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Integrated biotic and abiotic indicators for evaluating ecosystem health in the Qara-Su River, Iran

Ehsan Asadi-Sharif [1], Shima Rahim Pouran [2*], Amin Salimi Godeh-Kahriz [3], Abolfazl Bayrami [3*]

[1] Offshore Fisheries Research Centre, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Chabahar, Iran
[2] Department of Range and Watershed Management, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
[3] Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran

Abstract

Increasing anthropogenic pressures have intensified contamination in river ecosystems, highlighting the urgent need for comprehensive environmental evaluations. This study was designed to evaluate the ecological quality of the Qara-Su River in Ardabil Province, Iran, using a combination of biotic and abiotic metrics. Macroinvertebrate sampling was conducted across four stations from June 2021 to April 2022 using a Surber sampler, yielding a total of 5,092 specimens representing ten taxonomic orders. Water and sediment samples were analyzed for lead and cadmium concentrations, and macroinvertebrate communities were assessed to compute diversity indices (Shannon, Simpson, evenness, dominance) and biotic indices (HFBI, BMWP). Additional evaluations included bioconcentration (BCF), biota–sediment accumulation (BSAF), and contamination indices (I_{geo}, Er, RI, HPI). Correlation analysis was used to explore relationships between biotic and abiotic variables. The results revealed that the Pb and Cd content were elevated in both water and biota, particularly in Hydropsychidae, and exceeded permissible limits at downstream sites. Seasonal water-quality patterns showed higher nutrient loads and lower dissolved oxygen during warmer periods, along with consistently greater pollution at downstream stations exposed to cumulative agricultural, domestic, and aquaculture inputs. The strong correlations between abiotic and biotic indices confirmed the reliability of macroinvertebrate-based assessment. The combination of biotic and abiotic indicators revealed spatial variation in ecological health along the Qara-Su River, highlighting localized pollution risks masked by average conditions. These findings emphasize the importance of integrating multiple assessment tools to support targeted river management and mitigation strategies.

Keywords: Environmental monitoring; Macroinvertebrate community; Sediment quality; Qara-Su River

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** Corresponding author (communicator): rahimpooran@yahoo.com, abolfazlbayrami@gmail.com

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1. Introduction

Riverine ecosystems are essential for maintaining environmental stability and sustaining human well-being by providing freshwater, regulating hydrological cycles, and supporting diverse biological communities (Bănăduc et al., 2022). These ecosystems are among the most productive and ecologically important habitats on Earth, yet they are increasingly threatened by anthropogenic activities (Naiman & Dudgeon, 2011). Industrial discharges, agricultural runoff, urban development, and dam construction contribute significantly to the degradation of river health, causing chemical contamination, habitat alteration, and loss of biodiversity (Mobasher et al., 2023). Among these stressors, agricultural activities have been identified as the leading driver of riverine pollution, accounting for approximately 72% of overall impacts on freshwater systems (FAO, 2023). Additional contributing sources include municipal wastewater discharges, urban stormwater runoff, resource extraction, industrial effluents, hydrological modifications, silviculture, onsite sewage systems, and changes in flow regimes. While these influences vary by region, agriculture remains a consistent and dominant source of contamination, particularly through nutrient enrichment, pesticide infiltration, and sedimentation (Chakraborty, 2021). This widespread and multifaceted pollution highlights the urgent need for comprehensive water quality assessments that consider both chemical and biological components of aquatic systems.

Historically, the assessment of river water quality has evolved through three major phases, reflecting advances in ecological understanding and monitoring technologies. The earliest phase, extending from the 1800s to the 1960s, emphasized abiotic indicators such as biochemical oxygen demand (BOD), dissolved oxygen (DO), phosphate, and nitrate. These parameters were commonly used to classify water quality due to their direct association with pollutant concentrations (Starzecka, 1929). The second phase, from the 1960s to the 1980s, witnessed a paradigm shift toward biotic assessment, with the incorporation of bioindicators such as benthic macroinvertebrates, particularly aquatic insects, whose community structure and pollution sensitivity reflect the ecological condition of water bodies (Herman & Nejadhashemi, 2015) Biotic methods gained prominence not only due to their ecological relevance but also because of their cost-effectiveness and ability to reflect cumulative and long-term environmental stress. The current era, spanning from the 1980s to the present, is characterized by the integration of biotic and abiotic indicators, facilitating more comprehensive and accurate assessments of river health. This integrative approach provides a nuanced understanding of both immediate physicochemical conditions and longer-term biological responses, making it especially suitable for identifying localized risks and informing sustainable water management strategies (Asadi Sharif et al., 2021). Studies in various countries have shown that biotic indicators are effective tools for evaluating river ecosystem health, with applications ranging from diatom-based assessments of eutrophication to macroinvertebrate metrics for detecting hydromorphological disturbance (Ibáñez et al., 2010; Dong et al., 2023). In Iran, similar approaches have been applied to evaluate river conditions using macroinvertebrate communities and physicochemical parameters (Aazami et al., 2015; Shokri et al., 2014). Collectively, these studies demonstrate the widespread use of biological sampling and combined biotic–abiotic indices, providing a methodological foundation relevant to the present research on the Qara-Su River. Situated in northwestern Iran, the Qara-Su River is the longest and most voluminous inland river in Ardabil Province, which plays a crucial role in regional agriculture, livestock production, and rural livelihoods. However, over recent years, the ecological condition of the Qara-Su River has been increasingly compromised by a range of both point-source and non-point-source pollution, most notably the release of municipal, agricultural, and industrial effluents with insufficient treatment (Ghanbari et al., 2022). Additional pressure on the river’s water quality arises from the establishment of aquaculture facilities and the operation of an upstream wastewater treatment plant (e.g., Sabalan Dam), both of which contribute to altered hydrology and nutrient loading (Figure 1). Despite growing concerns and previous studies addressing localized pollution in Ardabil Province, no study to date has comprehensively evaluated the ecological health of the Qara-Su River using an integrated framework of biotic and abiotic indicators.

To fill this knowledge gap, the current study was designed to identify and characterize both point and non-point pollution sources affecting the Qara-Su River. Four monitoring sites were set up at key locations along the river. Heavy metal concentrations were measured in biota, water, and sediments, followed by ecological risk assessment using standard pollution indices. Additionally, biotic and abiotic indicators, including macroinvertebrate communities and a range of chemical and geochemical indices, were combined to evaluate the ecological health of the river. This comprehensive approach aimed to provide valuable insights into the spatial distribution of pollution, its impact on aquatic biodiversity, and to offer evidence-based recommendations for the sustainable management of the Qara-Su River ecosystem.

