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Identification Of The Macrobenthic Population In Three Tributaries Of The Kordan River (Senj, Aghasht, And Baraghan) And Water Quality Assessment Of These Rivers Using Biological Indices

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Abstract
Given that macrobenthic organisms are highly responsive to environmental changes—including alterations in the physical and chemical parameters of water, as well as the impacts of industrial and agricultural pollution—the study of benthic communities and the assessment of water quality according to biological indicators are widely regarded as one of the most prevalent and cost-effective approaches for monitoring river health. Therefore, utilizing this method with its unique and beneficial characteristics holds a very crucial position in water quality assessment discussions all over the world, including in more water-scarce countries like Iran. This research was conducted to identify the macrobenthic population and evaluate the health of the water in three key tributaries of the Kordan River—Senj, Baraghan, and Aghasht—through monthly sampling using a Surber sampler, with three quantitative replicates and one qualitative sample collected during the spring and summer of 2023. Alongside sampling at each branch, the parameters of PH, electrical conductivity (EC), dissolved oxygen (DO), and temperature were also recorded. The benthic organisms were identified to the genus level, belonging to 7 classes, 15 orders, 55 families, and 113 genera, with aquatic insects being the most diverse and dominant group. Among the three investigated rivers, the Baraghan River exhibited the highest diversity with 26 identified families, while the Senj River had the highest density, with 13,776 collected specimens. To assess the health of the three rivers, the Hilsenhoff, BMWP, ASPT, and EPT indices were calculated. According to the Hilsenhoff index, all three rivers were classified as "Good." The EPT index rated the condition of each branch as "Excellent," while the BMWP index indicated a "Good" status for all rivers. Based on the ASPT index, the Baraghan River was evaluated as "Very Good," while the Senj and Aghasht Rivers were rated as "Good." Although various agricultural, aquaculture, and tourism activities take place along the banks of these rivers, particularly Baraghan and Aghasht, the results indicate that the current self-purification capacity of the three rivers exceeds the impact of environmental pollutants.
Keywords— *Macroinvertebrates - Bioindicator- Kordan stream – bioassessment - HFBI, EPT richness*

I. INTRODUCTION

By examining the distribution of water resources globally, we find that the majority of water resources are either in the oceans or locked in the ice caps at the Earth's poles (about 99%), making them inaccessible for human use. Therefore, only a small portion of water resources (around 1%), which includes surface running waters, wetlands, and lakes, are available for direct human exploitation and use [1]. Despite freshwater representing a small share of total water resources, it has significant impacts on human societies. Unfortunately, over the past century, the expansion of various industries, the development of human communities, and the ever-growing global population have led to increased interference in aquatic ecosystems, resulting in pollution and sometimes rendering these waters unusable. The pollution resulting from human disturbances affects aquatic environments in two ways: as a "pulse" (limited and identifiable) or as a "press" (long-term sequence), both leading to a reduction in water quality. This decline in water quality has been one of the major concerns since the 1960s. [2]

Attention to preserving and maintaining water quality has led to the development of methods for assessing water bodies. Initially, physicochemical studies were used to determine water quality, but nowadays, limnological studies are conducted in three areas: physicochemical, bacteriological, and biological. This is because identifying physical and chemical pollutants alone does not fully represent water pollution. Among these methods, given the limitations of bacteriological approaches, the study of the biology of running waters is of particular importance. [3] , [4] The biological method was first used In Europe In the early 20th century, under the concept of biological monitoring based on the study of aquatic animals (such as benthic invertebrates, fish, and periphyton), which serve as indicators of water quality. [5] Although fish, phytoplankton, and aquatic plants can also be used in water quality control programs, benthic invertebrates are practically and widely used for assessments because they are abundant in all surface waters. These organisms respond to a wide range of physical and chemical stress factors affecting water quality, such as pH, temperature, oxygen, organic pollution, and others. Different species of benthic invertebrates show varied reactions to changes in water quality, their sampling is quick and inexpensive, and their identification is relatively affordable. They have limited migratory patterns, and due to their relatively short life cycles compared to other aquatic communities like fish, they quickly respond to stress factors. [6] , [7], [8] Another important role of benthic invertebrates is their contribution to the decomposition of organic materials in river systems. They act as a crucial link in the energy transfer chain within the food web. These organisms are a food source for many fish, amphibians, and birds, providing energy and nutrients for higher trophic levels.[9] As a key link in this chain, benthic invertebrates can influence the increase or decrease of energy production in this cycle.

Given the inherent characteristics of benthic invertebrates and their close interaction and sensitivity to environmental factors, their presence or absence, diversity, and population structure can be used for continuous and reliable assessments of running water quality [5]. By identifying benthic invertebrate populations with different resistance levels to environmental conditions, several biological indices can be used in further studies for water evaluation, including EPT (Ephemeroptera, Plecoptera, Trichoptera Index), HFI (Hilsenhoff Family-Level Index), BMWP (Biological Monitoring Working Party System), and ASPT (Average Score Per Taxon), all of which are based on the accurate identification of benthic invertebrates.

Numerous studies have been conducted on various rivers using macroinvertebrates for biological assessments, such as the study by Nasri et al on the Zayandeh Rud River in Isfahan Province. In this study, the HFI, BMWP, ASPT, and Shannon-Wiener indices were calculated, and the water quality of this river was ultimately reported as acceptable but unstable based on different indices. It is recommended that monthly sampling be conducted in significant rivers like the Zayandeh Rud to provide a clear perspective over a long period [10].

In the study conducted by Foomani on the Shanbeh Bazar River in Anzali, 17 families of macroinvertebrates were identified, and different stations showed pollution levels ranging from moderate to severe using biological indices[11]. In the studies by Taban, which were geographically close to the current research sampling locations and stations, 22 families of macroinvertebrates were reported in the Jajrud River and 24 families in the Karaj River, indicating significant diversity [12]. Repeating sampling in the following years will help clarify the management and maintenance of these rivers, which will be highly beneficial.

In the study by Aazami on the Tajan River, macroinvertebrates, fish, and physicochemical factors were simultaneously used to assess water health. Ultimately, using invertebrates and fish was found to be more appropriate for evaluation [13].

Among studies from different geographical regions, Ibezute's research in Nigeria examined the impact of brewery effluent on the macroinvertebrate population of a river receiving the effluent. Seven genera were identified, showing the impact of the effluent on the population, with significant differences from the populations identified in the present study. This indicates the effects of effluent on macroinvertebrate diversity [14]. In a study by L. Wang on the Fenhe River in China, the impact of urbanization on macroinvertebrate populations and water quality was investigated. Results showed that the habitats in the middle and upper parts of the river were severely deteriorating due to population growth and economic issues, with an increase in pollution-resistant benthic populations [15].

