The University of Tehran Press

Pollution

Print ISSN: 2383-451X
Online ISSN: 2383-4501

https://jpoll.ut.ac.ir/

Impact of Physical-Chemical Parameters on Benthic Macroinvertebrate Ecological Distribution: A Case Study of the Oum Er Rbia River

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Article Info

Article type:
Research Article

Article history:
Received: 3 November 2025
Revised: 18 February 2026
Accepted: 2 April

Keywords:
Biomonitoring,
Benthic macroinvertebrates,
IBGN,
Organic pollution,
PCA,
Oum Er Rbia River

ABSTRACT

The Oum Er Rbia River is an ecosystem that is essential for biodiversity and human activities, but it is threatened by the degradation of its aquatic habitats, particularly due to the discharge of domestic wastewater. The assessment of water quality, carried out using physicochemical and biological approaches, shows significant variations between sampling stations. Stations S1 and S2 exhibited optimal physico-chemical characteristics for biodiversity, complying with water quality standards (e.g., BOD_5 below 3 mg/L). This was reflected in the biotic communities, which showed high taxonomic diversity and a dominance of highly sensitive groups such as Ephemeroptera and Plecoptera. In contrast, station S3 has very poor water quality, with BOD_5 of 48 mg O_2/L and COD of 239 mg/L, as well as Dissolved Oxygen (DO) to 5 mg/L. The IBGN is very low 5 < IBGN < 8, with a dominance of polluent-tolerant taxa, such as Chironomidae, reflecting a significant loss of biodiversity. At the downstream station S4, BOD_5 decreases to 11 mg O_2/L and COD to 38 mg/L. The IBGN reaches 13 and the OPI is 3.25, indicating moderate pollution. Statistical analysis confirmed that organic pollution is the principal driver of water quality degradation and biological impairment along the river, with principal component analysis (PCA) clearly demonstrating the strong relationship between organic load, physicochemical parameters, and macroinvertebrate community structure. This study provides crucial information to guide conservation and restoration efforts for the Oum Er Rabia ecosystem.

Cite this article: Bouasria, H., Bahouar, E.H,, Mrabet, L., S'hih, Y., Loukili, A., & Abba, E,H. (2026). Impact of Physical-Chemical Parameters on Benthic Macroinvertebrate Ecological Distribution: A Case Study of the Oum Er Rbia River . *Pollution*, 12(2), 515-532.
https://doi.org/10.22059/poll.2026.405316.3185

DOI: https://doi.org/10.22059/poll.2026.405316.3185

INTRODUCTION

Water is a natural resource that is essential to life and the proper functioning of ecosystems (Tampo et al., 2015; Badil, 2024). However, the availability and quality of this resource is a major challenge for modern societies, which are faced with ever-increasing demand for water, the increasing scarcity of water resources, and various environmental challenges (El Houssaine et al., 2025). In this context, in order to meet the requirements of sustainable water resource management, monitoring the ecological status and quality of watercourses has become a priority in many countries (Sawadogo, 2018). Freshwater ecosystems are among the most threatened

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and vulnerable natural environments, subject to considerable human pressures, particularly related to urbanization, intensive agriculture, and domestic wastewater (Bahouar et al., 2025; Mrabet et al., 2025). These factors lead to significant alterations in the physical, chemical, and hydrological conditions of watercourses, with direct repercussions on aquatic biodiversity (Camara et al., 2014).Aquatic macroinvertebrates are living organisms that play a crucial and key role in freshwater ecosystems, actively participating in the nutrient cycle, the decomposition of organic matter, and the preservation of trophic balances(Macadam and Stockan, 2015)**.** They are reliable bioindicators of water quality due to their sensitivity to physico-chemical variations in the environment (Bouasria et al., 2025; Espinar-Herranz et al., 2025). In addition, the variety of responses of different species to environmental stress makes them particularly suitable for biomonitoring programs (Dolédec & Statzner, 2008; Yadamsuren, O et al., 2020)**.** The use of benthic macroinvertebrates as biological monitoring tools is common (Berrahou et al., 2001; Mabrouki et al., 2019; El Alami, 2021; Ouma et al., 2025). These organisms have varying sensitivity to pollution, which allows them to reflect contamination levels through their abundance. In unaffected watercourses typically exhibit high species richness, whereas increased anthropogenic pressures alter faunal composition and reduce biodiversity (Bouasria et al., 2025; Tampo et al., 2021; Nedeau et al., 2003). The Oum Er Rbia River is exposed to multiple pressures, including direct discharge of domestic wastewater. This likely affects the composition and distribution of macroinvertebrates due to spatial changes in water quality in Khénifra. This study aims to assess physicochemical variations in water from upstream to downstream of Khénifra. Also, the impact of these variations on the structure, distribution, and diversity of aquatic macroinvertebrate communities using standardized biological indices. Hypothesize that water quality deteriorates downstream, causing significant changes in the biological composition of the community.

MATERIALS AND METHODS

Study area

This study was conducted in the Khenifra region, which is part of the Beni Mellal-Khenifra administrative region, in the central Moroccan Middle Atlas. The Oum Er Rbia River is one of the most important rivers in Morocco, with a length of 550 km. It originates in the Middle Atlas, at an altitude of 1800 meters, and flows over a wide area before emptying into the Atlantic Ocean at the town of Azemmour (Figure 1). Due to its geographical location, the Khénifra region is characterized by its varied terrain and clay soils resulting from the weathering of basaltic rocks, which favors market gardening as the main activity of the population.

The Middle Atlas is characterized by a cold humid climate and is classified as a mountain Mediterranean climate (Martin, 1981). Precipitation varies greatly, ranging from 1100 mm/year in the upstream areas (Middle Atlas) to 300 mm/year downstream, with an overall average estimated at 550 mm/year (Barakat et al., 2016).

Sampling sites and stations

Four stations were chosen based on specific criteria, the choice of stations was made on the basis of a set of criteria that meet the objectives of our study, these are criteria of representativeness of the stations in relation to the section studied, their location upstream and downstream of potential sources of pollution that influence the ecological integrity of the river studied and finally the ease of access and sampling (Figure 1). *Station ST1, located in El Borj Khenifra, is situated at coordinates 5.623365° W and 33.016746° N. Station ST2, corresponding to the upstream section of Khenifra, is located at 5.659333° W and 32.981083° N. Station ST3, named Chellal, is located at coordinates 5.66249007° W and 32.94209312° N. Finally, station ST4, located downstream of Khenifra, has coordinates of 5.67718053° W and 32.92154584° N.*