2. Materials and Methods

2.1 Study area

The Qara-Su River, known as the largest and longest inland waterway in Ardabil Province, stretches about 255 kilometers through the region. As an important tributary of the Aras River, it helps drain into the Caspian Sea basin. The river starts in the Talesh Mountain range in eastern Ardabil and flows westward through various landscapes before merging with the Aras River, along with other major tributaries like the Darrehroud and Ahrchaei Rivers (Figure 1).

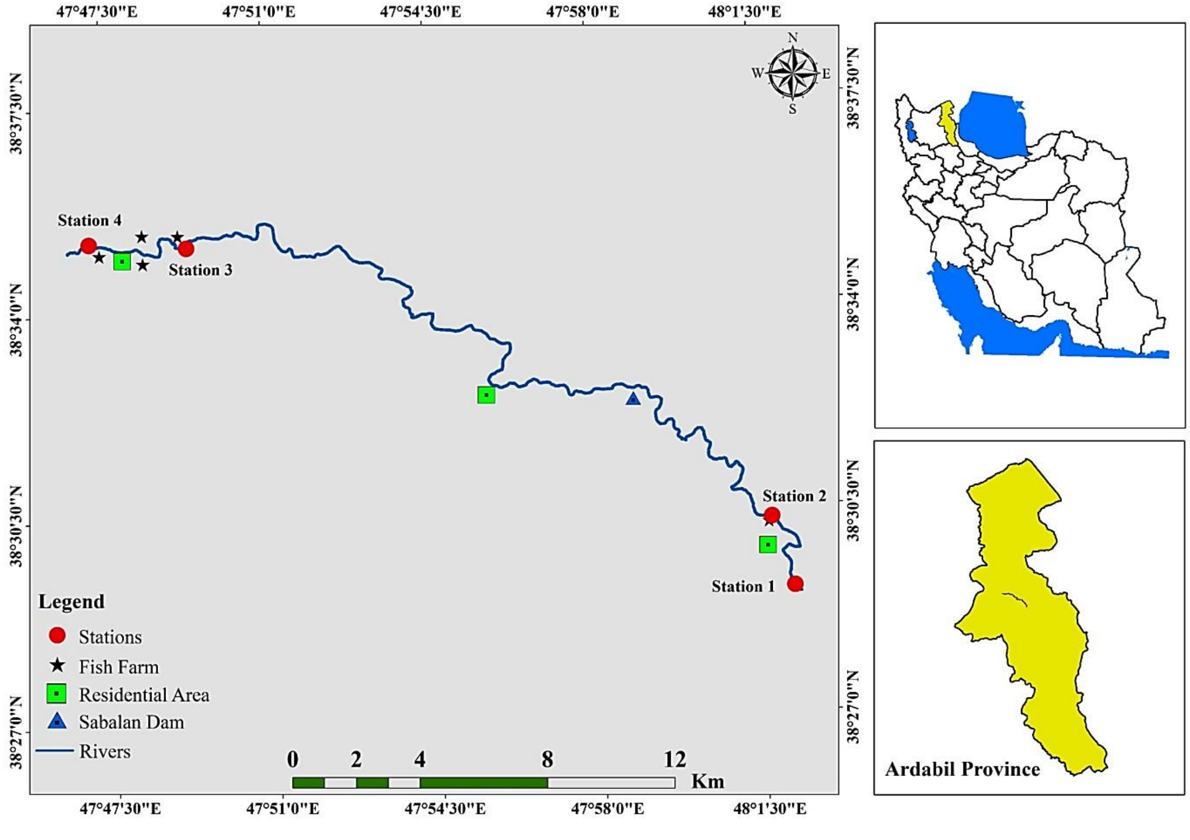

Figure 1. The locations of the sampling stations on the Qara-Su River (blue line), Ardabil, Northwest of Iran.

Four monitoring stations were strategically established along the Qara-Su River, following the identification of both point and non-point sources of pollution, to capture spatial variations in water quality: Station 1 (S1): Positioned upstream of Arbab Kandi village, this site serves as the reference station, as it is unaffected by nearby anthropogenic influences such as rural wastewater discharge, industrial effluents, or aquaculture operations. Station 2 (S2): Located downstream of Arbab Kandi, this station is subject to inputs from livestock operations and rural sewage, reflecting typical agricultural and domestic impacts. Station 3 (S3): Situated upstream of Kangarloo village, this site also receives runoff and wastewater from rural settlements and livestock activities, contributing to nutrient and organic loading. Station 4 (S4): Located in the downstream reaches of the Qara-Su River, this station is directly influenced by effluents from several upstream fish farms, making it a critical location for assessing aquaculture-related pollution. A detailed summary of the geographical and environmental characteristics of each sampling station is provided in Table 1.

Table 1. Geographic coordinates, environmental features, and pollution sources associated with the four monitoring stations along the Qara-Su River, Ardabil Province, Iran.

Station Location	Coordinates	Altitude (FT)	Substrate
Upstream of Arab Kandi	38°29' 8.46" N ;48° 2' 55" E	1133	Large cobbles, clay, and silt
Downstream of Arab Kandi	38°30' 17.2" N; 48°1' 44.2" E	1123	Large cobbles, clay, and silt
Upstream of Kangarloo	38°35' 7.12" N;47°49' 16.08" E	962	Large cobbles, clay, and silt
Downstream of Qara-Su River	38°35' 8.88" N;47°47' 8.92" E	953	Large cobbles, clay, and silt

2.2. Sampling overview

Samples were collected from four sites along the Qara-Su River (designated S1-S4) to assess biotic variables, including macroinvertebrate communities, and abiotic variables, such as heavy metal pollution in sediment and water. At each station, four water and four sediment samples were collected per season. Sediment samples were obtained at a depth of 0.5 m, and water samples were taken approximately 5 m from the riverbank. All samples were acidified with nitric acid and subsequently analyzed for cadmium and lead using a Varian AA220FS atomic absorption spectrometer. Quantitative sampling of benthic macroinvertebrates was carried out using a Surber sampler (0.3 m × 0.3 m; area: 0.09 m²), specifically targeting riffle and edge mesohabitats, which are known to support diverse invertebrate communities. Sampling procedures were based on the Rapid Bioassessment Protocols (RBP) developed by Barbour et al. (Barbour et al., 1999) and were replicated three times at each site to ensure data reliability and spatial representativeness. Sampling was conducted bimonthly over the course of one year. The collected materials were filtered through a 500 µm mesh sieve to retain macroinvertebrates and associated debris and were immediately preserved in plastic containers for transport. In the field, water samples were stabilized using 5% buffered formalin and later analyzed in the laboratory following standardized procedures described by the American Public Health Association (APHA, 1999). All macroinvertebrates retained in both sediment and water samples were carefully sorted and taxonomically identified to the family level using a dissecting microscope, based on the recorded identification keys (Fathi et al., 2022; Trigal et al., 2009).