Febriani & Harahap studied the diversity of macroinvertebrates in the New River Flow of Pinang City, Labuhan Batu Selatan, identifying 11 genera, which were reported as favorable[16]. Raghad Zidan conducted a study on the Shatt al-Arab River, where monthly sampling was carried out at three stations over six months, identifying 12 species. Given that biological indices were not calculated for water quality assessment, it would be advisable to calculate these indices [17].

In the research by Y. Wang conducted in the Heihe River Basin, China, a total of 50 species were identified, with macroinvertebrate populations being more influenced by human factors than natural conditions [18]. Lowe, in another study on a wet-dry tropical estuary in northern Australia, concluded

that benthic diversity is influenced by seasonal changes [19].In studies by Sajeeb on the Karnafully River estuary, eight groups of benthic organisms were identified. It would be beneficial to calculate additional biological indices and provide more detailed information in the article [20].
Finally, in a study by Musavi, conducted in the present study area during 2008-2009, the population and diversity of benthic organisms were shown at two stations, Baraghan and Aghasht. Fifty-six families were identified, and biological indices were calculated, providing a good research background for reassessing this region, which can offer a better understanding of the area over time.
Given the importance of the Kordan River and its tributaries in providing water for aquaculture, agriculture, and the development of Alborz Province and its watershed, continuous monitoring of water health is among the effective measures for their conservation. In a previous study in this region by Musavi, conducted on two tributaries, Aghasht and Baraghan, and the main course of the Kordan River, the benthic populations of these stations were determined, and the EPT and HFI biological indices were calculated [21]. Considering the significant time lapse since this research and that the Senj River, one of the important tributaries of the Kordan River, was not examined by them, a follow-up and comprehensive study was conducted after 14 years. The Senj River, along with the Aghasht and Baraghan Rivers (the three main tributaries of the Kordan River), was sampled to identify and compare benthic populations, so all three main tributaries of the Kordan River would be simultaneously evaluated in this research. The Aghasht and Baraghan Rivers were reassessed using the BMWP, ASPT, EPT, and HFI biological indices to complete the previous study in terms of both the number of tributaries and biological indices. As a result, this study offers a more comprehensive comparison of their health status with the inclusion of the three main tributaries.

II. METHOD & MEASURES
The Kordan River, the case study, is located north of Karaj city and approximately 40 kilometers west of Tehran, at an elevation of 1,408 meters within the Alborz Mountains. The river has an approximate length of 75 kilometers and its key tributaries are Aghasht, Senj, Baraghan, and Velian.
The Kordan River watershed spans an area of 838 square kilometers, situated between 35°55' to 36°05' N latitude and 50°45' to 51°05' E longitude. This watershed is bordered by the Alborz mountain range and the Taleghan watershed to the north, the ridge separating the Fashand River watershed to the west, the Karaj-Qazvin road in the Sardaran mountains to the south, and the Lik Joochal mountain range (Baraghan River watershed) to the east. According to the De Martonne climate classification, the study area features a macroclimate that ranges from semi-arid and Mediterranean to semi-humid and humid. The region receives an average annual precipitation of 450 millimeters, with snowfall occurring in winter and thunderstorms in summer. The average annual temperature is 13°C, with recorded absolute maximum and minimum temperatures of 38°C and -10°C, respectively.
To assess the water quality of the Kordan River, three of its most significant tributaries were selected for study: Aghasht Station, Baraghan Station, and Senj Station. The geographical details of these stations are provided in Table I.
Table I: Characteristics of Sampling Stations

latitude	Longitude	Sea Levl (m)	Station
N35° 59′ 21.4″	E050° 51′53.0″	1491	Aghasht
N35° 57′ 10.9″	E050° 55′ 53.3″	1795	Baraghan
N36° 00′ 00.9″	E050° 57′ 53.3″	1985	Senj

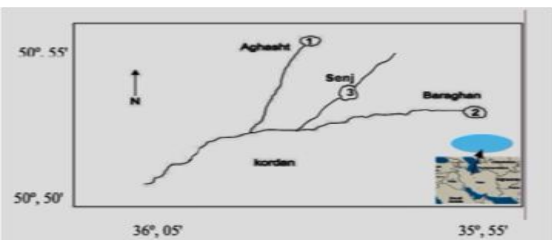

Fig .1: Schematic map of Kordan Stream and location of stations (not to scale). Source of photos & map: the writer

In order to collect samples and obtain the necessary data, during the spring and summer of 1402, macroinvertebrate samples were collected monthly from the rivers at three stations—Aghasht, Senj, and Baraghan. Sampling was conducted simultaneously at each station, with four replicates (three quantitative and one qualitative) collected each month. For a quantitative sampling of benthic invertebrates, a Surber sampler (30 cm × 30 cm, 250 µm mesh) was used. The Surber sampler consists of two metal frames, each 30 × 30 cm, with one frame placed perpendicularly on top of the other. A 250 µm mesh net is attached to one of the frames.

The Surber sampler was positioned on the riverbed, and all stones and objects within the frame were washed into the net. The net's contents were then passed through a sieve, with larger stones, branches, and leaves separated and placed into labeled containers, which were preserved using 4% formalin. This process was repeated three times to account for different habitats at each station (such as riffles, low-flow areas, and high-flow areas) to capture maximum benthic invertebrate diversity. For qualitative sampling, a hand net was used to collect samples from aquatic plants and calm sections of the riverbank, which were then transferred to their respective containers. In the laboratory, the samples were carefully sorted under a stereomicroscope (dissecting microscope) and stored in containers with 75% ethanol. The specimens were then identified to the genus level using relevant identification guides. [22], [23], [24], [25], [26], [27].

Alongside the sampling of benthic organisms, the most important physical and chemical water parameters were analyzed due to their significant impact on factors such as the distribution of benthic organisms, their living conditions, and overall water quality. Based on these criteria, water temperature, pH, electrical conductivity (EC), and dissolved oxygen (DO) were identified as the primary factors for analysis, and all related data were recorded.

Water temperature was measured using a mercury thermometer with an accuracy of 0.5°C. The thermometer was placed in the water without direct contact with the hand, and the temperature was read after 2 minutes. To measure the water's pH, a portable pH meter with the specifications of a Waterproof Double Junction PH TestR30 was used, and the readings were documented. Dissolved oxygen levels were measured using a portable DO meter, specifically the Jenway 970 DO meter. Electrical conductivity was measured with a portable EC meter, the Dual Range EC Test 11, and all data were systematically recorded.

