (3-7, 11-17, 21-23) was determined that the condition of surface water bodies is deteriorating, especially with regard to the reduction of species diversity, the presence of an increasing number of widespread and cosmopolitan species, invasive species, as well as an increase in nutrient, primarily nitrogen and phosphorus. It is important to emphasize that a larger amount of nutrient, primarily nitrogen compounds, reaches aquatic ecosystems from agricultural production, which consequently leads to the acceleration of the eutrophication process and the appearance of water blooms. In the southern part of the catchment area, seawater intrusion and salinization of water bodies, especially those used for water supply (for example, the Gabela water pumping station, which serves for the water supply of Neum and Ravno) and irrigation of agricultural land, are a problem. Eutrophication and blooms of water have been recorded in most of our lakes (e.g. Rama, Jablanica, Busko, Deran lake, etc.).

This natural, but also artificially induced process occurs over a long period of time in a closed water system by the gradual accumulation of nutrient salts, the formation of sediment and the change of biogeochemical cycles of elements necessary for the development of living organisms. Therefore, the increased degree of trophy of artificial lakes is an inevitable scenario due to the very accelerated creation of the water system in which, due to intense anthropogenic action, larger amounts of nutrient salts are released and accumulated, which favor the increased development of pioneering photosynthetic organisms. The development of phytoplankton biomass leads to a change in the relationship of the dominant species of plankton, where species that are not consumed by zooplankton most often dominate. Many of these species, especially from the group of Cyanobacteria, secrete endotoxins that are dangerous both for the living world of the aquatic system and for humans.

In addition to the change in water quality, the composition of species also changes, so species adapted to life in clean water are replaced by species that suit the newly created conditions. The appearance of eutrophication is therefore not a simple change of water, but a change in the metabolism of the entire ecosystem, and the result is a change in the ecosystem itself. Considering the state of surface water bodies, we are at the very beginning of facing the problems of algae blooms, eutrophication, salinization and other anthropogenically accelerated challenges and risks, and according to estimates, the situation will worsen in the time ahead. In solving such challenges, sustainable



water management in the future will have increasing importance in the processes of adaptation to increasingly certain changes in environmental factors, and therefore water management more than ever in history requires an integrated approach that includes environmental, social, economic and health dimensions.

Conclusion

According to the results of biological research carried out in 2022, a great diversity as well as the abundance of bioindicators were recorded for most of the researched locations, an oligo to betamesosaprobic or betamesosaprobic state was determined, i.e. water from I.-II., or II. quality categories and good ecological condition/potential. A good ecological condition/potential has been established for most standing water bodies. Despite the fact that we have satisfactory conditions in surface water bodies, we noted changes as challenges that we will face and present solutions in the near future; challenges such as eutrophication, water blooms, invasive species, reduction of biodiversity, disturbances in the trophic cascade, salinization, etc.

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