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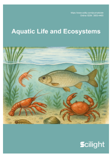

Review

Benthic Macroinvertebrates as Bioindicators of Aquatic Health: A Review and Comparative Analysis of Diversity Indices for Water Quality Assessment

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Abstract: River ecosystems are critical to biodiversity and provide essential ecological services, with benthic macroinvertebrates serving as reliable bioindicators of water quality as they provide an integrated indication of past and present ecological conditions of aquatic bodies. The ubiquitous distribution, relatively long-life cycles, high accumulating capacity and sedentary nature of benthic macroinvertebrates, make them a great tool in assessing the health condition of aquatic ecosystems. The present study involves a comprehensive review and comparison of diversity indices commonly used in river monitoring. Data for the analysis were sourced from peer-reviewed articles, environmental reports, and case studies on river monitoring. This paper summarizes the role of benthic macroinvertebrates as bioindicators to evaluate the water quality of fresh water bodies using different bioassessment approaches which include Classical diversity indices such as the Shannon-Wiener, Simpson, and Margalef indices, Saprobic index, HBI index, BMWP score, NSF-WQI score, taxa richness and EPT ratio. The presence or absence of specific macroinvertebrate taxa reflects their differential tolerance to pollution. The classification of benthic macroinvertebrates with respect to tolerance values indicates the status of freshwater bodies. The paper aims to highlight applications and the methods used in biomonitoring, guiding river management, and supporting sustainable conservation strategies needed for the sustainability of aquatic resources.

Keywords: macroinvertebrates; diversity indices; river monitoring; biodiversity; ecological health; river ecosystems; bioassessment

1. Introduction

1.1. Threats from Anthropogenic Activities

To protect, manage, and restore freshwater biodiversity and associated natural resources, the development and validation of diverse bioassessment methodologies are crucial [1,2]. To effectively conserve and manage freshwater resources, the development and application of reliable monitoring and assessment tools are essential. Traditional monitoring methods, primarily focused on physicochemical parameters, offer only a snapshot of water quality at the time of sampling and may overlook long-term ecological trends. In contrast, biological monitoring techniques provide a more integrated and temporally robust assessment of ecosystem health [3,4]. The key idea of bioassessment is that changes in biological communities found in different locations reflect the environmental conditions among sites [5]. But metacommunity theory suggests that the composition of biological communities is a combination of local (e.g., environmental filtering), regional (e.g., dispersal), and stochastic (e.g., random local disturbances) processes [6,7]. Therefore, there is a need to validate and compare traditional bioassessments based on species–environmental relationships to provide more accurate information on the ecological health of freshwater ecosystems.

Using aquatic organisms for freshwater ecosystem health assessment is a well-established approach, as they are affected by a wide range of stressors that reflect the effects of pollution conditions and changes in habitat and hydrological conditions. To date, bioassessment with various aquatic organisms (e.g., fish, macroinvertebrates, and algae) is widely used in many countries and organizations, such as the European Water Framework Directive (WFD) and the US National Biomonitoring Program (NBP). Bioassessment indices are the key tools for biomonitoring of freshwater ecosystems. Their usage proposed information on environmental health, which plays an important role in defining the objectives of environmental management and related policies. Among biological monitoring approaches, benthic macroinvertebrates have emerged as effective bioindicators for assessing the ecological status of freshwater ecosystems. Macroinvertebrates are widely used in bioassessment due to their suitable life history, habitat fixation, ease of collection, and ability to respond rapidly to pollution, habitat alteration, and other anthropogenic stressors. Consequently, their presence, absence, and community structure offer crucial insights into the environmental quality of aquatic systems. Several bioassessment indices have been developed to quantify macroinvertebrate community responses to environmental stress. The bioassessment indices for macroinvertebrates mainly include: (1) biodiversity indices such as the Shannon-Wiener Index(H′) and Simpson index; (2) taxonomic richness indices such as the EPT richness; and (3) indices based on tolerance or sensitivity values for macroinvertebrates such as the BI (biotic index), FBI (family-level biotic index) [8], BMWP (Biological Monitoring Working Party), and improved ASPT (average score per taxon) index based on BMWP [9,10]. Although these indices have demonstrated utility across various geographic and ecological contexts, they often overlook the influence of spatial processes and metacommunity dynamics. However, these traditional bioassessment indices all share a common problem: they ignore the impact of spatial processes on communities [11–13].

1.2. Importance of Freshw. Ecosystems

Freshwater ecosystems (Figure 1)—rivers, streams, lakes, wetlands, and ponds—are among the most valuable natural resources on Earth, providing a wide range of ecological functions critical to both environmental sustainability and human well-being [14]. They serve as habitats for a wide range of aquatic organisms such as fishes, zooplankton, benthic macroinvertebrates, macrophytes, and aquatic birds, while also delivering essential ecological services such as drinking water, sanitation, agriculture, hydropower, nutrient cycling, habitat provision, and support for biodiversity [15].

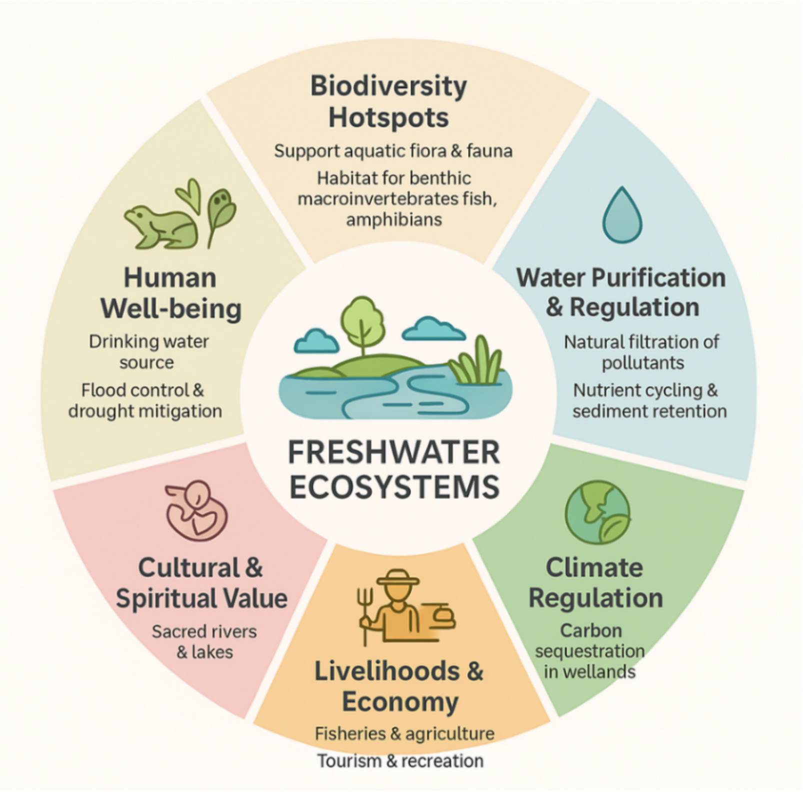

Figure 1. The multifaceted importance of freshwater ecosystems for environmental and societal well-being.

The ecological significance of freshwater ecosystems is profound, as rivers and streams are irreplaceable resources that play a pivotal role in human and societal development [16–18]. However, they face escalating threats from overexploitation, habitat fragmentation, agricultural activities, land use changes, pollution from agricultural runoff, industrial and domestic waste, and climate change [19–22]. These stressors accelerate biodiversity loss, degrade water quality and impair ecosystem functionality [23,24]. The fragmentation and destruction of habitats due to expanding human settlements and agricultural activities further intensify this ecological decline [25,26].

1.3. Ecological Significance of Diversity Metrics in Bioassessment

Diversity metrics play a central role in freshwater bioassessment by summarizing structural attributes of biological communities that respond predictably to environmental stress. Classical indices such as the Shannon–Wiener and Simpson indices are widely applied because they integrate information on taxonomic richness and relative abundance into interpretable measures of community organization. While Shannon diversity is sensitive to changes in rare taxa and evenness, Simpson's index places greater emphasis on dominance patterns and is therefore more robust to sampling variability and indicative of pollution-driven community homogenization [27,28]. Complementary metrics, including Pielou's Evenness and taxonomic distinctness, provide additional ecological resolution by isolating distributional equity and phylogenetic breadth within assemblages [29,30]. Such indices are particularly informative because ecological degradation often manifests initially as shifts in dominance and evenness rather than outright species loss, making them effective early indicators of stress associated with eutrophication, habitat modification, or selective pollution pressures [31]. The ecological value of diversity metrics lies not in individual indices but in their combined interpretative power. Richness-based measures (e.g., Margalef and Menhinick indices) are sensitive to habitat simplification and toxic disturbance, whereas evenness and dominance metrics respond more strongly to competitive exclusion and organic enrichment [32,33]. Consequently, integrated use of multiple diversity indices enables discrimination among stressor types and improves ecological inference.

Functional indices such as Rao's quadratic entropy link community structure directly to ecosystem functioning and resilience, while phylogenetic metrics capture evolutionary filtering under environmental stress [33–35]. These advances reinforce the consensus that no single index adequately captures ecological complexity, supporting the adoption of multi-index frameworks in contemporary bioassessment. Recent developments increasingly extend beyond purely taxonomic metrics to incorporate functional and phylogenetic diversity. Critically, diversity metrics not only serve as ecological indicators but also contribute to management and policy frameworks. For instance, indices like Shannon-Wiener Index, Simpson, and BMWP scores have been incorporated into the European Water Framework Directive (WFD) and other national monitoring programs, providing standardized benchmarks for ecological quality assessment [36,37]. In India, regional adaptations such as the Water Quality Biotic Index (WQBI) and the National Eutrophication Index (NEI) have been developed to account for the country's distinct hydrological regimes, climatic variability, and biogeographical characteristics [38]. These region-specific tools acknowledge that indices originally designed for temperate systems may not always perform reliably under tropical monsoon-driven flow variability, high biodiversity, and multiple stressor scenarios typical of Indian freshwater ecosystems. Such global and regional adaptations highlight the applied significance of diversity indices in shaping conservation, restoration, and sustainable management strategies.

In sum, the ecological importance of diversity metrics stems from their ability to bridge theory and application: they distill community-level responses into quantifiable indices that inform both ecological understanding and water resource governance. The continuing challenge is to integrate classical richness–evenness–dominance metrics with emerging functional and phylogenetic approaches, thereby ensuring that bioassessment tools remain both ecologically robust and operationally relevant.

1.4. Role of Bioassessment in Ecological Health Evaluation

Traditionally, water quality monitoring has relied heavily on physicochemical parameters such as dissolved oxygen, nutrient concentrations, pH, temperature, and salinity. While these indicators offer immediate and quantifiable data, they may not fully reflect the cumulative or chronic impacts of pollution events or ecological disturbances. Bioassessment overcomes this limitation by using biological communities as integrative indicators of environmental condition, reflecting the combined effects of pollution, hydrological alteration, and habitat change over time [39–41]. Among the most widely used biological indicators are benthic macroinvertebrates, which are particularly sensitive to environmental changes due to their varying pollution tolerances, limited mobility, and relatively long-life spans [42,43]. Their presence or absence can indicate the cumulative effects of pollutants, hydrological alterations, and habitat degradation. Macroinvertebrate-based indices therefore provide robust measures of ecological integrity, capturing multiple stressors that may not be evident from instantaneous chemical measurements alone [44,45]. A range of diversity- and tolerance-based indices—including Shannon, Simpson, Margalef, BMWP, Hilsenhoff Biotic Index, and EPT metrics—are widely applied in national and international monitoring frameworks to assess ecological status and pollution impacts [46–48]. When combined with physicochemical data, these biological indices enable a more holistic and diagnostically powerful assessment of freshwater ecosystems (Figure 2).

However, recent studies emphasize the need to incorporate spatial dynamics and metacommunity theory to improve the accuracy and ecological relevance of these assessments [3]. Such integrative assessments are crucial for identifying critical tipping points, formulating targeted restoration plans, and ensuring adaptive river basin management. In this context, the present review synthesizes current knowledge on the use of pollution-sensitive and pollution-tolerant benthic macroinvertebrates for bioassessment, evaluates commonly applied diversity and biotic indices, and highlights their applications, limitations, and potential for improving freshwater conservation strategies [49].

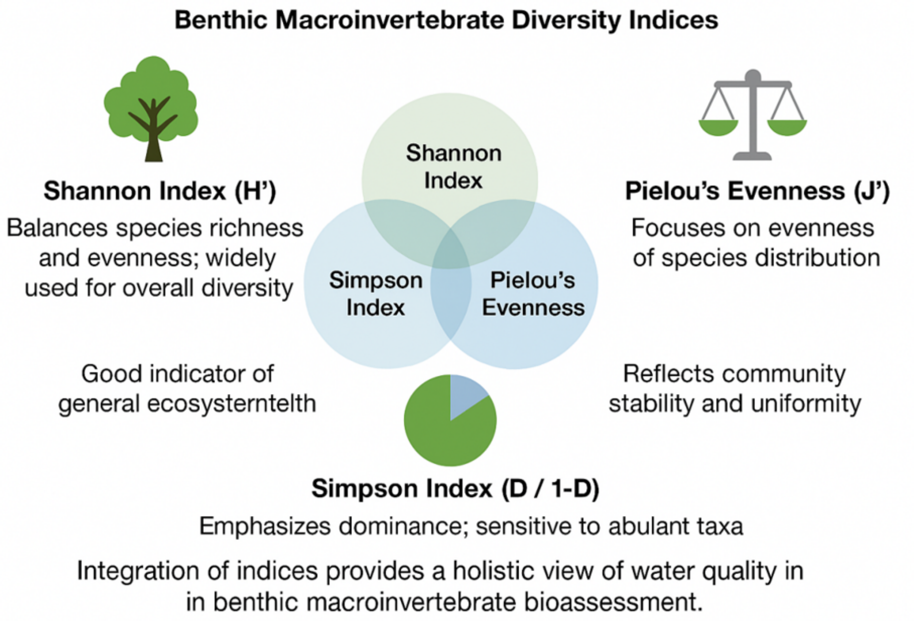

Figure 2. Benthic macroinvertebrate diversity indices.