In addition to these considerations, the water quality of the three discussed tributaries was classified using biological indices such as HFBI, EPT, ASPT, and BMWP. The EPT diversity index is equal to the total number of families from the three orders—Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)—present in the sample. To calculate the Hilsenhoff Family Biotic Index (HFBI) at the family level, the following formula was used:

$$HFBI = \Sigma (X_i \times T_i)/n$$

Where:

X_i= Number of individuals in a family

T_i= Tolerance value of that family

n = Total number of organisms in the sample

The Biological Monitoring Working Party (BMWP) index is calculated using the following formula:

$$BMWP=\Sigma t_i$$

Where:

t_i = Tolerance value of each family to organic pollution

In this study, due to the similarity in the diversity of various aquatic groups to those in North America, the tolerance values for each family were based on the North American reference, as revised by Mackie in 2001. Moreover, to estimate the Average Score Per Taxon (ASPT) index, the following formula is used:

$$ASPT=\Sigma t_i/n$$

Where:

t_i = Tolerance value of each family to organic pollution

n = Total number of organisms in the sample

Statistical calculations for data processing, plotting graphs, and analyzing correlations and significant relationships between physical and chemical variables and their impact on the diversity and density of benthic invertebrates were performed using SPSS 17.0 software. In SPSS, one-way ANOVA was used for statistical analysis to investigate significant differences in the population and distribution of benthic organisms across the studied tributaries and months. The LSD post-hoc test was employed to further examine these differences.

III. RESULTS

In line with the research objective of identifying the population of benthic invertebrates in the three tributaries of the Kordan River (Aghasht, Baraghan, and Senj) and subsequently determining the water quality of these rivers using biological indices, the physical and chemical parameters at these three stations were measured over a six-month sampling period. The results of these parameter measurements throughout the sampling period are presented in Table II.

Table II: Physical and Chemical Parameters Measured During the Research

station	Temperature(C)	Do(mg/L)	pH	EC(µs.cm-1)
Sa1	11.3	10.6	6.7	2.8
Sa2	10.5	10.7	6.71	2.4
Sa3	16.5	10.4	6.65	2.4
Sb1	11.2	10.4	7.63	3.4
Sb2	11.4	10.4	8.02	2.9
Sb3	11.5	10.9	8.31	2.7
Sc1	16.9	9.1	6.65	4.8
Sc2	17	9.2	6.8	4
Sc3	16.3	9.8	6.92	3.4
Sd1	23.8	8.3	7.62	4.9
Sd2	24.3	8.1	7.5	4.8
Sd3	24.7	8.2	8.1	4
Se1	19.8	8.9	7.84	5.5
Se2	16.2	9.3	7.01	4.5
Se3	20	9.1	8.23	3.9
Sf1	14.5	9.7	7.83	6
Sf2	14.3	9.8	8.63	5.1
Sf3	13.2	10.3	8.7	3.8
avg S1	16.3	9.5	7.37	4.6
avg S2	15.6	9.5	7.44	3.95
avg S3	16.5	9.7	7.81	3.36
avg a	12.8	10.6	6.7	2.5
avg b	11.3	1.5	7.9	3
avg c	16.7	9.3	6.79	4.06
avg d	24.2	8.2	7.74	4.5
avg e	18.6	9.1	7.69	4.63
avg f	14	9.9	8.38	4.96

Aghasht	**Baraghan**	**Senj**	**March**	**April**	**May**	**June**	**Juy**	**August**
S1	**S2**	**S3**	**a**	**b**	**c**	**d**	**e**	**f**

The range of water temperature variations observed in this study, as shown in Table II and Figure 2, indicates that in the three rivers—Senj, Baraghan, and Aghasht—the minimum temperature was recorded at 11.2±0.15°C in May, while the maximum temperature was 24.7±0.45°C in July. The temperature increased steadily until July, after which it began to decrease. The range of water temperature variations across the tributaries during the study period was from 15.6±4.96°C in Baraghan to 16.5±4.98°C in Senj, with an average temperature of 16.3±0.46°C throughout the sampling period.

Fig .2: The average water temperature in three stations during the sampling period (Centigrade)

In examining the dissolved oxygen levels in the Senj, Aghasht, and Baraghan rivers, the maximum concentration was recorded at 10.9±0.15 mg/L in May, while the minimum concentration was 8.1±0.1mg/L in July. The changes in this parameter showed a decreasing trend from April to July, followed by an increasing trend from July to September. The range of dissolved oxygen variations among the three rivers was from 9.50±0.89 mg/L in Aghasht to 9.78±0.98 mg/L in Senj, with an average of 9.58±0.14 mg/L measured in Baraghan. (Table II and Figure 3)

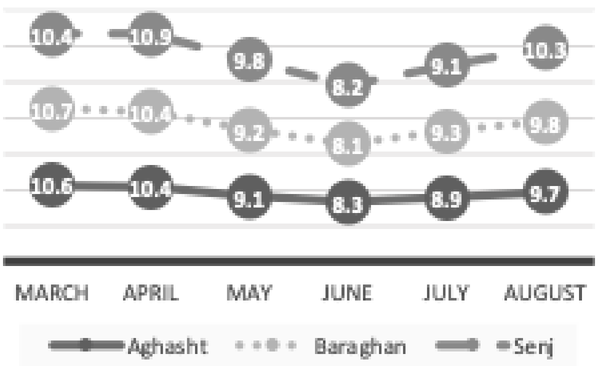

Fig.3: Average dissolved oxygen in water during the sampling period at three stations (mg/L)

The range of pH variations during the study period was recorded with a minimum of 6.65±0.034 in April and a maximum of 8.7±0.48 in September. The average pH across the three tributaries was 7.3±0.56 in Aghasht, 7.8±0.82 in Senj, and 7.44±0.75 in Baraghan. (Table II and Figure 4)

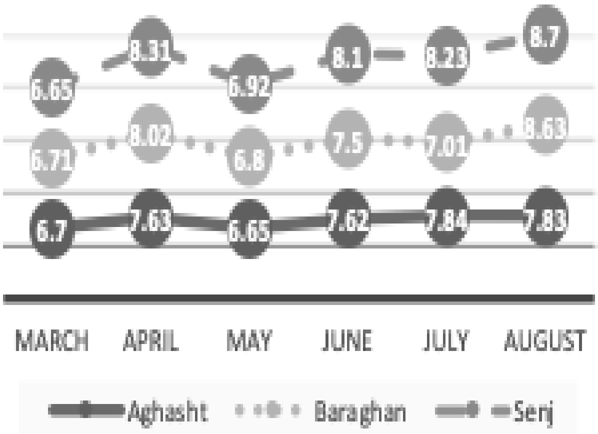

Fig.4: Average pH at three stations during the sampling period at three stations

Throughout this study, as indicated in Figure 5 and Table II, electrical conductivity ranged from a minimum of 2.4±0.36 millisiemens per centimeter (mS/cm) in April to a maximum of 6±1.1 mS/cm in September. Among the three rivers studied, the maximum electrical conductivity was 4.56±1.2 mS/cm in Aghasht, while the minimum was 3.36±0.67 mS/cm in Senj, with Baraghan recording 3.95±1.08 mS/cm.

