SCIENCE SET OPEN ACCESS PUBLISHERS
ISSN: 3066-9847
Research Article
Science Set Journal of Economics Research

Ecological Status Assesement of the River Basin CRNA 2021 – 2022 Case Study

Vesna Kechovska[1], Sheriban Ramani[1*], Maja Aleksovska[2], Nexhadi Idrizi[2], & Jelena Radosavljevic[2]

[1, 2]Hydrometeorologycal Service of the Republic of North Macedonia, Skupi 28, 1000 Skopje, R.N. Macedonia

[*]**Corresponding author:** Sheriban Ramani, Hydrometeorologycal Service of the Republic of North Macedonia, Skupi 28, 1000 Skopje, R.N. Macedonia.

Submitted: 06 May 2025 **Accepted:** 13 May 2025 **Published:** 19 May 2025

https://doi.org/10.63620/MKSSJER.2025.1077

Citation: *Kechovska, V., Ramani, S., Aleksovska, M., Idrizi, N., & Radosavljevic, J. (2025). Ecological Status Assesement Of the River Basin CRNA 2021 – 2022 Case Study. Sci Set J of Economics Res, 4(3), 01-10.*

Abstract

Maintaining good ecological status of water bodies is very important and also it is a challenge today, because of industrial influences, municipal wastewater as well as pollution from agricultural activities. Benthic diatoms as a part of phytobenthos and benthic macroinvertebrates are very sensitive to organic and inorganic pollution and as a biological quality elements (BQS) are used for ecological assessment of water quality. The river Crna, is a right tributary to the river Vardar. The aim of this study is to present the pollution of the River Basin Crna, based on the ecological status assessment of the water bodies. Ecological status assessment of water bodies in this basin was assessed by the physico-chemical parameters, metals, benthic diatoms communities and benthic macroinvertebrates. The research was conducted on 13 sampling stations in 2021 and on 12 sampling stations in 2022, in the spring and autumn. The ecological status of the water bodies according diatoms was assessed with IPS calculation and the corresponding ecological quality ratio (EQR). According to the benthic macroinvertebrates the ecological status was assessed with the indices SI, BMWP, ASPT and the Number of taxa. The research has shown differences in ecological status on the water bodies in the River Basin Crna and according to the assessment five of the water bodies (VT_3 - Prilepska River in front of the river inflow in Borotinsko Blato - Bakarno Gumno, VT_4 - Borotinsko Blato in front of the river inflow in Crna River - v. Chepigovo, VT_5 Dragor in front of the river inflow in Crna River in 2021, VT_6 - Crna River v. Novaci and VT_15 - Crna River v. Skochivir), have bad ecological status.

Keywords: River Basin Crna, Ecological Status, Chemical Parameters, Benthic Diatoms, Benthic Macroinvertebrates

Introduction

Pollution of surface bodies of water is a significant problem, especially anthropogenic pollution by industrial and municipal wastewater, agricultural activities and other influences on rivers [1]. Irrigation, regulation of riverbeds and gravel exploitation from riverbeds causes hydromorphological changes that influence the flora and fauna of the river, leading to change of ecological status of water bodies. Due to the pollution freshwater ecosystems have suffered and are among the most degraded on the planet, experiencing greater losses of species and habitat than terrestrial or marine ecosystems [2].

Polluted surface waters, such as rivers, can seep into groundwater and contaminate it. Irrigation with contaminated water leads to the pollution of agricultural activities, negatively impacting human health. The Water Framework Directive 2000/60/EC emphasize the need of maintaining good ecological status of the surface water bodies. To achieve this, continuous monitoring of water bodies for the assessment of both chemical and ecological status is essential [3].

The ecological status assessment of the water bodies by biological quality elements (BQEs) includes assessment according to phytobenthos and benthic macoinvertebrates, physico-chemical quality elements and other specific pollutants. Diatoms as an integral part of the phytobenthos and the benthic macroinvertebrates are the most important indicators for ecological status assessment of water bodies, because the development of benthic diatoms and macroinvertebrate communities are directly related to chemical, physical and hydromorphological conditions in the water body. They are a suitable indicator for increased concentration of nutrients in water bodies. Freshwater ecosystems have suffered and are among the most degraded on the planet, experiencing greater losses of species and habitat than terrestrial or marine ecosystems [2]. Biological status assessment of water bodies, in accordance with the composition of diatom communities is done using several indices. The IPS (Cemagref 1982) index is considered suitable for R. N. Macedonia, geographical area. Ecological status assessment for water bodies, expressed through the Ecological Quality Ratio (EQR) calculation based on the IPS index, is directly dependent on the relative abundance of species. The indices SI, ASPT, Number of taxa and BMWP were used for ecological status assessment based on the composition of benthic macroinvertebrates communities. In the study multiple physical and chemical parameters were included: pH, specific conductivity, DO, BOD5, COD, N - NH_4^+,N- NO_2^-, N- NO_3^-, PO_4^{3-}, Fe, Mn, Pb, Zn, Cd, Cr and Cu.

Until recently, there was little data regarding the ecological status of the water bodies in River Basin Crna, which is a part of River Basin Vardar in R. N. Macedonia. In accordance with the project document 'River Monitoring System in Macedonia,' three sampling points are included in the monitoring network of the National Hydrometeorological Service of North Macedonia (BT_15 Skocivir, BT_16 Palikura and BT_17 Eleska). Over a two-year period, from May 2021, to October 2022, surveillance monitoring was conducted at an additional ten sampling points in the Crna River Basin to gather more data on the ecological status of the water bodies. For this purpose, water samples were collected to determine biological and chemical parameters to assess the ecological status of the water, based on biological quality elements (BQEs).

