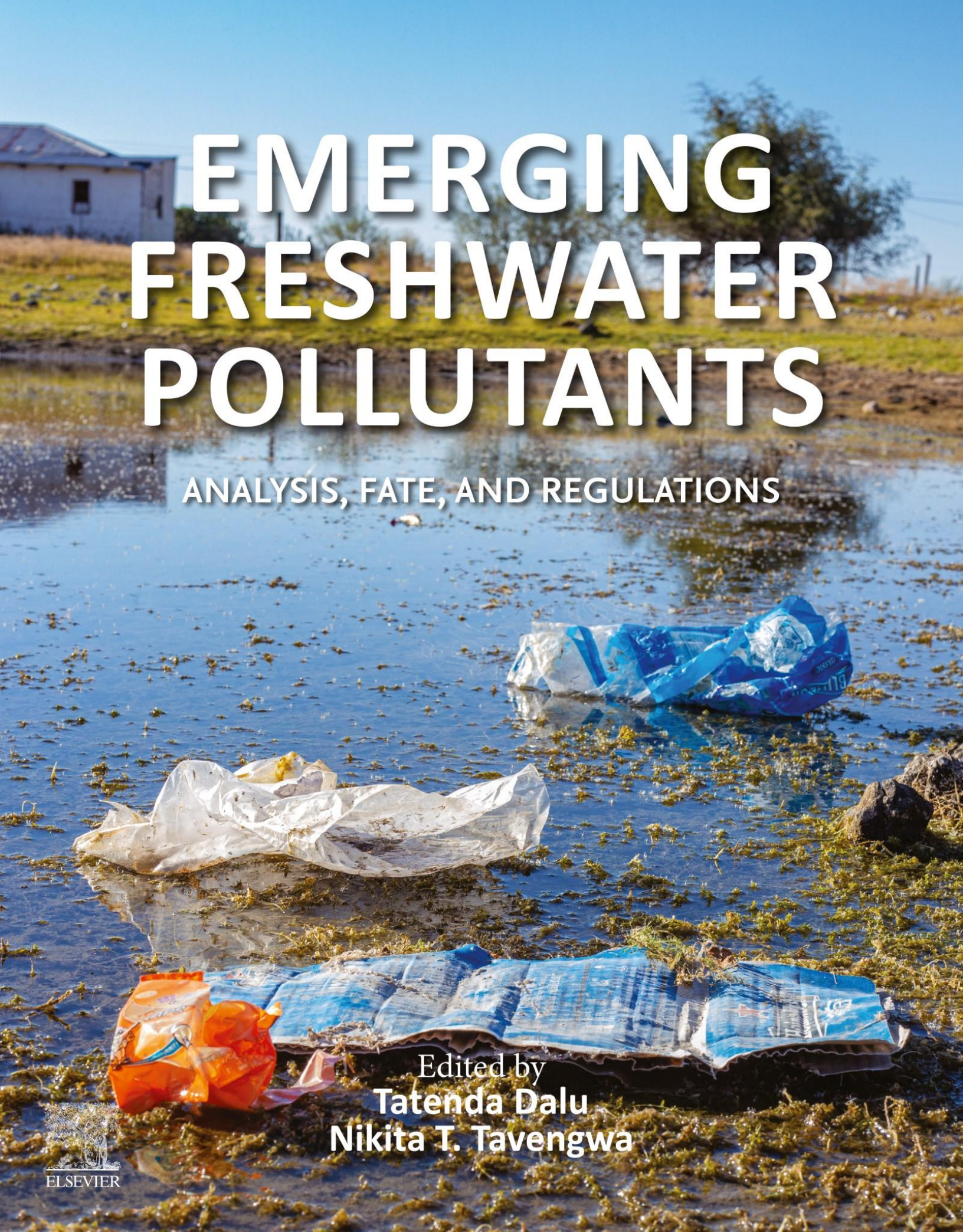
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Analysis, Fate, and Regulations

Edited by

TATENDA DALU
School of Biology and Environmental Sciences, University of Mpumalanga, Nelspruit, South Africa; South African Institute for Aquatic Biodiversity, Makhanda, South Africa

NIKITA T. TAVENGWA
Department of Chemistry, University of Venda, Thohoyandou, South Africa

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Front cover

1. Plastic polluted Khakhea Bray pan, South Africa (credit Chad Keates)

Back cover (from top)

1. Polluted Bloukrans River, Makhanda, South Africa (credit Tatenda Dalu)
2. Polluted Bloukrans River showing pollution forming due to bacterial activity, Makhanda, South Africa(credit Tatenda Dalu)
3. Fannie Masina collecting plankton samples from a pan in Khakhea Bray pan, South Africa (credit Chad Keates)
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CHAPTER

2

Stream biomonitoring: The role of diatoms, macroinvertebrates, and fish

Tongayi Mwedzi, Tinotenda Mangadze, Adroit T. Chakandinakira, and Taurai Bere

2.1 Introduction to biomonitoring

High population densities and multiplicity of industrial and agricultural activities expose most hydrographic basins to heavy and rising environmental impacts especially to pollution by domestic and industrial waste residues (Salomoni et al., 2006; see Tavengwa and Dalu, 2022, chapter 1). This increasing anthropogenic influence on lotic environments that parallels civilization has captured public interest because of the consequent deterioration of water quality, health problems, pest plants and animals, and other problems (Bere, 2007). Several techniques have been employed to assess the ecological integrity of lotic systems; chief among which is biological monitoring.

