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Bioindicators and biomonitoring: Review of methodologies applied in water bodies and use during the Covid-19 pandemic

Bioindicadores y biomonitorización: Revisión de las metodologías aplicadas en las masas de agua y su uso durante la pandemia de Covid-19

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Abstract

The objective of this review is to highlight the importance of the use and application of bioindicators in the evaluation of the quality of water bodies from their origins to the present Era; during and post Covid-19 Era (2019-2022). More than 800 papers were analyzed in relation to the theme and the discussion of the advantages and disadvantages of the application of biomonitoring studies, criteria for selection of methods and organisms. This review focuses on algae, benthic macroinvertebrates and fish in river ecosystems, reservoirs, and other water bodies. Different types of biomonitoring methodologies are addressed, including biotic and diversity indices, multimetric and multivariate approaches, integrative methods, functional metrics, and new generation biomonitoring or "biomonitoring 2.0". This review highlights the great importance of the use and application of bio-indicators and biomonitoring techniques within environmental management and environmental health sustainably.

Keywords: Macroinvertebrate; fish; algae; water quality; rivers; impoundments; biomonitoring 2.0; Covid-19.

Resumen

El objetivo de esta revisión es destacar la importancia del uso y aplicación de los bioindicadores en la evaluación de la calidad de los cuerpos de agua desde sus orígenes hasta la época actual; durante y después de la era Covid-19 (2019-2022). Se analizaron más de 800 trabajos en relación con el tema y la discusión de las ventajas y desventajas de la aplicación de los estudios de biomonitoreo, los criterios de selección de los métodos y los organismos. Esta revisión se centra en las algas, los macroinvertebrados bentónicos y los peces en ecosistemas fluviales, embalses y otras masas de agua. Se abordan diferentes tipos de metodologías de biomonitorización, incluidos los índices bióticos y de diversidad, los enfoques multimétricos y multivariantes, los métodos integradores, las métricas funcionales y la biomonitorización de nueva generación o "biomonitorización 2.0". Esta revisión resalta la gran importancia del uso y la aplicación de bioindicadores y técnicas de biomonitorización dentro de la gestión ambiental y la salud ambiental de forma sostenible.

Palabras clave: Macroinvertebrados; peces; algas; calidad del agua; ríos; embalses; biomonitorización 2.0; Covid-19.

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Introduction to bioindicators and biomonitoring

History of the development of biomonitoring

Superficial water bodies such as rivers and reservoirs provide the foundations for the development of aquatic ecosystems, as well as for many human activities. For this reason, they have also become some of the most threatened ecosystems around the world (Cairns & Pratt, 1993). With the increasing demands of human activities and the stress that they set on water resources, it is clear that the preservation and restoration of our aquatic ecosystems is of paramount importance. Biological monitoring can be defined as the observed responses that organisms manifest to determine if their environment is favorable for them to live (Aitio, 1994; Kushlan, 1993; Paoletti, 2012; Zonneveld, 1983). Environmental (natural) or anthropogenic (artificial) factors may disrupt the balance of an aquatic ecosystem, resulting in a biological response that can be studied in order to assess the condition of the ecosystem. These biological studies, in parallel with the traditional physical and chemical analysis, can lead to a more accurate understanding of the water quality status of a certain water body and, therefore, to a better discernment of the state of an ecosystem. Most likely, rudimental forms of biomonitoring began with the observations and conclusions of fishermen and keepers of rivers and lakes (Cain *et al.*, 1992; Kushlan, 1993; Oertel, 1998).

Aristotle first linked water pollution with observations of oxygen reduction (black decaying mud), a community of Beggiatoa sulfur bacteria (white slime), oligochaete sludge worms, and chironomids (red tubes) (Moog *et al.*, 2018). The modern history of biomonitoring began in the United States when Stephen Alfred Forbes, in 1887, introduced the concept of *biological community* to assess the degree of organic pollution in rivers (Adams & Rowland, 2003). Then, in the early 19th century, the German scientists Kolkwitz and Marsson developed the concept of *biological indicators of pollution*, also called the Saprobic System, for rivers and streams (Persoone & De Pauw, 1979). This system differed from the conventional chemical water analysis in the fact that it was solely based on the abundance and distribution of several biological species (Lovely, 1995). In the 1950s, the first biotic indices, which are numeric values assigned to resemble the sensitivity tolerance of an organism to anthropogenic stress, were developed in the United States and Europe in parallel (Cairns & Pratt, 1993). Also, around this time, several diversity indices were developed, which use species abundance, evenness, and richness to determine the health of a community of aquatic organisms (O'Keeffe, 1986). The multi-metric approach saw its roots in the early 1980s. These approaches use indices that relate a metric to the specific impacts caused by environmental stressors (Gammon & Simon, 2000). Around 1990, the first multi-variate approaches were developed in the United Kingdom. These methodologies are based on predictive systems that evaluate the difference between the expected composition of aquatic communities and the observations done in the field. Also, around this time, the first integrative assessment approaches that use a wide range of organisms were developed. In the late 1990s, functional approaches arose, these methodologies are based on observations about the mechanisms by which communities of organisms obtain their food and other functions during their life cycles. In the early 2010s researchers began to apply metabarcoding to assess the ecological status of an aquatic community. These most-recent approaches based on DNA analysis are also known as “next generation biomonitoring” or “biomonitoring 2.0” (Baird & Hajibabaei, 2012; Wikström *et al.*, 1999). Figure 1 shows the summary timeline of the use of bio-indicators.