1.5. Scope and Aim of the Review

Although benthic macroinvertebrates (BMIs) are widely recognized as robust bioindicators of freshwater ecosystem health, most existing reviews have focused primarily on taxon-level indicator roles, tolerance classifications, or regional case studies, often emphasizing descriptive patterns rather than comparative analytical performance of assessment tools. In particular, comparative evaluations of diversity and biotic indices across contrasting ecological contexts remain fragmented, and critical synthesis addressing their regional applicability, sensitivity to different pollution types, and methodological limitations is still lacking.

Previous reviews have generally treated diversity indices as interchangeable or supplementary metrics, with limited attention to how index performance varies under different disturbance regimes (e.g., organic vs. toxic pollution), hydrological settings (lotic vs. lentic systems), or taxonomic resolutions. Moreover, most global syntheses are strongly biased toward temperate river systems, resulting in insufficient guidance for tropical and subtropical regions, where biodiversity patterns, flow variability, and multi-stressor pressures differ substantially. As a consequence, practitioners often apply globally established indices without adequate contextual calibration, potentially leading to inconsistent or misleading ecological assessments.

The present review addresses these gaps by moving beyond a taxon-centric narrative to a condition-based comparative framework. Specifically, this review aims to:

- Critically evaluate the ecological relevance of benthic macroinvertebrates as bioindicators in relation to environmental gradients rather than species lists alone.
- Compare the performance of commonly used diversity indices (e.g., Shannon, Simpson, Margalef) and biotic indices across different pollution types, hydrological regimes, and biogeographic settings.

- Identify strengths, constraints, and sources of bias associated with index selection, taxonomic resolution, and methodological approaches.
- Assess the transferability of globally applied indices to regional contexts, with particular emphasis on tropical river systems and Indian freshwater ecosystems.

By synthesizing evidence across ecological conditions and regions, this review provides practical guidance for index selection and interpretation, thereby enhancing the reliability of bioassessment outcomes for freshwater monitoring, restoration planning, and adaptive water resource management. A key objective of the present review is therefore to critically examine the applicability, transferability, and limitations of globally used diversity and biotic indices when applied to local and regional contexts, particularly in India. By comparing the performance of widely adopted international indicators with regionally adapted indices, this review aims to highlight gaps, mismatches, and opportunities for contextual calibration, thereby enhancing the practical relevance of bioassessment frameworks for freshwater monitoring, conservation, and management in tropical river systems.

1.6. Hypothesis

This review is based on the hypothesis that different diversity indices used to assess macroinvertebrate communities capture unique but complementary aspects of water quality. It posits that no single index can fully represent ecological integrity, and therefore, a comparative, multi-index approach is essential to understand the complex relationship between benthic macroinvertebrates and freshwater health.

2. Literature Review and Trend Analysis (PRISMA-Based)

To ensure a comprehensive and multidimensional synthesis of existing knowledge on benthic macroinvertebrates and diversity indices in freshwater bioassessment, this review adopted a systematic and researcher-driven literature survey approach. Peer-reviewed literature was primarily identified through widely used scholarly search platforms, including Google Scholar and Semantic Scholar, to capture a broad spectrum of ecological, bioassessment, and water quality studies. While these platforms do not function as curated citation databases, they were used solely as discovery tools to ensure wide coverage of both classical and recent studies across disciplines.

To enhance organizational efficiency and conceptual structuring, AI-assisted tools (e.g., Elicit and SciSummary) were used only for bibliographic management, keyword clustering, and preliminary content mapping, and not for study selection, interpretation, or synthesis. All articles were manually screened, critically evaluated, and synthesized by the authors, with final inclusion based on relevance, methodological rigor, ecological context, and contribution to the objectives of the review. Visual mapping platforms (Napkin AI and Whimsical) were employed exclusively to aid in the development of conceptual frameworks and comparative schematics, without influencing analytical judgments.

The literature search employed combinations of keywords such as “Diversity Indices”, “Benthic Macroinvertebrates”, “Freshwater Systems”, and “Water Quality”, yielding an initial pool of 3785 research and review articles. Following title, abstract, and content-level screening, a refined selection of key publications was retained to support a focused, critical, and context-driven synthesis of diversity and biotic indices used in freshwater bioassessment.

2.1. Inclusion and Exclusion Criteria

The review included peer-reviewed journal articles published between 2000 and 2024, written in English, and explicitly addressing water quality monitoring through benthic macroinvertebrates. Studies that applied or compared diversity indices and biotic indices were prioritized. Conversely, studies were excluded if they were unrelated to water quality assessment, focused solely on physicochemical parameters without biological data, or constituted grey literature such as thesis, preprints, and non-peer-reviewed reports. In addition, only English-language publications were considered.

2.2. Article Screening and Selection

After deduplication, titles and abstracts were independently screened by two authors using Mendeley and Zotero to minimize subjective bias. A total of 310 articles were shortlisted for full-text evaluation. Full texts were subsequently assessed independently by both reviewers against predefined inclusion and exclusion criteria. Any discrepancies in study selection were resolved through discussion and consensus. Following this rigorous screening process, 120 articles were finalized and included in the review. A PRISMA flow diagram (Figure 3) summarizes the article selection procedure.

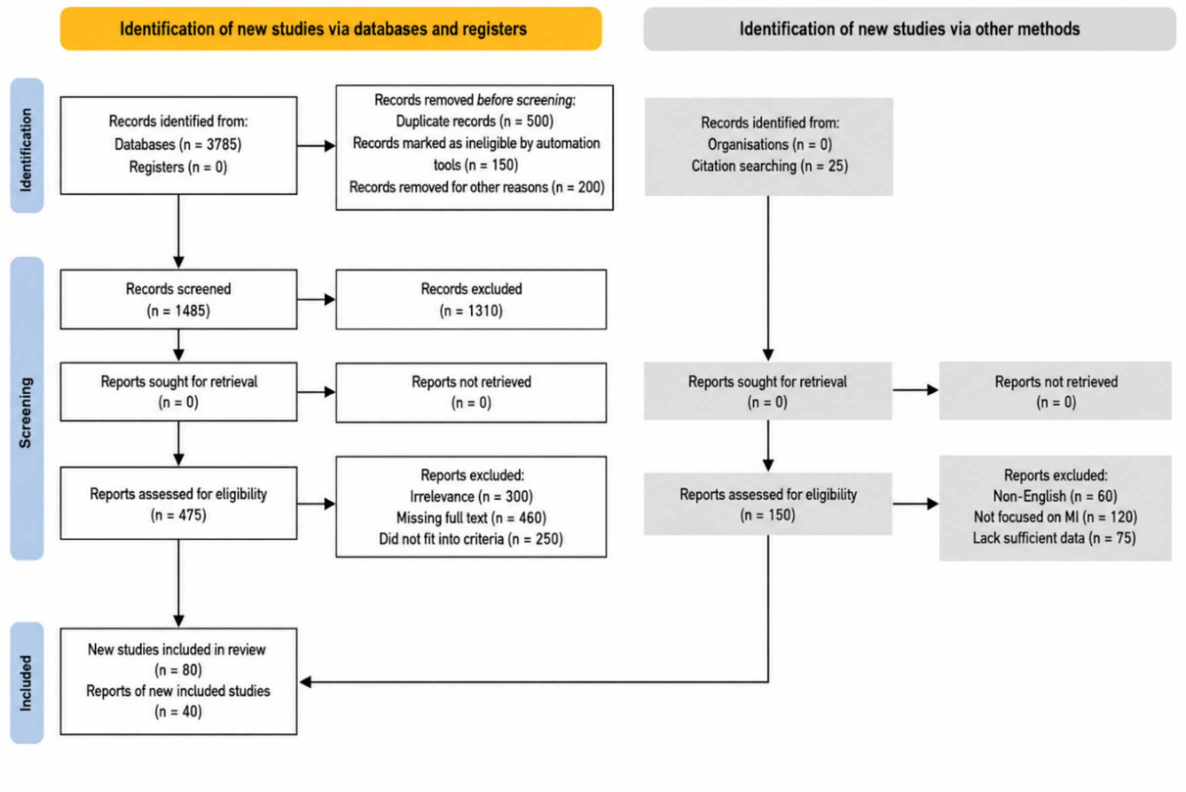

Figure 3. PRISMA flowchart illustrating the selection process of reviewed studies.

3. Geographic Distribution of Research

Over the past two decades, global research employing benthic macroinvertebrates as bioindicators for freshwater assessment has expanded considerably, reflecting their proven utility in capturing ecological responses to environmental stressors. However, this growth is characterized by marked geographical disparities. High-income regions such as North America, Europe, and parts of Oceania account for the majority of scientific output, largely supported by long-standing biomonitoring frameworks, advanced taxonomic capacity, and policy-driven mandates [20,49]. For instance, the European Union's Water Framework Directive (WFD) has standardized biological monitoring across member states, while the United States Environmental Protection Agency (USEPA) has institutionalized long-term biomonitoring under the National Aquatic Resource Surveys, ensuring continuous data generation and methodological consistency [50]. In contrast, developing regions in South Asia, Africa, and Latin America are still in the process of formulating region-specific bioassessment frameworks, where challenges such as limited taxonomic expertise, inadequate baseline datasets, and financial constraints hinder large-scale implementation [51,52]. Nonetheless, emerging economies including India, China, Brazil, and South Africa have demonstrated notable progress in recent years. Despite significant advances in biomonitoring frameworks, methodological heterogeneity and limited standardization remain major barriers to cross-regional comparability, particularly between temperate and tropical freshwater systems [53]. Importantly, this disparity is not solely a consequence of differences in financial resources or technical capacity, but also reflects fundamental ecological non-equivalence among regions. Bioassessment indices and reference conditions developed for temperate rivers in Europe and North America are often poorly transferable to tropical systems such as those in India and Brazil, where higher baseline species richness, distinct community composition, monsoon-driven hydrological variability, and pronounced seasonal cycles strongly influence macroinvertebrate assemblages and their responses to stressors.

As a result, the direct application of temperate-region indices in tropical contexts can lead to misclassification of ecological status or underestimation of anthropogenic impairment, even when methodological rigor is maintained. This uneven applicability highlights the need for regionally calibrated bioassessment frameworks that are grounded in local ecological realities while remaining conceptually aligned with international standards. Greater global collaboration should therefore prioritize not only capacity building, but also comparative ecological research and cross-biome validation of indices, enabling the development of standardized yet ecologically meaningful tools for freshwater assessment across biogeographical regions [22,39].

3.1. Publication Trends

The cumulative publication trend from 2013 to 2024 reveals a clear and sustained increase in research output, reflecting the progressive growth of scientific interest in the application of benthic macroinvertebrates, bioassessment methods using macroinvertebrates and diversity indices as bioindicators of water quality (Figure 4). The upward trajectory is consistent across the entire period, with no signs of plateauing, which underscores the enduring relevance and expansion of this research field. A noticeable acceleration in publication rate can be observed after 2019, suggesting that global environmental challenges such as climate change, pollution, and the demand for sustainable freshwater management have stimulated greater scholarly attention The number of relevant publications grew from an average of 125.5 articles in 2013 to 198.5 articles in 2024, reflecting growing research emphasis on biodiversity indices and. A particularly sharp rise is evident from 2016 onward, indicating a growing global emphasis on biodiversity-based assessments in aquatic ecology. The steepest rise occurred between 2021 and 2023, coinciding with heightened environmental awareness and policy-driven initiatives aimed at freshwater ecosystem conservation. This growth also mirrors the global prioritization of UN Sustainable Development Goal 6 (Clean Water and Sanitation), which emphasizes ecological monitoring and adaptive management [54,55]. By 2024, the cumulative number of publications had risen substantially compared to the early years, indicating both an expansion in methodological approaches and a widening recognition of the ecological importance of freshwater monitoring. This pattern not only highlights the academic value of biodiversity-based assessment tools but also reflects their increasing integration into applied ecological monitoring and policy frameworks. The consistent growth demonstrated by the cumulative trend suggests that research in this domain is likely to continue expanding, addressing both regional and global water quality concerns in the coming years. This trend underscores the increasing relevance of diversity indices—particularly in the context of water quality monitoring and environmental impact assessments—as essential tools in aquatic bioassessment protocols.