Most rivers in this basin are exposed to pollution from municipal and industrial wastewater, as well as from industrial and agricultural activities, particularly from the municipalities of Prilep and Bitola and other populated areas. The presence of solid waste also affects water quality, harming diatoms and macroinvertebrate communities in some water bodies. The water from the rivers is used to irrigate agricultural areas, especially during the summer months when there is a significant decrease in water flow, which contributes to the deterioration of the rivers' ecological status. However, the inflow of smaller mountain streams from the Mariovo area contributes to improve the ecological status of the Crna River at Rasimbegov Most before it flows into the Tikvesh reservoir [4].

Materials and Method

Study Area

River Crna is the largest right tributary to the river Vardar, with length of 207 km, it drains a catchment area of 5890 km2 of which 5130 km2 in N. Macedonia. The river source is at Crna Dupka near the village Zeleznik at 750 m altitude and it starts as a fast-flowing mountain river. It flows through four different morphological zones (Official Gazette RM, No. 61/2017).

Figure 1: The Map of the River Basin Crna with the Sampling Points

Downstream from the source is the measurement station in the village Dolenci. The river flows through Demir Hisar in a west - east direction, at Buchim village it enters in Prilepsko Pole in a south – east direction and it gains a character of a slow flowing low land river. On the left of the river Crna the river Blato is found which flows through Borotino. Downstream from Borotino on the left bank on river Blato, the Prilepska River flows in the river Blato which flows into river Crna at the village Chepigovo. At the village Topolchani, the river Crna enters Bitolsko Pole flowing in southerly direction, keeping the character of the low land river. Downstream from the village Topolchani, on the right bank side of the river Crna at Mogila village, the river Shemnica which flows through a Strezevo reservoir flows in to the river Crna. Further downstream on the right bank, the Dragor River, which flows from the city of Bitola and the village of Orizari, enters the Crna River, just downstream from the measurement station at Novaci. The Eleshka River flows from Greece into North Macedonia near the Medzitlia border crossing, and between the villages of Kremenica and Germian, it also joins the Crna River on the right bank. Then, river Crna makes a band in a south - easterly direction and in Skochivir village it turns again in a north - easterly direction flowing through a Skochivir canyon where it again flows as a fast-flowing river and it continuous to flow through Mariovo as a mountain river in a north - east direction (Official Gazette RM No. 6/2017). The measurement station at Rasimbegov Most is located just before the entrance to the Tikveshko Reservoir. At the outflow of the Tikveshko Reservoir, the sampling station in Vozarci village is located. The Palikura sampling station is situated at the confluence of the Crna River and the Vardar River. (Official Gazette RM, No. 6/2017).

Sampling Procedure

The ecological quality assessment of water bodies in the Crna River Basin was conducted over a two-year period, with samples taken in spring and autumn in accordance with the Water Framework Directive (WFD). A total of 129 samples of phytobenthos and 25 samples of benthic macroinvertebrates were collected to assess the ecological status of the water bodies at 13 sampling stations (Table 1). Sampling took place from May 4 to May 17, 2021, and from October 4 to October 20, 2021, followed by additional sampling from May 9 to May 18, 2022, and from October 4 to October 18, 2022. Of these, 117 representative samples of phytobenthos and all samples of macroinvertebrates were included in the analysis [5].

Samples of phytobenthos were taken from stones, macrophytes, sand, epipelon, and all suitable substrates in the riverbeds, following MKC EN 13946:2009. The samples were collected in 50 ml plastic containers and preserved with 4% formaldehyde.

Samples of benthic macroinvertebrates were collected and identified according to standards EN 16150:2012, which provides guidance on pro-rata multi-habitat sampling of benthic macroinvertebrates from wadeable rivers, and EN 27828:1994, which outlines methods for biological sampling and handnet sampling of aquatic benthic macroinvertebrates. The samples were collected in cylindrical plastic jars with tamper-evident cap, with 1000 ml volume and were preserved with 38 % formaldehyde or 75 % ethanol [6].

The pH was measured in the field with a portable pH meter WTW Multi 340i Set based to ISO 10523: 1994. Dissolved oxygen was determined based on the ISO 5813: 1983. BOD5 was determined by APHA AWWA 5210 B, and COD according to ISO 8467: 1993. To determine N-NH4, the Spectroquant Ammonium test (Merck reagents) was used, which is analogous to APHA AWWA - NH3 D. Nitrites were determined according to ISO 6777:1984, while nitrates were measured using the AQUANAL Plus method, which involves the formation of a red-violet azo dye with N-(1-naphthyl)-ethylene diammonium dichloride. Orthophosphates and total phosphorus were determined using the Spectroquant Phosphates test (Merck reagents), following the APHA AWWA 4500-P E. Metals were determined using the graphite AAS technique following method APHA AWWA 3113 B, as well as the flame technique according to method APHA AWWA 3111 B

To prepare permanent slides of diatoms, a pretreatment was first performed on a small quantity of subsamples using the acid digestion method with a saturated solution of KMnO4 and 37% HCl (Round et al., 1990). The permanent slides were then prepared using Naphrax as the mounting medium.