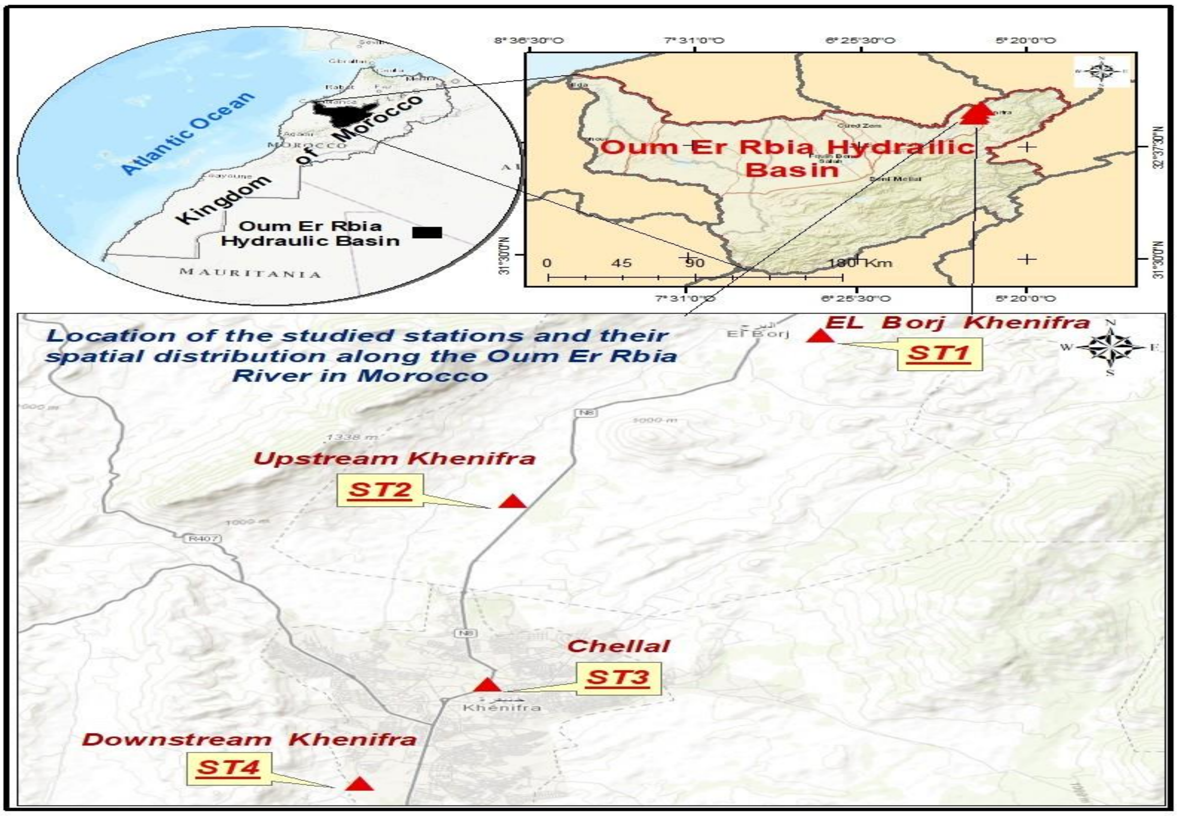

Fig. 1. Location of the different study stations.

Together, these stations provide representative spatial coverage of the studied watercourse and a relevant assessment of environmental variations along the longitudinal gradient.

Macroinvertebrate sampling

From June 2024 to May 2025, surveys were carried out in accordance with the IBGN sampling protocol (AFNOR NF T90-350) with one sample per season. All these samples should give an idea of the diversity of habitats and the greater faunal diversity of this station. In our study, macroinvertebrate collections were carried out in 4 stations (S1, S2, S3 and S4), using a Surber net and a trough net. The samples were immediately placed in marked plastic containers and fixed with 10 % formalin. The species to be identified are preserved in 70 % alcohol. In the laboratory, the macroinvertebrates collected are first washed in water, filtered through a binocular microscope and identified from reference books and keys for determining benthic macroinvertebrates (Moisan, 2010; Tachet et al, 2010).

Physicochemical and organic analyses

Abiotic variables

Physicochemical parameters were measured simultaneously during wildlife sampling and at the same stations. Parameters recorded in situ included water temperature (°C), pH, electrical conductivity (µS/cm), and dissolved oxygen concentration (% and mg/L), using a Hach HQ40 multiparameter device. *Additional laboratory analyses were performed according to the methods described by Rodier(2009) and included specific parameters such as BOD_5, COD, ammonium (NH_4^+),phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), Sulfate (SO_4^{2-}), chloride (Cl^-), total hardness (TH), and Total Alkalinity (TA).*

Organic Pollution Index (OPI)

This index was developed by (Leclercq, 2001), it allows water quality to be classified into five categories ranging from very good to very poor, according to specific thresholds for each parameter. Via a classification grid.

The Global Normalized Biological Index (IBGN)

Based on macroinvertebrate bioindicators, it is a valuable tool for assessing water quality by using macroinvertebrate fauna as an essential component of the environment, providing an overall quantitative overview of the environment. This measurement was used to establish the biological quality of the water. The AFNOR method (1992) was used to perform the calculation, with the following formula:

IBGN=GI+CV-1

This formula means that GI is the indicator group, which only groups taxa present in an appropriate quantity, and CV is the variety class, which groups all species identified in the sample. There are five quality levels for the IBGN, which can range from 0 to 20: very poor quality (less than 5), poor quality (5 to 8), average (9 to 12), good (13 to 16), and very good (17 or more). In the absence of indicator taxa, a score of 0 is assigned.

The IBGN is based on the analysis table comprising the 9 indicator faunal groups on the ordinate and the 14 taxonomic variety classes on the abscissa.

Data analysis

Principal component analysis (PCA) and linear regression were performed under R4.5.1 (https://cran.r-project.org/), to explore the relationships between physicochemical parameters, among themselves and with biological metrics, including richness-abundance clusters.

Linear regression was used to model the relationships between macroinvertebrate abundance groups and specific environmental variables, including organic pollution indicators (such as BOD_5), in order to assess the influence of physicochemical conditions on community structure.

RESULTS

Variation of Physical-Chemical Parameters at the Four Stations

The physical-chemical analysis results of water in different stations of the section studied are recorded in Figure 2.

Water temperatures recorded at the four stations ranged from 18.2°C to 20.42°C. The lowest temperature values were observed at stations S1 and S2, and the highest value was observed at stations S3.

The pH measured at the various stations along the Oum Er Rbia River ranged between 7.85 and 8.09. These values are within the optimal range for all the stations studied, indicating that the water exhibits slightly alkaline characteristics. This alkaline character is generally associated with the presence of carbonate rocks in contact with the water. According to Moroccan standards, acceptable pH values for surface waters are between 6.5 and 8.5 confirming the compliance of the analysed samples (ME-MCATUHE, 2002). The electrical conductivity recorded at the four stations ranged between 2545 and 2688 µS/cm. Although these values are relatively high, they remain below the limit set by Moroccan standard N.M 03.7.001, which establishes a maximum threshold of 2700 µS/cm for water intended for human consumption. Regarding Chloride concentrations show notable spatial variation. Station 3 recorded the highest value at 755 mg/L, classifying it as Class 4 (Poor). Stations 1 (604 mg/L), 2 (645 mg/L), and 4 (656 mg/L) belong to Class 3 (Medium). These results suggest a significant saline influence, particularly at Station