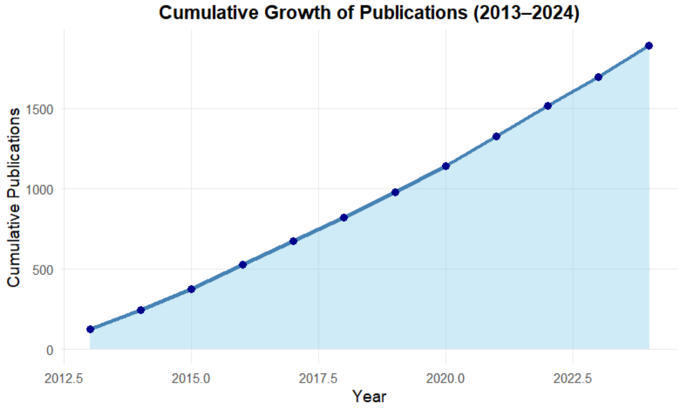

Figure 4. Annual trend in publications (2013–2024) on "diversity indices" and "benthic macroinvertebrates" across google scholar and semantic scholar.

3.2. Country-Specific Research Trends in Diversity Indices for Benthic Macroinvertebrates (2013–2024): A Global and Indian Perspective

The country-specific trend analysis from 2013 to 2024 highlights a substantial and growing global interest in using diversity indices of benthic macroinvertebrates for water quality assessment, with notable disparities across regions (Figure 5). The United States remains the leading contributor, driven by long-standing implementation of biomonitoring protocols under the USEPA's National Aquatic Resource Surveys, which heavily rely on indices such as Shannon-Wiener Index and Biotic Indices [56,57]. Similarly, the European Union's Water Framework Directive (WFD) has institutionalized standardized macroinvertebrate monitoring across member states, stimulating methodological harmonization [58]. In emerging economies such as Brazil and China, steady growth reflects large-scale river basin management and biodiversity-focused conservation initiatives. Brazil's trajectory highlights increasing ecological focus on Amazonian freshwater systems, while China's research has accelerated with environmental restoration projects such as the Yangtze River Protection Law [59,60]. Countries like Australia, Canada, and New Zealand also show consistent growth, tied to watershed-level biomonitoring programs. Global assessments highlight that water quality deterioration and resource stress are major challenges requiring integrated management and international cooperation [61,62]. Similarly, Germany's uptick can be linked to EU directives such as the Water Framework Directive (WFD), which mandates biological monitoring using benthic macroinvertebrates and associated diversity indices [58].

Significantly, India has demonstrated a remarkable increase in research output post-2018, reflecting heightened national awareness and policy-driven efforts under programs such as Namami Gange Programme. Indian contributions largely focus on classical indices (Shannon-Wiener Index, Simpson, EPT, Family Biotic Index), adapted for riverine systems such as the Ganga, Yamuna, and Brahmaputra [63–67]. Recent reviews emphasize the role of diversity indices in supplementing conventional physicochemical monitoring and advocate for integrated bioassessment in Indian water governance [68,69]. Despite infrastructural and taxonomic challenges, this upward trajectory signals a paradigm shift toward ecological monitoring frameworks that incorporate benthic macroinvertebrate metrics.

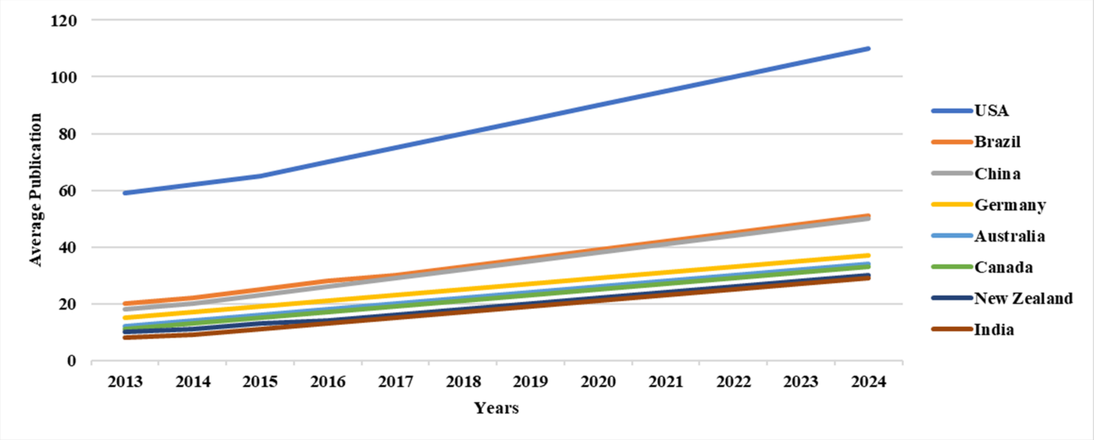

Figure 5. Country-specific trend analysis of publications on diversity indices for benthic macroinvertebrates (2013–2024).

This shift is not only vital for sustainable water resource management but also aligns with global objectives such as the UN SDG 6 (Clean Water and Sanitation). In conclusion, the increasing deployment of diversity indices across different countries reflects their universal applicability and robustness as indicators of aquatic ecosystem health. In the Indian context, their growing acceptance in both academic and applied research domains underscores a critical transformation in freshwater monitoring paradigms—moving from purely chemical analyses to holistic, ecosystem-based evaluations.

3.3. Semi-Quantitative Synthesis of Index Application Trends

Despite the extensive body of literature on macroinvertebrate-based bioassessment, quantitative synthesis of index application patterns remains limited. To address this gap, a semi-quantitative analysis was conducted based on the final set of 120 studies included in this review. The frequency of index usage, regional distribution, and dominant pollution contexts were evaluated to identify broader methodological trends.

Across the reviewed studies, diversity indices—particularly Shannon–Wiener and Simpson—were employed in approximately 65–70% of assessments, most commonly as supplementary indicators rather than standalone metrics. Tolerance-based indices such as BMWP, FBI, and EPT were applied in nearly 55% of studies, predominantly in relation to organic pollution and nutrient enrichment. Multimeric indices were reported in approximately 30% of studies, with a marked increase in publications after 2015, reflecting a shift toward integrative assessment frameworks.

Regionally, studies from Europe and North America showed greater reliance on multimeric and trait-based approaches, whereas assessments from South Asia, Africa, and parts of South America continued to Favor classical diversity and biotic indices. Tropical river studies frequently reported discrepancies between diversity metrics and ecological condition, reinforcing the need for regionally calibrated indices.

This semi-quantitative synthesis highlights a clear methodological transition from single-index reporting toward integrated frameworks, while also revealing persistent regional disparities in analytical complexity and calibration capacity.

4. Innovative Bioassessment Approaches Using Benthic Macroinvertebrates: A Systematic Integration of Methods

Benthic macroinvertebrates form the foundation of freshwater bioassessment because their community structure integrates the cumulative effects of chemical, physical, and hydrological stressors over time. However, no single analytical method is sufficient to capture the full complexity of ecosystem health. Consequently, contemporary bioassessment increasingly relies on integrated frameworks that combine taxonomic sensitivity, diversity patterns, functional responses, and rapid assessment tools into a coherent monitoring system (Table 1). In this section, macroinvertebrate-based bioassessment is conceptualized as a multi-layered and sequential process, in which different approaches function as complementary components rather than isolated techniques.

Table 1. Frequency of index use across regions and pollution contexts (%).

Index Category	Global (%)	Temperate (%)	Tropical (%)	Primary Stressor
Diversity indices	68	55	82	General disturbance
Biotic indices	54	60	48	Organic pollution
Multimeric indices	31	45	18	Multiple stressors
Trait-based indices	22	38	12	Toxic/hydro morphology

4.1. Pollution Sensitivity and Indicator Roles of Macroinvertebrate Taxa

Macroinvertebrate taxa exhibit varying degrees of sensitivity to pollution, making them highly effective biological indicators (Table 2) for freshwater ecosystem assessment. Sensitive taxa such as Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)—collectively known as the EPT group—are typically abundant in clean, well-oxygenated waters and are significantly reduced or absent in polluted environments, highlighting their utility in detecting ecological degradation [49,70]. In contrast, pollution-tolerant taxa including Chironomidae (non-biting midges), Tubificidae (sludge worms), and Physidae (snails) often dominate in organically enriched or hypoxic condition, signalling poor water quality [20,71]. Moderately tolerant taxa (e.g., Odonata, Coleoptera, Isopoda, Planariidae) indicate transitional ecological states (Figure 6) [72]. This gradient-based response underpins their use in both global indices (e.g., the Biological Monitoring Working Party (BMWP), Average Score Per Taxon (ASPT), and Stream Invertebrate Grade Number Average Level (SIGNAL)) and region-specific adaptations (e.g., WQBI in India), allowing for robust, cost-effective, and ecologically meaningful assessments of water quality.

Table 2. Macroinvertebrate groups, their pollution sensitivity, ecological indicator roles, and representative references.

Macroinvertebrate Group	Representative Families/Genera	Pollution Sensitivity	Indicator Role	Example References
Ephemeroptera (Mayflies)	Baetidae, Heptageniidae, Ephemerellidae	High (sensitive)	Indicators of clean, well-oxygenated water	[73,74]
Plecoptera (Stoneflies)	Perlidae, Nemouridae, Perlodidae	High (sensitive)	Indicators of clean, cold, oxygen-rich habitats	[73,75]
Trichoptera (Caddisflies)	Hydropsychidae, Limnephilidae, Leptoceridae	High (moderate–high)	Indicators of clean to slightly polluted water	[76]
Odonata (Dragonflies & Damselflies)	Libellulidae, Coenagrionidae	Moderate	Indicators of moderately polluted or degraded habitats	[77]
Coleoptera (Aquatic beetles)	Dytiscidae, Elmidae, Hydrophilidae	Moderate–low (family dependent)	Variable; some indicate clean water (*Elmidae*), others tolerate pollution	[70,72,78,79]
Chironomidae (non-biting midges)	*Chironomus, Tanytarsus*	Low (tolerant)	Indicators of organic enrichment, hypoxia, and polluted conditions	[80,81]
Oligochaeta (Aquatic worms)	Tubificidae, Naididae	Very low (highly tolerant)	Indicators of heavily polluted and anoxic habitats	[82]
Hirudinea (Leeches)	Glossiphoniidae, Hirudinidae	Low (tolerant)	Indicators of organic and chemical stress; nutrient enrichment	[83–85]
Gastropoda (Snails)	Lymnaeidae, Planorbidae, Viviparidae	Variable (taxon-dependent)	Indicators of nutrient enrichment and pollution gradients (pulmonates = tolerant, prosobranchs = sensitive)	[86–88]
Bivalvia (Clams, Mussels)	Unionidae, Corbiculidae, Sphaeriidae	Moderate–low	Indicators of organic pollution; filter-feeding bivalves sensitive to sedimentation and eutrophication	[89]
Isopoda & Amphipoda	Asellidae, Gammaridae	Moderate	Indicators of organic enrichment and habitat degradation	[90]
Hydracarina (Water mites)	*Unionicola, Arrenurus*	Moderate	Sensitive to habitat change and microhabitat quality	[91,92]
Planariidae (Flatworms)	*Dugesia, Planaria*	Moderate	Indicators of nutrient-rich and organically enriched environments	[93]

Note: Pollution sensitivity categories represent generalized tolerance levels and may vary with geographic region, habitat conditions, and taxonomic resolution. Color coding indicates sensitivity levels (High = Red, Moderate = Yellow, Low = Green &Variable= Blue). EPT taxa (*Ephemeroptera*, *Plecoptera*, *Trichoptera*) are widely recognized as indicators of good water quality. Interpretations should consider local hydromorphological variability and biogeographically calibrated thresholds.

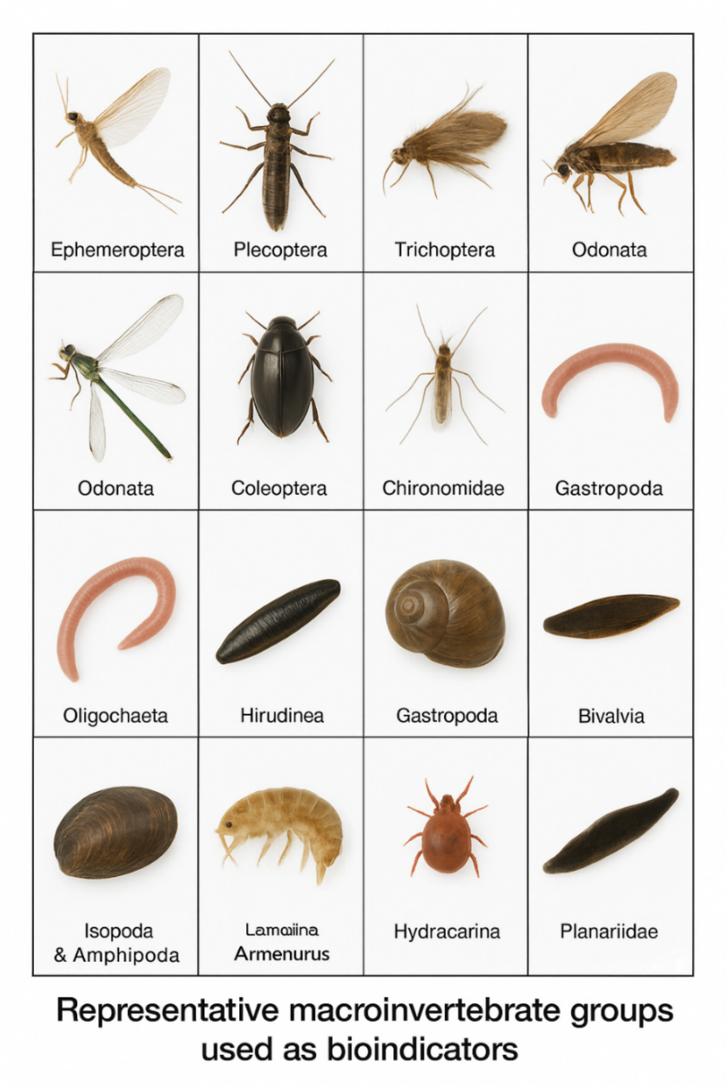

Figure 6. Representative benthic macroinvertebrate groups commonly used as bioindicators of freshwater ecosystem health.