The diatoms were identified and counted using a Leica DM 2000 LED light microscope. Species counting was performed on up to 400 valves, in accordance with standard MKC EN 14407:2009, which is equivalent to EN 14407:2004. Taxonomic identification was conducted using literature and taxonomic keys (Krammer & Lange-Bertalot, 1986, 1991 Band 2/3, 1991 Band 2/4, 1997 Band 2/2, etc.).

The macroinvertebrates were identified and counted using a Carl Zeiss STEMI 508 stereomicroscope. Taxonomic identification was performed using literature and taxonomic keys (I.D. Wallace, B. Wallace, and G.N. Philipson, 1990; Nilsson, 1996; Moog, 2003; Eiseler, 2005; Dobson, Pawley, Fletcher, and Powell, 2012; Glöer, 2015, etc.).

Table 1: Sampling Points, Code, Sampling Date, the Altitude and the Geographic Coordinates of the Water Bodies in the CRNA River Basin.

Sampling station	Code	Sampling data	Altitude [m]	Geographic coordinates
Crna River (v. Dolenci)	VT_1	04.05.2021	708	41°18'56" N
		04.10.2021		21°05'48" E
		09.05.2022		
		04.10.2022		
Crna River (v. Buchin)	VT_2	04.05.2021	605	41°16'70" N
		04.10.2021		21°18'75" E
		09.05.2022		
		04.10.2022		
Prilepska River (in front of the river inflow in Borotinsko Blato - Bakarno Gumno)	VT_3	04.05.2021	603	41°16'47" N
		04.10.2021		21°25'39" E
		09.05.2022		
		04.10.2022		
Borotinsko Blato (in front of the river inflowin Crna River - v. Chepigovo)	VT_4	04.05.2021	604	41°14'50" N
		04.10.2021		21°23'36" E
		09.05.2022		
		04.10.2022		

Dragor (in front of the river inflow in Crna River)	VT_5	06.05.2021	573	41°03'23" N
		06.10.2021		21°25'56" E
Crna River (v. Novaci)	VT_6	06.05.2021	578	41°02'47" N
		06.10.2021		21°26'34" E
		12.05.2022		
Shemnica (in front of the river inflow in Crna River - v. Mogila)	VT_7	06.05.2021	583	41°06'42" N
		06.10.2021		21°25'92" E
		12.05.2022		
		06.10.2022		
Crna River (v. Topolchani)	VT_8	06.05.2021	587	41°12'58" N
		06.10.2021		21°25'86" E
		12.05.2022		
		06.10.2022		
Crna River (Rasimbegov Most)	VT_9	17.05.2021	369	41°11'78" N
		19.10.2021		21°42'83" E
		18.05.2022		
		18.10.2022		
Crna River (on the outflowin Tikveshko reservoir - v. Vozarci)	VT_10	17.05.2021	172	41°24'98" N
		19.10.2021		21°55'80" E
		18.05.2022		
		18.10.2022		
Crna River (v. Skochivir)	VT_15	10.05.2021	576	40°58'31" N
		20.10.2021		21°38'34" E
		16.05.2022		
		13.10.2022		
Crna River (Palikura in front of the river inflow in river Vardar)	VT_16	17.05.2021	170	41°33'26" N
		19.10.2021		21°58'72" E
		18.05.2022		
		18.10.2022		
Eleshka River (in front of the river inflow in Crna River - v. Brod)	VT_17	10.05.2021	583	40°55'02" N
		20.10.2021		21°30'50" E
		16.05.2022		
		13.10.2022		

Statistical Analyses and Ecological Status Assessment

The ecological status of a water body according to the benthic diatoms communities as an integral part of the phytobenthos of the water body was calculated according to the biotic index IPS/5 - Indice de polutio sensibilité (Cemagref 1982) as a most suitable for our country. The metrics boundary values are presented in Table 2. This index is in direct correlation with relative species abundance in the samples. The IPS was calculated using the software program OMNIDIA 7 V8.1 (Lecointe at al. 1993).

The equation for IPS calculation is a modified Zelinka- Marvan equation (1961):

Where: Aj - abundance, total counted number of valves of species from the sample

- Ij - Indicator index for tolerance of a species j
- Vj - Indicator value for sensitivity (stenoecy degree) of a species j

Table 2: IPS Boundary Values for Ecological Status Assessment

Ecological status	IPS values
high	$17 \leq IPS \leq 20$
good	$13 \leq IPS < 17$
moderate	$9 \leq IPS < 13$
poor	$5 \leq IPS < 9$
bad	$IPS < 5$

Ecological status assessment for each water body was expressed as an ecological quality ratio (EQR) which was calculated on the base of IPS index values. In this study, normalized ecological quality ratio (EQRn) are presented in range from 0 (bad) to 1 (high).

The indices SI (Saprobic index, adapted Serbian version by Zelinka & Marvan), BMWP (Biological Monitoring Working Party), ASPT (Average Score Per Taxon) and the Number of taxa were calculated using software program ASTERICS V3.0 (www.aqem.de).