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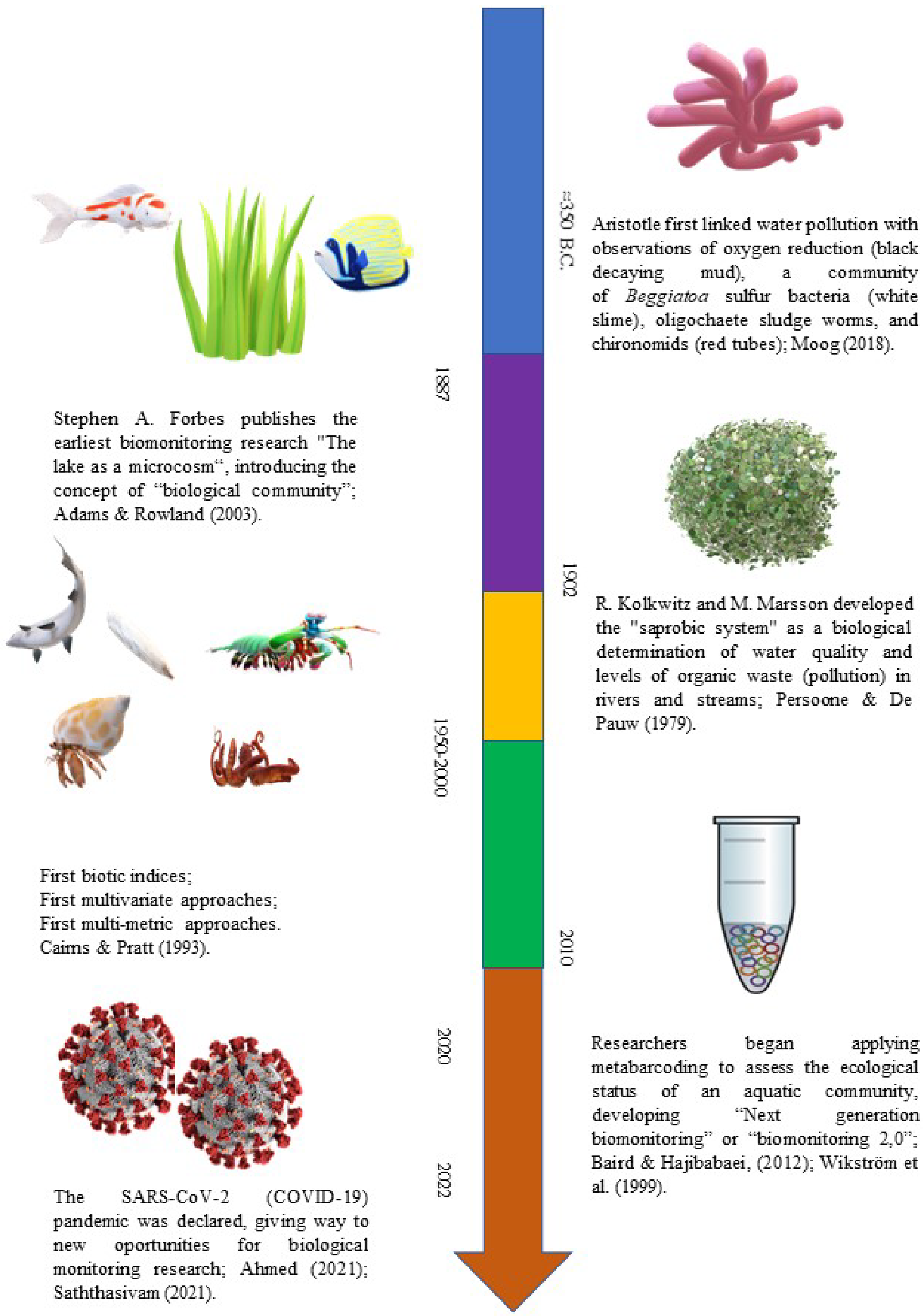

Figure 1. Timeline of the practical use of bioindicators and biomonitoring from the historical origin to our present Era.
Source: Authors' own elaboration on Paint 3D Software 5.1809.1017.0.

In our Era, the coronavirus disease (Covid-19) pandemic was declared, giving way to new opportunities for biological monitoring research (Ahmed *et al.*, 2021; Saththasivam *et al.*, 2021). For example, studies showed that honey bee (*Apis mellifera*) colonies are excellent biomarkers in SARS-CoV-2 surveillance for environmental detection of human airborne pathogens in densely urbanized areas (Cilia *et al.*, 2022). This research was conducted in Bologna on March 2021, in the third wave of the Italian pandemic (environmental conditions allowed high PM concentrations in the air). Results showed positive for the target bee genes of viral SARS-CoV-2 RNA. This experiment indicates a novel use of *A. mellifera* colonies in the environmental detection of airborne human pathogens during Covid-19 Era.

In another work, to assess the impact of measures taken on air quality in the Moscow region, *Pleurosium shreberi* mosses were collected in June 2020 from sites considered to be contaminated by metals (Yushin *et al.*, 2020). The results of two biomonitoring studies conducted in the Moscow region in 2019 and 2020 were compared. Moss sampling proved to be a suitable and low-cost bioindicator of heavy metal air pollution. The self-isolation period adopted to cope with the Covid-19 pandemic resulted in a decrease of Cd content in the Moscow region, while the content of other analyzed elements decreased or remained the same or even increased in satellite cities near Moscow.

Research strategy

To conduct the present literature review, a research strategy was devised aiming to introduce the reader to the evolution of the concepts of bioindicators and biomonitoring, along with the different techniques for water quality assessments and their application.

For this purpose, the software VOSviewer version 1.6.12 (van Eck & Waltman, 2017) was used to create and visualize bibliometric networks, which include individual publications, journals, and other research that can be based on citation occurrence, bibliographic coupling, co-citation, and co-authorship relations (Figure 2). Web of Science was the database used to search for the literature related to the concepts of bioindicators and biomonitoring in ecology. Once the bibliometric network maps were generated, a summary table was created to manage the most relevant literature in the form of books, manuals, articles, and previous literature reviews on the subject. Based on the bibliometric network research, some of the most cited and illustrative bibliography (including articles, book chapters, manuals, and other literature reviews) was selected as the core reference material for the writing of the present literature review (Table 1).

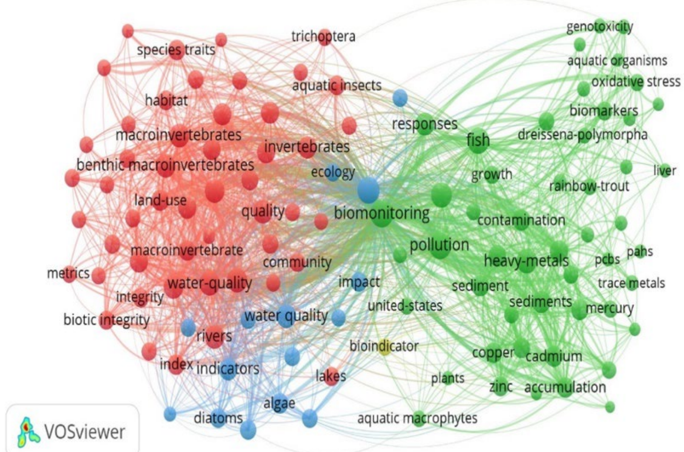

Figure 2. Bibliometric network map for biomonitoring related topics.
Source: Authors' own elaboration on the software VOS viewer version 1.6.15.

Table 1. Summary of biomonitoring methods for water bodies.