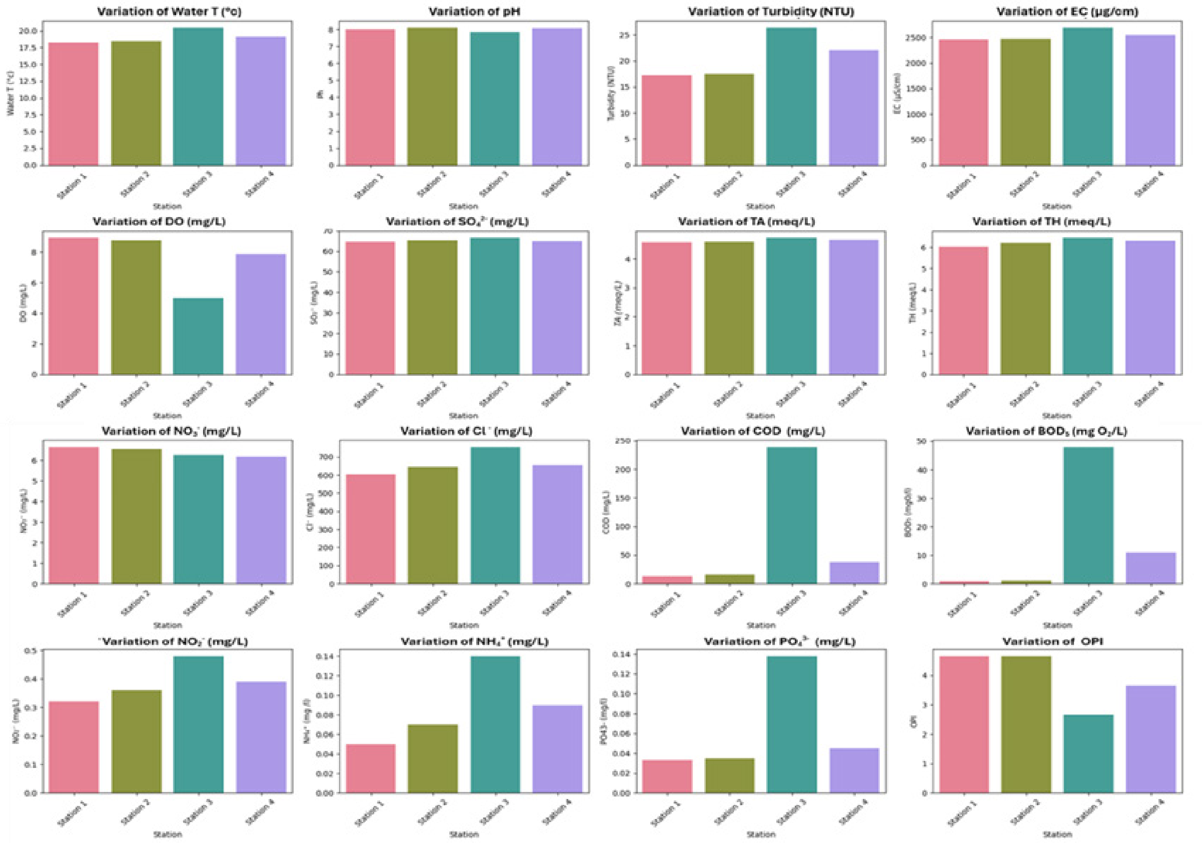

Fig. 2. Physicochemical parameters measured at the four study stations.

3, likely due to anthropogenic inputs.

Dissolved oxygen (DO) concentrations at Stations S1, S2, and S4 range between 7.88 mg/L and 9.95 mg/L. These high values classify these stations as Class 1 (Excellent) according to water quality standards. These results reflect a good level of oxygenation, indicative of a healthy aquatic environment with little impact from organic pollution. In contrast, station S3 recorded a significantly lower value of 5 mg/L. The marked drop in dissolved oxygen at station 3 indicates significant organic pollution, likely linked to the discharge of untreated wastewater directly into the aquatic environment.

The five-day biochemical oxygen demand (BOD) showed significant variation between stations. Stations 1 (1.0 mg O_2/L) and 2 (1.1 mg O_2/L) displayed low values, placing them in Class 1 (Excellent), indicating a low presence of biodegradable organic matter. Station 4, with a concentration of 9 mg O_2/L, belongs to Class 3 (Medium), indicating a moderate level of organic pollution. In contrast, station 3 recorded a critical value of 48 mg O_2/L, placing it in Class 5 (Very Poor). This exceptionally high value reflects a high organic load, probably due to the direct discharge of untreated wastewater, which constitutes a significant pressure on water quality and the ecological functioning of the environment.

A similar trend is observed for Chemical Oxygen Demand (COD). Stations S1 (13 mg/L) and S2 (16 mg/L) show low values, reflecting a low organic load. In contrast, COD increases sharply at station S3 (239 mg/L), indicating a high pollutant load. At station ST4, the value decreases to 38 mg/L, suggesting the beginning of recovery of the downstream environment.

Sulphate concentrations remain below 100 mg/L at all stations, placing them in Class 1 (Excellent), indicating a notable absence of sulphate pollution. Regarding nutrients, Stations 1 and 2 show low levels of nitrites (NO_2^-), ammonium (NH_4^+), and phosphates (PO_4^{3-}). At Station 3, these values increase significantly (NO_2: 0.48 mg/L; NH_4^+: 0.14 mg/L; PO_4^{3-}: 0.138 mg/L), suggesting the presence of nutrient-rich effluents, probably of domestic origin. These concentrations decrease slightly at Station 4, indicating partial dilution or the beginning of recovery.

Correlation Matrix between environmental parameters

The correlation analysis between environmental parameters reveals significant and consistent relationships between these variables measured at the different study stations (Figure 3).

Particularly noteworthy are strong positive correlations between turbidity and water temperature (r = 0.987), between electrical conductivity and water temperature (r = 0.998), as well as between (CAT, r = 0.996) and total hardness (TH, r = 0.940), indicating that these parameters evolve jointly under the influence of common environmental or anthropogenic factors. In addition, indicators of organic pollution such as BOD_5 (r = 0.987), COD (r = 0.965) and the Organic Pollution Index (OPI), show a strong correlation between them. There is a near-perfect negative correlation between OPI and turbidity (r = -0.999), which demonstrates the consistency of the water quality measurements. However, dissolved oxygen (DO) shows substantial negative correlations with most pollution parameters, including BOD_5 (r = -0.998), DOC (r = -0.988) and ammonium (NH_4^+, r = -0.978), signalling the deterioration of water quality associated with the increase in pollutant loads. This contradiction illustrates the standard phenomenon of reduced oxygenation of the environment in response to organic pollution. The results of this correlation between these parameters highlight a structured environmental gradient within which the increase in chemical pollution causes simultaneous changes in physicochemical conditions, thus confirming the human influence on the ecological dynamics of the aquatic environments explored.

Variation in the Composition of Benthic Fauna Among Four Stations

The study of aquatic fauna in the Oum Er Rbia River has highlighted a remarkable faunal

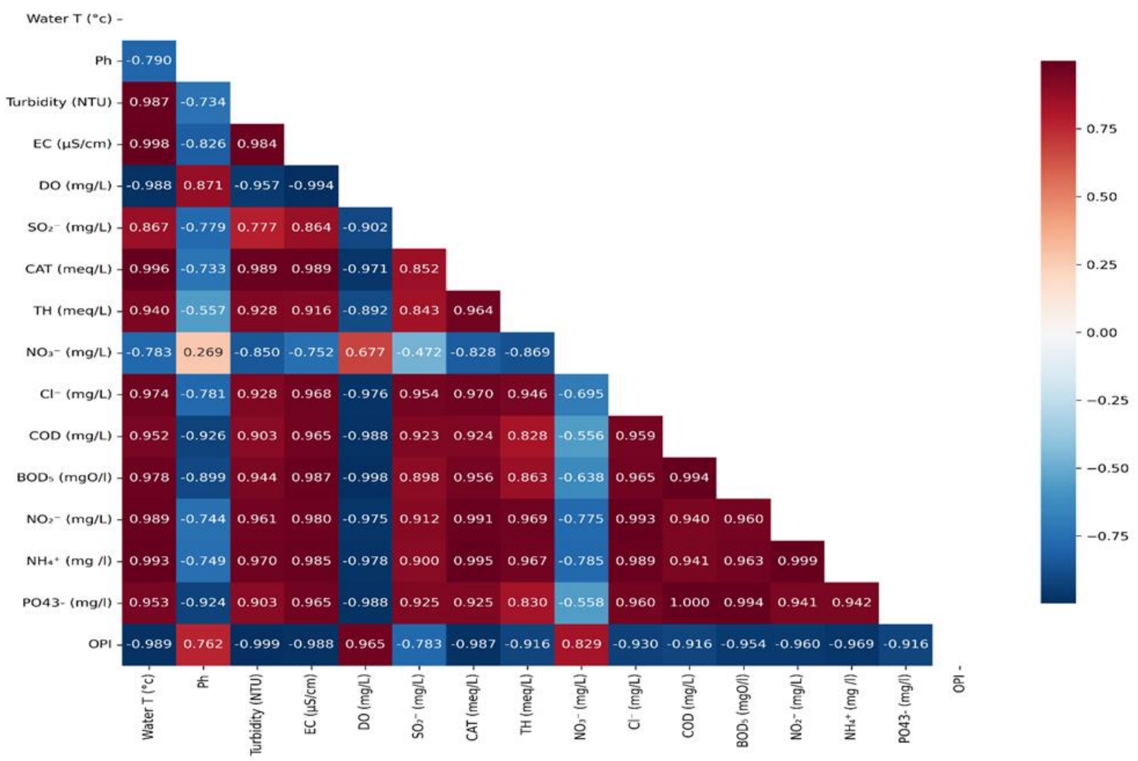

Fig. 3. Correlation between environmental parameters.

diversity distributed among several taxonomic groups. In total, 10 groups were identified, with varying proportions (Figure 4).