Results

Tables 3 and 4 present the annual mean values of the physico-chemical parameters used to assess the water quality of the water bodies in the Crna River Basin during the study period of 2021 and 2022, while table 5 presents the average values of the physico-chemical parameters for the water bodies in the Crna River Basin for the years 2021 and 2022. Regarding pH values, the highest average was recorded at VT_1 (Crna River, v. Dolenci) with a value of 8.20, while the lowest average was observed at VT_6 (Crna River, v. Novaci) 7.25. All water bodies examined showed high ecological status based on these pH measurements. The water body VT_3 (Prilepska River, at the inflow into Borotinsko Blato - Bakarno Gumno) exhibited the highest average conductivity of 525.25 µS/cm, indicating a moderate ecological status. While, the lowest average conductivity was recorded at VT_7 (Shemnica, at the inflow into the Crna River, v. Mogila) with a value of 187.25 µS/cm, which reflected a high ecological status [7].

The highest average DO concentration was 10.76 mg/L for water body VT_10 (v. Vozarci), which indicated a high ecological status. In contrast, the lowest average DO concentration was 4.96 mg/L in VT_15 (v. Skochivir), indicating a moderate ecological status.

For BOD5, the water body VT_6 (v. Novaci) had the highest average value at 9.44 mg/L DO, reflecting a pure ecological status. The lowest average BOD5 was 1.82 mg/L DO in VT_7 (Shemnica, v. Mogila), which indicated a high ecological status for this parameter.

The highest average COD concentration for both years was 5.84 mg/L DO in VT_3 (Prilepska River at Bakarno Gumno), indicating a moderate ecological status. While the lowest average COD concentration was 0.89 mg/L DO, reflecting a high ecological status.

Additionally, at the VT_3 was recorded the highest average NH4+ concentration, 2722.15 µg/L N, indicating a moderate ecological status. The lowest average NH4+ concentration was found in VT_10 (Vozarci), 39.20 µg/L N, which corresponded to a high ecological status.

The highest average NDO concentration was 254.0 µg/L N in water body VT_3, indicating a moderate ecological status. In contrast, the lowest average NDO concentration was 2.7 µg/L N in VT_1 (Crna River at v. Dolenci), reflecting a high ecological status.

For NO3 concentration, the highest average was 2146.9 µg/L N in VT_4 (Borotinsko Blato v. Chepigovo), which indicated a good ecological status. The lowest average NO3 concentration was 207.0 µg/L N in VT_1, also reflecting a high ecological status.

Water body VT_3 exhibited the worst ecological status based on average $PO4^{3-}$ concentration, with the highest value recorded 1490.5 µg/L $PO4^{3-}$, while the lowest average $PO4^{3-}$ concentration was 54.3 µg/L in VT_7 (Shemnica, v. Mogila), which indicated a good ecological status

Table 3: Annual Mean Values of the Physico-Chemical Parameters for the Water Bodies in the CRNA River During 2021.

Code of sampling point	pH	Specific Conductivity [µS/cm]	DO [mg/L]	BOD [mg/L DO]	COD [mg/L DO]	N-NH4+ [µg/L N]	N-NDO- [µg/L N]	N-NO3- [µg/L N]	P-PO43- [µg/L PO43-]
V_1	8.23	316	10.59	1.67	0.95	58.8	3.3	281.4	63
V_2	8.15	256.5	10.37	2.01	1.64	109.5	5.5	472.7	82.5
V_3	7.83	508.5	7.45	6.93	6.95	2583.4	282	1933.7	800
V_4	7.76	429.5	6.02	5.78	5.24	803.6	176	1854.5	322
V_5	7	248	3.36	18.47	7.76	1561.25	18.3	1492.3	258
V_6	7.23	276	6.14	13.19	4.15	770.85	41.3	1176.2	151
V_7	7.61	112.5	9.99	1.75	3.11	130.9	12.9	906.4	48
V_8	7.69	320.5	9.05	2.78	2.79	226.05	58.6	1499.5	142.5
V_9	8.25	319	10.58	6.14	4.47	85.15	85.4	1809.8	193
V_10	8.4	344	13.02	2.35	2.13	29.9	29.4	1079.7	133.5
V_15	7.57	348.5	5.31	6.37	4.72	546.4	68.8	1045.5	395
V_16	8.19	378	8.28	5.37	3.4	30.45	23.2	1073.1	100.5
V_17	7.73	201	10.36	3.09	2.54	191.9	49	1054.9	296

Table 4: Annual Mean Values of the Physico-Chemical Parameters for the Water Bodies in the CRNA River During 2022.

Code of sampling point	pH	Specific Conductivity [µS/cm]	DO [mg/L]	BOD [mg/L DO]	COD [mg/L DO]	N-NH4+ [µg/L N]	N-NDO- [µg/L N]	N-NO3- [µg/L N]	P-PO43- [µg/L PO43-]
V_1	8.18	307.00	10.38	3.99	0.84	46.45	2.10	132.60	46.00
V_2	8.03	260.00	9.47	3.80	1.65	46.90	4.95	427.15	74.00
V_3	7.83	542.00	7.59	3.10	4.73	2860.90	226.05	2356.85	2181.00
V_4	7.59	391.50	5.20	6.14	4.70	837.25	132.20	2439.25	1315.50
V_5	/	/	/	/	/	/	/	/	/
V_6	7.27	269.00	7.86	5.70	2.56	926.15	33.80	1489.35	804.50
V_7	7.76	262.00	9.51	1.89	2.02	63.50	26.65	1804.25	60.50
V_8	7.66	292.00	8.02	3.68	3.16	147.50	66.00	1010.20	248.00
V_9	8.05	444.50	8.74	3.60	2.76	51.90	57.55	1810.80	214.50
V_10	7.91	344.00	8.49	3.84	2.29	48.50	20.85	767.10	94.00
V_15	7.34	487.00	4.61	3.77	2.93	885.05	39.55	533.50	531.00
V_16	7.88	320.50	7.36	2.00	1.26	52.55	16.00	930.75	115.00
V_17	7.22	242.00	9.40	2.51	2.05	54.85	22.85	582.50	327.50

Table 5: Average Values of the Physico-Chemical Parameters in the Water Bodies of the CRNA River Basin During the Period 2021 to 2022.