A portable multi-parameter meter (WTW, Germany) was used to measure several in situ water quality parameters, including pH, temperature (°C), dissolved oxygen (DO, mg/L), and electrical conductivity (EC, µS/cm). In addition, grab samples of water were collected from each monitoring station for subsequent laboratory analysis of nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), and biological oxygen demand (BOD). Analytical procedures for these parameters followed APHA guidelines (APHA, 1999). To evaluate contamination from toxic metals, the cadmium (Cd) and lead (Pb) concentrations were measured in the selected macroinvertebrate specimens, water, and sediment using an atomic absorption spectrophotometer after acid digestion. In short, samples of dominant benthic macroinvertebrates (primarily Gammaridae and Simuliidae) were oven-dried and subsequently ashed. The resulting material was digested using concentrated nitric and perchloric acids following the procedure described by Tinggi et al. (1992). After digestion, the solutions were cooled, filtered, and brought to a final volume of 50 mL with distilled water. Concentrations of Cd and Pb in the digests were determined by atomic absorption spectrophotometry. These measurements provided insight into both environmental exposure and bioaccumulation potential within aquatic organisms.

2.3. Abiotic indices

To determine the degree of pollution of the Qara-Su River, multiple indices were applied to quantify heavy metal contamination and ecological risks.

2.3.1. Geo-accumulation Index (I_{geo})

This index, originally proposed by Muller (Lim et al., 2021), quantifies the extent of heavy metal accumulation in sediment samples. The Igeo is calculated as follows (Eq. 1):

$$I_{geo} = \log_2 \left(\frac{Ci}{1.5 \times Bi}\right) \qquad (1)$$

Where *Ci* represents the measured concentration of the *i*th heavy metal, and *Bi* denotes its corresponding geochemical background concentration. The multiplication factor 1.5 accounts for potential natural fluctuations in background levels. The pollution intensity is categorized into seven distinct classes based on the I_{geo} values, ranging from unpolluted ($I_{geo} \leq 0$) to extremely polluted ($I_{geo} > 5$), with intermediate classes including slight (0–1), moderate (1–2), and heavy pollution (3–4). These classifications facilitate a clearer understanding of sediment contamination levels and their potential environmental implications.

2.3.2. Potential Ecological Risk Index (RI)

This index was originally proposed by Hakanson (Hakanson, 1980), which evaluates the ecological threat posed by heavy metals in sediments. It aggregates individual metal risk factors as follows (Eq. 2):

$$RI = \sum_{i=1}^{n} E_r^i = \sum_{i=1}^{n} T_r^i P_i = \sum_{i=1}^{n} T_r^i \frac{Ci}{Bi} \qquad (2)$$

In this equation, E_r^i is the ecological risk factor for the i^{th} metal, T_r^i is the toxic response factor, reflecting the relative toxicity of each metal (with values of 5 for lead (Pb) and 30 for cadmium (Cd) as per Hakanson). P_i is the contamination factor defined by the ratio of measured concentration C_i to background concentration B_i. Based on *Er* values, metals are assigned to five ecological risk categories: very high (*Er* > 320), high (160–320), considerable (80–160), moderate (40–80), and low risk (< 40). The overall ecological risk (RI), calculated as the sum of all individual Er values, is classified into four levels: low (RI < 120), moderate (120 ≤ RI < 240), considerable (240 ≤ RI < 480), and very high risk (RI > 480).

2.3.3. Heavy Metal Pollution Index (HPI)

HPI serves as an indicator of the overall contamination level of heavy metals in water samples. This index incorporates weighted contributions of each metal, with weights (W_i) assigned between 0 and 1 based on their relative importance. The permissible concentration limits were adopted from the WHO guidelines for drinking water quality. The HPI is computed using the following formula (Eq. 3) (Rajan & Nandimandalam, 2024):

$$HPI = \frac{\sum_{ki=1}^{n} W_i \, Q_i}{\sum_{ki=1}^{n} W_i} \qquad (3)$$

Where Q_i is the sub-index value corresponding to the *i*th parameter, W_i is its unit weight, and n denotes the number of heavy metal parameters considered.

2.4. Biotic indices

2.4.1. Bioconcentration Factor (BCF)

BCF indicates how much a substance accumulates in an organism relative to its concentration in the surrounding medium, commonly aquatic ecosystems. It is determined by the following ratio (Eq. 4) (Kowalska et al., 2024):

$$BCF = \frac{\text{Concentration of the substance in the organism}}{\text{Concentration of the substance in the surroundings}} \qquad (4)$$

A higher BCF value indicates a greater propensity for the chemical to bioaccumulate, which may pose significant ecological and health risks.

2.4.2. Biota-Sediment Accumulation Factor (BSAF)

BSAF is a critical environmental parameter used to evaluate the bioaccumulation potential of contaminants from sediments into aquatic organisms (Arriola et al., 2024). This ratio compares the contaminant concentration within the organism to that found in the corresponding sediment (Eq. 5):

$$BSAF = \frac{\text{Concentration of the contaminant in the organism}}{\text{Concentration of the contaminant in sediment}} \qquad (5)$$

2.4.3. Hilsenhoff Family Biotic Index (HFBI)

HFBI assesses stream and river water quality by analyzing the composition and abundance of benthic macroinvertebrate families. It is calculated as the abundance-weighted mean of tolerance scores assigned to each family (Eq. 6) (Mobasher et al., 2023):

$$\text{HFBI}=\frac{\sum n_i \times t_i}{N} \tag{6}$$

Where n_i represents the number of individuals in the ith family, t_i is the corresponding tolerance value, and N is the total number of individuals sampled. Lower HFBI values indicate better water quality and reduced organic pollution, whereas higher values reflect deteriorated conditions.

2.4.4. The Biological Monitoring Working Party (BMWP)

The BMWP scoring system is widely employed in the United Kingdom. The BMWP index evaluates riverine and stream ecological health based on the presence and diversity of aquatic macroinvertebrates (Herman & Nejadhashemi, 2015). The overall BMWP score is the sum of individual taxonomic scores, with the occurrence of pollution-sensitive taxa contributing higher values, thereby indicating superior water quality.

2.4.5. Diversity Indices

Diversity Indices are employed to characterize biodiversity within biological communities. This study employed four key indices: the Shannon-Wiener diversity index (H), which measures species richness and evenness; the dominance index (D), reflecting the prevalence of the most abundant species; Simpson’s index (1-D), indicating the probability that two individuals randomly selected belong to different species; and the evenness index (E), assessing the equitability of species distribution (Strong, 2016).