Diptera largely dominate all stations (38.28%), with a strong representation of Chironomidae, Simuliidae, and Limoniidae, reflecting a strong adaptation of this group to the environmental conditions of the river. Ephemeroptera occupy the second place (26.57%), with a notable representation of the families Baetidae, Caenidae, and Heptageniidae, indicators of well-oxygenated waters and diversified substrates, well represented in the less impacted stations. Next come Odonata (8.21%), Hemiptera (7.81%), and Coleoptera (6.73%), reflecting heterogeneous hydro-morphological conditions. In contrast, Molluscs (18%), Crustaceans (3.87%), Trichoptera (2.15%), and Annelids (1.16%) are the least abundant. Plecoptera, represented only by the Nemouridae family, constitute the rarest taxon (0.05%), but their presence in certain stations suggests good ecological quality of the environment.

Upstream stations S1 and S2 reveal a high taxonomic richness (29 and 33 taxa), characterized by the presence of organisms very sensitive to pollution, such as Nemouridae, Philopotamidae, and Elmidae, which reflects an ecosystem in very good ecological condition. In contrast, station 3, located in an urban area and subject to domestic wastewater discharge, has a low taxonomic richness (only 14 taxa), it is dominated by tolerant taxa such as Chironomidae and Culicidae, reflecting high organic pollution and marked ecological degradation. Station S4, located downstream, is distinguished by a recovery in taxonomic richness (28 taxa), accompanied by the return of moderately sensitive indicator groups such as Hydropsychidae, Gomphidae, and Baetidae. This recovered diversity suggests the existence of a natural self-purification process in the river, allowing a partial improvement in ecological quality downstream, despite persistent anthropogenic pressure.

Heatmap of Families Grouped by Hierarchical Clustering

The heatmap based on the presence/absence of macroinvertebrate families and organized by hierarchical classification reveals a clear structuring of the communities into four taxonomic clusters, reflecting a marked ecological gradient from upstream to downstream of the city (Figure 5).

Sites 1 and 2, located upstream, are associated with Clusters 1 and 2, respectively. Cluster 1, composed of families such as Philopotamidae, Anthomyiidae, Dixidae, Noteridae, and

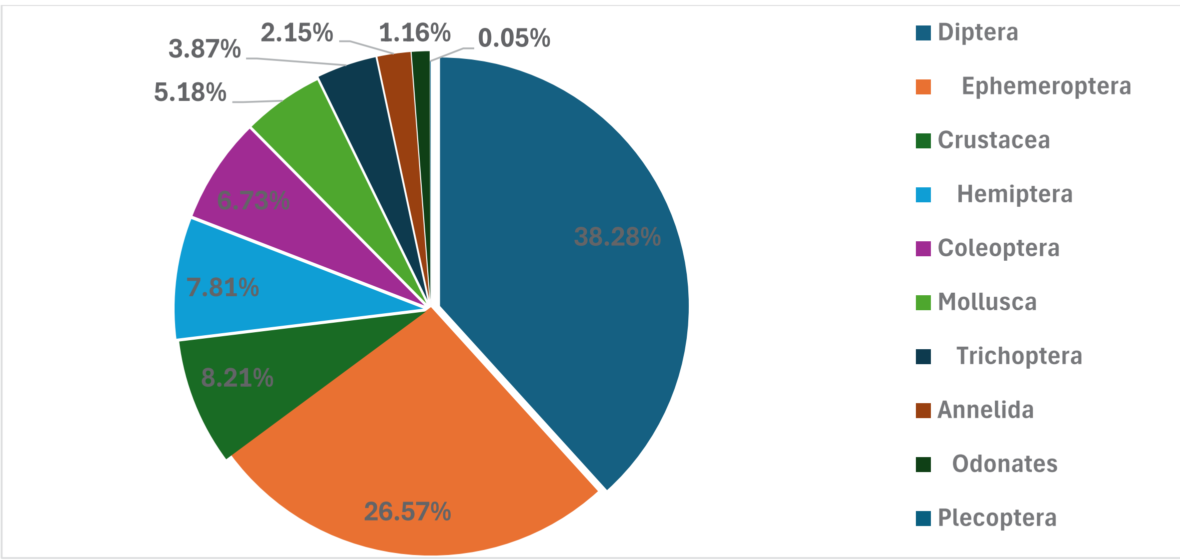

Fig. 4. Taxonomic Composition and Relative Abundance of Benthic Fauna in the Study Area.

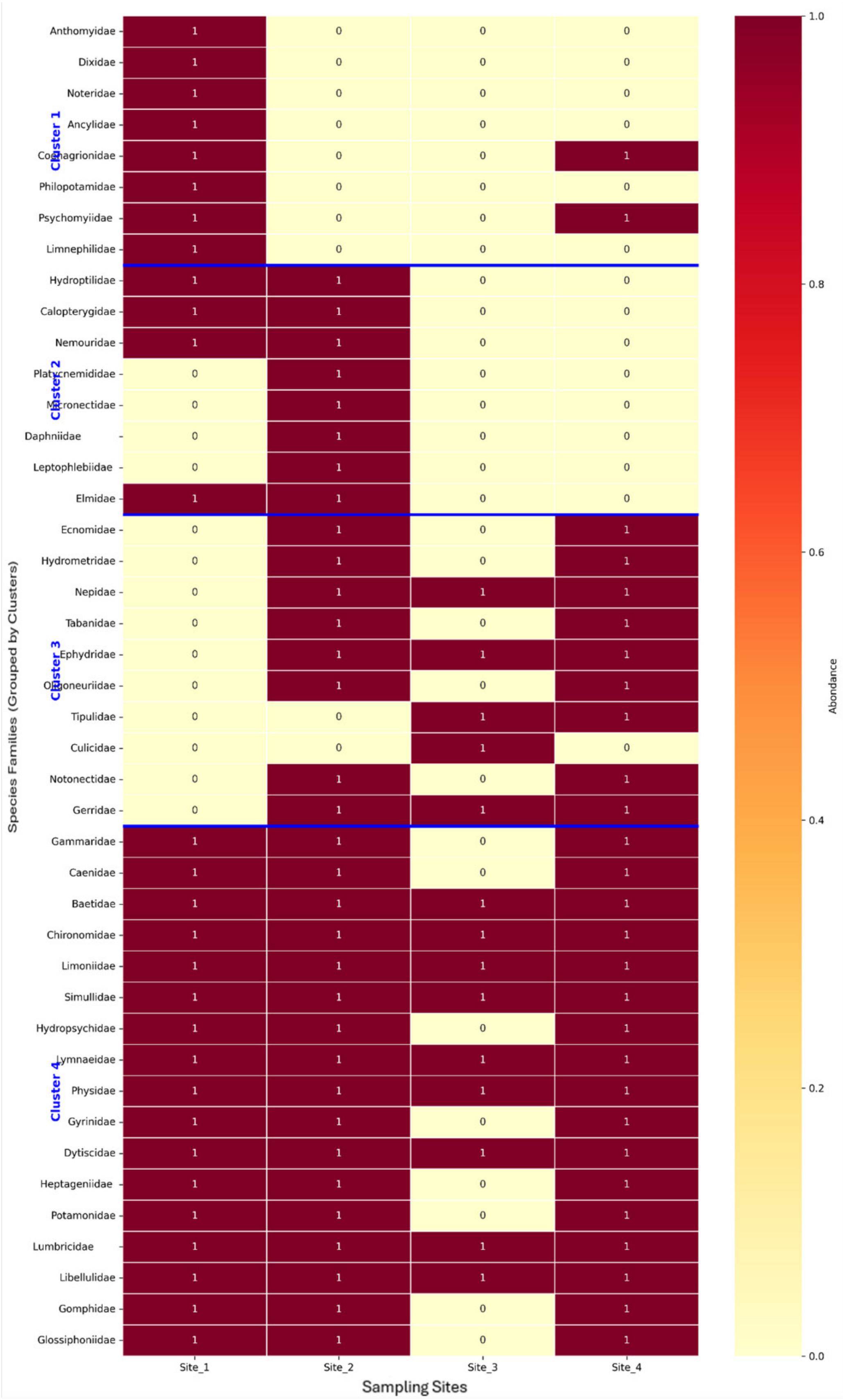

Fig. 5. List of macroinvertebrate taxa collected at the sampling sites.