Code of sampling point	pH	Specific Conductivity [µS/cm]	DO [mg/L]	BOD [mg/L DO]	COD [mg/L DO]	N-NH4+ [µg/L N]	N-NDO- [µg/L N]	N-NO3- [µg/L N]	P-PO43- [µg/L PO43-]
V_1	8.20	311.50	10.48	2.83	0.89	52.63	2.7	207.0	54.5
V_2	8.09	258.25	7.52	2.91	1.64	78.20	5.2	449.9	78.3
V_3	7.83	525.25	7.52	5.01	5.84	2722.15	254.0	2145.3	1490.5
V_4	7.67	410.50	5.61	5.96	4.97	820.43	154.1	2146.9	818.8
V_5	/	/	/	/	/	/	/	/	/
V_6	7.23	272.50	7.00	9.44	3.35	848.50	37.5	1332.8	477.8
V_7	7.69	187.25	9.75	1.82	2.56	97.20	19.8	1355.3	54.3
V_8	7.68	306.25	8.53	3.23	2.98	186.78	62.3	1254.8	195.3
V_9	8.15	381.75	9.66	4.87	3.61	68.53	71.5	1810.3	203.8
V_10	8.16	344.00	10.75	3.09	2.21	39.20	25.1	923.4	113.8
V_15	7.45	417.75	4.96	5.07	3.82	715.73	54.2	789.5	463.0
V_16	8.04	349.25	7.82	3.68	2.33	41.50	19.6	1001.9	107.8
V_17	7.47	221.50	9.88	2.80	2.30	123.38	35.9	818.7	311.8

The annual mean values for metal concentrations in the water bodies of the Crna River Basin for 2021 and 2022 are presented in Tables 6 and 7, respectively. Table 8 shows the average values for both years.

The highest average iron (Fe) concentration was 273.75 µg/L in VT_4 (Borotinsko Blato, at the inflow to the Crna River, v. Chepigovo), indicating good ecological status. However, in 2022, the mean iron concentration increased to 349.50 µg/L, resulting in poor ecological status for this water body. The lowest average iron concentration was 18.38 µg/L in VT_1 (Crna River at v. Dolenci), reflecting good ecological status.

For manganese (Mn), VT_4 also had the highest average concentration at 119.00 µg/L, showing poor ecological status, while the lowest average was 14.9 µg/L in VT_16 (Crna River at Palikura, at the inflow to the Vardar River), indicating good ecological status [8].

The highest average lead (Pb) concentration was 15.97 µg/L in VT_7 (Shemnica, at the inflow to the Crna River, v. Mogila), which showed poor status. In 2022, this mean annual value increased to 31.13 µg/L, indicating a decline to poor status for this water body. Conversely, the lowest average lead concentration was 0.31 µg/L in VT_15 (Crna River at v. Skochivir), reflecting good status.

VT_6 recorded the highest average zinc (Zn) concentration at 13.10 µg/L, while VT_8 (Crna River at v. Topolchani) had the lowest average concentration at 2.49 µg/L. Both values indicated good ecological status.

The highest average cadmium (Cd) concentration was 0.09 µg/L in several water bodies, including VT_1, VT_15, and VT_17, indicating good status. However, in 2021, the mean annual values for these water bodies showed poor status for this parameter. The lowest average cadmium concentration was 0.03 µg/L, which indicated good status in VT_4 and VT_7, and also in VT_15 in 2021.

For chromium (Cr), VT_7 had the highest average concentration at 0.06 µg/L, while VT_15 had the lowest at 0.87 µg/L. Both values indicated good ecological status.

Finally, the highest average copper (Cu) concentration was 5.40 µg/L in VT_2 (Crna River at v. Buchin), which indicated good ecological status. The lowest average concentration was 0.26 µg/L in VT_8 (Crna River at v. Topolchani), also reflecting good ecological status

Table 6: Annual Mean Values of the Metals in the Water Bodies of the CRNA River Basin During the Period 2021.

Code of sampling point	Fe [µg/L]	Mn [µg/L]	Pb [µg/L]	Zn [µg/L]	Cd [µg/L]	Cr [µg/L]	Cu [µg/L]
V_1	4.75	24.5	0.25	5.15	0.1	0.19	0.06
V_2	23.5	32	0.86	4.96	0.085	0.04	9.64
V_3	88	78.5	0.87	11.05	0.051	0.32	0.77
V_4	198	149	0.68	7.4	0.026	0.18	1.35
V_5	196	76	0.72	7.15	0.025	0.24	1.32
V_6	148	82.5	0.46	15.8	0.114	0.15	0.76
V_7	116.5	44.5	0.81	5.8	0.028	0.03	0.1
V_8	68.5	54.5	0.63	4.75	0.117	0.23	0.27
V_9	152	41	0.54	6.31	0.052	0.24	0.69
V_10	29	29.3	0.25	6.31	0.12	0.07	0.86
V_15	280.5	81	0.37	10.05	0.156	0.33	1.16
V_16	27	24.5	0.44	6.55	0.143	0.77	0.54
V_17	136	55.5	0.41	6.4	0.139	0.48	1.37

Table 7: Annual Mean Values of the Metals in the Water Bodies of the CRNA River Basin During the Period 2022.