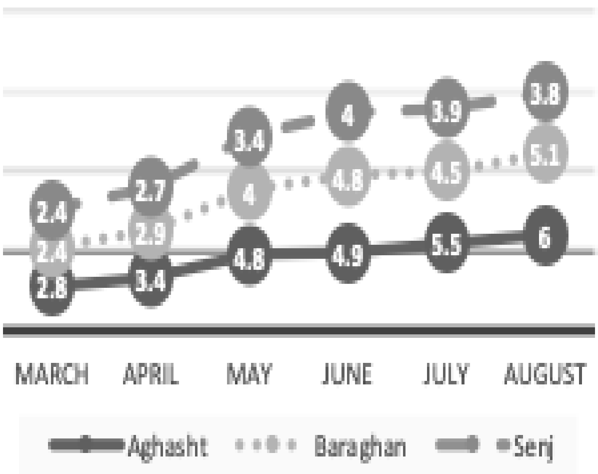

Fig.5: Average electrical conductivity at three stations during the sampling period

During the study of the benthic invertebrate community in the tributaries of the Kordan River, conducted over a six-month sampling period at three study stations, four phyla—Arthropoda, Annelida, Platyhelminthes, and Mollusca—were identified. These included 7 classes, 15 orders, 58 families, and 129 genera. The number of identified families, classes, and orders in this survey showed significant variation and greater diversity compared to the results obtained from studies on benthic fauna in the Karaj and Jajrud River beds, which are geographically close [28], [12], [11], [14]. The classification of these organisms is presented in Table III.

In this study, insects were the most diverse group of benthic organisms in the river, with 9 orders and 48 families. Among these, the order Diptera had the highest diversity, comprising 12 families and 7,563 specimens. This diversity within Diptera contrasts sharply with the findings of a study by Omoigberale et al., where only 3 families from this order were identified, highlighting a significant difference in diversity. In contrast, the classes Collembola, Turbellaria, Gastropoda, and Malacostraca, each with only one order and one family, showed the least diversity among the identified benthic fauna. Among the families identified, the family Chironomidae exhibited the highest diversity, with 39 genera identified, similar to observations by Omoigberale et al., where Chironomidae was the most prevalent family in the samples [30]. The least diversity and abundance were observed in the families Braconidae, Curculionidae, Coccinellidae, and Empididae.

According to Table IV, the order Ephemeroptera was most abundant in the Senj River, with 6,784 specimens (49%), followed by Diptera with 3,408 specimens (25%), and Trichoptera with 2,576 specimens (19%).

As shown in Table VI, in the Aghasht River, *Diptera* was the most abundant order, with 2,608 specimens (48%), followed by Ephemeroptera with 1,757 specimens (32%), and Lumbriculida with 372 specimens (9%). Within this order, the genus Prosimulium sp from the family Simuliidae was observed, characterized by a pollution tolerance score of 1 indicating its inability to survive in polluted waters[31]. This genus is typically found in mountain rivers with rocky substrates, especially those covered with moss. The Prosimulium sp found in this study precisely matched such habitats, as reported by Feld who also found Prosimulium species in mountainous river stations, with no records from non-mountainous river stations [32]. Notably, this genus was identified in Iran for the first time.

Table V shows that in the Baraghan River, the order *Ephemeroptera* had the highest abundance, with 1,819 specimens (35%), followed by *Diptera* with 1,547 specimens (29%), Trichoptera with 800 specimens (15%), and Plecoptera with 320 specimens (6%).

Table III: Classification of Benthic Invertebrates Identified

Phylum	Class	Order	Family	Species
Entoma	Collembola	Collembola	Isotomidae	Isotoma sp - Agrenia sp
	Insecta	Ephemeroptera	Baetidae	Baetis sp - Centroptilium sp
			Heptagenidae	Epeorus sp - Heptagenia sp
				Rhithrogena sp - Cinygmula sp
			Caenidae	Caenis sp
			Tricorythidae	Tricorythodes sp
			Ephemerellidae	Ephemerella sp
		Plecoptera	Perlidae	Claassenia sp - Macrogynoplax sp
			Chloroperlidae	Utaperla sp - Alloperla sp - Trizmaka sp
			Taeniopterygidae	Taenionema sp
			Perlodidae	Isoperla sp
			Leuctridae	Leuctra sp
		Trichoptera	Limnephilidae	
			Hydropsychidae	Hydropsyche sp
			Brachycentridae	Micrasema sp
			Glossosomatidae	Glossosoma sp - Anagapetus
			Hydroptilidae	Ochrotrichia sp
			Odontoceridae	Psilotreta sp
			Leptoceridae	Leptocerus sp
			Lepidostomatidae	Lepidostoma sp
			Philopotamidae	Chimarra sp
		Diptera	Athericidae	Atherix sp
			Tabanidae	Tabanus sp - Crysopos sp
			Tipulidae	Tipula sp - Dicranota sp
				Cyptolabis sp - Molophilus
				Hexatoma sp - Brachypremna sp
			Simuliidae	Simulium sp - Ectemnia sp
				Stegopterna sp - Prosimulium sp
			Stratiomyidae	Euparyphus sp
			Ceratopogonidae	Bezzia sp - Forcipomyia sp
				Atrichopogon sp
			Blephariceridae	Bibiocephala sp
			Dixidae	Dixa sp
			Muscidae	Limnophora sp
			Empididea	Hemerodromia sp
			chironomidae	Cricotopus sp - Nanocladius sp
				Eukiefferiella sp - Paralimnophyes sp
				Limnophyes sp - Rheocricotopus sp
				Paraborniella sp - Parakiefferiella sp
				Podochlus sp - Thienemannimyia sp
				Pirara sp - Pseudosmittia sp
				Thienemaniella sp - Clinotanypus sp
				Rheotanytarsus sp - Anzacladius sp
				Tanytarsus sp - Paraheptagyia sp
				Polypedilum sp - Xenochironomus sp
				Dan edward V15 sp - Cladopelma sp
				Stictochironomus sp - stictocladius sp
				Monopelopia sp - Paramerina sp
				Cardiocladius sp - Parametriocnemus sp
				Microtendipes sp - Batryocladius sp
				Echinocladius sp - Corynoneura sp
				Paratanytarsus sp - Austrochlus sp
				Ablabesmyia sp - Parachironomus sp
				paratendips sp - Genus Australia sp
			Psychodidae	
		Coleoptera	Elmidae	Narpus sp - Optiservus sp
				Stenelmis sp - Macronychus sp
			Dytiscidae	Agabus sp - Ilubius sp
			Gyrinidae	Gyrinus sp - Gyretes sp
			Hydrophilidae	Laccobius sp
			Curculionidae	Curculio sp
			Carabidae	Harpalus sp - Bembidion sp
			Scirtidae	Elodes sp - Prionocyphon sp
			Hydrochidae	Hydrochus sp
			Staphylinidae	Paederus sp - Stenus sp
				Bledius sp
			Coccinellidae	
		Hemiptera	Aphididae	Aphid sp
			Notonectidae	Notonecta sp
			Veliidae	Velia sp - Microvelia sp
			Delphacidae	
		Hymenoptera	Braconidae	Braconid sp
	Arachnida	Araneae	Pisauridae	
		Trombidiformes	Arrenuridae	
			Hygrobatoidae	
	Malacostraca	Amphipoda	Gammaridae	Gammarus sp
Mollusca	Gastropoda		Physidae	Physa sp
Annelida	Oligochaeta	Lumbriculida	Lumbriculidae	
		Megadrilacea	Lumbricidae	
Platyhelminthes	Turbellaria	Tricladia	Planaridae	Planaria sp