Limnephilidae, is strictly present in Site 1 and reflects a well-oxygenated, minimally disturbed environment characterized by high ecological quality. Cluster 2, shared between Sites 1 and 2, includes sensitive families such as Nemouridae (order Plecoptera), Hydroptilidae, Calopterygidae, and Elmidae, confirming good environmental quality and a certain ecological continuity between these two upstream stations.

Site 3, located in the immediate vicinity of domestic wastewater discharges, is characterized by Cluster 3, dominated by pollutant-resistant families such as Chironomidae, Culicidae, Simuliidae, and Lumbricidae. The predominant presence of these organic pollution-tolerant taxa indicates significant ecological degradation, linked to organic enrichment and the reduction of dissolved oxygen caused by domestic effluent. Finally, Site 4, located downstream and corresponding to Cluster 4, includes families such as Gammaridae, Caenidae, Hydropsychidae, Heptageniidae, Potamonidae, Gomphidae, among others, which were absent from Site 3. The presence of these families, even if they sometimes appear in low abundance compared to the upstream stations (Sites 1 and 2), indicates a partial recovery of water quality. This faunal composition reflects an intermediate ecological quality, illustrating the self-purification capacity of the environment downstream of the polluted area and the beginnings of restoration of environmental conditions.

The analysis focuses on statistically significant correlations, which highlight robust links between species clusters and environmental conditions (Figure 6). Among them, cluster 4 stands out for the most marked and significant correlations with the measured parameters. It presents very strong positive correlations with dissolved oxygen, both for specific richness and for species abundance (Pearson coefficient = 0.967, $p \leq 0.05$). This indicates that the species grouped in Cluster_4 are particularly adapted or favoured by well-oxygenated environments, which can be interpreted as a sign of good water quality, especially at sites 1 and 2. Furthermore,

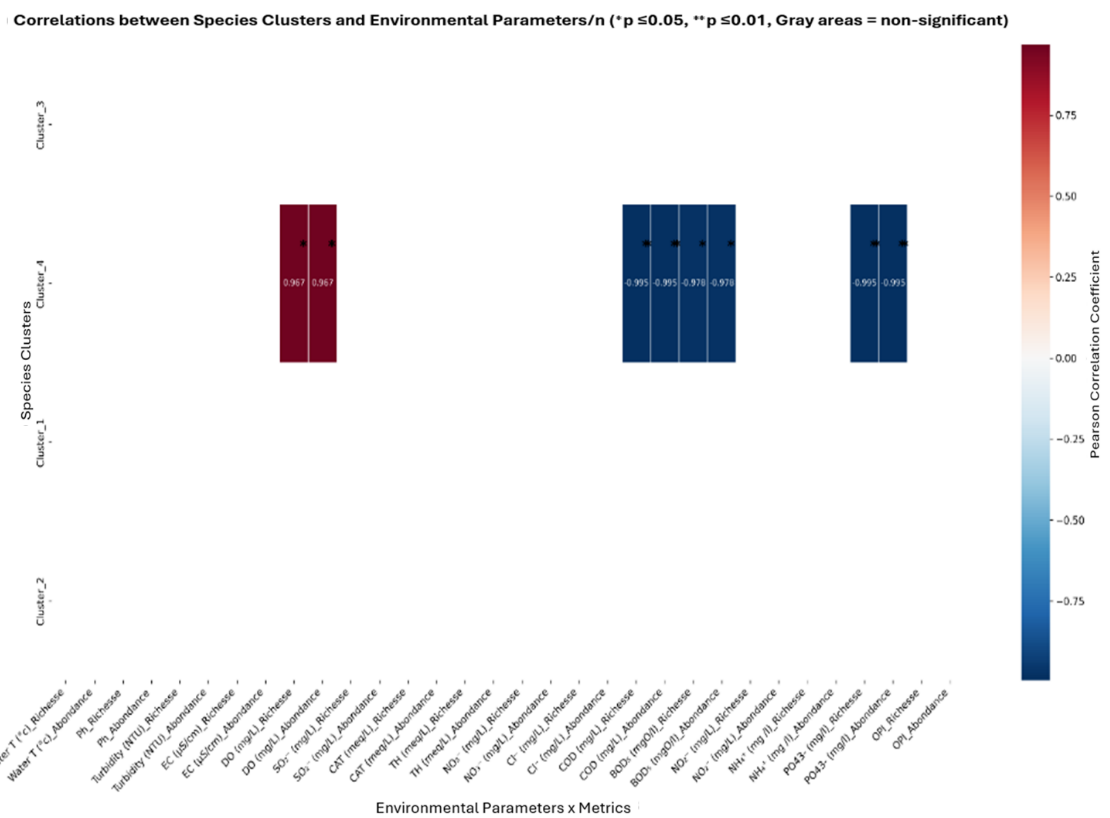

Fig. 6. Significant correlations between species clusters and environmental parameters.

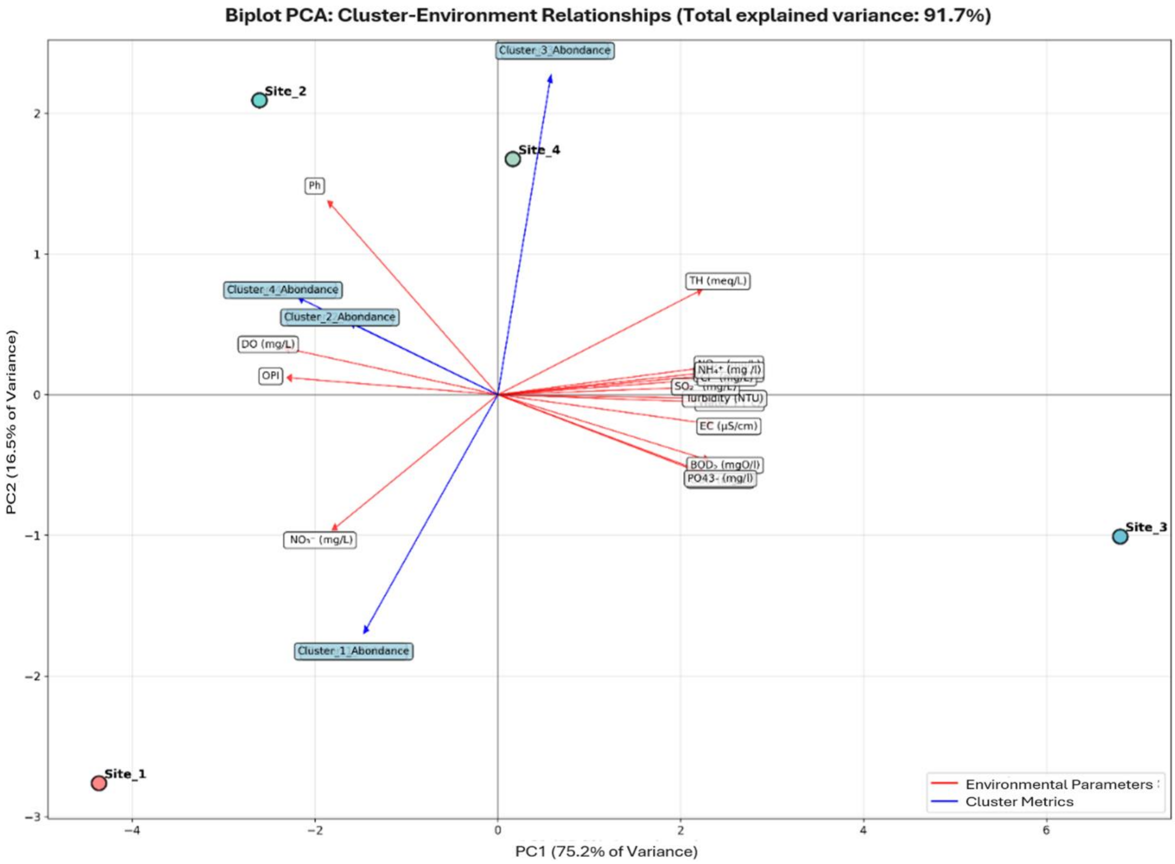

Fig. 7. Principal component analysis illustrating correlations between environmental variables and abundance clusters.