Aquatic biological monitoring (or biomonitoring) is the use of biotic components to assess changes in the condition of the rivers, lakes, and wetlands (Cairns and Pratt, 1993; Dallas, 1995; Li et al., 2010). Aquatic organisms are good indicators of the condition of the freshwater ecosystems as they are permanently present in water and assessment based on them integrates cumulative effects of changes in the physical and chemical constituents of the water body (Rosenberg and Resh, 1993). Biomonitoring is fast and cost-effective in assessing the effects of environmental stressors on aquatic ecosystems (Bere and Tundisi, 2010; Mangadze et al., 2019). The organisms used in biomonitoring are referred to as bioindicators. Periphyton, fish, and macroinvertebrates are the frequently used bioindicators in lotic systems globally (Bonada et al., 2006; Siziba et al., 2017). Each of these bioindicators has distinct advantages in terms of their sensitivity to different stressors, ability to indicate short- and long-term impacts, taxonomic needs, and sampling equipment requirements (Table 2.1) (Resh, 2007).

Biomonitoring is more established and has a long history in developed (mainly temperate regions) than in developing countries (mainly tropical regions) (Mezgebu, 2020). Biomonitoring in developed countries, therefore, tends to have a long tradition in the approaches used e.g., countries in Eastern Europe have used the Saprobien approach for at least a century (Resh, 2007). Developing countries on the other hand often modify and adopt sampling techniques and indices from developed countries (Bere et al., 2014; Mangadze et al., 2019). In many cases, these transfers are from different geographic regions i.e., temperate to tropical e.g., North America to India (Sivaramakrishnan et al., 1996), the United Kingdom (UK) to Thailand (Mustow 2002). This poses reliability and validity challenges as biomonitoring indices are usually developed based on species assemblages and associated environmental sensitivities within their respective regions (Ochieng et al., 2019). Furthermore, some taxa occur differently (in terms of diversity, abundance, and sometimes the niche they occupy) in different regions. For example, Plecoptera has low diversity in the tropics but high in temperate regions, shredders are rare in the tropics but common in the temperate regions (Mwedzi et al., 2016, 2020; Masese and Raburu, 2017).

Biomonitoring in developing countries is limited by incomplete taxonomical resolution and seldom known sensitivities of many tropical taxa, lack of research funding and priorities, lack of legislation (or lack of implementation), lack of clear political regional policies, and incomplete knowledge on how tropical rivers function (Resh, 2007; Odountan et al., 2018; Mangadze et al., 2019; Mezgebu, 2020). In most cases, there is no biomonitoring supportive decision-making process and there is little state support for research (Mezgebu, 2020). The objective of this chapter is to provide the history, current efforts, challenges, and future direction of biomonitoring with a special focus on developing countries (which are largely tropical). The chapter also

provides a practical guide of how the most commonly used bioindicators i.e., diatoms, macroinvertebrates, and fish can be used in biomonitoring.

TABLE 2.1 Advantages and challenges associated with different biotic groups used in aquatic biomonitoring.

Organism	Advantages	Challenges
Diatoms	• High species diversity • Pollution tolerances are well documented in books, webs, and articles. • Useful and quick indicators of eutrophication and increases in turbidity. • Easy and quick to collect (scraping, pipetting, using corers for soft sediments and sand). • Artificial substrates can be used. • Preservation of the siliceous cell wall (paleolimnological applications) and integrative power variable depending on the species	• Taxonomic identification is challenging, hence data collected by non-specialists is not reliable. • There are sampling and enumeration problems with certain groups. • Background life-history information is often lacking for many species • Not very useful for severe organic or fecal pollution. • Poorly sensitive to habitat disturbances • Results obtained are usually difficult to interpret into values meaningful to the general public. • The applicability of diatom indices across geographical regions differs yet most indices are used internationally despite the need for indices to be unique for a region.
Macroinvertebrates	• Many sedentary species can indicate effects at the site of sampling. • Whole communities can respond to change. • Long-lived species can indicate integrated pollution effects over time. • Qualitative sampling is easy. • Simple sampling equipment. • Good taxonomic keys.	They are difficult and time-consuming to sample, sort, and identify Quantitative sampling is difficult. background life-history information is often lacking for many species The substrate type is important when sampling. Species may drift in moving waters. Knowledge of life cycles is necessary to interpret the absence of species.
Fish	• Methods are well developed. • Immediate physiological effects can be obvious. • Can indicate food chain effects. • Ease of identification.	•Results obtained are usually difficult to interpret into values meaningful to the general public. •Ecological status evaluations do not take into consideration the presence of new non-native species in their calculations Fish are highly mobile- they may migrate to avoid disturbance/ pollution. Sampling gear can be very selective Seasonal time scales, •Relative tolerance of fish to substances chemically harmful, •Requires more manpower for field sampling. •The lack of ecological and life-history information makes the development of IBI difficult •A high degree of omnivory •Shifts in food items consumed by a given species is often determined by the habitat where the fish occur making assignations to trophic groups difficult and probable species extinctions in the rivers and streams caused by introduced species.

2.2 History of aquatic biomonitoring

The idea of using biota as indicators of environmental condition started with Kolkwitz and Marsson (1902, 1908, 1909) who developed the saprobic system in rivers as a measure of the degree of contamination. The term "saprobic system" was derived from the word saprobia which refers to the dependence of an organism on decomposing substances. It used the presence and/or absence of different aquatic taxa particularly bacteria, algae, protozoans, and rotifers to ascertain the degree of contamination by organic pollution primarily sewage (Rolauffs et al., 2004; Monaghan and Soares, 2012). Different organisms (identified at species level) were therefore assigned "saprobic values" based on their pollution tolerance. Values ranged from zero to eight with higher values being for those organisms which are tolerant to pollution.

2.3 Practical guide to biomonitoring

The saprobic system was criticized for prescribing subjective pollution tolerance limits for organisms, intensive sampling, and non-applicability of the saprobic values to other geographic locations and other types of pollution, e.g., inorganic and radioactivity (Metcalfe, 1989). Hence ecological studies shifted their focus to biodiversity indices (e.g., Shannon-Wiener diversity index) which use components of community structure (richness, evenness, and abundance) to describe the response of a community to the quality of its environment.