2.5. Data analysis

Statistical analyses were performed employing the PAST software package (version 3.25), with significance evaluated at a 5% threshold ($p < 0.05$). Data normality was examined via the Shapiro-Wilk test. To quantify species diversity within the biological communities, univariate diversity indices, including the Shannon-Wiener diversity index (H), Dominance index (D), and Simpson’s index (1–D), were employed, providing a detailed characterization of community structure. The relationships between biotic and abiotic variables were investigated using Spearman’s rank correlation coefficient, implemented through the PAST software.

3. Results

3.1. Heavy Metal Concentrations in Sediment, Water, and Aquatic Organisms

The concentrations of Pb and Cd were measured in sediment, water, and selected aquatic invertebrates to assess the extent of contamination and bioaccumulation within the study area. In sediment samples, the average concentrations of Pb and Cd were 0.02 mg/kg and 0.001 mg/kg, respectively. In the water column, mean Pb and Cd levels were found to be 0.133 mg/L and 0.009 mg/L, respectively. These values suggest a relatively higher mobility and availability of these metals in the aqueous environment compared to their sediment-bound forms. On the other hand, bioaccumulation analysis revealed notable uptake of both metals in benthic macroinvertebrates. In specimens belonging to the Hydropsychidae family, Pb and Cd concentrations were recorded at 0.70 mg/kg and 0.29 mg/kg, respectively. Similarly, in Hirudinea species, Pb and Cd concentrations were 0.58 mg/kg and 0.23 mg/kg, respectively. These findings indicate the potential of both taxonomic groups to serve as bioindicators of heavy metal pollution in freshwater ecosystems.

3.2. Evaluation of Abiotic Indices

3.2.1. Geoaccumulation Index (I_{geo}), Ecological Risk Index (E_r), and Potential Ecological Risk Index (RI)

The geo-accumulation index (I_{geo}) values for sediment samples indicated that the study area falls within the "unpolluted to moderately polluted" category ($0 \leq I_{geo} < 1$), suggesting a relatively low degree of heavy metal contamination in surface sediments. Consistent with these findings, the ecological risk factor (Er) also revealed a very low ecological risk across all stations, except for station S3, which exhibited a comparatively higher ecological risk than the other sampling sites. The potential ecological risk index (RI) further supported the above observations. The RI values for stations S1, S2, S3, and S4 were calculated as 0.419, 0.295, 0.404, and 0.411, respectively, indicating a small ecological risk at all locations. These results collectively suggest that while contamination levels are generally low, localized ecological risks, particularly at station S3, may warrant further monitoring.

The HPI was applied to assess the level of contamination in water samples across seasons and stations. The results revealed a seasonal increase in Pb concentrations at station S4, with the highest HPI value observed during autumn. Across most stations, the HPI values for Pb exceeded the threshold value of 20, indicating a significant pollution risk associated with this metal. In contrast, Cd contamination generally remained at moderate levels, although a rising trend was also observed at station S4, particularly in the autumn and winter seasons. Table 2 presents the seasonal HPI values for Cd and Pb at each station. Notably, in spring, Pb levels at station S3 peaked at 174, whereas in winter, the highest Pb value (133.2) was again recorded at station S4. The Cd contamination showed lower overall HPI values, with the highest reading (30.00) occurring at station S4 during autumn. These patterns stress the need for continued monitoring, especially at downstream sites with elevated pollution risks.

Table 2. Seasonal HPI values for Cd and Pb contamination at monitoring stations

HPI	S1		S2		S3		S4	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
Spring	0	0	28.02	174	19.98	68.40	24	76.80
Summer	0	66	16.02	78	12.00	60	28.02	84
Autumn	10.02	48	19.98	36	19.98	18	30.00	36
Winter	0	0	0	60	16.02	75.60	24.00	133.2

3.1.1. Biotic indices

3.3.1. Macroinvertebrate Community Composition

A total of 5,029 macroinvertebrate individuals were collected from the four sampling stations along the Qara-Su River, encompassing 11 orders and 16 families (Table 3). The family Chironomidae (Diptera) exhibited the highest abundance and was consistently present at all stations, reflecting its known tolerance to a broad range of environmental conditions, including degraded water quality. Conversely, pollution-sensitive taxa such as Ephemeroptera (Baetidae, Caenidae) and Trichoptera (Hydropsychidae) showed a declining trend in presence and abundance toward downstream stations. Notably, Caenidae was absent at station S4, and the overall richness of sensitive taxa decreased from S1 to S4. This spatial trend indicates increasing environmental stress and degradation downstream, likely linked to cumulative anthropogenic impacts. Additionally, families known for higher tolerance to pollution, such as Tubificidae (Oligochaeta), Glossiphoniidae (Hirudinea), and Simuliidae (Diptera), were also prevalent across stations, including S4. The observed shifts in community composition and the dominance of tolerant species at downstream sites indicate the influence of pollution gradients and the diagnostic value of macroinvertebrates as bioindicators of river health.

3.3.2. Seasonal Water Quality Variations

Water quality parameters were monitored in situ and ex-situ across all four seasons at each station (Table 4). Seasonal patterns showed clear fluctuations in dissolved oxygen (DO), nutrient concentrations, pH, temperature, and flow, which were closely linked to shifts in macroinvertebrate assemblages. DO generally peaked during spring and remained high in winter, conditions that favor oxygen-sensitive taxa. In contrast, DO was lowest in summer and declined again in autumn, reflecting increased temperatures, reduced flow, and higher nutrient loads. Nitrate and phosphate concentrations followed the opposite trend, showing their highest levels during summer and remaining elevated into autumn, consistent with seasonal agricultural runoff, livestock inputs, and aquaculture activity. Temperature displayed the expected pattern of warmer summer conditions and cooler winter values, while pH remained near neutral throughout the year but showed a slight seasonal depression during summer. On the other hand, flow was greatest in spring and lowest in summer, likely driven by hydrological factors such as precipitation patterns, evapotranspiration, and snowmelt dynamics.

Table 3. A list of macro-invertebrates recorded in four stations on the Qara-Su River.