Illustrated taxa include Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies), Odonata (dragonflies and damselflies), Coleoptera (aquatic beetles), Chironomidae (non-biting midges), Gastropoda (snails), Oligochaeta (aquatic worms), Hirudinea (leeches), Bivalvia (clams and mussels), Isopoda and Amphipoda (aquatic crustaceans), Laminina/Armenurus (water mites; Hydracarina), and Planariidae (flatworms). These groups span a broad gradient of pollution sensitivity, making them valuable indicators for assessing water quality and ecological integrity across freshwater systems.

4.2. Macroinvertebrate Diversity Indices and Ecological Quality Assessment

Macroinvertebrate-based diversity and biotic indices provide complementary insights into ecological quality, with indicative thresholds commonly used to distinguish between polluted and relatively undisturbed systems (Figure 7). Diversity indices such as the Shannon–Wiener Index (H′), Simpson Index (1–D), Margalef richness (d), and Evenness (J′) generally decline under degraded conditions, reflecting reduced taxonomic richness, uneven community structure, and dominance of pollution-tolerant taxa. In many temperate river systems, higher values of these indices (e.g., $H' \approx 3$, $1\text{–}D > 0.8$, $d > 3$, $J' > 0.7$) are often associated with good ecological status and greater representation of sensitive taxa [31,94]. However, these threshold values should not be interpreted as universal benchmarks. In tropical, monsoonal, or naturally stressed ecosystems, baseline diversity values may be inherently lower due to high seasonal variability, hydrological disturbance, or biogeographic constraints, even in minimally impacted systems. Consequently, diversity indices are most robust when applied in a relative or regionally calibrated framework, emphasizing spatial comparisons, temporal trends, and integration with biotic indices and local reference conditions rather than absolute cut-off values.

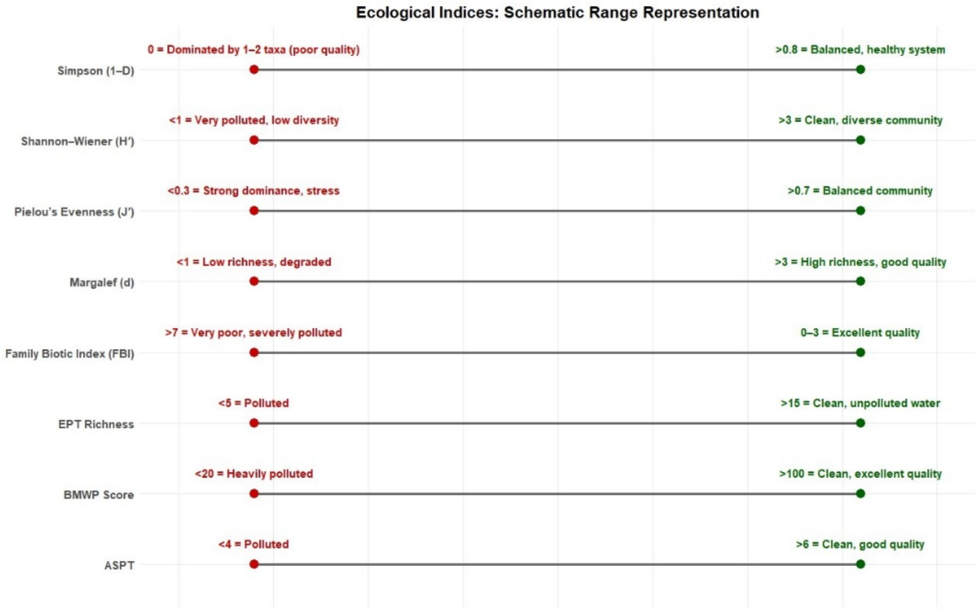

Figure 7. Macroinvertebrate diversity indices with ecological quality zones.

Biotic indices, however, demonstrate stronger discriminatory power in detecting pollution stress. The Family Biotic Index (FBI) increases under poor conditions due to the prevalence of tolerant groups, whereas lower values (<3) are indicative of excellent water quality. Similarly, the richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa is highly responsive to environmental degradation, with richness values exceeding 15 denoting unpolluted waters [73–76]. Composite scoring systems such as the Biological Monitoring Working Party (BMWP) and Average Score Per Taxon (ASPT) further integrate tolerance-weighted taxonomic responses. BMWP scores above 100 and ASPT values exceeding 6 are consistently associated with good ecological status, while lower scores reflect impaired systems dominated by tolerant assemblages [10].

Collectively, these indices underscore the value of using both diversity-based and biotic approaches in biomonitoring. While diversity indices provide a generalized assessment of community structure, biotic indices offer finer resolution in capturing ecological degradation linked to organic enrichment, habitat alteration, and pollution stress. The integration of multiple indices therefore ensures a robust and comprehensive evaluation of freshwater ecosystem health.

4.3. Innovative and Emerging Bioassessment Approaches: Opportunities and Constraints

Recent advances in freshwater bioassessment have expanded the toolkit for benthic macroinvertebrate–based monitoring through molecular, trait-based, and data-driven approaches (Figure 8). DNA barcoding and environmental DNA (eDNA) techniques enhance taxonomic resolution by enabling the detection of cryptic, rare, or early life-stage taxa and reducing dependence on expert morphological identification. These methods are particularly valuable in regions with high biodiversity or taxonomic uncertainty. However, molecular approaches also present notable limitations. Incomplete reference barcode libraries, primer bias, differential DNA shedding rates, and uncertainty in linking eDNA signals to organism abundance or ecological function constrain their interpretability. Moreover, high costs, infrastructure requirements, and the need for standardized protocols limit their routine application in long-term monitoring programs, particularly in resource-limited settings. Also, molecular detection alone does not convey ecological condition and is most effective when integrated with conventional indices

Trait-based approaches offer mechanistic insights into ecosystem functioning by linking biological responses to specific stressors (e.g., organic pollution, flow alteration, habitat degradation). Nevertheless, trait databases remain incomplete for many tropical taxa, and trait plasticity across life stages and environmental gradients can complicate interpretation. Further refinement is achieved through multimeric indices, multivariate statistics, machine learning, and GIS-based modeling, which integrate multiple biological dimensions and enable spatial and temporal scaling of assessments. Artificial Intelligence (AI) and Machine Learning (ML) tools show promise for automated species identification, predictive modelling, and large-scale pattern recognition. Yet, their reliability is strongly dependent on the quality and representativeness of training datasets, which are often biased toward temperate regions. Additionally, AI-driven outputs may lack ecological transparency, raising concerns about interpretability and reproducibility.

Taken together, these innovative approaches should be viewed as complementary rather than substitutive to conventional morphology-based and index-driven bioassessment. Their greatest value lies in hybrid frameworks, where molecular and computational tools enhance resolution and efficiency while traditional indices provide ecological context, comparability, and cost-effective applicability.

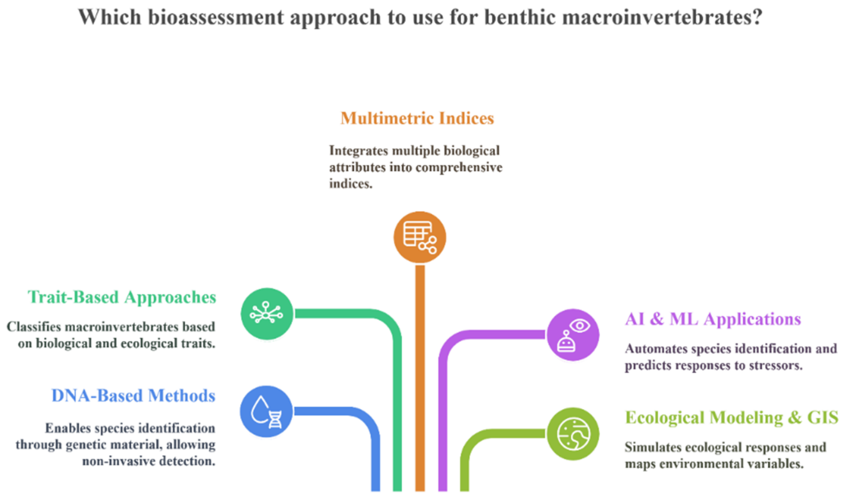

Figure 8. Key Bioassessment approaches for benthic macroinvertebrate.

4.4. Conceptual Framework for Integrated Bioassessment: Linking Metrics into a System

Rather than operating independently, the approaches described above are most effective when applied within a hierarchical and integrative bioassessment framework (Figure 9). In this system, indicator taxa provide the initial diagnostic signal, diversity and biotic indices quantify the magnitude of ecological change, and functional, molecular, and analytical tools refine interpretation by linking biological responses to underlying stressors and spatial patterns. This layered integration reduces uncertainty associated with single-index reliance, improves ecological realism, and supports adaptive management decisions. Such system-level integration is particularly critical in heterogeneous freshwater environments, where ecological responses are non-linear and strongly influenced by regional hydrology, biodiversity, and disturbance regimes

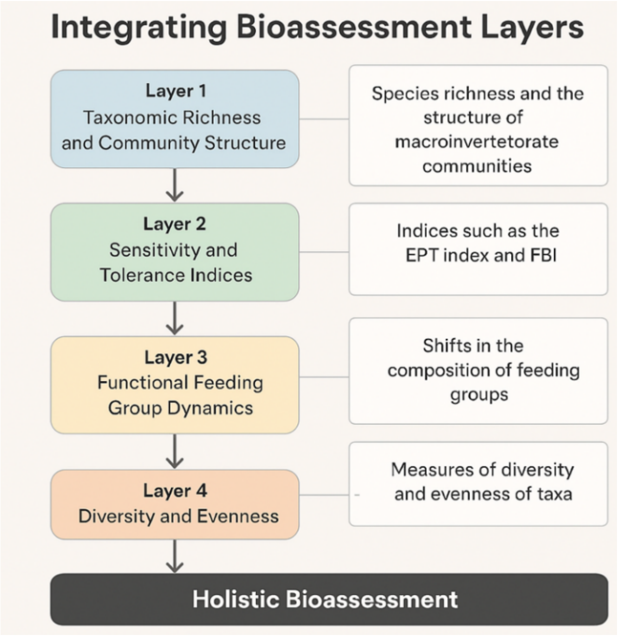

Figure 9. Conceptual framework illustrating the system-level integration of macroinvertebrate-based bioassessment methods, where taxonomic sensitivity provides diagnostic signals, diversity and biotic indices quantify ecological condition, advanced tools refine interpretation, and rapid bioassessment protocols operationalize the framework for routine monitoring.

4.5. Rapid Bioassessment Protocols (RBPs): Integrating Speed with Scientific Rigor

Rapid Bioassessment Protocols (RBPs) translate integrated bioassessment concepts into practical, field-ready monitoring tools. By emphasizing simplified sampling designs, family-level identification, and rapid computation of robust metrics such as EPT richness and FBI, RBPs balance scientific rigor with logistical efficiency. Unlike physicochemical measurements that capture instantaneous conditions, RBPs integrate biological responses over time, reflecting both chronic and episodic disturbances [39,56].

RBPs function as an operational entry point for integrated bioassessment frameworks and are widely applied in large-scale monitoring programs, including the European Water Framework Directive and USEPA bioassessment initiatives, as well as in resource-limited regions where cost-effective yet ecologically meaningful assessment tools are essential [94,95]. When embedded within a broader integrative framework, RBPs provide timely, scalable, and policy-relevant insights into freshwater ecosystem health [50].

4.6. System Integration of Macroinvertebrate-Based Bioassessment Approaches

Although individual bioassessment methods are discussed separately in Sections 4.1–4.5, they collectively form a stepwise and interdependent monitoring system rather than isolated analytical tools (Figure 9). The integrated framework begins with the identification of pollution-sensitive and pollution-tolerant taxa (Section 4.1), which provides a biological signal of ecosystem condition. This signal is subsequently quantified through diversity and biotic indices (Section 4.2), allowing standardized comparison across sites and temporal scales. Advanced and innovative approaches, including trait-based analyses, molecular tools, and data-driven techniques (Section 4.3), enhance taxonomic resolution and diagnostic accuracy, particularly in complex or biodiversity-rich systems. These multiple biological outputs are then synthesized within a conceptual framework (Section 4.4) that links organismal responses to ecological processes and environmental stressors, reducing uncertainty in interpretation. Finally, Rapid Bioassessment Protocols (Section 4.5) operationalize this integrated system into a practical, time-efficient methodology suitable for routine monitoring and management applications.

Together, this system-based integration ensures that macroinvertebrate bioassessment captures both ecological complexity and management relevance, bridging detailed biological information with scalable monitoring needs.

5. Diversity Indices: Concepts and Applications

Diversity indices, often referred to as bio-indices, are powerful tools in aquatic ecology that integrate both quantitative and qualitative aspects of biological communities. They are numerical expressions that reflect not only species diversity (richness and evenness) but also the ecological sensitivity of individual taxa to environmental stressors, thus providing a more comprehensive assessment of water quality [96]. By condensing complex ecological information into measurable values, these indices allow researchers and policymakers to monitor ecosystem health, detect environmental degradation, and evaluate the effectiveness of restoration efforts.