Biodiversity indices were favored for their quantitative and dimensionless nature, independence of sample size, and the fact that they lend themselves to statistical analysis (Metcalfe, 1989). In this approach, less disturbed environments are expected to be characterized by a high species diversity and richness. Species diversity is expected to decrease in stressed environments as sensitive organisms are lost. However, biodiversity indices tend to have varying values depending on the taxonomic resolution used (e.g., species or genetic diversity). Furthermore, individual species are reduced to anonymous numbers disregarding pollution tolerances (Chutter, 1972). For these reasons modern indices were developed which combined the indicator value concept with the biodiversity of different selected groups (Li et al., 2010).

The ecological indices commonly used to evaluate river health broadly fall into three groups; biotic, multivariate, and multimetric indices. Biotic indices evaluate river health based only on organisms' tolerance to pollution e.g., the Hilsenhof Biotic Index, Biological Monitoring Working Party (BMWP) scoring system, fish species biotic index, etc. Individual taxa are assigned sensitivity weightings to environmental characteristics such as organic pollution. The values for all taxa in a sample are then summed or averaged. Biotic indices have the advantage of being simple and rapid, measuring only one disturbance (e.g., organic pollution tolerance). However, organisms usually do not respond to only one disturbance as stressors do not occur in isolation (Mwedzi et al., 2017). Furthermore, biotic indices are unlikely to be universally applicable as indicator organisms vary from region to region (Taylor et al., 2007a; Rimet, 2012; Bere et al., 2014; Mangadze et al., 2019). For that reason, multimetric and multivariate statistical approaches are being considered for the rapid bioassessment of river ecosystems (Rosenberg and Resh, 1993; Stevenson and Smol, 2003; Mueller et al., 2014). These approaches are a more objective way of identifying groups of reference sites with which monitoring sites can be compared (Mangadze et al., 2016).

The multimetric approach combines several variables associated with structural and functional attributes of a biological community (e.g., number of families, functional feeding groups, life history strategies, pollution tolerance, etc.) into a single index e.g., the index of biotic integrity (IBI). This provides a broader understanding of assemblage responses to different stressors. Furthermore, unlike any other ecological index, multi-metric indices can identify the cause of degradation. They are therefore the most recommended ecological indices (Fierro et al., 2017).

Multivariate indices use statistical analysis to infer relationships between faunal patterns and environmental stressors. This includes ordination analysis (e.g., Principal components analysis, canonical correspondence analysis, etc.) and clustering analysis (e.g., hierarchical clustering, multidimensional scaling, etc.). The most advanced multivariate indices are predictive and compare the observed with the expected faunal patterns in the absence of environmental stress (Bird and Day, 2010; López-López and Sedeño-Díaz, 2015). Examples include the River InVertebrate Prediction And Classification System (RIVPACS) and the Australian River Assessment System (AusRivAS). Predictive modeling approaches are cheaper and provide a holistic assessment of anthropogenic effects on rivers. While multivariate indices offer a higher predictive power compared to other indices, they were not developed to find patterns and therefore do not establish impacts.

2.3.1 Diatoms

2.3.1.1 The use of diatoms in biomonitoring

Diatoms (Bacillariophyceae) are unique microscopic unicellular micro-organisms, found in all aquatic biotypes, and play a vital role in the food web structure of aquatic ecosystems as they are the chief primary producers in these ecosystems (Smucker and Vis, 2011; Dalu and Froneman, 2016; Kock et al., 2019; Pajunen et al., 2020; Dalu et al., 2020). Diatom species have a cosmopolitan nature, shortest generation times, and quick response to environmental perturbations (Stevenson et al., 2010). Unlike animal components of an ecosystem (e.g., macroinvertebrates and fish), diatom species have specific optima and tolerance ranges for nutrients as well as pH and have therefore been used successfully in studies of monitoring eutrophication, organic and metal pollution in streams (e.g., Bellinger et al., 2006; Potapova and Charles, 2007; Pandey and Bergey, 2016). Diatoms constitute an extremely diverse phylum (approximately 100,000 species) (Guiry, 2012; Mann and Vanormelingen, 2013). A large number of taxa allows redundancies of information in the data sets and increases the assurance of environmental inferences (Ovaskainen and Soininen, 2011). Therefore, many diatom-based biotic indices have been developed worldwide on species specific sensitivities, or tolerances to infer specific environmental conditions in lotic systems and to estimate the status of river ecosystems (Li et al., 2010). These indices have universal applicability since they are based on the ecology of widely distributed or cosmopolitan taxa. However, due to the scarcity of information on ecological preferences and tolerances of diatoms in developing countries, diatom-based biotic indices developed in Europe are often applied to assess ecological conditions in tropical context (Bere et al., 2014). Nonetheless, floristic and faunal differences among regions may contribute to differences in the water quality characteristics of rivers and may lead to variation in diatom taxa composition (Taylor et al., 2007a; Bere et al., 2014; Mangadze et al., 2019). Different regions may also have endemic diatom species, therefore there is a need for the development of region-specific diatom indices (Harding and Taylor, 2011).

Diatoms were first used to assess water quality conditions in freshwater habitats by Kolkwitz and Marsson (1908). Their study demonstrated the potential and robustness of diatoms that could enable their use to monitor river quality. They developed the saprobe system (the Saprobity index) which is based on the classification of diatom taxa according to the resistance, sensitivity, or indifference to pollution (e.g., Lange-Bertalot, 1979). These saprobe systems led to the direct development of numeric biotic indices. For example, in Austria, this method has been the basis of a diatom index that is the only index routinely applied on a national scale in Europe (Prygiel et al., 1999). After these first approaches, the use of indices to assess water quality was also attempted by Zelinka and Marvan (1961), Slàdecek (1973, 1986), Coste and Leynaud (1974), and Descy (1979).