Cluster_4 shows very strong negative correlations with several pollution indicator parameters, such as Chemical Oxygen Demand (COD), 5-day Biological Oxygen Demand (BOD_5), and phosphates (PO_4^{3-}), with coefficients reaching -0.995 for COD and PO_4^{3-}, and -0.978 for BOD_5 (all with $p \leq 0.01$). Cluster 4 (sensitive) showed a strong positive relationship with dissolved oxygen (DO) and strong negative relationships with COD, BOD5, and PO_4^{3-}. These results suggest that Cluster 4 is composed of species sensitive to pollution and low water quality conditions, often considered indicator species of good water quality. The corresponding cells are greyed out, indicating that the p-values are above the significance threshold ($p > 0.05$). However, referring to (Figure 5), the principal component analysis (PCA) highlights overall trends in the multivariate data, suggesting the existence of potential associations between some abundance clusters and physicochemical parameters. These observations could reflect complex, possibly non-linear, ecological interactions that are not captured by the Pearson correlation test, which assumes of a bivariate linear relationship. This highlights the interest of combining complementary statistical approaches to better interpret ecosystem dynamics.

Correlation Matrix between environmental variables and abundance clusters

The correlation analysis between environmental parameters and abundance clusters reveals significant and consistent relationships between these variables measured at the different study stations (Figure 7).

Principal component analysis (PCA) explored the relationships between physicochemical parameters and biological metrics (abundance clusters) and effectively discriminated sites according to their level of anthropogenic impact (Figure 7). The first two factorial axes accounted for 91.7% of the total variance, demonstrating excellent representativeness of the factorial design. The first axis (75.2%, PC1) mainly reflects a pollution gradient. From the least impacted sites to the most impacted sites by pollution, while the second axis (16.5%, PC1) allowed us to distinguish more subtle variations related to the pH and oxygenation of the environment. Site 1 is located at the negative end of the first principal axis (PC1), indicating that it is strongly correlated with high dissolved oxygen (DO) values and distant from pollution vectors (NH_4^+, PO_4^{3-}, BOD_5, etc.). This site is associated with Cluster 1, whose abundance is also positioned in this area, reflecting a community rich in sensitive taxa. This allows us to distinguish more subtle variations related to pH and oxygenation of the environment. These results confirm that this site is little disturbed since it is located upstream, away from anthropogenic activities, reflecting a good ecological status.

Site 2 is positioned in the upper left part of the graph, with a positive value on the PC2 axis. It is moderately influenced by pH, slightly basic, and weakly affected by other pollution parameters. This site is linked to Clusters 2 and 4, whose abundance remains moderate but is associated with a still diverse fauna, including both sensitive taxa and some tolerant taxa. It represents a relatively preserved ecological transition environment, although less favourable than Site 1. The water quality remains good, with a still functional ecological balance.

Site 3 is positioned at the right end of the first main axis (PC1), strongly correlated with the typical parameters of significant organic pollution, including high concentrations of ammonium (NH_4^+), phosphates (PO_4^{3-}), BOD_5, turbidity and conductivity. It is associated with Cluster 3, whose abundance is oriented in the same direction as these environmental vectors. However, this abundance does not reflect high species richness, but rather a dominance of tolerant, opportunistic species with low bioindicator value. The results from this site show a low overall abundance in relation to a strong degradation of aquatic fauna following the impact of domestic wastewater, which causes an increase in the organic load, leading to a major alteration of water quality. These results suggest a disturbed ecosystem in which the physicochemical and organic pollution conditions are unfavourable, which limits aquatic diversity and favours the dominance of some tolerant taxa, such as Chironomidae and Tubificidae. Site 4 is in the central right part of the graph near cluster 3, this site and translates into moderate pollution probably less intense than that of site 3. The site is influenced by several pollution parameters (NH_4^+, PO_4^{3-}, BOD_5), but in lesser proportions, which could be explained by self-purification mechanisms. The fauna that develops there remains partially altered, with a diversity slightly higher than that observed at Site 3 but less than sites 1 and 2. This site is influenced by several pollution parameters (NH_4^+, PO_4^{3-}, BOD_5), but in lesser proportions, which could be explained by natural dilution or self-purification mechanisms.

The analysis of the correlation of faunal groupings (cluster) and environmental variables makes it possible to highlight an ecological gradient influencing the composition of macroinvertebrate communities according to the intensity of disturbance of physicochemical parameters (Figure 7). These results are supported by significant linear relationships between biological indicators and key physicochemical parameters such as BOD_5, COD and dissolved oxygen (DO) (Table 1).

The results of the linear regression show that organic pollution strongly influences biological indicators in the Oum Er Rbia River. The Organic Pollution Index (OPI) decreases significantly with increasing BOD5 ($R^2 = 0.911$; $p = 0.046$), indicating a deterioration in biological quality, while the relationship with COD is not significant ($R^2 = 0.839$; $p = 0.084$). Taxonomic richness and the Shannon index are strongly influenced by organic pollution in the Oum Er Rbia River. Taxonomic richness decreases significantly with increasing BOD5 ($R^2 = 0.94$; $p = 0.031$) and COD ($R^2 = 0.938$; $p = 0.032$), while the Shannon index also decreases with increasing BOD5 ($R^2 = 0.98$; $p = 0.01$). Conversely, both indices increase with dissolved oxygen, highlighting the positive effect of oxygenation on biodiversity. The sensitive Ephemeroptera, Plecoptera, and

Table 1. Linear relationships between biological indicators and physicochemical parameters (BOD_5, COD and DO)

Regression	Variable Y	Variable X	Equation	R (correlation)	R² (determination)	P-value	significance
1	OPI	BOD_5 (mg/L)	y = -0.0409x + 4.536	-0,954	0,911	0,046	* (significant)
2	OPI	COD (mg/L)	y = -0.0081x + 4.526	-0,916	0,839	0,084	ns
3	Richesse Taxonomique	BOD_5 (mg/L)	y = -0.320x + 49.938	-0,969	0,94	0,031	*
4	Richesse Taxonomique	COD (mg/L)	y = -0.0655x + 50.062	-0,968	0,938	0,032	*
5	Richesse Taxonomique	DO (mg/L)	y = 3.857x + 15.564	0,955	0,911	0,045	*
6	Indice de Shannon	BOD_5 (mg/L)	y = -0.0229x + 2.859	-0,99	0,98	0,01	**
7	Taxons EPT sensibles	BOD_5 (mg/L)	y = -0.1695x + 9.089	-0,999	0,998	0,001	*** (very highly significant)
8	Taxons EPT sensibles	COD (mg/L)	y = -0.0344x + 9.130	-0,989	0,978	0,011	** (highly significant)
9	Taxons EPT sensibles	DO (mg/L)	y = 2.074x - 9.357	0,999	0,998	0,001	*** (very highly significant)