A wide variety of biotic indices have been developed over the last several decades to assess aquatic ecosystem condition, each tailored to specific ecological contexts and monitoring objectives. Early frameworks primarily emphasized species presence and absence, but subsequent developments incorporated abundance patterns, tolerance values, and functional traits, thereby improving diagnostic capacity [97]. Today, indices range from traditional diversity-based approaches such as the Shannon-Wiener and Simpson Index, to taxon-specific metrics such as the EPT richness index and biotic indices like BMWP, ASPT, and Hilsenhoff’s FBI. The continued refinement and application of diversity indices underscore their ecological and policy significance. They not only serve as diagnostic tools for identifying impaired water bodies but also guide adaptive management strategies, offering a bridge between ecological theory and applied conservation practice.

5.1. Commonly Used Diversity Indices in Aquatic Bioassessment Using MI

Diversity indices are essential for evaluating the composition and structure of benthic macroinvertebrate communities, reflecting varying responses to environmental gradients and anthropogenic pressures. Table 3 summarises commonly used indices, including global tools like Shannon-Wiener, Simpson’s, and Pielou’s Evenness, along with region-specific indices such as the Indian Biotic Score (IBS) and NEI. The accompanying flow diagram (Figure 10) categorizes these indices into Richness, Evenness, Dominance, and Functional groups, offering a comprehensive visual summary for comparative evaluation.

Table 3. Comparison of commonly used diversity and biotic indices.

Index	Category	What It Measures	Best Used When	Formula	Strengths	Weaknesses	Key Reference
Shannon-Wiener Index (H′)	Diversity Index	Species diversity (richness + evenness)	Sites with moderate to high species richness	$H' = -\sum (pi \times \ln pi)$	Captures both richness and evenness; sensitive to environmental changes	Sensitive to sample size; requires accurate species-level identification	[31]
Simpson's Index (D)	Diversity Index	Dominance (probability that two individuals belong to the same species)	High-dominance communities (polluted sites)	$1 - D = \sum (pi^2)$	Simple to interpret; less sensitive to rare species	Underestimates diversity in communities where rare taxa are important	[32]
Margalef's Richness Index (d)	Richness Index	Species richness relative to sample size	Comparing sites with different sample sizes	$d = (S - 1)/\ln(N)$	Adjusts for sample size; straightforward calculation	Considers only richness, ignores evenness	[98]
Pielou's Evenness (J′)	Evenness Index	Distributional evenness of individuals among taxa	Sites with similar richness but different abundance patterns	$J' = H'/\ln(S)$	Standardizes diversity by evenness; complements Shannon-Wiener Index	Dependent on Shannon-Wiener Index; not meaningful as standalone	[33]
EPT Index	Indicator-based Index	Richness of sensitive taxa (Ephemeroptera, Plecoptera, Trichoptera)	Streams/rivers where EPT are reliable indicators	EPT = Number of EPT taxa	Strong bioindicator of organic pollution; field-friendly	Less effective in regions naturally poor in EPT taxa	[55]
Biotic Indices (e.g., BMWP, FBI, ASPT)	Indicator-based Index	Pollution tolerance-weighted scores	Assessing gradients of organic pollution	Varies (e.g., BMWP = $\sum$ taxon scores; FBI = $\sum (ni \times ti)/N$)	Incorporates ecological sensitivity of taxa; widely used in regulatory monitoring	Requires regional calibration and good taxonomic resolution	[10,76]
Hill's Numbers (N_1, N_2)	Diversity Index	"Effective number of species"	Quantifying true diversity at different sensitivity levels	N_1 = e^H′; $N_2 = 1/\sum (pi^2)$	Intuitive; unifies richness and evenness; widely applicable	Less commonly used; may require transformations	[99]

Figure 11 illustrates the frequency of use of various diversity indices in aquatic ecological research. This distribution visualizes the proportional application of various diversity indices in aquatic ecological research, particularly in studies employing benthic macroinvertebrates as bioindicators. The Shannon-Wiener Index dominates usage at 55%, underscoring its status as the most preferred and versatile diversity metric. Its popularity is rooted in its dual sensitivity to both species' richness and evenness, enabling a comprehensive characterization of ecological complexity offering a holistic view of community diversity. Several studies e.g., ref. [100] have emphasized its utility in detecting anthropogenic disturbances, nutrient enrichment, and habitat degradation.

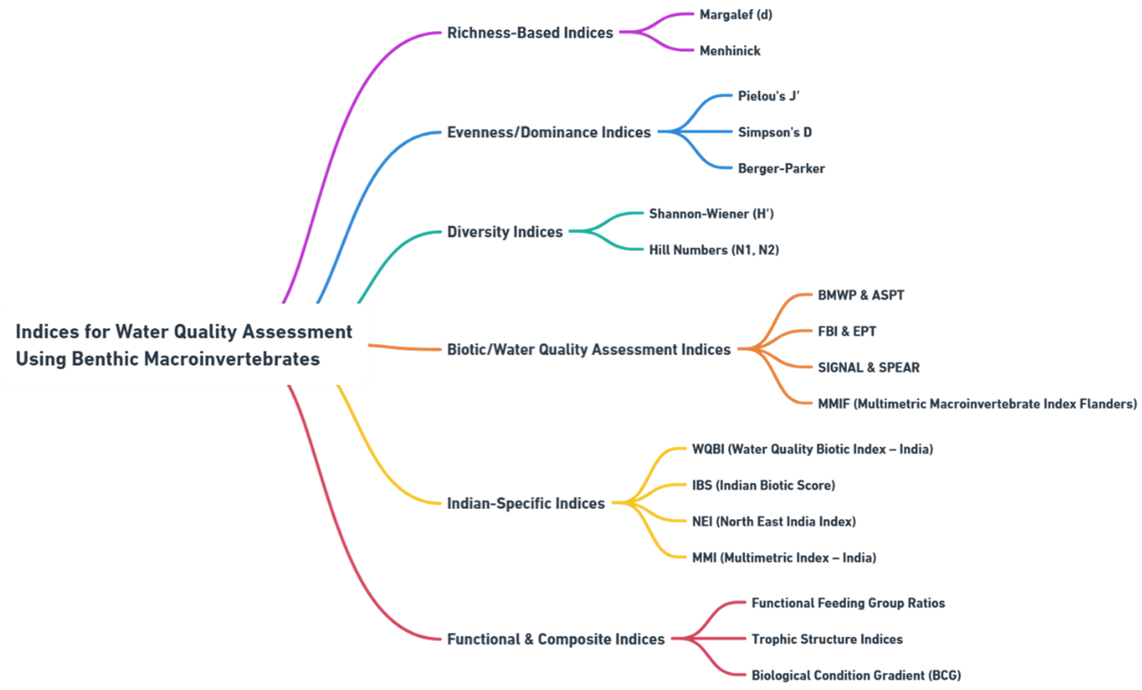

Figure 10. Flow representation of different diversity indices for benthic macroinvertebrates.

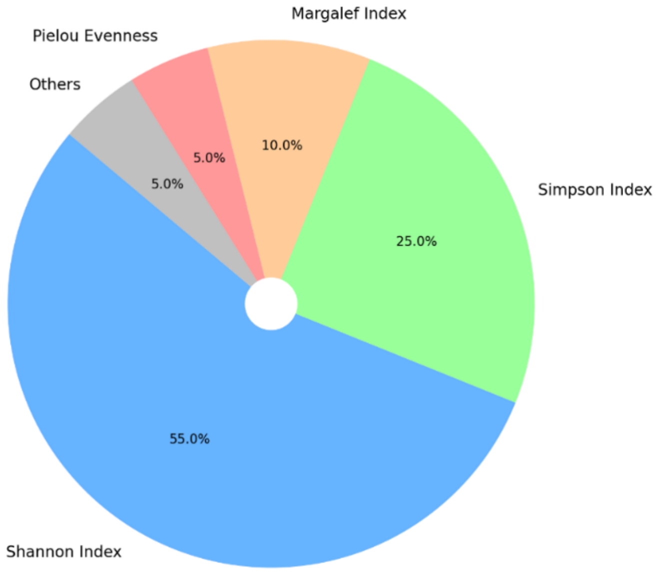

Figure 11. Distribution of diversity index utilization in aquatic research.

The Simpson Index, used in 25% of studies, is particularly valued for capturing species dominance and community stability. It tends to be less sensitive to sample size and rare species, making it a robust measure in studies where dominant taxa are indicative of ecological stress [95,101,102]. Both Shannon-Wiener and Simpson indices are widely embedded in standardized bioassessment protocols, such as those guided by the EU Water Framework Directive (WFD) and US EPA Rapid Bioassessment Protocols, due to their simplicity, reliability, and interpretability [103]. The

Margalef Index (10%) is often used to highlight species richness, particularly in environments where species count variations are more ecologically significant than evenness. Its application is common in initial biodiversity surveys and environmental impact assessments [101]. Pielou's Evenness Index (5%), though less frequently used independently, is often employed in conjunction with Shannon-Wiener Index or Margalef indices to assess distributional equity among species. This is particularly valuable in habitats where pollution tends to skew species abundances toward dominance by tolerant taxa. The remaining 5%, labelled "Others", includes less commonly used indices like the Brillouin, McIntosh, or Berger-Parker indices. These are typically applied in specialized research settings or comparative studies to assess methodological robustness or index sensitivity.

Overall, the clear dominance of the Shannon-Wiener Index and Simpson indices reflects a methodological preference in ecological studies for indices that capture both richness and evenness—key dimensions of biodiversity that respond sensitively to environmental stressors. Their widespread use is not only a testament to their statistical robustness but also to their long-standing presence in academic literature and monitoring guidelines.

5.2. Critical Appraisal of Diversity Relationships and Multi-Index Interpretation

Pairwise comparison of Shannon-Wiener Index, Simpson, and Pielou's Evenness indices (Figure 12) illustrates that widely used diversity metrics reflect partially overlapping but ecologically distinct dimensions of macroinvertebrate assemblages. The moderate positive association between Shannon-Wiener Index and Simpson indices confirms that both respond to overall community heterogeneity; however, their differential weighting of rare versus dominant taxa leads to divergent interpretations under disturbed conditions. The weak and occasionally negative relationship with Pielou's Evenness demonstrates that increases in taxonomic richness or dominance do not necessarily correspond to equitable individual distribution, particularly in organically enriched systems where tolerant taxa proliferate disproportionately.

The large confidence intervals surrounding these relationships highlight the instability of single-index inference across habitats and seasons. Community responses to stressors such as nutrient loading, flow alteration, and habitat simplification often decouple richness from evenness, producing conflicting signals among indices. These results reinforce the reviewer's concern that reliance on any individual metric can be misleading and that diversity indices must be interpreted within a multi-dimensional framework.

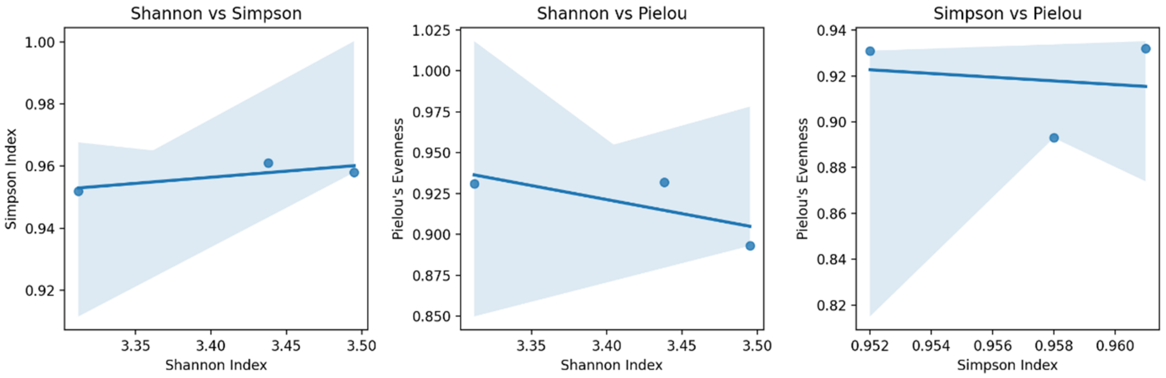

Figure 12. Correlation trends among Shannon-Wiener, Simpson, and Pielou's Evenness indices depicting diversity patterns in macroinvertebrate communities.

Relationships among Shannon-Wiener, Simpson, and Pielou's Evenness indices illustrating partial decoupling of richness, dominance, and evenness signals in macroinvertebrate assemblages. The figure supports the argument that single-index interpretation is unstable across ecological contexts.

To clarify the complementary roles of major indices, Figure 13 provides a synthesis-oriented evaluation based on six operational criteria: richness sensitivity, evenness responsiveness, dominance detection, ecological interpretability, computational simplicity, and taxonomic dependence. This comparison demonstrates that:

- Shannon-Wiener index offers balanced sensitivity to richness and evenness but may overestimate integrity in dominance-driven communities;
- Simpson index is robust for detecting dominance shifts yet relatively insensitive to loss of rare taxa;
- Margalef index responds strongly to taxon number but lacks structural insight;
- Pielou's index isolates evenness and is meaningful only when paired with richness metrics;
- Berger–Parker index rapidly identifies dominance but provides minimal ecological breadth.