Methodology	Results	Reference
Description of biomonitoring techniques: ecological methods, physiological and biochemical methods, controlled biotests, contaminants in biological tissues, histological and morphological methods.	The use of ecological methods based on indicator species accompanied by physical and chemical analyses have been developed into water quality or ecosystem indices. Proper interpretation of the results is of paramount importance.	(Bartram & Ballance, 1996)
Diversity indices: e.g., Shannon Index, Simpson Index, Margalef Index. Biotic indices: e.g., BMWP, ASPT. Multimetric approaches: e.g., Index of Biotic Integrity. Multivariate approaches: e.g., RIVPACS. Functional approaches: Functional Feeding Groups (FFG), Multiple Biological Traits.	Trends in biomonitoring in river ecosystems include increasing application of functional measures (e.g., microbial enzyme activity, bacterial luminescence, photosynthesis, respiration) and Molecular Techniques (e.g., DNA-based methods).	(Li et al., 2010)
Physico-chemical parameters. Surber sampling. Family level identification. Ecological features and physical evaluation of habitats for ES. Principal Component Analysis (statistics). Shannon Diversity Test (Hlog10).	The richness of the benthic macro invertebrate taxa was high in all subbasins, indicating a status of good for the substrate heterogeneity on the riverbed as well as several food sources. In the case of algae species, some rivers showed high richness, which represents good hydromorphological quality conditions.	(Caro-Borrero & Carmona-Jiménez, 2019)
Analysis of the following questions: What Is a Bioindicator? Isn't it Called Biomonitoring? Why Are Bioindicators Better Than Traditional Methods? What Makes a Good Bioindicator? Benefits and Disadvantages of Bioindicators.	The use of bioindicators uses the biota to assess the cumulative impacts of both chemical pollutants and habitat alterations over time. Bioindicators add a temporal component corresponding to the life span or residence time of an organism in a particular system, allowing the integration of current, past, or future environmental conditions.	(Holt & Miller, 2011)
Semi-quantitative sampling approach for soft-bodied algae: multihabitat sampling, single targeted-habitat sampling. European standard semi-quantitative method (taxonomic id.). Biotic Indices (BI), Multimetric indices of biotic integrity (IBI).	Algal growth can be limited by scarcity of macronutrients and micronutrients, but the most frequent limiting factors are nitrogen (N) and phosphorus (P), because demand is high relative to their availability.	(Stancheva & Sheath, 2016)
Physico-chemical parameters, Metal Index (MPI), Metal Pollution Index (HPI), Biological Accumulation Factor (BAF), Statistical Package for Social Sciences (SPSS),	Pb concentrations in the macrophytes were in the toxic range. Biomass concentrations of Cd were relatively low. Biomass concentration of Ni also varied according to plant species. The macrophytes accumulated Mn within normal growth range.	(Ogunkunle et al., 2016)
Measurement of phosphorous, nitrogen, metal particles, alkalinity, hardness, conductivity, oxigen, temperature, salinity, pH.	Mechanical fermentation brought on a reduction in the quantity of zooplankton species and changes in species strength, both of which were influenced as pH decreased from 7.0 to 3.8.	(Rawtani et al., 2016)
Method of collection: site selection, rocky & muddy bottom sampling. Identification categories: sensitive, somewhat sensitive, tolerant.	Provide information regarding impacts that continuously influence aquatic life. Macroinvertebrate assessments should be conducted twice a year (spring and fall). Rocky bottom sampling (streams), muddy bottom (lakes/dams).	(Conrad & Hilchey, 2011)
Creation of artificial substrate bags (stones in nets), 10-day colonization. Multivariate procedures: principal component analysis, Cluster analysis: Ward's method, Multidimentional scaling: correlation matrix. South African Scoring System for Dams (SASSD) Index, Belgian Biotic Index (BBI), boxplots.	Sampling the natural substrate indicates the resident biota and is impacted by the available habitat, whereas artificial substrates measure the colonization potential and are indicative of the water quality but not of the natural invertebrate fauna.	(Thirion, 2000)
Macroinvertebrates were collected using a Surber sampler (quantitative) and a concave mesh kitchen strainer (qualitative). Analysis of biotic index BMWP-CR. Determination of mean and standard deviation.	Results show that sampling method selection has a large influence on the outcome of the BMWP-CR index. Intensive sampling with a Surber sampler resulted in much higher BMWP-CR index scores and different water quality classifications compared to qualitative sampling with a strainer.	(Gutiérrez-Fonseca & Lorion, 2014)
Multi-HabitatSampling XP T 90-333' sampling protocol, Surber sampler, physico-chemical analysis, Standardized Effect Size normalization, Metric normalization, Metric selection, IBMA calculation, Ecological quality class boundaries, Tests of the IBMA.	The IBMA biomonitoring tool significantly improves the detection of impaired reaches, it also fulfills the WFD requirements. As a generalist index, the IBMA is sensitive to the current range of potential disturbances. It considers both taxonomic characteristics and biological traits of benthic macroinvertebrates.	(Touron-Poncet et al., 2014)
Surber net: quantitative, fluvial ecosystems, shallow depths, fine-medium substrates. Kicknet: more species richness, all kind of ecosystems, shallow-medium depths, all types of substrates. Dredge: expensive, high depths, lake and wetland ecosystems, fine substrates. Core: wetlands, soft substrates, high-medium depths. Artificial substrates: great diversity of habitats and facilitates the study in areas of high depths.	1. Cover all the diversity of habitats present at the sampling sites (spatial variability), in order to capture most of the biological diversity. 2. Define control sites (low or no anthropic activity), which allow a comparison with the impacted sites. 3. Consider the temporal variability, including sampling in dry and rainy seasons.	(Correa-Araneda, 2016)

Source: Authors' own elaboration

Bioindicators

The term *bioindicator* refers to an organism that indicates the presence of an environmental stressor (e.g., pollutants, excess nutrient) by manifesting a physical, chemical, or behavioral response (Hee, 1993). Bioindicators provide qualitative or quantitative data about the effects of the different pollutants present in the ecosystem, as well as information of how long they have been present at the site of study (Gerhardt, 2002). Animals (fish, birds, macroinvertebrates, etc.), plants and fungi (mosses, lichens, tree rings, etc.), and microorganisms (algae, diatoms, etc.) are all examples of commonly used bioindicators in environmental assessment studies (Bonanno *et al.*, 2020; Gerhardt, 2002; Hinojosa-Garro *et al.*, 2020; Prazeres *et al.*, 2020). Bioindicators can be grouped into accumulation indicators: those that can store pollutants without any visible changes in their metabolism (e.g., fish), and response indicators: those that present symptoms of environmental stress when taking up small amounts of harmful substances (e.g., diatoms) (Witt, 1996). According to Holt & Miller (2011), regardless of the environment, geographic region, organism, or type of disturbance, a good bioindicator always presents certain characteristics: a) they are abundant and common, b) they are of economical/commercial importance, c) they are a good indicator ability, and d) they are well-studied.

Biomonitoring

This is defined as the observation of biological communities or individual organisms and their responses to physical or chemical changes in their environment over time. Biomonitoring can provide qualitative assessment by observing and recording such changes, or it can provide quantitative evaluations by carrying chemical analyses of substances present in the tissues of organisms. Biological monitoring can be divided into active biomonitoring, including all methods that put organisms under controlled conditions into the site of study, and passive biomonitoring, using organisms and communities of organisms that are a natural component of the ecosystem and appear spontaneously (Witt, 1996). An early example of the application of biological indicators can be traced back to the early years of the Industrial Revolution. At that time, canaries were kept in underground coal mines to obtain early-warning signals for the miners in the United Kingdom (Pollock, 2016). Given the hypersensitivity of these birds to small concentrations of carbon monoxide and methane gas, they served as a biological indicator of unsafe conditions for workers.

Advantages and disadvantages of biomonitoring methods

Given that biomonitoring methods evaluate the cumulative impacts of physical and chemical changes over time, they offer several advantages over the traditional physical-chemical analysis for water quality assessments. Mainly, bioindicator organisms have a life cycle or residence time in certain environments; thus, this allows for the integration of present, past, and future habitat conditions. Another advantage is that bioindicators offer a range of tolerance to pollutants, and they can reflect even tiny biologically meaningful levels of contaminants. However, these biological methods also have disadvantages (Wepener, 2013). For example, it can be difficult to relate observed effects to specific aspects of environmental disturbance, such as contamination or natural changes. Another disadvantage is that the use of a single species or group of species (e.g., periphyton) to assess the overall quality of an ecosystem may eventually lead to unwanted results, undermining the complexity of an environment (Holt & Miller, 2011).

Selection of biomonitoring methods

When selecting a biological monitoring method, one must consider certain factors to meet the desired objectives of the ecological assessment. Selecting an adequate biomonitoring technique from the existing methods will mostly depend on the scope of the study and availability of resources (Bartram & Ballance, 1996). The following are the principal methods used to conduct a biomonitoring study:

- Biological tissue analysis: to determine the concentration of certain substances in living organisms.