Code of sampling point	Fe [µg/L]	Mn [µg/L]	Pb [µg/L]	Zn [µg/L]	Cd [µg/L]	Cr [µg/L]	Cu [µg/L]
V_1	32.00	6.30	0.44	0.22	0.075	0.14	0.59
V_2	84.50	5.30	0.43	0.22	0.041	0.27	1.16
V_3	203.50	48.00	0.52	8.85	0.040	0.51	0.06
V_4	349.50	89.00	0.42	1.55	0.042	0.40	0.72
V_5	/	/	/	/	/	/	/
V_6	202.50	35.50	0.42	10.40	0.031	0.13	0.59
V_7	172.00	14.30	31.13	0.22	0.024	0.09	0.56
V_8	112.00	14.80	0.43	0.22	0.020	0.49	0.26
V_9	202.00	5.30	0.59	0.22	0.028	1.16	0.11
V_10	55.00	7.30	0.66	0.22	0.019	0.10	1.04
V_15	171.50	14.00	0.25	4.21	0.026	1.41	1.26
V_16	38.50	5.30	0.25	0.96	0.012	0.51	1.29
V_17	164.00	22.50	0.25	3.46	0.034	1.17	1.13

Table 8: Average Values of Dissolved Metals in the Water Bodies of the CRNA River Basin During the Period 2021 to 2022.

Code of sampling point	Fe [µg/L]	Mn [µg/L]	Pb [µg/L]	Zn [µg/L]	Cd [µg/L]	Cr [µg/L]	Cu [µg/L]
V_1	18.38	15.40	0.35	2.69	0.09	0.16	0.32
V_2	54.00	18.65	0.64	2.59	0.06	0.16	5.40
V_3	145.75	63.25	0.69	9.95	0.05	0.41	0.41
V_4	273.75	119.00	0.55	4.48	0.03	0.29	1.04
V_5	/	/	/	/	/	/	/
V_6	175.25	59.00	0.44	13.10	0.07	0.14	0.68

V_7	144.25	29.40	15.97	3.01	0.03	0.06	0.33
V_8	90.25	34.65	0.53	2.49	0.07	0.36	0.26
V_9	177.00	23.15	0.57	3.27	0.04	0.70	0.40
V_10	42.00	18.30	0.46	3.27	0.07	0.09	0.95
V_15	226.00	47.50	0.31	7.13	0.09	0.87	1.21
V_16	32.75	14.90	0.34	3.76	0.08	0.64	0.92
V_17	150.00	39.00	0.33	4.93	0.09	0.82	1.25

The ecological status of the water bodies in the Crna River Basin, based on IPS and EQR values, is summarized in Tables 9 and 10. Data for water body VT_5 is only available for 2021, as it was identified as highly polluted, with bad ecological status. The dominant diatom genus found there was Nitzschia sp., which is characteristic of highly polluted waters, indicating poor ecological status. In September 2021, a sample of epilithon from VT_5 showed 99% Nitzschia palea and 1% Craticula accomoda.

Water bodies VT_1 and VT_2 were classified as having good ecological status, while VT_3 and VT_4 according IPS and EQR n were assessed with poor ecological status. In October 2021, VT_6 was noted to have bad ecological status, although it exhibited moderate ecological status in the spring. In the following year, due to changes in the river's course and hydromorphology, the ecological status of VT_6 could not be determined [9].

Water body according diatoms communities, VT_15 had poor ecological status in 2021, Also, previous measurements indicated that it had experienced bad status during some periods.

Table 9: Annual Mean Values for IPS for Diatoms Communities in the Water Bodies of the CRNA River Basin During the Period 2021 and 2022 year.

Code of sampling point	VT_1	VT_2	VT_3	VT_4	VT_5	VT_6	VT_7	VT_8	VT_9	VT_10	VT_15	VT_16	VT_17
IPS (annual mean values for 2021)	15.83	14.80	7.05	9.90	3.20	8.20	12.25	12.54	9.87	/	7.90	13.12	11.08
IPS (annual mean values for 2022)	15.12	13.73	6.94	8.55	/	/	11.28	11.97	12.48	12.67	10.20	12.50	11.60
IPS average Value	15.48	14.27	7.00	9.23	/	/	11.77	12.26	11.18		9.05	12.81	11.34

Table 10: Ecological Status, Expressed as Normalized Ecological Quality Ratio (EQR n), Communities in the Water Bodies of the CRNA River Basin During the Period 2021 / 2022 According Diatoms Communities.

Code of sampling point	VT_1	VT_2	VT_3	VT_4	VT_5	VT_6	VT_7	VT_8	VT_9	VT_10	VT_15	VT_16	VT_17
EQRn (annual mean values for 2021)	0.74	0.69	0.3	0.45	0.13	0.36	0.56	0.58	0.44	/	0.35	0.61	0.5
EQRn (annual mean values for 2022)	0.71	0.64	0.3	0.38	/	/	0.51	0.55	0.57	0.58	0.46	0.58	0.53
EQRn average	0.73	0.67	0.3	0.42	/	/	0.54	0.57	0.51		0.41	0.6	0.52

The results based on indices calculated from the presence of benthic macroinvertebrates are presented in Tables 11 and 12. Table 13 shows the average values for the water bodies over both years of examination. The data indicate that water bodies VT_3, VT_4, VT_5 in 2021, VT_6, and VT_15 all exhibited bad ecological status.