Phylum	Class	Order	Family	S1	S2	S3	S4
Arthropoda	Insecta	Ephemeroptera	Baetidae	*	*	*	*
			Caenidae	*	*		
Arthropoda	Insecta	Trichoptera	Hydropsychidae	*	*	*	*
Arthropoda	Insecta	Diptera	Chironomidae	*	*	*	*
			Simuliidae	*	*	*	*
			Tabanidae	*	*	*	*
			Dicranota	*	*	*	
Arthropoda	Insecta	Isopoda	Aselidae	*	*	*	*
Arthropoda	Insecta	Coleoptera	Hydraenidae	*		*	*
Arthropoda	Copepoda	Cyclopoida	Cyclopidae	*	*	*	*
Arthropoda	Collembola	Entomobryomorph	Isotomidae	*			*
Mollusca	Gastropoda	Prosobranchiata	Viviparidae	*	*	*	
		Hygrophila	Lymnaeidae	*	*	*	*
Annelida	Clitellata	Hirudinida	Glossiphoniidae	*	*	*	*
Annelida	Oligochaeta	Haplotaxids	Tubificidae	*	*	*	
			Lumbricina	*	*	*	*

A comparison among stations within each season revealed a consistent spatial gradient in water quality. The upstream reference station (S1) demonstrated the lowest nutrient concentrations and highest DO, indicating minimal anthropogenic influence. Conditions progressively shifted at S2 and S3, where moderate increases in nutrients and corresponding declines in DO reflected inputs from rural settlements, livestock activity, and household wastewater. The downstream station (S4), directly influenced by aquaculture discharges and cumulative upstream effects, consistently exhibited the highest nutrient levels and lowest DO across all seasons. This longitudinal pattern, combined with seasonal variation, likely contributed to the proliferation of pollution-tolerant macroinvertebrate taxa at downstream reaches, particularly during warm, low-flow periods.

Table 4 Physicochemical parameters of Qara-Su river by season and station (Mean ± SD).

Season	Station	Temp (°C)	PO_4^{3-} (mg/L)	NO_3^- (mg/L)	Flow (m^3/s)	pH	DO (mg/L)
Summer	S1	13.9 ± 0.5	1.87 ± 0.3	54.93 ± 7.5	1.69 ± 0.3	6.90 ± 0.1	11.4 ± 0.9
	S2	14.2 ± 0.6	2.19 ± 0.4	67.96 ± 9.2	2.21 ± 0.4	6.85 ± 0.1	10.6 ± 1.0
	S3	14.4 ± 0.7	2.43 ± 0.5	76.73 ± 11.0	2.38 ± 0.5	6.73 ± 0.2	9.7 ± 1.1
	S4	14.7 ± 0.6	2.79 ± 0.5	84.82 ± 13.5	3.16 ± 0.5	6.77 ± 0.2	8.5 ± 0.8
Autumn	S1	11.0 ± 0.4	1.39 ± 0.3	41.86 ± 6.5	3.98 ± 0.5	7.10 ± 0.1	12.4 ± 1.2
	S2	11.1 ± 0.5	1.92 ± 0.4	57.93 ± 9.8	4.29 ± 0.6	6.90 ± 0.2	11.1 ± 1.1
	S3	11.3 ± 0.6	2.17 ± 0.5	71.77 ± 11.7	4.51 ± 0.6	6.85 ± 0.2	10.2 ± 1.0
	S4	11.5 ± 0.5	2.92 ± 0.6	88.40 ± 14.2	5.10 ± 0.7	7.00 ± 0.1	8.9 ± 0.9
Winter	S1	7.9 ± 0.5	2.11 ± 0.3	2.83 ± 1.0	3.41 ± 0.4	6.90 ± 0.1	11.2 ± 1.0
	S2	8.0 ± 0.5	2.42 ± 0.4	5.92 ± 1.3	3.82 ± 0.4	6.85 ± 0.2	10.9 ± 0.9
	S3	8.3 ± 0.6	2.59 ± 0.5	8.37 ± 1.7	4.07 ± 0.5	6.78 ± 0.2	9.9 ± 0.8
	S4	8.1 ± 0.5	2.72 ± 0.5	11.24 ± 1.8	4.42 ± 0.5	6.82 ± 0.1	9.2 ± 0.7
Spring	S1	11.7 ± 0.5	0.52 ± 0.1	13.87 ± 2.8	3.92 ± 0.5	7.73 ± 0.1	10.8 ± 1.0
	S2	12.0 ± 0.6	0.69 ± 0.2	23.94 ± 3.9	4.36 ± 0.5	7.61 ± 0.1	10.4 ± 0.9
	S3	12.3 ± 0.5	0.81 ± 0.2	31.68 ± 4.6	4.61 ± 0.6	7.55 ± 0.2	9.6 ± 0.8
	S4	12.4 ± 0.6	0.98 ± 0.3	34.43 ± 5.1	4.99 ± 0.7	7.67 ± 0.2	9.0 ± 0.8

3.3.3. Bioaccumulation Indices

To evaluate the bioaccumulation potential of heavy metals in aquatic organisms, two widely used indices, Bioconcentration Factor (BCF) and Biota–Sediment Accumulation Factor (BSAF), were calculated for the most frequently encountered macroinvertebrates: Hydropsychidae and Hirudinea species (Table 5). Hydropsychidae exhibited higher BCF values for both Pb (5.26) and Cd (3.22), compared to lower values in Hirudinea (Pb: 3.75; Cd: 2.55). This pattern aligns with reported trends where filter-feeding taxa such as Hydropsychidae accumulate metals more efficiently than deposit feeders like Hirudinea, due to their constant exposure to suspended particulates and dissolved contaminants in lotic systems(Evans et al., 2006; Watson et al., 2024). Similarly, the BSAF values, reflecting the accumulation of metals from sediments, were also higher in Hydropsychidae (35 for Pb and 29 for Cd) than in Hirudinea (29 and 23, respectively). The findings indicate that both taxa accumulate heavy metals from the water column and benthic substrates. Importantly, all recorded BCF and BSAF values exceeded commonly accepted ecological safety thresholds, indicating a significant risk of metal transfer through aquatic food webs and possible adverse effects on higher trophic levels.

Table 5. BCF and BSAF of Pb and Cd in macroinvertebrates

Organism	BCF (Pb)	BCF (Cd)	BSAF (Pb)	BSAF (Cd)
Hydropsychidae sp.	5.26	3.22	35	29
Hirudinea sp.	3.75	2.55	29	23

3.3.4. Biotic Water Quality Indices: HFBI and BMWP

The Hilsenhoff Family Biotic Index (HFBI) and the Biological Monitoring Working Party (BMWP) index were used to evaluate ecological quality across stations and seasons based on macroinvertebrate assemblages (Table 6). Together, these indices revealed spatial and temporal trends reflecting organic pollution levels and habitat conditions in the Qara-Su River. The HFBI scores generally indicated good to very good water quality across stations, with occasional classification as excellent in upstream reaches. A gradual increase in HFBI values was observed from upstream (S1–S2) to downstream (S3–S4) stations, particularly during the autumn and winter seasons, suggesting elevated organic load or reduced oxygen availability at the lower reaches of the river. This trend is consistent with observed drops in DO and increased nutrient concentrations downstream, indicating a relationship between abiotic stressors and macroinvertebrate community response.