- Morphological studies: observations of cellular and structural changes in living organisms.

- Controlled environments: measurements of beneficial or toxic effects on living organisms under controlled conditions *in situ* or in a laboratory.

- Ecological methods: based on community structure and diversity.

- Physiological and biochemical methods: based on community metabolism or biochemical effects in individuals or communities.

Ecological methods involve the use (and adaptation) of biotic and diversity indices and have been historically the most used methodologies for biomonitoring studies. Again, it is up to the researchers to inform themselves on which is the most suitable methodology for their objectives.

Selection of bioindicator organisms

When it comes to the selection of organisms to conduct a biomonitoring study, it is important to keep in mind that the organism must reflect the local conditions of the environment under study.

Macroinvertebrates, algae, and fish are the most common type of bioindicators used in river and reservoir ecosystems. The selection of a bioindicator is complicated and difficult, and it greatly depends on the objectives of the study (Han *et al.*, 2015). Some guidelines have been suggested to facilitate the selection process of organisms. According to Li *et al.* (2015) and Han *et al.* (2010), an "ideal" bioindicator must have:

- Taxonomic soundness: wide or cosmopolitan distribution.
- Low mobility: reflect local conditions.
- Well-known ecological characteristics.
- Suitability for laboratory experiments: high sensitivity to environmental and anthropogenic stressors.
- Economic, cultural, and social value.
- Quantification and standardization characteristics.

Indicator groups

Indicator groups refers to individual organisms or communities of organisms that are used to carry out a biomonitoring study. The most frequently used groups are macroinvertebrates, periphyton, and fish. The selection of an indicator group will depend on the aim of the study and on the ecosystem where it will be carried out. This review focuses on algae, benthic macroinvertebrates, and fish, as they are the most frequently employed type of bioindicators due to their suitability for carrying out biomonitoring studies in river and impoundment ecosystems.

Macroinvertebrates

Aquatic macroinvertebrates refer to a diverse group of insects and non-insects such as crustaceans, larvae, snails, and worms which are easy to see with the naked eye (macro), lack a backbone (invertebrates), and live in saline or freshwater environments (Collier *et al.*, 2018). The term *benthic macroinvertebrates* refer to the organisms that live in, on, or near the bottom of the seabed, rivers, and lakes (benthic zone). They usually dwell among sediments, stones, and aquatic plants. They are an extremely important link in the food chain of aquatic environments. In terms of food webs, they may be considered as the intermediaries between the lower and higher trophic levels of the food chain. They feed on plants, algae, or other macroinvertebrates, and they in turn become a food source for fish, birds, and reptiles (Nieto *et al.*, 2017).

Some of the factors that affect the health of macroinvertebrate communities are (Juvigny-Khenafou *et al.*, 2021; Lin *et al.*, 2020):

- Dissolved oxygen: low levels of dissolved oxygen in water can affect macroinvertebrates in their developing phase during which they require high levels of oxygen.
- Nutrient excess: eutrophication may limit the amount of dissolved oxygen needed by macroinvertebrates to develop.
- pH: low pH levels (i.e., acidic water) can dissolve exoskeletons and kill macroinvertebrates.
- Removal of riparian vegetation destroys their breeding and reproductive grounds.
- Seasonality: in winter, the number of available algae and other food sources decreases.

The use of benthic macroinvertebrates has been widely documented, and they are the most common type of organisms used to conduct biomonitoring studies, since they possess many desirable selection traits mentioned in the previous section (section 1.7): they have limited mobility and are good integrators of past environmental conditions; they are extremely diverse; they are ubiquitous; they are easy to collect and relatively easy to identify (Bartram & Ballance, 1996; Li *et al.*, 2010). For these reasons, benthic macroinvertebrates communities have been widely used in ecological approaches and biotic and diversity indicators for nearly 100 years (Cairns & Pratt, 1993; Johnson *et al.*, 1993; Juvigny-Khenafou *et al.*, 2021; Lin *et al.*, 2020). Due to their benthic nature, macroinvertebrates are considered very reliable and effective bio-indicators for biomonitoring in the assessment of ecosystem status in rivers and streams.

Several developing regions show growing interest in including biomonitoring of benthic macroinvertebrates for water resource assessments (Mathuriau *et al.*, 2012). These studies require low-cost methodologies to rapidly collect, process, and interpret data of sufficient quality over large spatial areas. However, professional monitoring of freshwater impacts are costly and time-consuming, and they require extensive technical and professional training, which limits the capacity for adequate and continuous support, especially in developing regions of the world. One solution is through the support of organizations.

Global Water Watch (GWW) has carried out studies dedicated to betony macroinvertebrates (Deutsch *et al.*, 2010; Flores-Díaz *et al.*, 2013). This organization has formed a worldwide network of community-based water monitoring groups including stream biomonitoring, teaching citizens the principles and practice of using macroinvertebrates to assess stream water quality through standardized monitoring techniques for physicochemical data and benthic macroinvertebrates. Training is done through actual field collection and assessment of macroinvertebrate communities.

Thus, GWW enhances the potential of citizen groups by training, certifying and equipping watershed residents to take an active part in stream surveys and monitoring programmes to provide baseline data on water resources.

The data generated can be used by teachers, policy makers, the scientific community, and the general public to support improved drinking water quality, river and lake conservation, and public education, while helping to develop local and regional natural resource plans and policies within and between watersheds (Deutsch *et al.*, 2010).

In the same context, several studies have reported on surveillance in communities. One study conducted sampling in the Pixquiac River in southern Veracruz, Mexico, by training community volunteers to monitor water conditions by collecting data using standardized, simplified, and inexpensive biomonitoring method (Campbell, 2007). Volunteers divided macroinvertebrates in each category into subgroups (category 1, mayflies, stoneflies, fruit flies not in the family Hydropsychidae, bank beetles, feather beetles: Psephenidae family, and aquatic snails; category 2, hellgrammites, dragonflies, fruit flies, filter-flies of the family Hydropsychidae, crayfish, amphipods, isopods, and black flies; category 3, maggots, mosquitoes, air-breathing snails). The results revealed expected declines in downstream cumulative index values as human presence increased.

Periphyton

It comprises a complex mixture of autotrophic and heterotrophic microorganisms such as algae, cyanobacteria, diatoms, and protozoa, all rooted in a matrix of organic material (Bae *et al.*, 2019; de la Peña & Barreiro, 2009; Murdock *et al.*, 2013). They are well-adapted at living on most submerged substrates such as rocks, sand, and other sediments in most aquatic ecosystems. The German term *Aufwuchs*, in this context, means “surface grow”, and it refers to floating periphyton that is adhered to rooted plants and other substrates in open aquatic surfaces. These *Aufwuchs* are usually found in environments with calm waters (e.g., dams, lakes, and ponds) (Rawtani *et al.*, 2016).

These conglomerates of organisms are primary producers within the trophic web and are, therefore, very sensitive to physical and chemical alterations (Ceschin *et al.*, 2020). They also have short life cycles and rapid reproductive rates, which make them prime candidates for studies focusing in short-term and abrupt changes in the environment (Hosmani, 2013; Rawtani *et al.*, 2016).