Coste and Leynaud (1974), developed the diatom-based stream quality assessment which targets water quality parameters such as phosphorus, nitrogen, biological oxygen demand (BOD), and chemical oxygen demand (COD). These parameters, varying at different scales in time and space, can impact diatom assemblages differently. In France, Coste (in CEMAGREF, 1982) proposed an index known as the Specific Pollution Sensitivity Index (SPI), which is based on 189 surveys conducted from the year 1977 to 1980 at the Rhone- M^ editerranee-Corse basin national monitoring network. The index has been updated since 1982 to incorporate changes in taxonomy and new knowledge of diatom ecology. Several studies have shown that the SPI index is the most widely used in Europe and water quality class boundaries have been set by several authors for rivers in Sweden, Finland, Belgium, and France (Prygiel and Coste, 2000; Eloranta and Soininen, 2002). Currently, in South Africa, the South African Diatom Index (SADI), a modified version of the Specific Pollution Sensitivity Index (SPI) which includes indicator and tolerance values for South African endemic species is being employed (Harding and Taylor, 2011).

Following the SPI, other indices were also developed, e.g., the Generic Diatom Index (GDI; Coste and Ayphassorho, 1991); Artoise-Picardie Diatom Index (APDI; Prygiel et al., 1996) was proposed (Coste and Ayphassorho, 1991); Biological Diatom Index (BDI; Lenoir and Coste, 1996) and Eutrophication/Pollution Index (EPI; Dell'Uomo, 1996). However, principal components analysis of data collected from the Artois-Picardie Region of France indicated a stronger correlation between most of these diatom indices and variables associated with organic pollution compared to eutrophication (Prygiel and Coste, 1993). This prompted the first attempts to develop a purpose-designed index to monitor eutrophication in rivers, the Trophic Diatom Index (TDI; Kelly and Whitton, 1995). This TDI index uses 86 epilithic diatom taxa (species and/or genus level) and each taxon is given a sensitivity value (1–5) and an indicator value (1–3). The final result of the TDI value ranges from 1 (very low nutrient levels) to 5 (very high nutrient levels).

In addition to these diatom-based auto ecological indices, other multimetric indices such as Metzmeier's diatom index of biotic integrity have also been developed in the United States of America (Kentucky Division of Water, 1993). This index includes taxa richness, Shannon diversity (Shannon and Weaver, 1949), the pollution tolerance index (Lange-Bertalot, 1979), and the proportion of species sensitive to pollution. Other studies in Europe (Barbour et al., 1999; Karr and Chu, 1999; Hering

et al., 2006a) have also proved these multimetric indices as effective tools for assessing lotic systems, lakes, and wetlands at varying geographic scales (Delgado et al., 2010).

2.3.2 Diatoms' sampling protocols

The need to monitor water quality has led to the development of standardized sampling protocols for diatoms since the end of the nineties (Kelly and Whitton, 1998; Afnor, 2007; Morin et al., 2016). Two Rapid Bioassessment Protocols are commonly used for diatom sampling. The first approach involves the assessment of species composition and/or biomass performed in the laboratory. The second approach involves a field-based rapid survey of diatom biomass and coarse-level taxonomic composition. These two approaches are discussed briefly below.

2.3.2.1 Laboratory-based approach

Field sampling procedures and sampling habitats

Benthic diatoms occur in four major and distinct habitats: (1) attached to macrophytic plants supporting the epiphyton; (2) gravel, bedrock, and stone surfaces supporting the epilithon; (3) sand surfaces supporting the epipsammon and (4) the epipelon, mobile taxa growing among deposited inorganic and organic sediment particles (Round, 1991). Differences in species composition of diatoms among these habitats are often evident as changes in color and texture of the diatom biofilms (Bahls et al., 2018). For this bioassessment protocol, multihabitat sampling or single habitat sampling may be used. However, many studies have shown that multihabitat sampling provides a more complete assessment of all taxa at a site, which potentially is a better characterization of biodiversity and bio integrity than assemblages from targeted habitats (Stevenson and Smol, 2003; Taylor and Cocquyt, 2016). Diatom samples are collected from all available substrates and habitats to form one composite sample, which is proportionally representative of the periphyton assemblage in the sampling area. The diatom suspension will be fixed with ethanol or 4% buffered formalin to a final concentration of 20% by volume. (APHA, 1995) (refer to Box 2.1 for all field equipment for diatom sampling). The techniques for the collection of diatoms in the different habitat types are described in the table below (Table 2.2).

Although many scientists prefer sampling natural substrates whenever possible to reduce field time and improve the ecological applicability of the information, artificial substrata can also be used to assess benthic diatom assemblages (Wojtal and Sobczyk, 2012). These include typically uniform substrata (e.g., glass slides, clay tiles, acrylic, or wooden dowels) that can be used across many water body types (streams, rivers, wetlands, lakes). Artificial substrates are placed in aquatic habitats and colonized over a period of time. Some of the advantages of using artificial substrates include; decreased habitat disruption; substantially improved sampling precision and reduced effects of small-scale habitat variations that inevitably exist in relation to natural substrata (Lane et al., 2003; Wojtal and Sobczyk, 2012). However, artificial substrates are time-consuming and require more attention during collection. Moreover, they have the risk of being vandalized by non-scientific people or loss of the artificial substrates from unforeseen events like floods (Bere and Tundisi, 2010; Morin et al., 2016). For that reason, many large national and state programs in Europe have chosen to sample natural substrata (e.g., Hering et al., 2006a; Johnson et al., 2006).