The critical implication is that no single diversity metric is universally valid across pollution gradients or habitat types. Indices optimized for detecting organic enrichment may fail under toxic or hydromorphological stress, while richness-based measures can misrepresent conditions in naturally uneven tropical assemblages. Therefore, Figure 13 is retained not as a descriptive illustration but as an integrative decision-support schematic guiding context-specific index selection.

This synthesis directly addresses the need for analytical depth by demonstrating how different indices respond under contrasting ecological scenarios and why metric pluralism is essential for credible bioassessment. Importantly, the radar chart represents relative conceptual performance rather than empirical effect sizes, serving as an integrative framework to summarize recurring trends reported across multiple studies rather than to rank indices definitively. Future applications should prioritize combinations of complementary indices rather than isolated reporting, particularly when assessments inform management decisions.

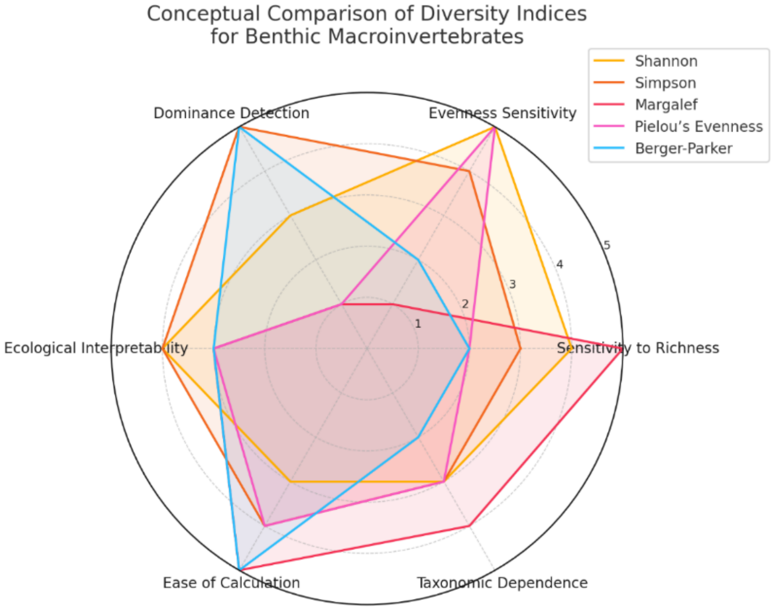

Figure 13. Conceptual visualization of macroinvertebrate diversity indices using a radar chart.

5.3. Basis for Conceptual Scoring and Literature Support

The relative scoring (1–5 scale) used in Figure 13 is derived from qualitative consensus reported in foundational and comparative ecological studies, rather than from a single dataset or meta-analysis. Scores reflect the directional strength or limitation of each index as consistently described in the literature. For example, Shannon-Wiener and Margalef indices are widely recognized for their sensitivity to species richness but are less effective at detecting dominance patterns, particularly under organic enrichment or intermediate disturbance [94,102] in contrast, the Berger–Parker index explicitly emphasizes dominance by the most abundant taxon and has been repeatedly shown to respond strongly to community simplification under pollution stress, albeit with limited sensitivity to rare taxa [104,105].

Similarly, Simpson's index has been documented as relatively robust to sample size variation and effective at detecting dominance shifts, while Pielou's evenness index provides complementary insight into community structure but lacks standalone diagnostic power for water quality assessment [94,106]. Taxonomic dependence scores were informed by studies demonstrating that most diversity indices yield stable patterns at family-level resolution, though fine-scale sensitivity improves with genus- or species-level identification [103].

Accordingly, the radar chart should be interpreted as a conceptual synthesis grounded in published ecological theory and applied biomonitoring literature, intended to guide index selection and integration rather than to replace site-specific calibration or quantitative validation.

6. Comparative Performance of Diversity and Biotic Indices Across Ecological Contexts

The effectiveness of diversity and biotic indices for freshwater bioassessment is strongly context dependent, varying with pollution type, hydrological regime, taxonomic resolution, and biogeographic setting. Individual indices rarely demonstrate universal applicability, and their diagnostic power fluctuates according to dominant stressors and habitat characteristics [49,107]. A condition-based evaluation is therefore essential for meaningful interpretation of bioassessment outcomes.

6.1. Performance Across Pollution Gradients: Organic Versus Toxic Stress

Diversity indices such as Shannon–Wiener and Simpson are widely used as general indicators of ecological condition; however, their sensitivity is often limited under organic enrichment characterized by gradual or intermittent disturbance. Moderate nutrient loading frequently increases the abundance of tolerant taxa without an immediate decline in richness, leading to inflated diversity values. Studies from tropical rivers have shown that Shannon-Wiener diversity may remain high despite substantial organic pollution due to dominance shifts rather than ecological recovery.

Biotic indices incorporating tolerance scores—such as BMWP and the Family Biotic Index (FBI)—generally respond more directly to organic loading and oxygen depletion [20,86]. Nevertheless, these indices were primarily calibrated against organic pollution and may underestimate impairment caused by heavy metals, pesticides, or salinity. Under toxic stress, mortality can affect both sensitive and tolerant groups, weakening the discriminatory capacity of tolerance-based metrics. Multimeric indices that integrate richness, composition, and functional traits have demonstrated improved performance across mixed pollution gradients. Comparative studies indicate that such approaches outperform single indices in rivers experiencing multiple co-occurring stressors, although their success depends on robust regional calibration [108].

6.2. Influence of Flow Regime: Lotic Versus Lentic Systems

Hydrological regime strongly shapes macroinvertebrate assemblages and consequently index performance. In lotic systems with stable flow, EPT richness and BMWP-type metrics reliably reflect habitat heterogeneity and dissolved oxygen conditions [49]. However, lentic and low-flow environments naturally favor tolerant taxa such as Chironomidae and Oligochaeta, which depress EPT-based scores even in minimally disturbed conditions. Diversity indices also show reduced diagnostic power in ponds and reservoirs where community structure is driven more by macrophyte cover and sediment texture than by water chemistry alone. Consequently, direct comparison of index values between lotic and lentic habitats often leads to systematic bias unless habitat-specific benchmarks are applied.

6.3. Role of Taxonomic Resolution in Index Sensitivity

Taxonomic resolution represents a critical trade-off between precision and feasibility. Family-level identification, widely adopted in national monitoring programs, enables rapid assessment but may mask ecologically meaningful changes. Several investigations have shown that genus-level data improve detection of early or low-intensity disturbance, particularly for diversity-based metrics [104].

Conversely, tolerance-based indices such as BMWP and FBI were originally designed for family-level application and often show limited additional benefit from finer resolution [86]. A hybrid strategy—family-level screening supplemented by genus-level indicators for sensitive groups—has been proposed as a pragmatic compromise, especially in regions with limited taxonomic capacity.

6.4. Biogeographic Applicability: Temperate Versus Tropical Rivers

Most classical indices were developed in Europe and North America and do not fully reflect the ecological dynamics of tropical rivers [61]. Tropical systems exhibit higher baseline diversity, continuous recruitment, and naturally greater dominance of tolerant taxa, reducing the discriminatory power of Shannon-Wiener Index and EPT richness Index. Direct transfer of BMWP or FBI scores to South Asian and African rivers has produced inconsistent assessments. Studies from India report that unmodified BMWP often underestimates degradation in monsoon-driven rivers where sensitive taxa are seasonally absent [105,106]. Regionally calibrated multimeric indices and trait-based approaches have therefore shown superior reliability in tropical context.

6.5. Synthesis and Implications for Bioassessment

Evidence from multiple regions confirms that no single index provides universally reliable assessment of freshwater health. Diversity indices capture broad community patterns but may misrepresent conditions under moderate disturbance or in naturally diverse tropical systems. Biotic indices offer stronger diagnosis of organic pollution yet perform poorly under toxic or hydro morphological stress as summarised in Table 4. Multimeric and trait-based frameworks provide the most balanced performance across ecological contexts, provided that regional calibration and appropriate taxonomic resolution are ensured [109].

An integrative strategy that matches index selection to pollution type, habitat, and biogeographic setting is therefore essential for credible bioassessment.

Table 4. Comparative performance of major diversity and biotic indices across ecological contexts.

Organic Pollution Gradient	**Shannon–Wiener, Simpson**	**Reflect Overall Community Heterogeneity; Easy Comparison across Studies**	**Often Remain High under Moderate Enrichment due to Dominance Shifts; Low Sensitivity to Early Degradation**	**Preliminary Screening, Reference Site Comparison**
Toxic pollution (metals, pesticides)	BMWP, FBI	High responsiveness to oxygen depletion and nutrient loading	Calibrated mainly for organic stress; weak for toxic contamination	Municipal wastewater, agricultural runoff
	Multimeric (MMI)	Integrates richness, tolerance, and composition	Requires regional calibration and reference conditions	Mixed land-use catchments
	Diversity indices	Detect catastrophic species loss	Poor discrimination at sub-lethal levels	Severe contamination events
	BMWP/FBI	Limited applicability	Tolerance scores not metal-specific	Not recommended alone
	Trait-based/MMI	Better linkage to mechanism (feeding, respiration traits)	Data intensive	Mining and industrial effluents
Flow regime—lotic	EPT richness, BMWP	Strong relation with habitat and DO	Seasonal bias in monsoon rivers	Mountain and perennial streams
Flow regime—lentic	Shannon/Simpson	Capture macrophyte-driven heterogeneity	EPT naturally low; false poor status	Ponds, reservoirs with habitat metrics
Taxonomic resolution—family	FBI/BMWP	Rapid, cost-effective	Misses' cryptic species replacement	National routine monitoring
Taxonomic resolution—genus/species	Diversity & traits	Higher sensitivity to subtle stress	Requires expertise, time	Research and impact studies
Biogeography—temperate	Classical indices	Well calibrated	—	Europe, North America
Biogeography—tropical	Adapted MMI/traits	Accounts for high baseline diversity	Direct transfer unreliable	South Asia, Africa, Neotropics

7. Global and Regional Indices for Benthic Macroinvertebrate Diversity: A Comparative Framework

The application of diversity indices in aquatic bioassessment varies significantly across geographical and ecological contexts, reflecting differences in biodiversity patterns, pollution types, and methodological preferences. Globally recognized indices such as the Shannon-Wiener Index, Simpson's Index, BMWP (Biological Monitoring Working Party), and ASPT (Average Score per Taxon) are extensively used in temperate regions, owing to their robustness and widespread validation. However, their direct applicability in tropical and subtropical regions, like India, often requires regional calibration due to ecological variability and differing taxonomic compositions. To address this, several region-specific indices have been developed. For instance, India has adopted localized indices such as the Water Quality Biotic Index (WQBI), the Indian Biotic Score (IBS), and the North East India (NEI) Index, which consider endemic species and local hydrological conditions. These indices offer improved sensitivity to region-specific stressors and habitat conditions. Collectively, the availability of both global and regional indices allows for a more tailored and accurate assessment of freshwater health, enabling ecologists and resource managers to make informed decisions based on context-specific ecological responses.

7.1. Global Perspective

In global freshwater biomonitoring efforts, diversity indices have become cornerstone tools for assessing ecological integrity and water quality. Indices such as the Shannon-Wiener Index and Simpson's Index are widely utilized for quantifying species richness and evenness, thereby reflecting the structural complexity of aquatic communities. Additionally, biotic indices like the BMWP score and ASPT score integrate species-specific pollution sensitivity to detect anthropogenic impacts, especially in European and North American freshwater systems. These indices are favoured in temperate regions due to their consistent performance across varied pollution gradients and their incorporation into legislative frameworks, including the EU Water Framework Directive (WFD). However, their generalized scoring systems often assume a taxonomic uniformity that may not translate well across ecozones. Consequently, the direct application of these indices in ecologically distinct regions such as the tropics may yield misleading or under representative assessments unless carefully recalibrated [49,84].

7.2. Indian Perspective

In contrast, the application of these global indices in India and other tropical countries often requires contextual adaptation due to ecological variability, diverse climatic zones, and unique macroinvertebrate faunal composition. In the Indian subcontinent, where freshwater ecosystems are influenced by unique climatic, topographic, and anthropogenic factors, regionally adapted indices have become essential for effective ecological assessment. Macroinvertebrate communities in India exhibit high endemism and are subjected to dynamic hydrological regimes driven by monsoons, seasonal droughts, and localized pollution sources. Recognizing these distinct ecological realities, researchers have developed and refined indices such as the Indian Biotic Score (IBS), Water Quality Biotic Index (WQBI), and the North East India Macroinvertebrate Index (NEI Index). These tools incorporate indigenous taxa and region-specific ecological tolerances, offering greater accuracy in detecting water quality changes in Indian rivers and streams [110]. Notably, studies conducted in biodiversity-rich regions like the Western Ghats, Brahmaputra basin, and Ganga-Yamuna River systems have demonstrated the effectiveness of these indices in capturing subtle ecological responses to land-use change, organic pollution, and hydrological alterations [109,111]. The growing body of Indian literature on macroinvertebrate-based biomonitoring signifies a shift toward context-driven ecological assessment and underscores the need for ongoing refinement of these indices through collaborative regional research. The parallel application of global and regionally adapted indices in India thus offers a hybridized approach, promoting both scientific rigor and ecological relevance.

7.3. Popular Indices Used in Different Regions

Different countries and regions exhibit preferences for specific diversity indices based on available resources, ecological context, and regulatory requirements (Figure 14).