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Periphyton growth is determined by abiotic and biotic factors (Wu, 2016), for instance:

- Light: the amount of solar energy that the conglomerate of organisms can absorb and turn into organic matter.
- Nutrients: especially nitrogen and phosphorus.
- Space availability: periphyton blooms require a substrate to attach to stable-flow conditions to improve growth.
- Temperature: generally warmer temperatures favor periphyton growth.

Their eligibility as bioindicators is based on the following characteristics: their pollution tolerances are well documented; there are mobile and sessile (attached) species; and they are species-rich and spatially compact (Bartram & Ballance, 1996; Burger, 2006). Simple non-taxonomic methods, like chlorophyll-α concentration determination, have been developed to assess the total biomass of algae present in water samples (Bartram & Ballance, 1996; Burger, 2006; Steinman *et al.*, 2017). Some of the taxonomic methods include taxa composition, as well as diversity and richness determination. This offers a wide range of reliable, relatively simple, low cost, early-warning biomonitoring methodologies (Burger, 2006). Given that periphyton can be mobile or sessile, they are a great bioindicator choice in both rivers and reservoir environments (Larned, 2010). In the case of river ecosystems, sessile species attached to hard surfaces species, such as diatoms, are preferred (Kelly *et al.*, 1998). Diatoms are microalgae and have the advantage of being easily identifiable (to the species level) (Medlin, 2018). They are preferred for riffle/run habitats because they remain in their location and, therefore, represent the conditions present at the sampling point (Gillett *et al.*, 2011; Medlin, 2018). For reservoirs, *Aufwuchs*, and especially phytoplankton, are preferred as a bioindicator for environments like lakes and ponds. Phytoplankton consists of a large variety of algae; some are benthic and some float in the surface. In balanced nutrient conditions, phytoplankton will dominate over cyanobacteria and diatoms, which thrives in eutrophic conditions (Elliott, 2010).

Fish

Fish are abundant in many aquatic environments, and they have always been an important dietary component for humans (Elliott, 2010). For this reason, biomonitoring in its most rudimentary form probably originated in the minds of lake and river keepers when they started to connect the decrease of fish population with factors external to the ecosystem (anthropogenic activities) (Cairns & Pratt, 1993). As primary and secondary consumers at different levels, fish can reflect the integrated trophic conditions in an aquatic environment (Anderson & Cabana, 2007). The term bioaccumulation refers to the gradual accumulation of toxic substances (pesticides and heavy metals, for example) in the tissue of living organisms (Van der Oost *et al.*, 2003). The concentration of these toxic substances accumulates, and it is increased at successively higher levels in the food chain through a process called biomagnification or bioamplification (Alonso *et al.*, 2008; Daley *et al.*, 2014). Given their place in the food web, fish can provide a great amount of information about the severity in which these bioaccumulation processes are occurring in a particular aquatic ecosystem (Chovanec *et al.*, 2003). Furthermore, fish are an important food source for humans, and monitoring their trace levels is important to ensure food safety.

The following are some of the habitat disturbances that negatively impact on fish communities (Wilson *et al.*, 2010):

- Eutrophication: radical decrease of dissolved oxygen levels.
- Invasive species: the introduction of alien species in aquatic ecosystems can displace native species.
- Pollution: the increase of toxic substances is one of the main reasons for fish depletion.
- Water flow: changes to physical habitat, nutrient distribution, and community composition.

Some characteristics that make fish good bioindicators are that they are easy to identify; they have a high sensibility to habitat disturbances; their size allows a variety of analytical procedures to be carried out (e.g., tissue analysis); and they have a strong economic, cultural, and social importance (Bartram & Ballance, 1996). For these reasons, fish resistance to certain pollutants have often formed the basis of ecological water quality standards. Due to their longevity and mobility, fish are ideal indicators in long-term ecological assessments in large areas such as river stretches, lakes, and ponds over large periods of time (years) (Whitfield & Elliott, 2002). These assessments have been useful in policymaking regarding biological integrity and protection of aquatic ecosystems (Figure 3).

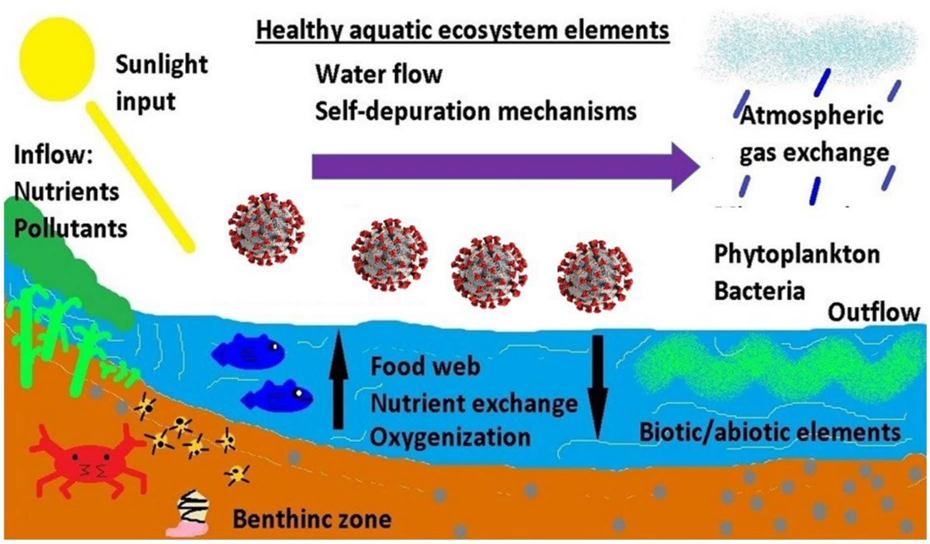

Figure 3. Elements present in a balanced aquatic ecosystem.
Source: Authors' own elaboration on the software VOS viewer version 1.6.15.

Biomonitoring methodologies

Since the mid-1900s, several biomonitoring indices have been developed in order to evaluate the status of aquatic ecosystems (Burger, 2006; Cairns & Pratt, 1993); these indices can be either quantitative or semi-quantitative. Some of these indices have been entirely developed anew since then, and some are adaptations of existing indices for specific ecosystem conditions. These biomonitoring methods include biotic indices, diversity indices, multi-metric indices, multivariate approaches, functional feeding groups, multiple biological traits, and DNA-Metabarcoding methodologies.

Biotic indices

A biotic index can be defined as a numerical value representing the tolerance of organism assemblages to pollution stress. A score is assigned to an ecological indicator status that can be used to calculate an index. The basis of biotic indices is to assign different types of indicator species to different levels of environmental disturbance, where the most sensitive species disappear, and more tolerant species increase in abundance (Burger, 2006). Some examples of biotic indices are the Trent Biotic Index (Cairns & Pratt, 1993), the Belgian Biotic Index (1983) (De Pauw *et al.*, 1986; Gabriels *et al.*, 2005), and the Hilsenhoff Biotic Index (Hilsenhoff, 1988). However, the Biological Monitoring Working Party Score System (BMWP, 1980) (Bartram & Ballance, 1996) along with the Average Score Per Taxon (ASPT) (Ballentes *et al.*, 2006) have been standardized by the International Organization for Standardization (ISO) and are a recommended methodology for the ecological assessments of river ecosystems by the Water Framework Directive in Europe. The principle of the BMWP is that invertebrates can be collected from representative habitats in a riverbed and then identified in the family taxonomic level. Each family is then given a score between 1 and 10 (1 being the most resilient organisms and 10 being the most sensitive), representing their sensitivity to environmental pollution. The score of each family is then added to obtain the BMWP score. After the BMWP score is obtained, the average score per taxon (ASPT) is also calculated. The ASPT represents the average of tolerance scores of the macroinvertebrate families identified, ranging from 0 to 10. A BMWP score higher than 100 and an ASTP value higher than 5 represent good or excellent water quality (Bartram & Ballance, 1996).