Laboratory procedures: Diatom preparation, identification, and counts

Diatom samples have to be cleaned through sedimentation (removing mineral debris) and oxidation (removing organic matter) before they can be identified. This is done to improve conditions for observation of the morphological elements (e.g., the shape and number of chromatophores and the shape and type of colony formation) in the microscope (Hasle and Fryxell, 1970). The detailed methods for diatom cleaning have been summarized by APHA (1995) and Taylor et al. (2007b). After cleaning, valves are mounted in a resin (Naphrax) or another high refractive index medium to make permanent slides. Diatoms are then counted and identified using a light microscope (1000 magnification) (see Box 2.1 for all laboratory equipment needed for diatom processing and identification). The abundances of all observed taxa are expressed as relative counts. The diatoms will be identified to the lowest possible taxonomic level using the best available keys (e.g., Krammer and Lange-Bertalot, 1986; Taylor et al., 2007b).

BOX 2.1

FIELD AND LABORATORY EQUIPMENT FOR DIATOM SAMPLING, PROCESSING, AND IDENTIFICATION (STEVENSON AND BAHLS, 1999).

Field equipment for diatom sampling–natural substrates

- Wide-mouth sampling bottle 100mL.
- Preservative [ethanol (final concentration of 20% by formalin (APHA, 1995)]
- Turkey baster; useful for collecting sediment samples.
- Pencil and labels, ethanol does not dissolve pencil
- Forceps for picking up filamentous algae and detritus.
- Water-proof marking pen.
- Plastic Pasteur-pipette are useful for collecting small amounts of sediment.
- Toothbrushes for scrubbing solid substrata.
- Knife for cutting the stems of aquatic vegetation
- Fine-mesh plankton net. (<10μm)

Laboratory equipment for diatom processing and identification

- Micro-pipette 1mL with disposable tips volume) or 4% buffered
- Glass and plastic Pasteur-pipettes (2–3mL)
- Forceps
- Microscope slides markings.
- Coverslips
- Diatom mountant
- 10mL plastic graduated centrifuge tube
- 15mL glass test tube
- 4mL glass sample storage bottles with a rubber seal inside the cap
- Waterproof fine marking pen
- White plastic tray with a lip
- Heat-resistant glass beaker 100mL
- Watch glass.

TABLE 2.2 Summary of collection techniques for diatoms in different habitat types.

Substrate type	Collection technique
Hard substrates (removable) e.g., stones, gravel, cobble, and woody debris	Randomly collect the representative substrates along each sampling stretch; brush or scrape representative area of diatoms from the surface and rinse into a sample jar.
Soft removable substrates e.g., macrophytes (submerged/emergent), mosses, and macroalgae	Select different species of plants. Put a portion of the plant in a sample container with some distilled water. Shake it vigorously and rub it gently to remove the resulting diatom suspension. Remove the plant from the sample container.
Large substrates (not removable) e.g., logs, boulders, roots, bedrock	Place PVC pipe with a neoprene collar at one end on the substrate so that the collar is sealed against the substrate. Brush or scrape algae in the pipe with a toothbrush or scraper. Remove the diatom suspension from the pipe with a pipette
Sediments e.g., sand, clay/silt, fine particulate organic matter	Invert petri dish over sediments. Trap sediments in a petri dish by inserting spatula under the dish. Remove sediments from the stream and rinse into a sampling container. Diatom samples from depositional habitats can also be collected with spoons, forceps, or pipette.

Adapted from Kentucky DEP Kentucky Division of Water, 1993. Methods for Assessing Biological Integrity of Surface Waters. Kentucky Natural Resources and Environmental Protection Cabinet, Frankfort, Kentucky, 139; Bahls L.L., 1993. Periphyton Bioassessment Methods for Montana Streams. Water Quality Bureau, Department of Health and Environmental Services Helena, MT.

However, diatom species-level identifications can be difficult because of their tremendous diversity and the increasing interest in taxonomy leading to the description of numerous new species and incessant taxonomic arrangements (Rimet, 2012). Zampella et al. (2007) also highlighted that the great diversity of diatom species and identification difficulties limit their use in routine monitoring and identification mistakes are numerous at the species level. Conversely, other studies have highlighted that it is necessary to have precise determination for a good environmental assessment (e.g., Ponader and Potapova, 2007). Nonetheless, investigations in sub-tropical rivers in Taiwan showed that identification of diatoms at genus level gave similar results as species level in assessing the effects of river regulation (Wu and Kow, 2002). The application of higher taxonomic level identification should not be indiscriminate, as it depends on physicochemical parameters and the study region (Chen et al., 2016).

2.3.2.2 Field-based rapid periphyton survey-viewing bucket survey method

This approach is a semi-quantitative rapid periphyton survey of algal biomass and taxonomic composition (Box 2.2). The advantages of this approach are that it reduces field and laboratory time; it can easily be employed in all sampling programs and enables rapid assessment of algal biomass over larger spatial scales than substrate sampling and laboratory analysis. This technique (i.e., processing done in the field) allows visual characterization of algal type, percentage cover, filament length, and periphyton mat thickness along multiple transects in streams (Stevenson and Bahls, 1999). Secchi-disc transparency is also a semi-quantitative approach for assessing plankton biomass in lakes (Wetzel and Likens, 1991). Coarse-level taxonomic characterization of communities is also possible with this technique; thus they require little taxonomic expertise (Stevenson and Bahls, 1999).

2.4.1 The use of aquatic macroinvertebrates in biomonitoring

Aquatic macroinvertebrates are water-dwelling invertebrates with a body size greater than 5mm (Ochieng et al., 2020). Their communities perform a variety of functions including accelerating detrital decomposition, transferring energy between primary producers and consumers, and translocation of nutrients from upstream to downstream river sections (Wallace and Webster 1996). Macroinvertebrates are the most widely used bioindicators (both in developed and developing countries) because they have a well-known life history and ecology (Rosenberg and Resh, 1993; Resh,

BOX 2.2

FIELD-BASED RAPID PERIPHYTON SURVEY (BARBOUR ET AL., 1999; STEVENSON AND BAHLS, 1999).