Similarly, the BMWP index showed the highest ecological quality in the upstream stations, particularly during spring and summer, corresponding with the presence of pollution-sensitive taxa such as Ephemeroptera and Trichoptera. In contrast, downstream stations (S3 and S4) consistently recorded lower BMWP scores, especially in winter, pointing to localized pollution or habitat degradation. These results align with previous findings on heavy metal accumulation and reduced biotic diversity at lower stations. Notably, both indices responded similarly to environmental gradients but offered complementary strengths: HFBI was more sensitive to organic pollution, while BMWP reflected broader ecological integrity, including habitat quality and diversity. Seasonal shifts, particularly the decline in index values during summer and winter, highlight the influence of flow reduction, temperature variation, and pollution loading on benthic macroinvertebrate structure.

Overall, the biotic indices provided strong evidence of declining ecological conditions from upstream to downstream, likely driven by the cumulative impacts of organic enrichment, reduced oxygen levels, and contaminant accumulation. These patterns reinforce the need for integrated biomonitoring approaches and suggest that Hydropsychidae, as a moderately pollution-tolerant taxon, can serve as reliable indicators for long-term ecological assessments in this river system.

Table 6. Seasonal variation in BMWP and HFBI scores across stations in the Qara-Su River (2021-2022)

Season	Station	HFBI	Class	BMWP	Class
Summer	S1	4.27 ±0.01^a	G	34.80±0.2^a	FP
	S2	4.61±0.01^b	G	35.30±0.3^a	FP
	S3	3.89±0.01^c	VG	27.80±20^b	FP
	S4	4.57±0.00^c	G	27.85±0.85^b	FP
Autumn	S1	3.87±0.00^a	VG	27.05±0.05^a	FP
	S2	3.65±0.06^b	E	26.75±0.25^a	FP
	S3	4.61±0.01^c	G	21.55±0.45^b	P
	S4	4.67±0.00^c	G	19.15±0.15^c	P
Winter	S1	4.42±0.02^a	G	25±0.00^a	P
	S2	3.96±0.01^b	VG	26.1±0.1^b	FP
	S3	4.27±0.02^c	G	20.0±0.00^c	P
	S4	4.82±0.02^d	G	17.15±0.05^d	P
Spring	S1	3.89±0.00^a	VG	35±0.00^a	FP
	S2	4.52±0.02^b	G	32.0±0.10^b	FP
	S3	4.15±0.01^c	VG	28.15±0.15^c	FP
	S4	4.57±0.01^b	G	23.10±0.20^d	P

E: Excellent, G: Good, P: Poor, VG: Very Good, FP: Fairly Polluted.

3.3.5. Diversity indices

The taxonomic structure of macroinvertebrate communities was further evaluated using the Shannon–Wiener diversity index (H′), Simpson's index (1–D), dominance index (D), and evenness index (E), with results illustrated in Figure 2. The analysis revealed that S1 and S3 consistently exhibited higher diversity, evenness, and ecological stability, while S2 and S4 demonstrated signs of reduced diversity and greater dominance by a few taxa. Specifically, higher values of the Shannon and Simpson indices at Stations 1 and 3 reflect greater taxonomic richness and a more balanced species distribution, suggesting favorable habitat conditions and lower environmental stress. In contrast, lower Shannon and Simpson values at Stations 2 and 4 coincide with higher dominance index scores, indicating that macroinvertebrate communities at these sites were more uneven and likely dominated by a few tolerant species. This pattern suggested that biotic homogenization could be occurring in response to environmental degradation, possibly driven by increased pollutant loads or habitat simplification downstream.

Evenness values further reinforce this interpretation, with higher evenness observed at the more ecologically stable Stations 1 and 3, while reduced evenness at Stations 2 and 4 signals competitive exclusion or environmental filtering, where only a limited number of taxa thrive under stressful conditions. Taken together, the diversity metrics presented in Figure 2 demonstrate a clear spatial gradient in ecological integrity, with upstream and midstream stations supporting more diverse and evenly distributed macroinvertebrate communities, and downstream sites showing signs of community simplification. These findings are consistent with the observed declines in water quality, biotic index scores, and increased metal bioaccumulation at downstream stations, reinforcing the utility of diversity indices as sensitive tools for ecological assessment in riverine environments.