Diversity indices

Univariate diversity indices relied on the Saprobity system, i.e., the capacity of self-purification of a water body resulting in zones of decreased pollution. These are considered the most commonly used indices in the past (Cairns & Pratt, 1993). A variety of diversity indices have been used to assess the condition of benthic communities given certain environmental variations (Bartram & Ballance, 1996). Nevertheless, the use of diversity indices has declined with time, and they are now combined with other metrics to produce more accurate approaches for aquatic ecosystem assessments. Examples of these indices, such as the Shannon-Wiener Index (Strong, 2016), the Simpson Index (Somerfield *et al.*, 2008), the Brillouin Index (Bandeira *et al.*, 2013), and the Margalef Index (1958) (Gamito, 2010; Guerold, 2000), are based on community structure indicators like abundance (total number of individuals), equitability (uniformity in the distribution of individuals of different species), and richness (number of species present). These indices are best applied when physical pollution is present, which imposes environmental stress on organisms. Stable ecosystems generally present a high species diversity (Morris *et al.*, 2014).

Multimetric indices

Multimetric indices are monitoring and assessment quantitative tools of ecosystem integrity (Burger, 2006). The objective of these methods is to create an index that can serve as an indicator of anthropogenic environmental stress to allow researchers identify highly preserved natural areas, to identify likely sources of pollution, and to define restoration measures for affected ecosystems (Schoolmaster *et al.*, 2012). To achieve this, diverse community structural and functional metrics or variables (for instance, abundance, equitability, tolerance to pollution, functional feeding groups and richness) are considered (Hering *et al.*, 2006). An example of a multimetric approach is the Index of Biotic Integrity (Karr, 1981), which has been widely adapted for different aquatic ecosystems around the world (Burger, 2006; Cairns & Pratt, 1993). These indices are generally used in systems where the underlying causes of pollution, and pollution processes, are not well understood (Schoolmaster *et al.*, 2012). They have nearly endless adaptation possibilities, and despite the questions regarding their usefulness on both interpretability and usefulness, they are widely used for benthic macroinvertebrates approaches (Morris *et al.*, 2014).

Multivariate indices

Multivariable approaches are statistical models designed to predict the biota that should be present at a riverine environment unexposed to anthropogenic stress. First, the model is based on several reference environmental parameters (e.g., Reference Condition Approach) (Bowman & Somers, 2005). Then, the modeled results are compared with the observed biota at the study site (Burger, 2006). Finally, if the observations are like the biota predicted, then the site is in "good condition" and vice versa. To accurately calibrate the model through knowledge of the biota, seasonal distribution and reference conditions in the area of interest are a prerequisite. The River Invertebrate Prediction and Classification System (RIVPACS) was the first large scale application of a multivariate model for a biomonitoring assessment (Wright *et al.*, 1998). It was developed in Great Britain during the 1980s and, after some adaptations (Burger, 2006; Cairns & Pratt, 1993), it is still widely used in that country to assess the status of freshwater ecosystems. Other examples are the Canadian Aquatic Biomonitoring Network (CABIN) (Curry *et al.*, 2018), the Australian River Assessment System (AUSRIVAS)(Sudaryanti *et al.*, 2001), and the Benthic Assessment of Sediment (BEAST) (Reynoldson *et al.*, 1995).

Functional metrics

Functional approaches are gaining popularity among researchers. They are a viable alternative when a biomonitoring study is restricted by the level of detail in taxonomic identification (Merritt *et al.*, 2017). Understanding community functional traits and structural elements is equally advantageous to achieve a better understanding of the overall aquatic ecosystem.

The Index of Trophic Completeness has shown that the pattern of Functional Feeding Groups (FFGs) distribution has been related to the environmental gradient in aquatic systems (bij de Vaate & Pavluk, 2004). The Benthic Index of Biotic Integrity (Kerans & Karr, 1994), the Florida Stream Condition Index (Barbour *et al.*, 1996), and the Rapid Bioassessment Protocols (Boonsoong *et al.*, 2009) are other examples of functional approaches.

Functional Feeding Groups (FFGs)

Classification approaches such as the FFGs are based on mechanisms used by organisms to obtain their food, rather than on taxonomic classifications. Feeding strategies of organisms under natural or anthropogenic stress reflects their capacity for adaptation. An advantage of the FFGs method is that a small number of groups of organisms can be studied collectively based on the way they process energy resources in aquatic ecosystems (Merritt *et al.*, 2017). The principal feeding groups are scrapers or grazers, which feed on periphyton; shredders, which feed on dead leaves or other coarse particulate organic matter; collectors or gatherers, which feed on fine particulate organic matter at the sediment; filterers, which feed on fine particulate organic matter by filtering it from water; and predators, which feed on other consumers.

Multiple Biological Traits

Multiple biological traits approaches are based on the concept that the status of an ecosystem can be quantified through the functional diversity of communities of organisms (Nock *et al.*, 2016). Biological traits describe a species physiology, morphology, life history, and behavior, capturing both inter-specific interactions and the connections between species and their environment. Multiple biological traits (e.g., size, number of descendants per reproductive cycle, parental care, and mobility) can be combined with multimetric approaches in order to identify different types of human impact (Dolédec *et al.*, 1999).

Next generation biomonitoring or Biomonitoring 2.0

Traditional biomonitoring assessments such as the use of biotic and diversity indices are based on direct observations of organisms, which have been proven to be resource- and time-consuming (Baird & Hajibabaei, 2012). However, new approaches based on molecular analysis have been developed in recent years. These methods have several advantages over traditional approaches in terms of comparability, costs, and speed; additionally, they have the potential to include new bioindicators, thereby improving the assessment quality of aquatic ecosystem systems (Wikström *et al.*, 1999).

DNA-Metabarcoding

High-throughput amplicon sequencing (HTS), also known as DNA-Metabarcoding, is an emerging technology in the field of environmental biomonitoring. It allows for the identification of individual species as well as whole communities of organisms. Analysis can be performed in parallel from many samples at the same time. DNA can be analyzed form living cells (e.g., diatoms) and tissue samples (e.g., from fish), as well as from water samples or sediments (environmental DNA or eDNA) (Baird & Hajibabaei, 2012; Wikström *et al.*, 1999).

Some of the biomonitoring methodologies that have been developed include PCR amplification, direct bulk DNA extraction, COI genes sequencing, and taxonomy-free approaches. These methods have been proven successful for microbial datasets; however, their application for macroinvertebrates is less reliable because of the possible biomass variation within different species. The amount of literature researching molecular approaches has increased exponentially in recent years. The Canadian Aquatic Biomonitoring Network (CABIN) is a pioneer in the use of genetic data for aquatic ecosystems biomonitoring. Examples of taxonomy and clustering-free approaches based on molecular methods have been developed by Pawlowski *et al.* (Apothéloz-Perret-Gentil *et al.*, 2017) and Tapolczai *et al.* (2019).