7.4. Comparative Utilization of Bioassessment Indices: Bridging Global Practices with Indian Applications

The comparative heat maps (Figure 15) illustrate significant disparities in the adoption and thematic diversity of macroinvertebrate-based bioassessment indices between global and Indian contexts. Globally, the emphasis is placed on biotic/water quality indices, notably the EPT Index, FBI, BMWP score, and ASPT, which are foundational tools for ecological integrity assessment across diverse biogeographical regions. These are complemented by functional, richness-based, diversity, and evenness indices—highlighting a multidimensional, ecosystem-level approach that integrates taxonomic, structural, and functional perspectives. In contrast, Indian applications are skewed toward region-specific indices such as the Indian Biotic Score (IBS), Water Quality Biotic Index–India (WQBI), and MMI–India, reflecting an ongoing effort to localize assessment frameworks for indigenous river systems.

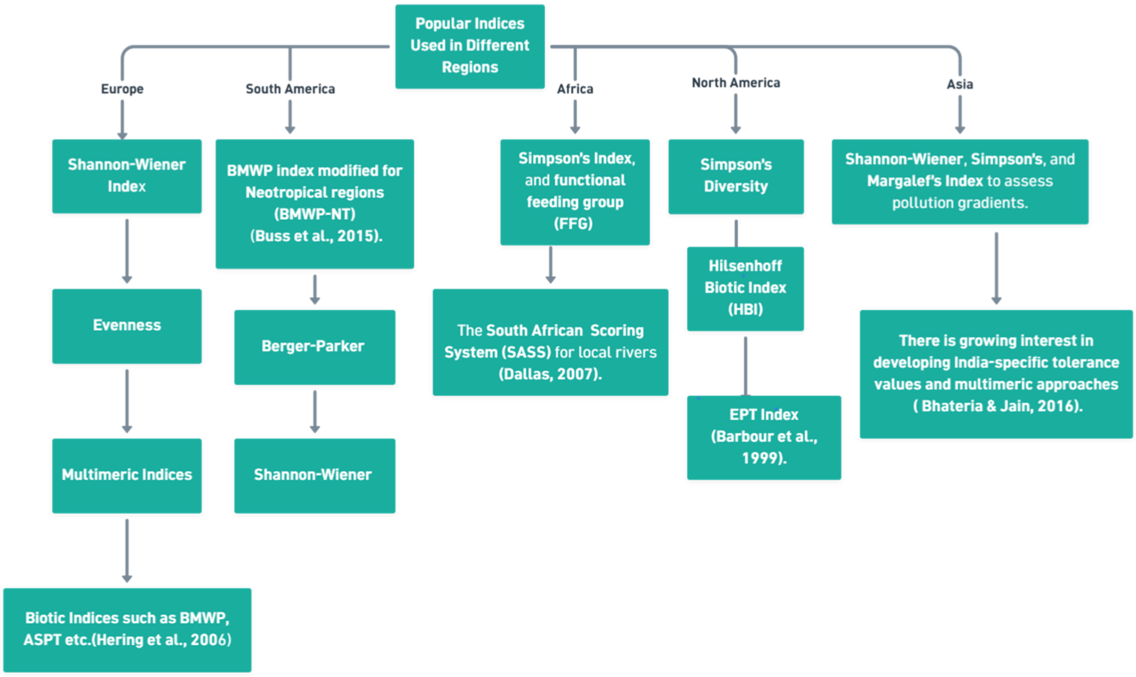

Figure 14. Regional patterns in the application of diversity indices for benthic macroinvertebrate assessment. Information synthesized from Buss et al. [7], Barbour et al. [58], Hering et al. [41], Dallas (2007) [89] and Bhateria and Jain [112].

Figure 15. Heatmap of biodiversity indices usage.

However, the limited adoption of advanced global tools—such as SPEAR (targeting species at risk), MMIF (Flanders' multimeric approach), and the Biological Condition Gradient (BCG)—in Indian studies points to methodological conservatism and potential gaps in capacity or ecological calibration. The underrepresentation of functional and trait-based indices, despite their value in assessing ecological processes and responses to stressors, suggests a need for capacity-building and methodological expansion in Indian bioassessment protocols. This divergence is further emphasized by the modest use of composite indices and evenness-based metrics in India, which globally play a critical role in capturing community-level imbalances due to anthropogenic disturbances.

Collectively, these heat maps reveal that while India has made notable strides in developing localized indices, the broader adoption of globally validated, multidimensional metrics remains limited. Bridging this methodological divide will require harmonizing indigenous approaches with internationally recognized standards—thereby enhancing the diagnostic power, comparability, and ecological relevance of bioassessment strategies in the Indian subcontinent.

7.5. Policy Integration

(1) European Union—Water Framework Directive (WFD)

The WFD (Directive 2000/60/EC) is one of the most comprehensive legislative frameworks integrating biological indicators, including macroinvertebrates, into surface water quality assessments. It mandates that "good ecological status" be determined through metrics like taxonomic composition, abundance, and diversity indices of macroinvertebrates, among other biological quality elements (BQEs) [113].

(2) United States—USEPA Guidelines

The USEPA supports the use of biological monitoring in its Rapid Bioassessment Protocols (RBPs) and promotes the use of metrics such as Shannon-Wiener, EPT taxa richness, and HBI. These are integrated into state-level assessments and Clean Water Act reporting [65].

(3) India—National Guidelines and CPCB Protocols

In India, the Central Pollution Control Board (CPCB) has initiated bioassessment pilot studies incorporating macroinvertebrates under the National Water Quality Monitoring Programme (NWMP). Although currently limited to case studies, efforts are underway to develop a national biotic index and incorporate indices such as Shannon-Wiener, Simpson, and EPT richness into river health assessments [114,115].

8. Integrating Biological and Physicochemical Indices for Comprehensive Water Quality Assessment

Traditionally, water quality monitoring has relied predominantly on physicochemical measurements—parameters such as pH, dissolved oxygen (DO), nutrient concentrations, salinity, and suspended solids. These indicators are vital for identifying the current status of a water body but represent only a "snapshot" of conditions at the time of sampling [67,116]. However, such measurements often fail to capture cumulative or long-term ecological impacts, especially in dynamic freshwater systems influenced by fluctuating environmental stressors. In contrast, biological indices, especially those based on benthic macroinvertebrates, provide a "moving picture" of aquatic ecosystem health. These organisms respond to a range of stressors over time and space, offering a more integrated assessment of water quality [117,118]. Biological methods have proven effective in detecting both natural and anthropogenic disturbances, as aquatic biota reflect the structural and functional integrity of ecosystems and integrate stressor effects over longer periods [119,120].

Research has consistently shown that the inclusion of aquatic organisms in water quality assessments provides more accurate ecological interpretations than physicochemical indicators alone. Biotic indices such as the BMWP score, EPT richness, and region-specific multimeric indices are increasingly used worldwide for river health assessment [46,65]. Refs. [112,121–123] emphasized that these tools often outperform conventional chemical monitoring methods in detecting ecosystem degradation. Despite advances in biomonitoring, literature still lacks comparative evaluations of biological assessment tools across different geographical and ecological contexts. Furthermore, ecological thresholds—specific values or ranges of biotic metrics beyond which significant ecological change occurs—are increasingly being applied in resource management frameworks to guide restoration and conservation efforts [116,124].

Concurrently, water quality indices (WQIs) based on physicochemical variables simplify complex datasets by aggregating multiple parameters into a single, interpretable value [125,126]. These indices vary widely in terms of calculation methods, indicator selection, and classification systems (e.g., number/color-coded scales), leading to differences in reported water quality status [127,128]. Therefore, choosing a reliable and context-appropriate WQI is essential for accurate environmental assessment. Increasingly, researchers and water managers acknowledge that no single index can capture the full complexity of aquatic systems. The integration of biological and physicochemical indices is now recognized as essential for a comprehensive and ecologically meaningful assessment of river conditions (Figure 16). While physicochemical indicators respond to immediate environmental changes, biological indices provide insight into chronic exposure and ecosystem-level responses [112,121,122].

Anthropogenic activities such as urban expansion, deforestation, agricultural runoff, and industrial discharges continue to degrade aquatic ecosystems [123]. Such activities alter habitat structures, contribute to forest fragmentation, and jeopardize aquatic biodiversity. Consequently, frequent monitoring of rivers is not only essential for protecting freshwater ecosystems but also for safeguarding human health, as an estimated 80% of global diseases are linked to water quality [129].

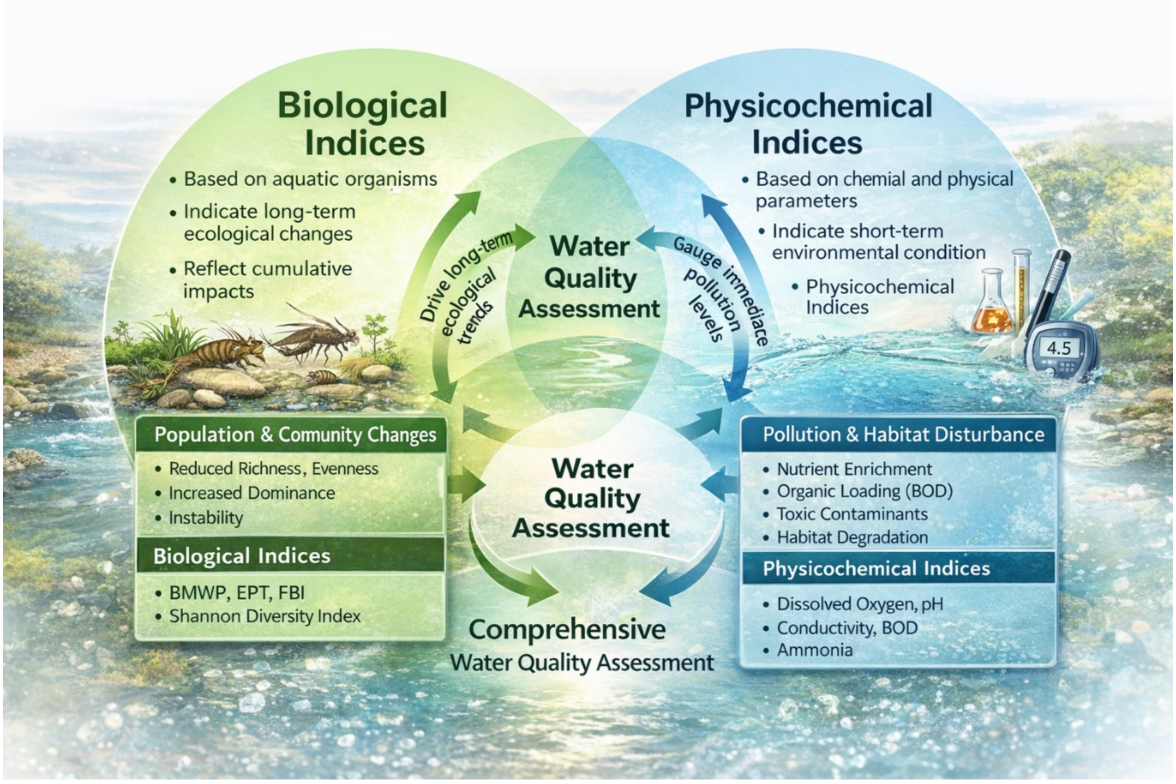

Figure 16. Conceptual model: Integration of biological and physicochemical indices.

9. Synthesis: Linking Benthic Macroinvertebrates and Diversity Indices in Water Quality Assessment

The effectiveness of benthic macroinvertebrates as bioindicators arises from fundamental ecological mechanisms governing species tolerance, life-history traits, and habitat specialization along environmental gradients (Figure 17). Across freshwater systems globally, shifts in macroinvertebrate assemblage structure reflect the integrated effects of physicochemical stressors—such as organic enrichment, nutrient loading, oxygen depletion, and habitat modification—rather than isolated water quality parameters. Diversity and biotic indices capture these biologically mediated responses by translating complex community changes into interpretable metrics of ecological condition. Recent studies (2020–2024) further substantiate their utility in ecological monitoring by establishing robust correlations between macroinvertebrate assemblages and physicochemical water parameters. For instance, in the Mutshundudi River, South Africa, sensitive taxa like Ephemeroptera were confined to upstream zones with minimal anthropogenic influence, while pollution-tolerant groups such as Chironomidae and Corixidae dominated midstream segments affected by agricultural runoff [130]. Similarly, a biomonitoring study in the Chishui River, China, observed substantial declines in sensitive macroinvertebrates under eutrophic conditions, reflecting reduced dissolved oxygen and elevated nutrient loads [131]. One key mechanism underpinning this strong correlation between macroinvertebrate assemblages and water quality is differential pollution tolerance. Sensitive taxa such as Ephemeroptera, Plecoptera, and Trichoptera possess high oxygen demands, narrow thermal tolerances, and limited physiological plasticity. As a result, they decline rapidly under eutrophication, organic loading, or toxic stress, leading to reduced richness and evenness in impacted reaches. In contrast, tolerant groups such as Chironomidae and Oligochaeta exhibit physiological adaptations to hypoxia and organic enrichment, allowing them to dominate degraded habitats. This replacement of sensitive taxa by tolerant forms drives consistent declines in Shannon–Wiener diversity and EPT richness while increasing dominance-based metrics, a pattern reported across rivers in Africa, China, Europe, and India [132,133]. Numerous global and Indian studies consistently demonstrate the efficacy of benthic macroinvertebrates as robust indicators of aquatic ecosystem health, reinforcing the central objective of this review—to evaluate and compare diversity indices in bioassessment frameworks.