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Table IV: The Density of Identified Orders in Senj River During the Sampling Period

Order	March	April	May	June	July	August	Total	Avg
Plecoptera	5	0	0	0	85	208	299	50
Ephemeroptera	139	48	512	3136	1349	1600	6784	1131
Coleoptera	5	5	0	5	11	16	43	7
Diptera	21	91	811	1317	560	608	3408	568
Hmiptera	0	0	11	0	5	0	16	3
Hymenoptera	0	0	0	0	0	5	5	1
Entomobryomorpha	0	0	0	0	27	0	27	4
Trichoptera	21	91	32	816	5	1611	2576	429
Tricladida	0	21	0	0	0	128	149	25
Lumbriculida	32	0	0	0	181	75	288	48
Haplotaxida	16	21	0	0	123	16	176	29
Amphipoda	0	0	0	0	0	0	0	0
Araneae	0	0	0	0	0	0	0	0
Trombidiformes	0	0	0	5	0	0	5	1
Heterostropha	0	0	0	0	0	0	0	0
Ind.m^{-2}	240	277	1365	5280	2347	4267	13776	2296

Table V: The Density of Identified Orders in Baraghan River During the Sampling Period

Order	March	April	May	June	July	August	Total	Avg
Plecoptera	5	5	0	43	165	101	320	53
Ephemeroptera	64	80	32	213	411	1019	1819	303
Coleoptera	0	0	0	5	59	0	64	11
Diptera	37	21	496	347	288	357	1547	258
Hmiptera	0	0	0	0	11	11	21	4
Hymenoptera	0	0	0	0	0	0	0	0
Entomobryomorpha	0	0	0	0	5	11	16	3
Trichoptera	27	293	21	16	352	91	800	133
Tricladida	0	5	0	0	0	27	32	5
Lumbriculida	0	0	5	5	91	160	261	44
Haplotaxida	0	0	0	16	48	53	117	20
Amphipoda	0	0	0	0	32	32	64	11
Araneae	0	0	0	0	0	0	0	0
Trombidiformes	0	0	0	0	0	5	5	1
Heterostropha	0	0	0	0	91	21	112	19
Ind.m^{-2}	133	405	555	645	1552	1888	5179	863

Table VI: The Density of Identified Orders in Aghasht River During the Sampling Period

Order	March	April	May	June	July	August	Total	Avg
Plecoptera	5	0	0	32	16	0	53	15
Ephemeroptera	45	11	43	581	464	613	1757	502
Coleoptera	0	0	0	11	11	0	21	6
Diptera	32	64	389	336	1035	752	2608	745
Hmiptera	0	5	0	0	11	32	48	14
Hymenoptera	0	0	0	0	0	0	0	0
Entomobryomorpha	0	0	0	11	0	5	16	5
Trichoptera	5	21	37	0	0	69	133	38
Tricladida	0	0	0	0	0	16	16	5
Lumbriculida	4	0	11	16	165	176	372	106
Haplotaxida	11	0	11	27	53	107	208	59
Amphipoda	0	0	0	5	5	16	27	8
Araneae	0	0	0	0	0	0	0	0
Trombidiformes	0	0	5	0	0	5	11	3
Heterostropha	0	0	0	0	123	60	183	52
Ind.m^{-2}	102	101	496	1019	1883	1852	5453	909

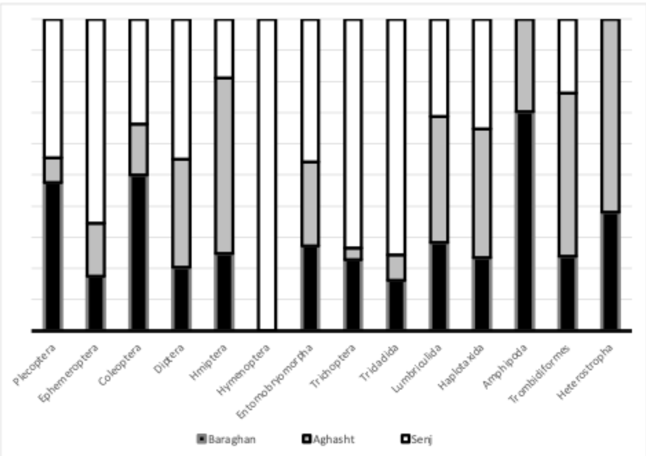

Fig.6: The density of identified orders in three rivers during the research period

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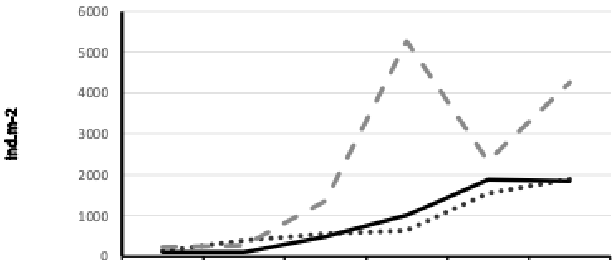
Based on the study of the benthic invertebrate populations in the Senj, Aghasht, and Baraghan tributaries, as indicated in Table IV, the highest abundance in the Senj River was recorded in July, with 5,280 specimens, while the highest diversity was observed in September, with 24 identified families. In the Aghasht River, the peak diversity and abundance were both observed in September, with 23 families identified and 1,852 specimens collected, as shown in Table V. Similarly, in the Baraghan River, the highest diversity and abundance were also recorded in September, with 26 identified families and 1,888 specimens collected, as presented in Table VI.
According to Chart 7, the trend of benthic invertebrate population changes in the Senj River showed an increase up until July, followed by a sharp decline in August, and then a subsequent increase in September. In contrast, the Baraghan River displayed a consistent increase in population from April to September. In the Aghasht River, the population showed an increasing trend from April to August but then experienced a slight decline in September compared to August.