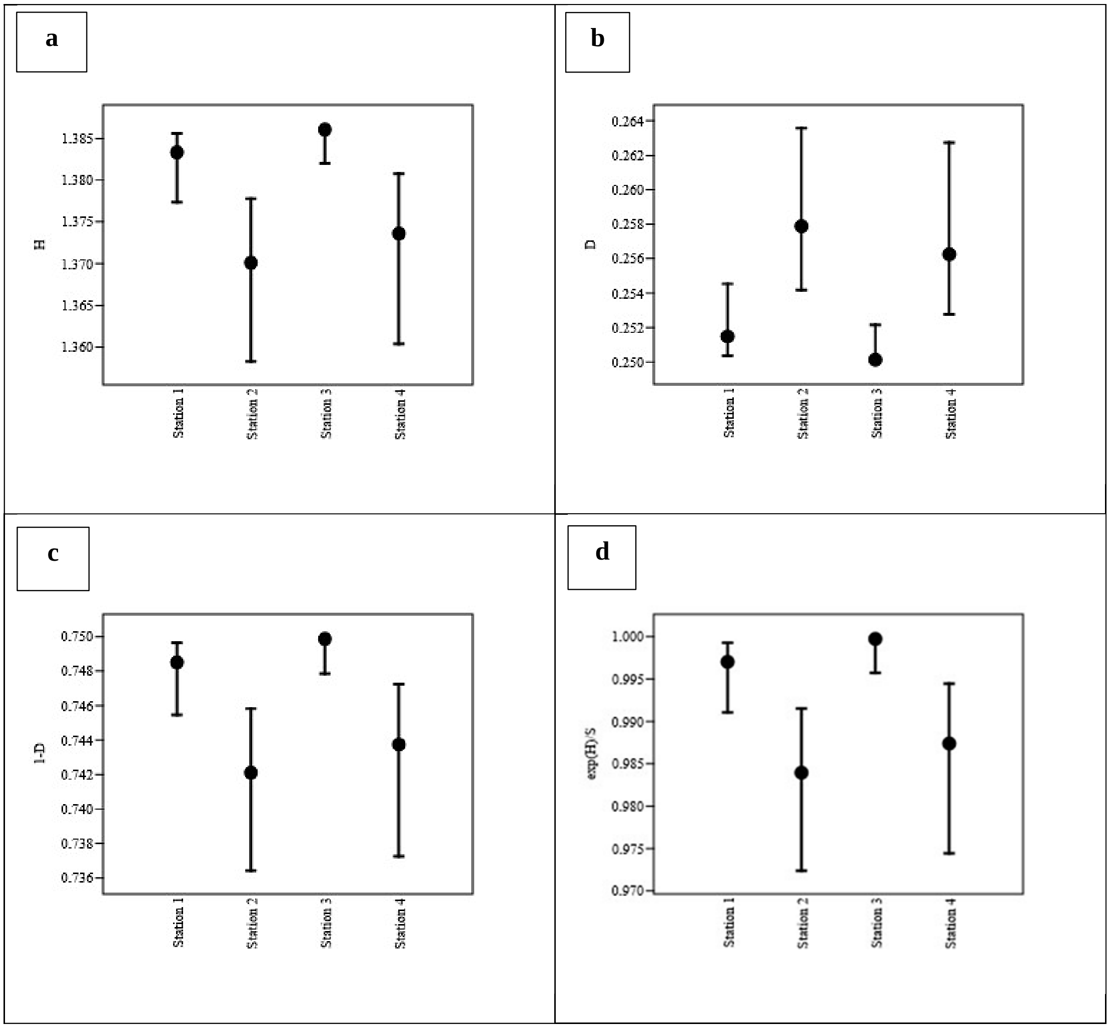

Figure 2. Taxonomic diversity (a Shannon-Wiener, b Dominance, c Simpson, d evenness index) at the four sampling sites in the Qara-Su River

3.4. Correlation Between Biotic and Abiotic Indices

Correlation analysis was conducted to explore the relationships between biotic indices (HFBI and BMWP) and abiotic pollution indicators (HPI, Igeo, and RI), with the results illustrated in Figure 3. The HFBI index exhibited a positive correlation with both HPI and Igeo, suggesting that increases in heavy metal contamination and sediment-associated pollution are reflected in the macroinvertebrate community structure, particularly in response to organic and chemical stress. This correlation supports the use of HFBI as a sensitive indicator for detecting anthropogenic impacts in freshwater systems. Similarly, the BMWP index showed positive correlations with Igeo and HPI, particularly for Cd, indicating that this biotic index is responsive to sediment-bound and waterborne metal contamination. Interestingly, BMWP displayed a negative correlation with HPI for Pb (r = –0.409), implying that Pb contamination could exert a more specific or distinct ecological pressure not linearly reflected in BMWP scores. The potential ecological risk index (RI) showed a weak positive correlation with HPI, but a stronger correlation with Igeo, reinforcing the interpretation that sediment quality is a key determinant of ecological risk, particularly in benthic habitats. Among all relationships, the strongest correlation was observed between BMWP and Igeo, highlighting a robust linkage between sediment-associated contamination and biotic responses. Additionally, a direct positive correlation was identified between HFBI and BMWP, confirming internal consistency between the two biotic indices in tracking ecological integrity along the pollution gradient. These patterns underscore the interdependence of biological and physicochemical indicators and highlight the importance of integrating multiple indices for a comprehensive assessment of river health.

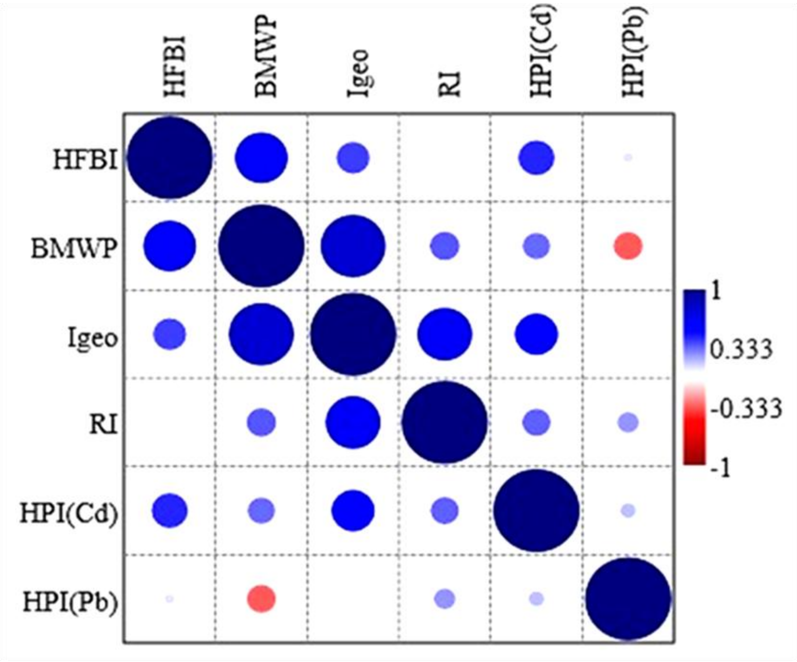

Figure 3. Correlation between biotic and abiotic indices.

4. Discussion

In recent years, integrating biotic and abiotic indicators has become a widely adopted approach in aquatic ecosystem monitoring, offering a holistic perspective on ecological health and pollution dynamics (Dong et al., 2023; Modley et al., 2020). This study applied such an integrative framework to assess the environmental quality of the Qara-Su River by combining chemical, ecological, and biological indices to uncover pollution trends, identify site-specific risks, and elucidate drivers of ecological degradation. Heavy metal analysis revealed that average sediment concentrations of Pb and Cd remained below internationally recognized freshwater sediment thresholds (35.8 mg/kg for Pb and 0.99 mg/kg for Cd) (Long et al., 2013), indicating that sediment-associated contamination does not pose a widespread environmental threat. However, further evaluation using abiotic indices, namely Igeo, Er, and RI, offered a more nuanced perspective. While the majority of stations fell within “unpolluted” or “low ecological risk” categories, station S3 emerged as a distinct outlier, exhibiting consistently elevated values across all three indices. This localized enrichment is likely associated with the presence of the Sabalan Dam, which alters sediment transport, hydrological flow, and geochemical conditions upstream and downstream. The influence of dams on sedimentation dynamics and metal mobilization is well established (Lee et al., 2022). They tend to trap fine sediments enriched with metals, alter redox conditions that favor metal remobilization, modify flow regimes that destabilize sediment structure, and promote chemical shifts that enhance metal solubility and bioavailability (Hahn et al., 2018; Schmutz & Moog, 2018). Such changes are conducive to the localized accumulation of pollutants, particularly in slow-moving or anoxic zones.