A second mechanism involves changes in community evenness and dominance structure under moderate disturbance. In nutrient-enriched or agriculturally influenced systems, intermediate levels of stress may initially promote opportunistic taxa, resulting in relatively stable richness but increased dominance by a few tolerant species. This explains why Shannon-Wiener diversity may remain moderate or even elevated in some impacted tropical systems despite ecological degradation, whereas indices emphasizing dominance (e.g., Simpson, Berger–Parker) and tolerance-weighted biotic indices (e.g., BMWP, FBI) more accurately reflect impairment. Such non-linear responses underscore the importance of interpreting diversity indices within their ecological and biogeographic context.

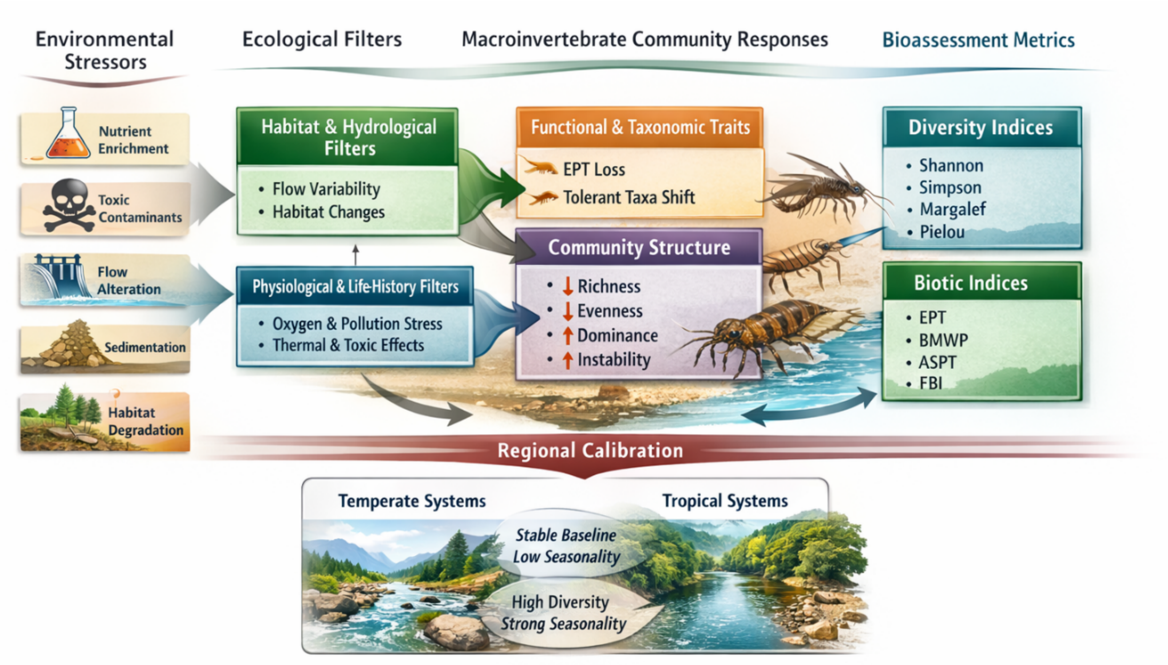

Figure 17. Conceptual synthesis illustrating the ecological mechanisms linking environmental stressors, benthic macroinvertebrate assemblage responses, and diversity/biotic indices in freshwater bioassessment.

In the Indian context, emerging evidence increasingly mirrors global bioassessment paradigms while revealing hydro-ecologically distinct response patterns. Multimetric indices like the ICI integrate multiple ecological attributes of macroinvertebrate communities to evaluate environmental stress [134]. Sarkar [135] documented a pronounced dominance of pollution-tolerant Chironomidae and associated taxa in impacted stretches of the Yamuna River, accompanied by reduced taxonomic richness and skewed community evenness, indicative of organic enrichment and habitat perturbation. Similarly, Goel et al. [136] demonstrated that benthic macroinvertebrate assemblages in the River Ganga exhibited depressed biotic integrity, with index-based assessments (e.g., Biotic Index scores) coherently tracking physicochemical deterioration and elevated anthropogenic stress.

Complementarily, Mishra and Nautiyal [137] observed marked downstream shifts in assemblage organization within the Bhagirathi River, where hydroelectric interventions altered substrate stability, flow heterogeneity, and habitat connectivity, collectively mediating reductions in ecological integrity.

Recent findings by Dutta and Borah [138] provide quantitative corroboration of these relationships, revealing robust correlations between macroinvertebrate diversity metrics (Family Biotic Index, Margalef richness, and related indices) and key pollution indicators, including biochemical oxygen demand and organic loading. In a comparable urban effluent gradient, Nautiyal and Mishra [139] documented significant declines in EPT diversity and Shannon–Wiener index values in the Ken River, concomitant with increased dominance coefficients and expansion of tolerant assemblages.

Collectively, these investigations affirm the cross-regional diagnostic robustness of macroinvertebrate-based bioassessment frameworks while emphasizing the necessity for biogeographically calibrated interpretive thresholds. In monsoon-driven tropical systems, hydrological stochasticity, sediment flux dynamics, and seasonal recolonization processes interact synergistically with pollution gradients to shape community trajectories. Consequently, downstream reductions in sensitive taxa and compensatory increases in tolerant guilds reflect not solely contaminant stress but also modified habitat templates and altered disturbance regimes. This ecological complexity underscores the need for regionally optimized indices that integrate natural hydromorphological variability with signals of anthropogenic disturbances in Indian freshwater ecosystems.

10. Gaps in Literature and Future Directions

While the application of benthic macroinvertebrates as bioindicators has significantly advanced freshwater biomonitoring, several persistent and emerging gaps hinder the development of a globally robust and predictive bioassessment framework (Figure 18). One of the foremost challenges is the geographic and taxonomic bias in existing studies. Large river systems in South Asia, Africa, and parts of South America remain underrepresented, and lesser-known taxa—particularly meiofauna and cryptic invertebrate species—are routinely excluded from assessments, leading to spatially skewed and taxonomically narrow insights.

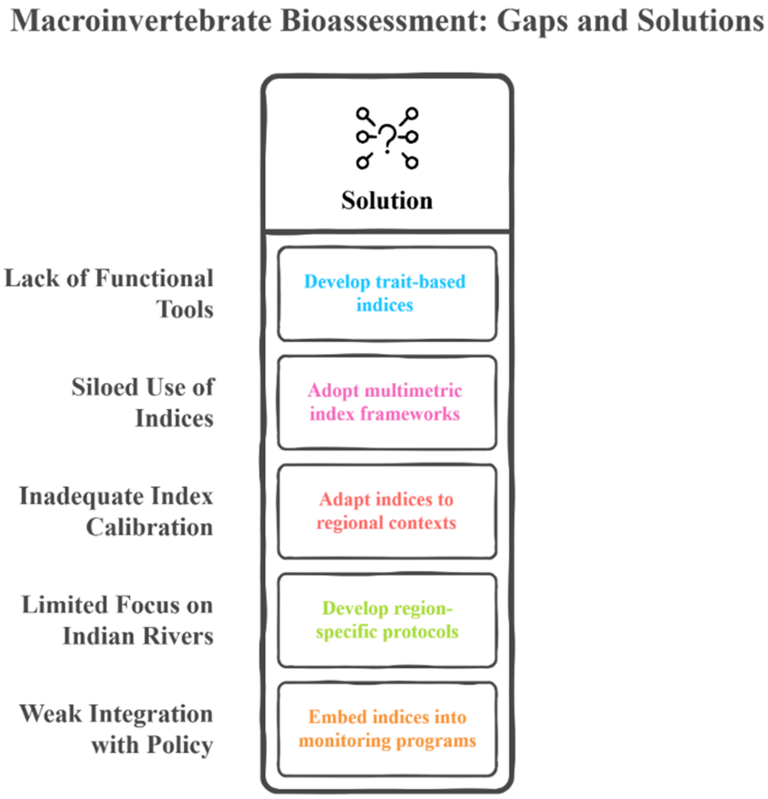

Figure 18. Bridging literature gaps in bioassessment practices.

Another major gap is the lack of standardized sampling protocols and analytical frameworks, especially across diverse ecological regions. Standardized laboratory protocols ensure consistency and accuracy in macroinvertebrate-based bioassessment studies. The processing of benthic macroinvertebrate samples, including sorting and identification, followed internationally accepted EPA guidelines to maintain methodological consistency. While numerous indices exist globally (e.g., BMWP, FBI, EPT), their inconsistent application and modification for regional contexts impede cross-comparability and data harmonization. A unified, tiered protocol that accommodates local ecological nuances while maintaining global compatibility is urgently needed. The rapid evolution of artificial intelligence (AI), computer vision, and machine learning (ML) presents transformative opportunities in macroinvertebrate bioassessment. Automated image-based identification systems, unsupervised classification of trait groups, and predictive modelling of water quality parameters based on macroinvertebrate data are emerging frontiers. However, these tools require large, high-quality, annotated datasets and ecological metadata to be effective—resources currently limited or fragmented across institutions.

Ecological modelling and forecasting also remain underutilized. There is limited integration of macroinvertebrate data with hydrological models, land-use change scenarios, and climate projections, despite their potential to predict ecosystem responses to future stressors. The development of coupled bio-physicochemical models, supported by long-term monitoring datasets, would significantly enhance scenario-based planning and water resource management.

Furthermore, the future of bioassessment lies in real-time, sensor-integrated biomonitoring platforms and remote or in-situ automated samplers. Current bioassessment remains largely manual and periodic; integrating macroinvertebrate indices into smart monitoring networks—potentially in combination with eDNA, telemetry, and Internet of Things (IoT)–based sensors—has the potential to transform water quality surveillance into a more continuous and responsive system. Such integration could enable near–real-time detection of ecological change and improve early-warning capabilities. However, this transition also presents significant challenges related to the storage, management, and interpretation of large volumes of heterogeneous biological data. High-throughput sequencing outputs, long-term sensor streams, and spatially explicit ecological datasets demand robust data infrastructures, standardized data formats, and advanced analytical frameworks. Without appropriate bioinformatics capacity, data governance strategies, and interdisciplinary expertise, the growing scale of biological "Big Data" may limit practical implementation rather than enhance monitoring efficiency.

Lastly, the need for capacity building and knowledge dissemination is paramount. Many regions lack trained taxonomists, digital infrastructure, or institutional support to adopt advanced methodologies. International collaborations, open-access platforms for macroinvertebrate trait databases, and citizen science initiatives should be fostered to democratize access to tools and knowledge.

11. Conclusions

This review highlights the central role of benthic macroinvertebrates as cost-effective, reliable, and ecologically relevant indicators of water quality. By synthesizing taxonomic, functional, and multimeric approaches, the article underscores how macroinvertebrate communities offer integrative insight into both acute disturbances and long-term ecological degradation in freshwater systems. The included heat maps reveal significant spatial variability in diversity indices across sampling sites, with high concentrations of pollution-tolerant taxa correlating with degraded zones, and more diverse, balanced communities present in relatively undisturbed habitats. These visualizations affirm the sensitivity of macroinvertebrate-based metrics—such as the EPT richness and functional feeding group distributions—in detecting environmental stress gradients. The flowchart on integrating bioassessment layers further illustrates how layering taxonomic richness, sensitivity scores, and functional traits yields a multidimensional and holistic understanding of water quality.

Macroinvertebrates not only reflect current environmental conditions but also integrate historical impacts, making them valuable for both reactive assessments and proactive management. Their use in Rapid Bioassessment Protocols (RBPs), as visualized in the conceptual diagrams, emphasizes their applicability in real-time decision-making and large-scale monitoring programs. Despite their potential, this review identifies persistent gaps—particularly in underrepresented regions such as South Asia, including India, where site-specific indices and calibration protocols remain underdeveloped. Additionally, a global inconsistency in the application and standardization of indices limits data comparability across river systems. Moving forward, broader adoption of trait-based and multimeric frameworks, as well as the integration of AI, machine learning, and real-time monitoring technologies, is crucial. The proposed "Integrated Future Pathways" flowchart offers a strategic roadmap for enhancing bioassessment practices, calling for the convergence of traditional taxonomy, big data analytics, ecological modelling, and policy integration. The integration of these innovative bioassessment methods offers a more complete picture of water quality, one that can accommodate the complex interactions between various environmental factors and aquatic life. By combining traditional and modern approaches, we can create a more resilient and adaptive monitoring framework, ensuring that aquatic ecosystems are protected and preserved for future generations.

In conclusion, macroinvertebrate-based bioassessment, when combined with physicochemical tools and supported by standardized, tech-enhanced protocols, represents a powerful, adaptive strategy for safeguarding freshwater ecosystems. As demonstrated through empirical visualizations and analytical frameworks in this review, diversity indices are not only diagnostic tools but also vital instruments for conservation planning, regulatory decision-making, and sustainable water resource management on a global scale. This review underscores the importance of utilizing both biological and physicochemical tools in river assessment frameworks. Their complementary strengths enable more accurate diagnosis of ecosystem health, facilitate evidence-based environmental management, and support the conservation of freshwater biodiversity.

Author Contributions

S.S.: conceptualization, methodology, literature review, data curation, formal analysis, visualization, writing—original draft preparation; A.S.: literature review, validation, writing—review and editing; J.V.: conceptualization, supervision, project administration, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interests.

Use of AI and AI-Assisted Technologies

No AI tools were utilized in the preparation of this paper.

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