In 2021, water body VT_5 demonstrated bad ecological status according to the ASPT, Number of taxa, and BMWP score, corroborating the findings from the IPS index. The ASPT and BMWP score indices were correlated; according to the ASPT index, both VT_3 and VT_5 were classified as having bad ecological status in 2021. Additionally, water body VT_4 also showed bad ecological status in 2022.

The BMWP score for 2021 and 2022 indicated bad ecological status for VT_3, VT_4, VT_6, and VT_15. Furthermore, according to the BMWP score, VT_7 was assessed as having poor ecological status [10].

Table 11: Annual Mean Values for Following Indices According Benthic Macroinvertebrates in the Water Bodies of the CRNA River Basin During the Period 2021.

Indices / Code of sampling point	VT_1	VT_2	VT_3	VT_4	VT_5	VT_6	VT_7	VT_8	VT_9	VT_10	VT_15	VT_16	VT_17
SI	1.85	2.05	2.8	2.8	/	/	2.4	2.35	2.1	/	2.75	2.25	2.3
ASPT	6.5	5.8	2.85	3.3	1.25	3.8	4.65	5.55	6.5	/	3.75	5.5	4.95
Number of taxa	20.5	21	8.5	12	2	11	11.5	21	20.5	/	10	13	14
BMWP Score	95	97	19	27	2	35	40	82	94	/	32	55	54

Table 12: Annual Mean Values for Following Indices According Benthic Macroinvertebrates in the Water Bodies of the CRNA River Basin During the Period.

Indices / Code of sampling point	VT_1	VT_2	VT_3	VT_4	VT_6	VT_7	VT_8	VT_9	VT_10	VT_15	VT_16	VT_17	VT_17
SI	1.95	2.05	2.8	2.8	2.95	2.45	2	1.95	2.4	2.8	2.3	2.2	0.5
ASPT	6.45	5.95	3.05	3.15	4	4.6	6.05	5.9	4.75	3.6	5.25	5.5	0.53
Number of taxa	19.5	17.5	10	6	7.5	11	19	20	10	9.5	13.5	14.5	0.52
BMWP Score	/	90	25	16	24	37	99.5	89	44	27	55	64	

Table 13: Average of Indices Values for 2021 and 2022 According Benthic Macroinvertebrates to the Investigated Sampling Points on the CRNA River Basin as a Part of River Basin Vardar in 2021 and 2022 year.

Indices / Code of sampling point	VT_1	VT_2	VT_3	VT_4	VT_6	VT_7	VT_8	VT_9	VT_15	VT_16	VT_17	VT_17	VT_17
SI	1.9	2.05	2,8	2.8	/	2.43	2.18	2.03	2.78	2.28	2.25	2.2	0.5
ASPT	6.48	5.88	2.95	3.23	3.9	4.63	5.8	6.2	3.68	5.38	5.23	5.5	0.53
Number of taxa	20	19.25	9.25	9	9.25	11.25	20	20.25	9.75	13.25	14.25	14.5	0.52
BMWP Score	/	93.5	22	21.5	29.5	38.5	90.75	91.5	29.5	55	59	64	

Discussion

The ecological status of water bodies changes throughout the year. During the spring months, abundant rainfall and increased water flow from snowmelt in mountain tributaries contribute to improved ecological conditions. However, in the summer and autumn, lower water flow combined with anthropogenic influences from agricultural, industrial activities, and municipal wastewater leads to a decline in ecological status. Additionally, solid waste in some water bodies negatively impacts their ecological quality [11].

In the upper reaches of the Crna River, water bodies demonstrate good ecological status based on the benthic diatom community and macroinvertebrates. For instance, VT_1 experiences minimal pressure from a few households and agricultural activities. Physical-chemical parameters and metal concentrations support this assessment, although in 2021, the annual mean cadmium concentration indicated poor status for this water body. VT_2 faces similar, albeit slightly higher, pollution pressure. Its ecological status is good according to both biological quality elements (BQEs), although $PO4^{3-}$levels indicate a moderate ecological status. On the right bank, indicators of organic pollution, such as Mentha sp. and Urtica sp., are present. The old copper mine near VT_2 contributes to elevated copper levels during heavy rain; in 2021, the mean annual copper concentration was 9.64 µg/L, indicating good ecological status despite the pollution source.

VT_3 has a regulated riverbed and displays signs of high pollution pressure from industrial and municipal wastewater from Prilep city, as well as agricultural influences from the Prilepsko Pole area. Physical-chemical parameters confirm this, particularly with average $PO4^{3-}$ values from 2021 and 2022 indicating poor ecological status. Elevated concentrations of oxygen, BOD, COD, and other nutrients, except nitrates, also point to pollution. Urtica sp., an indicator of organic pollution, dominates the riverbanks.

VT_4 is similarly affected by agricultural activities and municipal wastewater, with slow water flow leading to significant sedimentation of pollutants. This water body often floods the surrounding agricultural land. VT_5 suffers heavy pollution due to substantial industrial and municipal wastewater discharge from Bitola city and Orizari village. According to BQEs, this water body has a bad ecological status, which is further confirmed by physical-chemical parameters. Iron and manganese concentrations were higher in 2022, indicating poor ecological status. The inflow from the Dragor River into the Crna River resembles fecal wastewater, with visible layers of white mucus on stones and debris.