In addition to dam-induced effects, diffuse pollution from agricultural runoff, livestock operations, and rural sewage likely contributed to the elevated contaminant load observed at S3. Although S1 was selected as the reference site, the unexpectedly higher RI values at this station reflect the influence of diffuse upstream inputs and seasonal hydrological variations, which can increase metal accumulation even in relatively undisturbed segments of the river. Evidence from similar fluvial systems suggests that the combined application of Igeo, Er, and RI indices is effective in distinguishing broader pollution trends from site-specific ecological risks (Nakhaei et al., 2024). With regard to water quality, Pb concentrations in the water column (0.133 mg/L) exceeded regulatory standards for both aquatic life (0.05 mg/L) and surface water (0.005 mg/L), with the highest levels recorded at S3 and S4 (Shanbehzadeh et al., 2014). These elevated concentrations appear to be driven by a combination of sediment resuspension due to dam operations and direct inputs from nearby aquaculture facilities. Variability in metal concentrations downstream of dams has been linked to fluctuations in discharge and metal solubility, while aquaculture effluents, particularly from protein-rich feeds, have been identified as important contributors to organic and metal enrichment in freshwater systems (Hahn et al., 2018; Liu et al., 2024).

Bioconcentration (BCF) and Biota-Sediment Accumulation (BSAF) factors derived from macroinvertebrates provided additional insight into the bioavailability and trophic transfer potential of Pb and Cd in the river. Both metrics exceeded acceptable ecological thresholds, with the Hydropsychidae family displaying substantially higher accumulation levels compared to Hirudinea. This disparity is consistent with known ecological and physiological traits: Hydropsychidae, as filter feeders, continuously process large volumes of water and particulates, increasing their exposure to dissolved and suspended metals, whereas Hirudinea feed more intermittently on detritus and organic matter, resulting in lower uptake. These findings align with ecological assessments that emphasize the influence of trophic strategies, feeding modes, and habitat use on contaminant bioaccumulation in benthic communities (Kalantzi et al., 2013; Kowobari et al., 2024). Biotic indices further reinforced these patterns. The Hilsenhoff Family Biotic Index (HFBI) generally classified water quality as “good,” though a decline in ecological condition was detected at downstream stations in parallel with increased metal concentrations and nutrient enrichment. In contrast, the Biological Monitoring Working Party (BMWP) index, more sensitive to shifts in pollution-intolerant taxa, offered a more critical classification, indicating poor water quality at stations S3 and S4. This divergence stresses the limitations of relying on single indices and highlights the added value of multi-index approaches. Observed shifts in macroinvertebrate assemblages, including increased representation of tolerant taxa such as Chironomidae, Simuliidae, and Tubificidae, support the interpretation that effluent discharge and metal stressors are driving biotic degradation at downstream locations (Hatami et al., 2011).

On the other hand, the correlation analyses revealed strong interconnections between chemical and biological indicators. Significant relationships between Igeo and both HFBI and BMWP confirm that sediment-associated contamination was the major determinant of macroinvertebrate community structure. The observed negative correlation between BMWP and the Pb-based Heavy Metal Pollution Index (HPI) suggests that Pb, in particular, exerts disproportionate effects on sensitive taxa, reducing richness and community evenness. Meanwhile, the positive correlation between HFBI and BMWP indicates that these biotic metrics capture overlapping but distinct aspects of ecological condition. To summarize, these findings affirm the value of an integrated monitoring framework that synthesizes abiotic and biotic indicators. Such an approach enables early detection of environmental stress, enhances diagnostic capacity, and informs adaptive river management strategies by linking chemical signals to biological outcomes.

Conclusion

The integrated assessment of the Qara-Su River, based on both abiotic and biotic indicators, revealed a predominantly medium-risk contamination profile with localized pollution hotspots. While abiotic indices, including I_{geo}, Er, and RI, generally classified sediment quality as unpolluted, station S3 emerged as a notable exception, exhibiting elevated risk levels. This localized risk is likely driven by hydrological alterations associated with the Sabalan Dam, which can reduce flow velocity and enhance contaminant deposition. Consistent upstream-to-downstream increases in nutrient concentrations and declines in dissolved oxygen further reflected cumulative agricultural, domestic, and aquaculture pressures along the river. Pb concentrations exceeded surface water quality standards at downstream stations, particularly S3 and S4, reinforcing concerns about pollutant accumulation in these reaches.

Biological assessments complemented these findings. Bioaccumulation factors (BCF and BSAF) indicated substantial uptake of Pb and Cd by macroinvertebrates, with Hydropsychidae exhibiting greater metal accumulation than Hirudinea, likely due to their feeding behavior and habitat exposure. The observed negative correlation (r = –0.409) between the BMWP index and the Pb-based HPI emphasized the ecotoxicological impact of Pb, linking elevated concentrations with reduced macroinvertebrate diversity and sensitivity. Moreover, the degradation of both the HFBI and BMWP indices at downstream stations (S3 and S4) further indicated water-quality deterioration, consistent with the observed spatial gradients in nutrient enrichment, reduced oxygen availability, and increased contaminant exposure.

These findings highlight the need for targeted pollution control strategies, particularly in dam-influenced zones and near fish-farm discharge points. Without timely and location-specific interventions, ongoing contamination could impair aquatic food webs, reduce biodiversity, and lead to broader ecological imbalances. Future studies should prioritize regular ecological health assessments through long-term biomonitoring programs to support effective evaluation, adaptive management, and early detection of emerging pollution threats.

Recommended actions include implementing sediment-flushing or controlled flow releases downstream of the Sabalan Dam to help reduce pollutant buildup, and enforcing stricter waste-management and effluent-treatment practices for fish farms operating near stations S3 and S4. Establishing routine biomonitoring programs, combining macroinvertebrate indices with periodic metal assessments, is also advised to enable early detection and timely mitigation of emerging pollution hotspots.

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Author Contributions:

Ehsan Asadisharif: Software/statistical analysis, writing the initial version of the article.

Shima Rahim Pouran: Conceptualization, Guidance, analysis of data, Statistical analysis, Writing the initial version of the manuscript, Editing and reviewing the article, and Controlling the results.

Amin Salimi: Sampling and field visits, Investigation, and laboratory experiments.

Abolfazl Bayrami: Conceptualization, Guidance, Resources, Editing and reviewing the article, and Controlling the results.

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The authors declare no conflict of interest regarding the authorship or publication of this manuscript.

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The datasets are available upon a reasonable request to the corresponding author.

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