Integrative methods

Integrative methods are considered the most sophisticated approaches for aquatic ecosystems quality assessments (Burger, 2006). They are based on the integrated analysis of multiple bioindicator organisms, including macroinvertebrates, periphyton and fish, to evaluate the status of the ecosystem (Markert, 2007). The Water Framework Directive in Europe has proposed an integrated assessment system for freshwater bodies based on physicochemical analysis, hydromorphological characteristics, and bioindicators (Birk & Hering, 2006). The Southern African Scoring System (SASS) is another example of an integrative river ecosystem assessment (Burger, 2006; Dickens & Graham, 2002). To save resources, it is important that the researcher knows which bioindicators are the most suitable for the conditions present at the study site; for example, macroinvertebrates are good bioindicators for organic pollution, as well as for hydromorphological stress at the micro-habitat scale. Algae are good indicators to assess the effect of nutrients and eutrophication. Finally, fish are good indicators for hydromorphological deficits at the macro-habitat scale (Bartram & Ballance, 1996; Cairns & Pratt, 1993).

Use of Bioindicators during the Covid-19 Pandemic

The year 2020 marks the beginning of an atypical era at the international level, where humanity faced a new way of life. On March 11 of that same year, the World Health Organization (WHO) declared coronavirus disease (Covid-19) a global pandemic (CDC Covid-19 Response Team, 2020; Monroy-Torres *et al.*, 2021). The Covid-19 had a major negative impact on human health and economies around the world. To prevent the spread of infection in many countries, public life was restricted. New regulations adopted in many countries to help curb the spread of Covid-19 resulted in a decrease in the negative impact on the environment in some regions of the world (Abu-Rayash & Dincer, 2020; Bienkowska *et al.*, 2020; Chua *et al.*, 2020). During (2019-2021) and post Covid-19 Era (2022), the use of bioindicators for the study of environmental impact has been an essential tool (Figure 4).

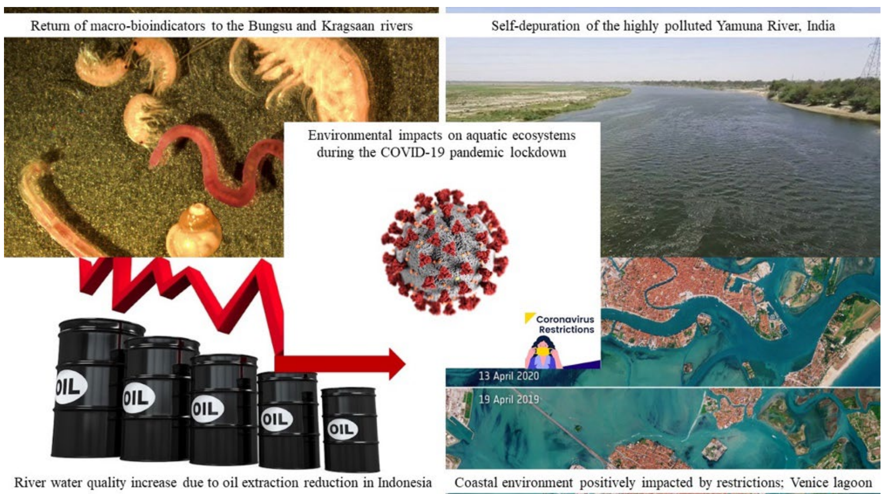

Figure 4. Examples of the use of bioindicators during the Covid-19 Era.
Source: Authors' own elaboration based on Rahmawati *et al.* (2021), Asim & Rao (2021), and Braga *et al.* (2020).

A recent study conducted an analysis of the pollution of the Bungsu and Kragsaan rivers caused by oil extraction in Wonocolo during the Covid-19 pandemic (Rahmawati *et al.*, 2021). It should be noted that conventional oil extraction decreased because of temporary closure of wells. The study, which observed that river quality improved during the Covid-19 pandemic, examined macrozoobenthos community structure as a bioindicator of water quality. The research used the observational method by purposively selecting sampling points. The macrozoobenthos samples were analyzed using the Shannon-Wiener diversity index, species evenness index, and dominance index. According to the Shannon-Wiener index, both rivers pollution status varied from the "very polluted" category to the "medium polluted". Even though there was a decrease in the levels of chemical pollutants in the sampling locations, which means an increase in the quality of water bodies, it turned out that the river ecosystem had not been able to restore its condition during the Covid-19 pandemic period.

Another study focusing on the use of diversity indices to assess the impacts derived from the COVID-19 pandemic on aquatic ecosystems was carried out in the Mediterranean Sea (Essid *et al.*, 2020). In this study, meiobenthic nematodes were exposed to three different doses of the drug ivermectin, which was confirmed as a Covid-19 treatment drug at the end of March 2020. The Mediterranean Sea was selected because it is a water body of confluence between three epicenters of the pandemic: Spain, France, and Italy. Also, it presents a high potential of water and sediment contamination with the drug because it is a semi-closed ecosystem characterized by a low renewal rate of its waters. The study's results, using the Trophic Diversity and a suggested Amphideal Diversity index, suggest that high concentrations of the drug ivermectin in water and sediments could result in an ecotoxic effect in aquatic environments around the world, leading to a significant reduction of abundance and taxonomic diversity in the nematode communities, as well as a high bioaccumulation potential of the drug in seafoods.

A similar study carried out in the Mediterranean Sea focused on the environmental impacts of another drug recommended as a treatment for Covid-19, hydroxychloroquine (HCQ) (Ben Ali *et al.*, 2021). This drug is also widely used worldwide, and it is also anticipated that high concentrations will be detectable in marine costal ecosystems. This study also focused on meiobenthic nematodes, which were exposed to different concentrations of HCQ for 30 days. The results indicated a marked decrease in abundance and assemblages using the Shannon-Wiener Index, whereas the individual mass and the Trophic Diversity Index increased at the highest concentrations. The results also suggest a bioaccumulation risk of the drug HCQ in seafood during and post the Covid-19 crisis. In the same context, fluoroquinolone antibiotics have been used in the treatment of Covid-19 (Chedid *et al.*, 2021; Karampela & Dalamaga, 2020).

One of the most polluted river areas in the world is the stretch of the Yamuna in India (Asim & Rao, 2021). A study analyzed the degree of contamination of the river during the shutdown due to the Covid-19 pandemic (Patel *et al.*, 2020). Within their study, the authors carried out an analysis of algal characteristics based on multi-temporal Landsat-8 images from previous and current closure periods in 117 areas of the channel, based on algal blooms and mineral content in water bodies. These algal blooms arise due to mixing of sewage and industrial effluent in the canal, and the most common types observed are *Chlorophyceae* and *Myxophyceae* (Madhusudhan, 2012). Within their results, it was observed that the increase in algal blooms decreased, and it was recorded a significant impact on water quality of the Yamuna in its stretch of the NCT of Delhi, with an improvement in water quality indices and a significant decrease in the biological oxygen demand (BOD) and chemical oxygen demand (COD) levels.