	March	April	May	June	July	August
Aghasht	102	101	496	1019	1883	1852
Baraghan	133	405	555	645	1552	1888
Senj	240	277	1365	5280	2347	4267

Fig.7: Changes in the population of three rivers during the research period

According to Chart 8, the Baraghan River had the highest diversity, with 26 identified families, while the Senj River had the highest density, with 13,776 specimens collected. The lowest diversity was observed in the Aghasht River, with 23 identified families, and the lowest density was recorded in the Baraghan River, with 5,179 specimens collected.

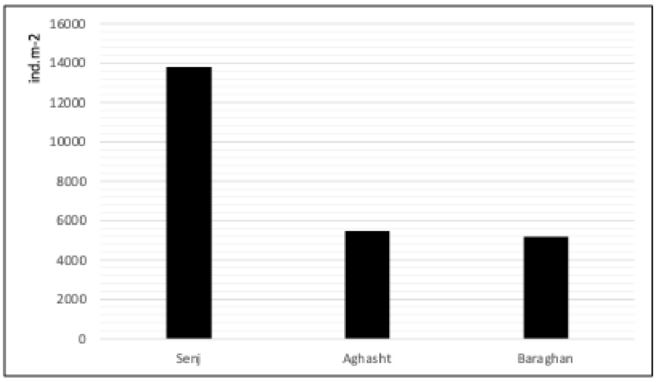


Fig.8: Benthic population density identified in three rivers

In this study, the Hilsenhoff Biotic Index (HBI) was examined to determine the water quality of the Senj, Aghasht, and Baraghan rivers using biological assessment indices. The results of the HBI index variations throughout the study period, as shown in Table VII and Figure 9, indicate that the minimum value of this index occurred in April at the Baraghan station, with a value of 1.84, while the maximum value was observed in June at the same station, with a value of 5.88.
The trend in the HBI index shows an increase from April to July, followed by a decrease after July. This pattern suggests the seasonal and potentially environmental and ecological factors influencing water quality in these regions.

Table VII: The Changing Trend of the Hilsenhoff Index in Different Stations of the Sampling Period

Station	March	April	May	June	July	August	Avg
Senj	5.33 Fair	3.55 Excellent	2.76 Excellent	5.44 Fair	4.5 Good	4.39 Good	4.32 Good
Aghasht	3.39 Excellent	4.45 Good	5.22 Fair	5.65 Fair	4.42 Good	5.45 Fair	4.76 Good
Baraghan	1.84 Excellent	2.19 Excellent	5.88 Fairly poor	5.2 Fair	3.77 Very Good	3.81 Very Good	3.78 Very Good
Avg	3.52 Excellent	3.39 Excellent	4.62 Good	5.43 Fair	4.23 Very Good	4.55 Good	4.29 Good

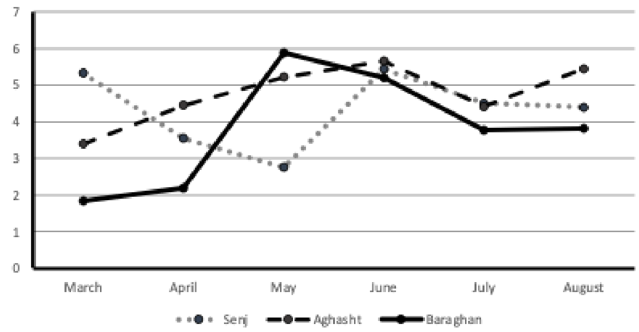


Fig .9: The changing trend of the Hilsenhoff index in three stations during the research period

The mean Hilsenhoff Biotic Index (HBI) for the three tributaries—Senj, Aghasht, and Baraghan—was estimated at 4.29 ± 0.75 during the study period. The minimum HBI, indicating a "Very Good" water quality status, was recorded at Baraghan with a value of 3.78 ± 1.59. The maximum HBI, indicating "Good" water quality, was observed at Aghest with a value of 4.76 ± 0.84, as detailed in Table VII. Among the sampling stations over the six-month sampling period, the water quality status was observed as follows: 33.3% Fair, 27.7% Excellent, 22.2% Good, 11.1% Very Good, and 5.5% Fairly Poor.

The assessment of the EPT (Ephemeroptera, Plecoptera, Trichoptera) diversity index across the study stations in the Senj, Aghest, and Baraghan rivers showed the maximum EPT value in the Senj River with 21 genera and the minimum in the Aghest River with 12 genera, with an overall average of 17 genera. These findings are illustrated in Figure 10 and Table VIII.

Table VIII: EPT Diversity Index Changes in Three Stations

Order	Senj	Aghasht	Baraghan	Total avg
Plecoptera	5	2	5	4
Ephemeroptera	7	6	9	7
Trichoptera	9	4	5	6
EPT Richness	21	12	19	17

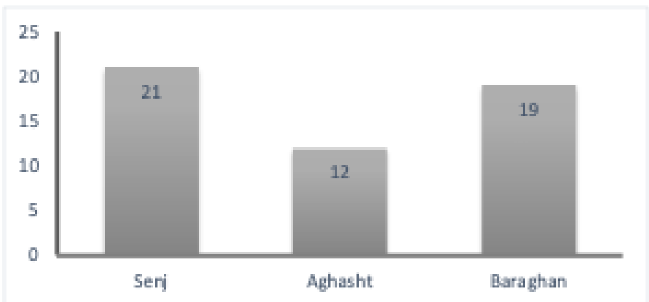


Fig.10: EPT diversity index changes in Senj, Aghasht, and Baraghan rivers during the research period

According to Table IX and Figure 11, the highest BMWP (Biological Monitoring Working Party) score was recorded in September at the Senj River station, with a value of 130. The lowest BMWP score was recorded at the same river in May. Regarding the ASPT (Average Score Per Taxon) index, the highest value was observed in July at the Baraghan station, while the lowest was recorded in April at the Senj station.