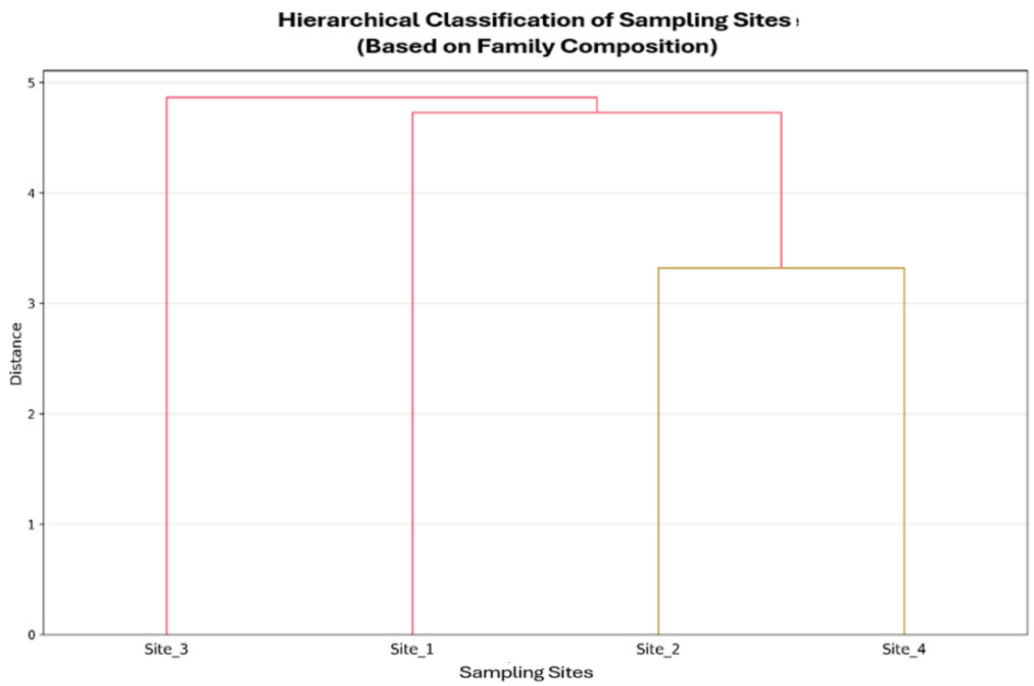

Fig. 8. Hierarchical classification of sampling sites

Trichoptera (EPT) taxa appear to be the most reactive indicators, showing the most pronounced responses. Their abundance decreases sharply with BOD5 and COD ($R^2 = 0.978–0.998$; $p \leq 0.011$) and increases with high oxygen concentrations ($R^2 = 0.998$; $p = 0.001$). These results confirm that BOD5 is the best indicator of biological degradation and that EPTs constitute reliable bioindicators for assessing the ecological quality of the Oum Er-Rbia River. These findings were further confirmed by hierarchical classification using Euclidean distance (Figure 8).

The distribution of macroinvertebrate families across the four sites of the Oum Er-Rbia River reveals significant heterogeneity in ecological conditions, clearly visualized by the hierarchical classification *(Figure 8)*. Site 3 is the most distinct and isolated element in the dendrogram, which is ecologically confirmed by its low taxonomic richness, with numerous absences in all clusters, suggesting that it may be subject to significant environmental stresses (pollution, physical alteration of the habitat, low oxygenation). This site could indicate degraded water or habitat quality. In contrast, Sites 2 and 4 form the closest cluster, indicating that they support the most similar communities. Site 1 is then grouped with them, sharing a generally better ecological status than Site 3. These sites (1, 2, 4) probably support greater diversity, including indicators of good water quality such as Baetidae and Heptageniidae. Although Site 4 shows intermediate conditions with a decrease in the diversity of certain taxa and the absence of others, which could indicate localized disturbances or fluctuating environmental conditions, its strong biological similarity to Site 2 suggests acceptable conditions.

Contribution of IBGN and IPO Indices in Water Quality Monitoring
Analysis of the variation of the Normalized Global Biological Index (IBGN) at the different study stations

Analysis of the results reveals significant variations in biological quality between the sampled stations (Figure 9).

Analysis of the results reveals significant variations in biological quality between the sampled stations. Stations 1 and 2 exhibit good ecological quality, with respective IBGN scores of 16/20 and 15/20. These high values, combined with significant taxonomic richness (29 and 33 taxa), reflect a relatively preserved environment, home to a diverse and sensitive macroinvertebrate fauna indicative of a well-balanced ecosystem. The water quality at these two stations is good *(Figure 9)*.

Station 3 is distinguished by extremely deteriorated ecological quality, with an IBGN score of 6/20 and low faunal richness (14 taxa). This situation is closely linked to the direct discharge of domestic wastewater, which results in considerable organic pollution and significant anthropogenic impact. These pressures lead to a remarkable simplification of the benthic community, indicative of poor water quality. Station 4 is located downstream and has an ecological score of 13/20. Although the taxonomic richness remains relatively high (28 taxa), the moderate value of the indicator group (5) reflects an average quality, which may indicate the beginnings of an ecological imbalance or increasing environmental pressure; the quality of their water is Average.

Analysis of the variation of the Organic Pollution Index (OPI) at the different study stations

The analysis of the results of the organic pollution parameters that were measured in the 4 study stations reveals a significant difference in organic pollution, highlighted by the variation

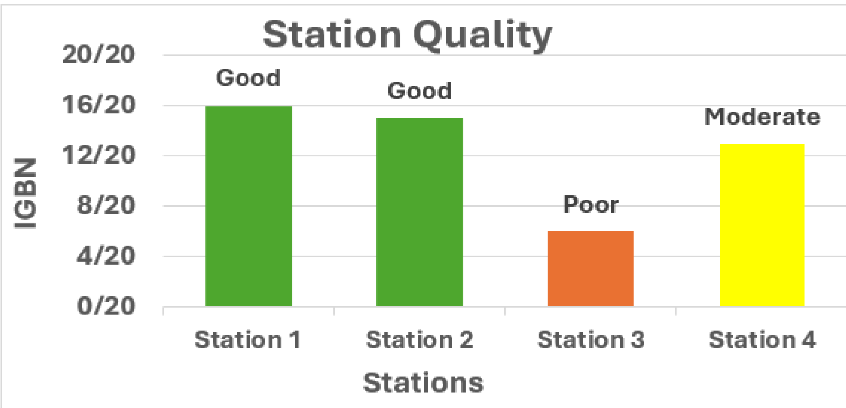

Fig. 9. IBGN values for the different stations of the Oum Er Rabia river.

Table 2. Results of Organic Pollution Index (OPI) and associated classification by station.

Stations	BOD5 (mg O_2/L)	NH_4^+ (mg /L)	PO_4^{3-} (mg/L)	OPI	Organic Pollution	Water Class
ST1	1	0.05	0,033	4,66	Low	Good
ST2	1,1	0.07	0,035	4,66	Low	Good
ST3	48	0.14	0,138	2,66	High	Bad
ST4	11	0.09	0,045	3,66	Moderate	Medium

in the organic pollution index (Table 2).

The upstream stations S1 and S2 show low values for the concentration of BOD_5, ammonium (NH_4^+) and phosphate (PO_4^{3-}), which reflects good water quality, favourable to aquatic life, especially macroinvertebrates, and presents high values of OPI (4.66), indicating low organic pollution.

However, at station 3, there is a significant deterioration in water quality, with high concentrations of BOD_5 (48 mg O_2/l), ammonium NH_4^+ (0.14 mg O_2/l), and phosphate (PO_4^{3-}) (0,138 mg/L), reflecting poor water quality. This station also has an organic pollution index of 2.66, indicating high organic pollution classified in the poor category.

In contrast, the downstream station S4 presents an intermediate situation, although the levels of BOD_5, ammonium (NH_4^+), and phosphate (PO_4^{3-}) are less alarming than at station S3. The organic pollution index is 3.66, indicating moderate organic pollution with average water quality.

DISCUSSION

The Oum Er Rbia River represents a crucial aquatic ecosystem for biodiversity and human activities. However, its preservation is increasingly threatened by the alteration of its aquatic habitats due to pollution caused by human activity (Benamar et al., 2019). One of the main causes of this alteration is the discharge of domestic wastewater, which seriously impacts aquatic fauna, particularly macroinvertebrates, which are known for their sensitivity to water quality (Camara et al., 2014).