At least three transects are established across a habitat accumulation. If different types of algae are present (e.g., being sampled (e.g., riffle or run, with water clarity such diatoms, blue-green algae, or other microalgae), each type that algal accumulation can be observed readily and char- is assessed separately. After the visual assessment is comacterized). Three locations along each transect will be pleted, statistically characterize the mean density and the selected (e.g., right bank, middle, and left bank). At each average percent cover by each type of algae.

selected sample location, a viewing bucket (0.5m diame-ter) containing a grid of 50 dot grid (77+1), is immersed Field equipment into the water. At each of these nine locations, the viewing bucket with a 50-dot grid bucket is submerged so that the diatoms are visible through meter stick the clear bottom of the bucket. Algal biomass is roughly pencil

characterized by counting the dots that occur over macro-
Rapid Periphyton Survey Field Sheet algae and by measuring the length and thickness of algal

2007). Furthermore, macroinvertebrates are ubiquitous, diverse (with a wide range of feeding habits and pollution tolerances), have long life spans (integrating short and long-term effects of environmental variations), have different sensitivities in their life stages, and rapidly recover from repeated sampling (and hence easily generate a lot of data for effective biomonitoring) (Rosenberg and Resh, 1993; Ochieng et al., 2020). Moreover, in the presence of well-developed taxonomic keys, macroinvertebrates are relatively easy to identify and their sampling protocols involve simple equipment (e.g., kick net) (Ochieng et al., 2020).

The Trent Biotic Index (TBI) (Woodiwiss, 1964) developed in the UK; is seen as the origin of most modern macroinvertebrate biotic indices. The TBI used two main aspects of benthic communities; i.e., biological richness and the presence of some key groups (with different levels of tolerance to environmental degradation). The index ranged from zero (polluted condition) to ten (clean waters). However, being the first index of its kind, it was criticized for being insensitive and providing erroneous results. It was modified to different indices in different countries e.g., the Indice Biotique in France (in 1968), Chandler's score in Scotland (in 1970), Chuttter's Biotic index in South Africa (in 1972), Extended Biotic Index in the UK (in 1978) (Dallas, 1995), Hilsenhoffs Biotic Index (in 1977) and Family-level Biotic Index (in 1987) in the United States (Li et al., 2010).

The Biological Monitoring Working Party protocol (BMWP) (Hellawell, 1986) evolved from Chandler's score and assumed particular importance. In this method, taxonomic identification was easier, because it was performed at the family level (Bo et al., 2017). In developing countries, modification of the BMWP led to several indices including the development of the South African Scoring System (SASS) (Chutter, 1994, 1998) and the Ethiopian Biotic scoring system (ETHbios) (Aschalew and Moog, 2015). Several southern African countries have subsequently modified and standardized the SASS protocol e.g., to Namibian Scoring System in Namibia, Okavango Assessment System in Botswana, and Zambian Invertebrate Scoring System in Zambia (Aschalew and Moog, 2015; Mangadze et al., 2019).

Given that differences in ecoregions lead to a wide array of habitat types, there are significant differences between sampling protocols and tools used across the globe (Bonada et al., 2006). This is expected as different habitat types will require different assessment methods and different bio criteria. However, the use of different tools and techniques (e.g., mesh size, time of collection, range of habitat type sampled) introduces bias making the comparison between biotic indices challenging (Brown, 2001). Universally consistent sampling and processing techniques are important in addressing global issues such as climate

change. Even though biomonitoring programs following standard methods could aid international data sharing and interpretation, internationally accepted standard methods for collecting and processing benthic macroinvertebrate samples do not exist as yet (Buss et al., 2015). However, there have been efforts to develop standardized protocols in Europe since the introduction of the European Water Framework Directive in 2000.

The development and testing of an integrated Assessment system for the ecological Quality of streams and rivers throughout Europe using benthic Macroinvertebrates (AQEM) project and Standardization of River Classifications: Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive (STAR), the project have facilitated the development of the AQEM/ STAR (Haase et al., 2004) and modified AQEM/STAR (MAS) protocols respectively (Clarke et al., 2006). While great efforts have been made in developing the AQEM/STAR and MAS protocols in Europe, most European Union countries have retained their methods that they have used for decades. There is greater potential for easier adoption of standardized regional protocols in geographic regions without a long history of comprehensive biomonitoring, such as Asia, Latin America, and Africa as new protocols are more acceptable among communities who do not have a history with their biomonitoring protocol (Buss et al., 2015). These countries will not have to deal with issues of national pride, prejudice, etc. (Resh, 2007). However, such political regional policies (like the European Water Framework Directive) are lacking in developing countries and would be a good starting point if regional protocols are to be developed. There is a general lack of regional collaboration to improve biomonitoring tools among stakeholders and governments of developing countries (Mangadze et al., 2019).

2.4.2 Macroinvertebrate sampling tools

Several different tools have been developed for collecting macroinvertebrate assemblages; e.g., grab nets, stovepipe coring devices, substrate Hess samplers, Surber samplers, and kick nets, (Fig. 2.1) (Merritt et al., 2008; Elias et al., 2014). Coring and dredging devices are used when sampling soft sediments or in deep waters where sampling has to be done from a boat.