Based on the results of these indices and according to the Water Framework Directive (WFD) guidelines, the ecological status of each station was categorized as High (H), Good (G), Moderate (M), Poor (P), or Bad (B), as shown in Table X. According to the BMWP index, 27.7% of the river stations were classified as having High ecological status, 33% as Good, and 33% as Moderate. In contrast, the ASPT index results indicated that 33% of the stations had High ecological status, 50% had good status, 5.5% had Poor status, and 16.6% were classified as Moderate.

Table IX: BMWP and ASPT Index During the Sampling Period in the Stations

station /month	Sa1	Sa2	Sa3	Sb1	Sb2	Sb3	Sc1	Sc2	Sc3	Sd1	Sd2	Sd3	Se1	Se2	Se3	Sf1	Sf2	sf3	Avg S1	Avg S2
Bmwp	105	89	36	67	62	62	112	83	60	53	95	88	60	103	96	90	122	130	81.1	92.3
Aspt	6.5	5.9	4.5	5.5	5.6	5.6	5.6	6.2	5	5.3	6.7	5.5	5	6.43	5.3	5.2	6.1	6.5	5.5	6.1

Table X: Water Quality Classes in Each Month and Each Station Based on the BMWP Index

station /month	Sa1	Sa2	Sa3	Sb1	Sb2	Sb3	Sc1	Sc2	Sc3	Sd1	Sd2	Sd3	Se1	Se2	Se3	Sf1	Sf2	sf3	Avg S1	Avg S2
Bmwp	H	G	P	M	M	M	H	G	M	M	G	G	M	H	G	G	H	H	G	G
Aspt	H	G	M	G	G	G	G	H	M	G	H	G	M	H	G	G	H	H	G	H

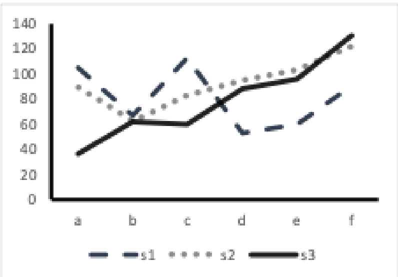


Fig.11: BMWP index changes in three stations during the sampling period

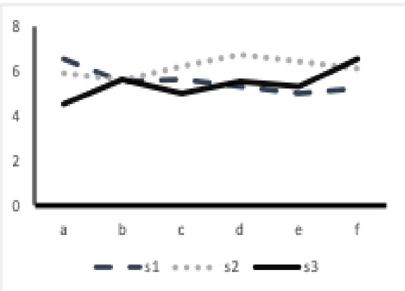


Fig.12: ASPT index changes in three stations during the sampling period

IV. **DISCUSSION**

In this study, a total of 24,408 macroinvertebrate specimens were identified over six months of sampling at three stations, representing four major groups: Arthropoda, Annelida, Platyhelminthes, and Mollusca. These specimens included 7 classes, 15 orders, 58 families, and 129 genera, demonstrating significant biodiversity. This high diversity likely reflects favorable living conditions for benthic organisms in the studied rivers. The diversity noted here contrasts sharply with findings from other studies. For example, Banagar, Febriani, and Taban reported the identification of only 10, 16, and 24 families, respectively. This suggests that the rivers examined in Banagar, Febriani, and Taban's research are likely under relatively greater environmental stress [33], [16], [12]. In the identified macroinvertebrate population, aquatic insects were the most dominant and diverse group, consistent with the results reported by Wang [18].

The identified benthic macroinvertebrate populations are noteworthy for both their diversity and density. Regarding the first characteristic, aquatic insects, which are predominantly associated with freshwater environments, represented the most frequently identified group. Within the identified insect orders, Diptera exhibited the greatest diversity, with 12 families identified. Among these families, Chironomidae stood out with 39 genera, marking a significant increase in diversity compared to the findings of Ertas, who identified only 7 genera within Chironomidae. Within this family, the highest diversity was observed in the subfamily Orthocladiinae, which prefers cold, flowing waters. This preference aligns with the geographical location and water temperatures observed at the three stations, explaining the high diversity of this subfamily in the study. These findings are consistent with those of García et al., where Orthocladiinae also showed significant diversity [34].

Additionally, Chironomidae is known to be resilient to pollution, a trait likely linked to their feeding habits as filter feeders of fine organic particles suspended in the water. The increase in the population of this family, reaching its peak in July, could indicate environmental stress factors such as decreased oxygen levels, increased temperatures, and the introduction of pollutants into the river. Although Chironomidae is generally considered a pollution-resistant family, there are genera within it, such as Tanytarsini, that are sensitive to pollution and are eliminated from contaminated waters [21]. The absence of Tanytarsini in any of the sampling months in the Aghasht River and in five out of six months in the Baraghan River, compared to its presence in five months in the Senj River, suggests that the pollution levels in the Senj River were low enough to support the survival of this genus.

In terms of the second characteristic, density, the order Ephemeroptera accounted for the highest density in both the Senj and Baraghan rivers, with 49% and 35% of the total, respectively. This order includes genera that are eliminated even with minimal environmental pollution, as well as those that are relatively resistant to pollution, making Ephemeroptera a valuable indicator in water quality assessments. Following Ephemeroptera, the order Diptera ranked second in density in Senj and Baraghan, with 25% and 29%, respectively, similar to findings observed by Liu [35]. In the Aghasht River, Diptera had the highest density at 48%, followed by Ephemeroptera at 32%.

Furthermore, the density of benthic macroinvertebrates varied among the sampling stations, decreasing from Senj to Aghasht. The high diversity and density observed at the Senj station seem to result from factors such as highwater flow, suitable depth, favorable vegetation cover, rocky riverbeds, and abundant riffle habitats. Additionally, limited tourist access to the river has likely helped maintain the pristine and undisturbed condition of the benthic fauna.

In contrast, the Baraghan River exhibited the lowest density throughout the study, which can be attributed to factors such as the high organic load from rural sewage, agricultural and gardening runoff, and increased tourist access. Baraghan also had higher flow rates and discharge compared to the other two stations in all sampling months, which could destabilize benthic habitats and result in lower densities.

In the Aghasht River, a decrease in the density of the orders Ephemeroptera and Trichoptera was observed, while there was an increase in the density of the order Lumbriculida and the family *Chironomidae*. These groups are known for their resilience to pollution, and their increased density suggests that environmental pressures, such as organic pollution and stressors, are more frequent or intense in Aghasht compared to the other stations. This could be related to the impact of construction and the presence of riverside gardens, which contribute to environmental stress in the area.