VT_6 has only slightly better pollution levels than VT_5 so that, benthic macroinvertebrate communities indicate bad ecological status. In October 2022, changes to the river's course made it impossible to assess the ecological status of VT_6. These hydromorphological changes are expected to impact the river's flora and fauna, and consequently, the ecological status of the water body.

The riverbed of VT_7 is a narrow channel primarily covered with Typha sp., a plant known for its ability to thrive in highly organic-polluted waters. During the assessment period, a fish population was noted in this water body, which is influenced by agricultural activities and water inflow from the Strezevo reservoir. Benthic macroinvertebrate communities indicate poor ecological status, corroborated by average lead concentrations for both years.

VT_8 has a moderate ecological status according to BQEs, influenced by agricultural activities from the upper part of Pelagonia. VT_17, before inflowing into the Crna River at VT_15, exhibits moderate ecological status according to BQEs but poor ecological status due to high $PO4^{3-}$ concentrations, also influenced by agricultural activities.

At the sampling station VT_15, pollution from industrial and municipal wastewater is evident, along with contributions from agricultural activities throughout the Pelagonia region. The river's flow accelerates as it enters the Skochivir canyon, and the water in VT_15 during summer and autumn often has a fecal odor, indicating bad ecological status according to benthic macroinvertebrates.

Downstream from VT_15 towards the Mariovo region, the river takes on a mountain character, collecting water from several clean mountain streams. This section experiences minimal agricultural pressure, resulting in moderate ecological status at sampling station VT_9. As the river flows into the Tikvesh reservoir, it is expected to exit with fewer pollutants. However, no improvement is noted at sampling station VT_10 near Vozarci village, where ecological status remains moderate. The low water levels in the reservoir, utilized for irrigation, along with the reservoir's age, likely contribute to this status.

Finally, sampling station VT_16 is located at the confluence of the Crna and Vardar rivers, where ecological status is moderate, primarily influenced by agricultural activities.

Conclusion

The pollution of Crna River is mostly from industry and municipal waste waters from larger and smaller municipalities and agricultural activities of the population. Litter in somewater bodies influences their ecological status. With properly operated and maintained waste water treatment plants and control of fertilizer and pesticide use it is possible to improve the ecological status of Crna River which is a part of the River Basin Vardar.

References

1. Ramani, S., Dragun, Z., Kapetanović, D., Kostov, V., Jordanova, M., Erk, M., & Hajrulai-Musliu, Z. (2014). Surface water characterization of three rivers in the lead/zinc mining region of northeastern Macedonia. Archives of environmental contamination and toxicology, 66, 514-528. Doi: 10.1007/s00244-014-0012-z.
2. Revenga, C., Brunner, J., Henninger, N., Kassem, K., & Payne, R. (2000). Pilot Analysis of Freshwater Ecosystems: Freshwater Systems. Washington, DC, USA: World Resources Institute.
3. Poikane, S., Kelly, M., & Cantonati, M. (2016). Benthic algal assessment of ecological status in European lakes and rivers: Challenges and opportunities. Science of the Total Environment, 568, 603-613.
4. Pinheiro, C., Oliveira, U., Rodrigues, T., Oliva-Teles, L., & Vieira, M. N. (2020). Assessing the ecological status of a Mediterranean river: benthic invertebrates and diatoms as complementary bioindicators. Limnetica, 39(1), 299-315.
5. Stenger-Kovács, C., Lengyel, E., Crossetti, L. O., Üveges, V., & Padisák, J. (2013). Diatom ecological guilds as indicators of temporally changing stressors and disturbances in the small Torna-stream, Hungary. Ecological Indicators, 24, 138-147. https://doi.org/10,1016/j.ecolind.2012.06.003.
6. Levkov, Z., Blanco, S., Krstic, S. V. E. T. I. S. L. A. V., Nakov, T. E. O. F. I. L., & Ector, L. (2007). Ecology of benthic diatoms from Lake macro Prespa (Macedonia). Algological Studies, 124(1), 71 - 83.
7. Jakovljević, O. S., Popović, S. S., Vidaković, D. P., Stojanović, K. Z., & Krizmanić, J. Ž. (2016). The application of benthic diatoms in water quality assessment (Mlava River, Serbia). Acta Botanica Croatica, 75(2), 199-205.
8. Eloranta, P., & Soininen, J. (2002). Ecological status of some Finnish rivers evaluated using benthic diatom communities. Journal of Applied Phycology, 14, 1-7.
9. Simić, S., Mitrović, A., Đorđević, N., & Radosavljević, S. (2021). Ecological status assessment of the Samokovska river-2018 case study. Kragujevac Journal of Science, (43), 167-181.
10. Van de Bund, W., & Solimini, A. G. (2007). Ecological Quality Ratios for Ecological Quality Assessment in Inland and Marine Waters. EUR 22722 EN, Luxembourg: Office for Official Publications of the European Communities ISSN 1018 - 5593.
11. Žutinić, P., Kulaš, A., Levkov, Z., Šušnjara, M., Orlić, S. Kukić, S., Goreta, G., & Gligora Udovič, M., 2020. Ecological status assessment using periphytic diatom communities – case study Krka River. Macedonian Journal of Ecology and Environment, 22 (1). 29-44.