Surber and Hess samplers are generally used in shallow streams (i.e., less than 15cm depth) with small substrata (i.e., sand, gravel, and very small cobble) (Buss and Borges, 2008). Kick nets are preferred in front of Surbers or Hess samplers especially when the substratum is large (Bonada et al., 2006). Kick nets are the tools of choice in rivers with fast flowing riffles (>50cm/s), typical of third to sixth-order rivers (Hauer and Resh, 2017). In the case of kick nets, macroinvertebrates are generally collected by disturbing bottom sediments and catching organisms swept into the net

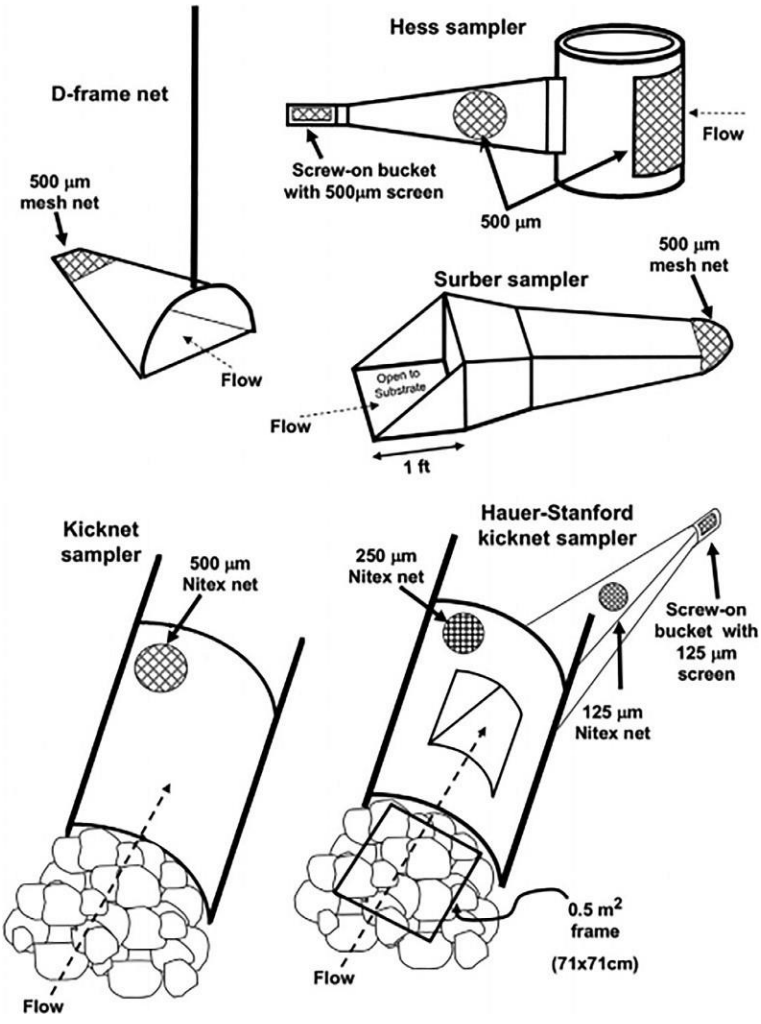

FIG. 2.1 Illustration of some of the most common macroinvertebrate sampling devices. Adapted from Hauer, F.R., Resh, V.H., 2017. Macroinvertebrates. In: Gore, J.A., Banning, J. (Eds.) Methods in Stream Ecology. Elsevier.

downstream of the disturbance. The major limitation of kick nets is the fact that they rapidly fill with material clogging the net and resulting in back welling (Hauer and Resh, 2017).

Ideal mesh size is the one with favourable cost/ effectiveness ratio, in which sample processing time is reduced and the sample is representative of the macroinvertebrate fauna at the site. A range of sizes (from 200 to 1000μm) has been used worldwide (Bonada et al., 2006). The standardization normative recommends a mesh size of 500μm in Europe (AENOR, 1995). This is also the most commonly used mesh size in the United States of America (Bonada et al., 2006). In Africa, a kick net (Fig. 2.2) with a mesh size of 1000μm is commonly used following the recommendations of the SASS protocol from which other Southern African protocols have been derived. Generally, literature recommends a mesh size of approximately 500μm as it is the most cost-effective retaining the most macroinvertebrate genera per unit of effort despite losing smaller specimens and earlier instars that are often difficult to identify accurately (Bonada et al., 2006; Buss et al., 2015).

2.4.3 Macroinvertebrates' sampling habitats

The habitats to be sampled in a macroinvertebrate biomonitoring exercise varies among sampling protocols. A single sample from the "most productive habitat" is usually recommended (Bonada et al., 2006). Riffles or stones generally seem to be the most productive habitat for macroinvertebrates and are thus emphasized in different protocols. However, anthropogenic impacts can be specific to a particular habitat, and/or sometimes the most productive habitat is not evident. Consequently, other protocols emphasize sampling all available habitats (Bonada et al., 2006; Blocksom et al., 2008; Buss et al., 2015). In that sense, a multi-habitat protocol integrating all habitats, as in SASS5 (Dickens and Graham, 2002) (Box 2.3) is desirable (Bonada et al., 2006). The SASS5 protocol is highlighted here as it is the most used protocol in Africa and the index from which most African protocols are derived.

2.4.4 Sample processing and identification of macroinvertebrates

Separating invertebrates from detritus and other debris and identifying them are the most time-consuming components of invertebrate-based monitoring studies. Sample sorting may be done in the field (Fig. 2.3) or samples can be collected and preserved for later processing in the laboratory. Field sorting saves time, allows easy location of moving live organisms (e.g., the stick-dwelling caddies), and reduces human exposure to preservatives such as formaldehyde (ethanol is usually a better alternative). However, field sorting can be biased toward selecting large, active, or conspicuous organisms and may not be practicable for large aquatic systems such as the Congo River. Some protocols e.g., SASS emphasizes field sorting and returning the samples to the river after identification and enumeration while as in other protocols e.g., the European Union's AQEM/STAR, sorting is done completely in the laboratory and requires that a defined subsample is taken before sorting (Haase et al., 2004).