During the sampling period, considering both diversity and density characteristics at all three stations, the highest diversity and density of benthic macroinvertebrates were observed simultaneously during the summer season. In summer, the water is typically clearer, the flow rate is lower, and aquatic organisms are more abundant due to the slower flow, optimal temperature, and high oxygen levels [36].In contrast, during spring, many benthic habitats are destroyed due to flooding. Therefore, an inverse relationship exists between benthic macroinvertebrate abundance and river discharge. Higher river discharge prevents the establishment of benthic organisms, as increased flow can result in the scouring of old riverbeds and increased turbidity. Additionally, findings by Mackenzie have shown that benthic macroinvertebrates exhibit greater species diversity at higher temperatures, as lower temperatures reduce metabolism, reproduction, movement, and ultimately their abundance and density [37].

The genus Prosimulium sp. from the family Simuliidae was observed as a new record in Iran. Prosimulium sp. has a pollution tolerance score of 1, indicating its inability to survive in polluted waters [31]; it is eliminated from the environment with even minor pollution. This genus prefers mountainous rivers with rocky substrates, ideally covered with large stones and moss [32]. The Prosimulium sp. found in this study, which was observed at the Senj station, was indeed in such an environment, consistent with Feld's findings.

Feld observed Prosimulium in stations located in mountainous rivers, while no occurrences of this genus were reported in stations situated in non-mountainous rivers.

After identifying the benthic macroinvertebrate populations, and determining the diversity and density of different groups based on stations and sampling months, the importance of using biological indices to monitor the stations becomes evident. The results of the average HFBI index during the study period showed that compared to a study conducted in 2011 by Mousavi, the mean value in Aghasht has not significantly changed, from 4.67 to 4.76, remaining in a 'Good' condition. However, in Baraghan, a notable positive change was observed, with the mean value improving from 5.04 to 3.78, reaching a 'Very good' status, which could indicate a reduction in pollution entering the river [21].

The second index used for biological monitoring is the diversity index of genera from the three orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). Members of these three families are highly sensitive to water oxygen levels and can be influenced by other factors such as environmental stress and seasonal changes. The EPT index shows a decreasing trend from the Senj station to the Aghasht station, with 21 genera in the Senj River, 19 genera in the Baraghan River, and 12 genera in the Aghasht River. This can be attributed to better health conditions, limited human access to the Senj River, and subsequently less pollution entering this river. In comparison with the results of Mousavi & Ramezani (2011), it is evident that this index has increased from 8 and 9 to 12 and 19 for the Aghasht and Baraghan rivers, respectively, indicating a reduction in environmental stress. The maximum EPT index was recorded in the summer when the highest diversity and density of benthic macroinvertebrates were observed, likely due to the increased availability of nutrients. The minimum was recorded in the spring.

The values of the water's physical and chemical variables largely reflect the ecological conditions of aquatic ecosystems during different seasons, as well as agricultural, urban, and industrial activities along the river. Each of these factors significantly impacts the distribution and population of benthic macroinvertebrates [38], [39]. In the present study, water temperature showed seasonal and daily variations, influencing macroinvertebrate populations. During the sampling period, the highest temperature was recorded in the summer, specifically in July, coinciding with the highest abundance and diversity of benthic macroinvertebrates. One of the factors contributing to this increase is the rise in temperature, which creates favorable conditions for the production of phytoplankton, a crucial food source for benthic organisms. According to the results reported by Sharbati, the rise in temperature, which subsequently leads to increased biological activities such as feeding and reproduction, results in higher diversity and density of benthic macroinvertebrates in summer [40].

The relationship between temperature and dissolved oxygen (DO) in water is inverse; as temperature increases, DO levels decrease. Therefore, in July, when the highest temperature was observed, the lowest level of dissolved oxygen was recorded. During this time, the population of the family Chironomidae, known for its resilience to pollution, oxygen deficiency, and a wide range of environmental parameters, increased. This population growth may indicate a departure from optimal conditions, consistent with the findings of Song Y and Jolejole [41], [42].

Another parameter that significantly impacts river health and the population of macroinvertebrates is water pH, which was recorded with an average value of 7.54 during the sampling period. This finding indicates a slight alkalinity in the flowing water at these stations, closely resembling the pH levels observed in most natural waters, which typically range between 5 and 9 [43]. The mild alkalinity could be due to the discharge of wastewater into the water and recreational activities around the river.

Finally, to establish the relationship between electrical conductivity (EC) and the benthic population, EC levels were measured. The concentration of ionic components in the river water, as indicated by electrical conductivity (EC), was consistently highest in the Aghasht River across all sampling months. This could be a sign of pollution, as the high level of construction activities, agricultural practices, and sewage from villas around this river contribute various pollutants to the water. The results from Shieh & Yang also support the link between pollution and electrical conductivity [44]. Although electrical conductivity is influenced by temperature, in the present study, it was also dependent on water discharge and flow rate. EC was lowest in April, during the high-water period, and highest in September, during the low-water period, which aligns with the findings reported by Kominoski [45]. One of the relationships observed between EC and the benthic population is that the density of pollution-sensitive families, such as Plecoptera, decreases with increasing electrical conductivity. As evident in this study, in the Aghasht River, where the highest EC of 6 mS/cm was recorded in September, no genus from the Plecoptera order was observed during this month.

V. CONCLUSION

In this study, the benthic macroinvertebrate communities of three tributaries of the Kordan River were collected and identified. Sampling was conducted monthly from April to September. Based on the results, aquatic insects were the largest group identified. Among the three rivers studied, the Senj River had the highest density of macroinvertebrates. Additionally, Prosimulium sp. was observed and identified for the first time in Iran in this river. A total of 58 families and 129 genera were identified over the six-month sampling period. The highest density of benthic macroinvertebrates was collected during the summer due to sufficient nutrients, favorable temperatures, appropriate flow rates, and other factors. Based on the identified populations, biological indices such as HFBI, BMWP, EPT, and ASPT were calculated to assess the health of the rivers. According to the HFBI index, the health of all three rivers was evaluated as "Good." The EPT index rated all three rivers as "Excellent." According to the BMWP index, the condition of all three stations was "Good." Finally, the ASPT index assessed the health of the Baraghan River as "Very Good," while the Senj and Aghasht Rivers were rated as "Good."

Given the unregulated construction along the riverbanks, the discharge of wastewater from gardens and villas into the water, and other human interventions, the health of these rivers is likely to be at risk in the future, exceeding their self-purification capacity. Considering the importance of the Kordan River for various activities in the region, it is recommended that human activities, wastewater discharges, and unregulated construction along the riverbanks be strictly managed and controlled. To ensure the health of the rivers, continuous monitoring of physical and chemical parameters, as well as biological assessments, should be conducted. Moreover, river assessment activities should be expanded to cover all tributaries of the Kordan River. It is hoped that this research will provide a valuable perspective for future monitoring and evaluations.

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