Stations S1 and S2, located upstream, characterized by a high IBGN index, demonstrate a rich biodiversity, represented by groups of pollutant-sensitive macroinvertebrates (Ephemeroptera, Trichoptera, and Plecoptera). The rarity of the latter taxon at these stations indicates well-oxygenated water, probably linked to the absence of a direct source of pollution (Ilham et al., 2022). Corroborating with the results of several research projects conducted at the national level (Berrahou et al., 2001; Lamri et al., 2016; Zuedzang Abessolo et al., 2021), these researchers attributed the rarity of this taxon to its high sensitivity to thermal fluctuations. These stations also hosted a wide variety of ephemeroptera, including Baetidae, known environmental indicator taxa, as well as Trichoptera, Diptera and Odonata. The latter, often observed at the water surface, are good indicators of ecosystem health (Hart, 2014). Regarding the physicochemical quality, these two stations present physicochemical characteristics typical of a healthy and little impacted environment, because they are in an area little affected by human activity. The temperatures are moderate, the pH is slightly alkaline, and the nutrient concentrations (NH_4^+, PO_4^{3-}) as well as the BOD_5 (< 1.1 mg/L) and COD (< 16 mg/L) values are low. Dissolved oxygen in the upstream stations (S1 and S2) reaches high levels respectively (8.95 mg/L at S1 and 8.75 mg/L at S2). According to the Moroccan surface water classification standards grid (Kanga Idé et al., 2021), these values are favourable to the respiration of aquatic organisms, placing the water in class 1 (excellent quality) for most parameters. Furthermore, the Organic Pollution Index reached an average value of 4.66, corresponding to zero to very low organic pollution (class 5 in the Leclercq, 2001 grid). These results are consistent with the abundance and diversity of macroinvertebrates observed, particularly sensitive groups such as Plecoptera, Ephemeroptera (Baetidae), Trichoptera, and Odonata.

Station S3, located in the urban area of Khenifra, shows a significant degradation of aquatic biodiversity. The fauna is dominated by pollutant-tolerant taxa, notably Chironomidae, known for their ability to survive in highly disturbed environments. These taxa play a major role in the resilience of aquatic communities, particularly in nutrient-poor environments, due to their ability to withstand degrading conditions (Hvitved-Jacobsen et al., 1998; Serra et al., 2017). Other tolerant taxa observed at this station included some Coleoptera and Oligochaetes, which are known to thrive in polluted sediments such as wastewater ponds (Crespo-Cebada et al., 2020). This station is severely polluted due to direct discharge of domestic and agricultural wastewater (Barakat et al., 2016; Bhadarka et al., 2024). These pressures lead to severe aquatic habitat degradation and biodiversity erosion (Derradj et al., 2007). Odonates are absent at Station 3, clearly indicating that water quality is degraded. For the physicochemical parameters, the dissolved oxygen concentration is 5 mg/L, while the BOD_5 and COD record high values of 48 mg O_2/L and 239 mg/L respectively, which confirms a high organic pollution of the water. These values are well beyond the thresholds admitted by the Moroccan standards for surface water quality. The correlation between the physicochemical parameters and the biological indices is very marked: the high organic load measured results in a significant drop in the Normalized Global Biological Index (IBGN < 8) and an extreme simplification of the faunal structure, dominated by resistant species, this is demonstrates clearly the impact of the pollution on the composition of aquatic communities (Wang et al., 2024).

Station S4, located downstream from Khenifra, shows signs of ecological resilience, albeit partial. Ecologically, macroinvertebrate diversity has improved significantly compared to station S3, which was severely degraded.

At this downstream station, sensitive indicator taxa such as Trichoptera, Ephemeroptera, and some Odonata have reappeared. These faunal groups indicate the beginnings of ecological restoration (Castelain et al., 2018). They also demonstrate a partial improvement in water quality and a reduction in the most intense anthropogenic pressures. However, taxa most sensitive to pollution, such as stoneflies, remain absent, reflecting a still partial resilience. Physicochemically, the data confirm this improvement dynamic.

The recorded BOD5 (11 mg/L) and COD (38 mg/L) values, as well as those of ammonium (NH_4^+) and phosphate (PO_4^{3-}), remain at intermediate levels. These results indicate moderate organic pollution, consistent with the biological disturbances still visible. In addition, the Organic Pollution Index reached a value of 3.25, indicating moderate organic pollution at class 3 according to (Leclercq, 2001). This assessment is also confirmed by the Moroccan surface water quality grid (ME-MCATUHE, 2002). The Normalized Global Biological Index (IBGN), for its part, reached 13, which reflects a specific diversity in the process of recovery but still limited compared to the upstream stations (S1 and S2).

Hierarchical clustering showed that stations S2 and S4 formed a group, while S3 remained isolated. This proximity reflected the relative ecological similarity between S2 and S4 rather than identical water quality. Although S2 maintained good water quality and was clustered with S4, which is in a phase of partial recovery, the two stations share several sensitive macroinvertebrate taxa. At S2, pollution levels are low, and the macroinvertebrate community is dominated by pollution-sensitive taxa (Trichoptera, Ephemeroptera, Odonata). However, S4 exhibited moderate pollution and intermediate diversity: sensitive taxa were gradually reappearing, while some tolerant taxa persisted, indicating partial recovery. In contrast, S3 remained highly degraded and dominated by tolerant taxa, confirming the high anthropogenic pressure at this station.

The findings of the PCA analysis (Figure7) provide a robust statistical validation of the observed ecological gradient. The first principal component (PC1), accounting for 75.2% of the total variance, clearly represents the organic pollution axis. Sites S1 and S2 are positioned at the negative end of PC1, indicating high DO and low pollution, whereas Site S3 is strongly correlated with the positive end, dominated by pollution vectors (BOD_5, COD, NH_4^+, PO_4^{3-}). Furthermore, the PCA definitively showed that the sensitive macroinvertebrate Cluster 4 is directly opposed to the pollution vectors, confirming that organic pollution is the single largest determinant of the benthic community structure in the Oum Er Rbia River. This aligns with findings from (Zuedzang Abessolo et al. (2021)) where nutrient loading dictated macroinvertebrate distribution.

This situation illustrates the positive effects of natural self-purification processes, such as those promoted by the riffles and the high flow of the river downstream of Khenifra, which improve water oxygenation and promote the degradation of organic matter (Namour et al., 2012). Although self-purification is crucial, it does not always guarantee a rapid return to original conditions. Additional efforts are needed for proactive management of pollution sources and to ensure sustainable recovery of the ecosystem (Chawla et al., 2023).

CONCLUSION

This study provided an in-depth assessment of the water quality of the Oum Er Rbia River using an approach combining physicochemical and biological parameters based on macroinvertebrates. The results obtained through this study demonstrate a clear spatial pollution gradient, with the upstream stations (S1 and S2) exhibiting good water quality, followed by a marked deterioration at station 3, which receives domestic wastewater, causing significant organic pollution, and a partial recovery downstream at station 4. Water quality indices such as the IPO index and the IBGN index confirm this diagnosis. The PCA results indicate that organic pollution is the primary factor controlling the structure of the aquatic ecosystem. Our study highlights the need for rigorous management of domestic wastewater to prevent the deterioration of aquatic ecosystems.

Moreover, this research provides a valuable basis for the development of bioindication tools adapted to the national context, and for the implementation of integrated management and ecological monitoring strategies at the watershed scale.

FUNDING

This work is part of the preparation of a doctoral thesis and is partly funded by the project "Response of freshwater biodiversity (bio-indicator macroinvertebrates and river trout, an endangered endemic species) to the combined impacts of climate change and anthropogenic activities in the Atlas Cedar Biosphere Reserve," led by the Ifrane Model Forest Association (AFMI). It also receives financial support from the French Embassy in Morocco.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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