Another study conducted in India has also concluded that lockdown measures have helped improve the water quality and overall ecosystem status of the Demodar River area due to the total or partial closure of many local industries (Chakraborty *et al.*, 2021). A total of 55 water samples were treated with methods such as WQI, Trophic State Index (TSI), Pearson's correlation coefficient, as well as *t* test to evaluate the physical, chemical, and biological status of river water. Results show that the nutrient enrichment status changed from "High" during the pre-lockdown period to "Low" or "Moderate" during the lockdown period, reflected in the reduction of eutrophication areas.

Another paper studied how the reduction of people's daily activities, living near rivers and coastal areas due to social distancing, can decrease the discharge of residue materials and nutrients (PO_4 and SO_4) to a water body during the Covid-19 contingency (Adwibowo, 2020). In the study, the selected bioindicator for nutrient concentration in the coast of Jakarta was chlorophyll-a. This area of study was selected due to it being surrounded by a highly dense metropolitan area. The methodology involved the measurement of chlorophyll-a (in mg/m3) using remote sensing data gathered during the period before and after the implementation of social distancing measures (January-April 2020), using the sea's surface water temperature as the environmental determinant (0 °C). The results showed that the anthropogenic activities in the coastal areas are strongly associated with nutrient levels, and therefore water quality, as indicated by chlorophyll-a concentration.

Several tourist water bodies were studied in relation to the environmental impacts with the use of bioindicators during the Covid-19 lockdown period, such is the case of the Venice lagoon (Braga *et al.*, 2020). Researchers took advantage of the sudden interruption of urban water traffic to analyse water transparency. Composites of satellite imagery were used to carry out a quantitative analysis of suspended matter patterns (turbidity) before and during the lockdown period. The study concluded that, during the pandemic, the environmental impacts were positive for Venice canals and lagoon, although water transparency will decrease as an effect of peak phytoplankton growth in summer.

Following this same context, a study carried out in 29 urban tourist beaches from seven Latin American countries evaluated the environmental responses to lockdown measures on anthropogenic stressors such as pollution, noise, human activities, and user density (Soto *et al.*, 2021). The influence of these stressors on bioindicators like plants and animals was assessed using standardized protocols. In addition, multivariate approaches were implemented to compare the environmental conditions of the beaches and found remarkable positive changes in the biological components and a decrease in anthropogenic stressors. These results suggest that the ecosystems in tourist beaches can recover in a short period of time, providing adequate conservation and remediation strategies.

Another research work was aimed to determine whether the coronavirus SARS-CoV-2 ribonucleic acid genome could be detected in zebra mussel (*Dreissena polymorpha*) to study its potential as a bioindicator of human pathogens (Le Guernic *et al.*, 2021). The mollusks were exposed to treated and raw wastewater from two wastewater treatment plants (WWTP) in France, under controlled conditions. Analysis of the mussels' digestive tissue showed the presence of the SARS-CoV-2 genome, whether exposed to raw or treated wastewaters. These results encourage the further development of biomonitoring techniques using macroinvertebrates for the detection of infectious pathogens in urban water distribution systems and natural ecosystems.

In another study, new generation biomonitoring methods used molluscs (clams) as biomarkers for the detection of SARS-CoV-2 in marine environments (Polo *et al.*, 2021). Ruditapes molluscs communities and surrounding sediments were analysed using next generation biomonitoring techniques such as viral RNA detection, using the RT-qPCR method targeting three genomic regions (IP4, E and N1). SARS-CoV-2 RNA traces was found in 9 out of 12 digestive tissue samples for two of the target regions, while three out 12 sediments samples were positive for only the IP4 target region. The PMAxx-triton viability by RT-qPCR assay showed that the RNA signals disappeared, indicating non-infectious potential. Furthermore, in this same study, the recently discovered human-specific gut associated bacteriophage crAssphage was also quantified and detected in all the samples, revealing the presence of human-derived wastewater contamination in the study area.

Table 2. Summary of water body biomonitoring methods used during the Covid-19 contingency in water bodies.

Site of study	Biomonitoring method	Bioindicator /indicator used	Reference
Coastal zone of Jakarta	Remote sensing data interpolation	Chlorophyll-a	(Adwibowo, 2020)
Yamuna River, India	Heavy Metal Pollution Index, and GIS spatial distribution	Algae: Chlorophyceae and Myxophyceae	(Asim & Rao, 2021)
Yamuna's River stretch, India	Class C Water Quality Index, and GIS spatial distribution	Faecal Coliform, BOD, COD	(Patel et al., 2020)
Damodar River, India	Water quality index, Trophic State Index, GIS, and Pearson's correlation coefficient analysis	Chlorophyll-a, Total Nitrogen (TN), Total Phosphorus (TP), BOD, COD	(Chakraborty et al., 2021)
Lagoon of Venice	Qualitative visual interpretation and quantitative analysis with GIS	Suspended matter patterns and turbidity	(Braga et al., 2020)
Urban tourist beaches (29) in seven Latin-American countries	Gower Similarity Index, Analysis of Similarities (ANOSIM)	Crabs, lizards, turtles, iguanas, birds; seaweed, seagrasses, beachgrass, shrubbery, vines, mangroves	(Soto et al., 2021)
Reims, France	Ribonucleic acid genome detection in wastewater treatment plants	Zebra mussels (Dreissena polymorpha)	(Le Guernic et al., 2021)
Galicia, Spain	Viral RNA detection by RT-qPCR, and PMAxx-triton viability RT-qPCR assay	Bivalve molluscan species from the genus Ruditapes	(Polo et al., 2021)
University of Florida	eDNA-based monitoring	Herpesvirus from a sea turtle, and eRNA-based detection of the SARS-CoV-2	(Farrell et al., 2021)
South-eastern Alps, France	Viral eDNA analysis	Common frogs (Rana temporaria), and insects	(Miaud et al., 2019)
Ghar El Melh Lagoon, Tunisia	Trophic Diversity Index, and Amphideal Diversity Index, multivariate analysis, ANOVA test	Meiobenthic nematodes	(Essid et al., 2020)
N/S	Chlorophyll-a, microcystin, and carotenoid analysis	Cyanobacterium (Microcystis aeruginosa)	(Wan et al., 2021)
Bizerte Bay, Tunisia	Hydroxychloroquine (HCQ) analysis, Shannon-Wiener Index, Trophic Diversity Index	Meiobenthic nematodes	(Ben Ali et al., 2021)

Source: Authors' own elaboration

Conclusions

Macroinvertebrates, periphyton, and fish are the most used bioindicators for water quality assessment in freshwater ecosystems such as rivers and reservoirs. There are several biomonitoring approaches that can be selected and combined, depending on the specific characteristics of the study, to determine the best course of action. Integrative physical-chemical and biological approaches allow a better understanding of how natural processes of aquatic ecosystems are altered by human activities and, therefore, how best to preserve and restore them. From the analysis of the main methodologies applied during and after the Covid-19 pandemic, the potential and important use and application of biondicators and biomonitoring in the relevance of contingency studies can be observed. Therefore, programs that incorporate biomonitoring and biomarker studies will allow a more proactive and preventive public health and environmental management.

From a sustainable perspective, bioindicators offer better advantages than physicochemical tests. Since they provide information relevant to both human and environmental health, these can provide early warning of any changes that may pose a significant risk to individual species, populations, communities, or ecosystems.

Finally, this study contributes to the potential relevance of the use and application of biomarkers within environmental management and environmental health sustainably.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Table 2 summarizes the water body biomonitoring methods used during the Covid-19 contingency in water bodies.

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