Identifying samples to species-level improves the sensitivity of biomonitoring, especially to subtle impacts (Campbell, 2002). However, identification to species level can be costly (in terms of time and money) and is usually

FIG. 2.2 Picture of South African Scoring System kick net during sampling, mesh size 1000μm on a 30-cm square frame with stout handle. Manyame river, Zimbabwe (Photo Credit: Tongayi Mwedzi).

BOX 2.3

THESOUTHAFRICANSCORINGSYSTEMVERSION5(SASS5)PROTOCOL.

A kick net of 3030cm and 1000µm (1mm) mesh size is Taxa are sorted and identified for 15min in the field or until used in three different biotopes : stones (both in and out of nonewtaxahavebeenseenafter5minofsorting.Identificacurrent); vegetation (both marginal and aquatic); and tion is at the family level except for Hydropsychidae and gravel, sand, and mud (GSM). For stones in current, kick Baetidae which are identified to species level (these families samplingisperformedfor2minor5minifthestonesaredif- have pollution tolerant and intolerant species). Approxificulttomove. Forstonesout of the current, kick-sampling is mately1minis assigned to hand-picking/noting specimens performed for 1min. For the marginal vegetation, the net is that may have been missed by the sampling procedure e.g., pushed vigorously over a total length of 2m, spread over snails and fast-moving pond skaters. Each macroinverteoneormorelocationsindifferentflowvelocities.Foraquatic brate taxon is awarded a score (from 1 to 15) in increasing vegetation, the net is pushed repeatedly over an area of order of the sensitivity to the water quality changes. The approximately one square meter. Gravel, sand, and mud final SASS5 score is calculated by summing the preare stirred by scraping with the feet (for approximately determined taxa tolerance values of all macroinvertebrates 1min), while continuously sweeping the net over the dis- within a particular sample. The Average Score Per Taxon turbed area to catch dislodged biota. All collected material (ASPT) is calculated by dividing the total SASS5 score by separated by biotope is poured into three different trays. the number of taxa.

FIG.2.3 Sorting and identification of macroinvertebrates in the field. Photo credit: Tongayi Mwedzi.

difficult due to taxonomic training required (Campbell, 2002; Bonada et al., 2006). Resources and taxonomic expertise are often limiting in developing countries and hence family level is often the only option for bioassessment (Ochieng et al., 2019). Other authors have argued that while species-level identification implies better precision in reporting the state of the aquatic environment, in most cases the general patterns reported at the family level usually convey similar information (Bowman and Bailey, 1997; Nielsen et al., 1998). Hence, various biotic indices identify samples at a family level making the process uncomplicated and cost-effective e.g., the BMWP and SASS5 (Hewlett, 2000). The limitation remains that of taxonomy field guides as in many cases the guides developed in temperate regions are being used in tropical regions. There is a need to develop local taxonomic guides (particularly in developing countries) based on species assemblages of the region.

2.5.1 Biomonitoring using fish

The use of fish in the biomonitoring of water quality dates back to the 1970s (Karr, 1981; Harris, 1995). Fish communities are valuable components of the freshwater ecosystems as they are on the top of the aquatic food web (Barbour et al., 1999). Fishes possess a suite of advantages, which make them good indicators of water quality e.g. they live in water all their life; have a long lifespan (about 2–10years), and can reflect both long-term (several years) and current

water quality and are less affected by natural microhabitat differences (López-López and Sedeño-Díaz, 2015, Raburu and Masese, 2012). More importantly, fish can easily be used to evaluate the societal costs of degradation as their economic and aesthetic values are well known. This makes it easy to use citizen science in which case taxa with societal and economic values must be targeted to involve local communities in data collection.

Biomonitoring using fish entails the collection of fish for the assessment of the quality of a site based on species presence (Karr, 1981; Culp et al., 2011) as well as tissue collection from fish samples for toxicological evaluation (Shailaja and D'Silva, 2003). Behavioral and physiological (cardiac and respiratory rates) responses of fish can also be used to indicate contamination (Morgan, 1978; Ma et al., 2010; Kuklina et al., 2013).

2.5.2 Collection of fish species samples

It is important to note that many countries require a permit for the collection of fish samples. As such researchers must consult the relevant authorities. The collection of fish samples can be done from different aquatic environments ranging from streams to rivers. Many gear types (active and passive) can be routinely used to sample fish, however, the most commonly used collection methods in freshwater habitats are electrofishing equipment, seine, fyke, and gill nets. The type of gear used is generally dependent on the size of the water body.

2.5.2.1 Active fishing gear

Electrofishing can be used in a wide range of habitats where safe wading or boating is possible. Electrofishing is considered the most effective method for capturing moderate to large-size fish, but when negative uncontrollable impacts are seen, its use should be severely restricted (Snyder, 2003). Seines and portable electro fishers are generally used in smaller streams. Catch efficiency is dependent on the conductivity and temperature of the habitat. Mortality rates with DC are low although spinal injuries are common in large fish (Portt et al., 2006). Seining cannot be used in habitats with a lot of macrophytes and stumps, in fast currents, or deep water. Fish mortalities can occur especially when the catch is being processed and the fish are subject to the stress of capture (Bayley and Herendeen, 2000; Portt et al., 2006).

2.5.2.2 Passive fishing gear

Fyke nets are used in habitats with water that is deeper than the height of the net as this helps maintain the shape of the frame of the net. Fish survival is high in fyke nets although larger fish tend to prey on small fish inside these nets (Mccombie and Berst, 1969; Portt et al., 2006). Gill nets can be used in most habitats with depths that allow the mesh to be extended between the float and lead lines and where the current is not strong (Portt et al., 2006). Gill nets however cannot be set in habitats with emergent and floating vegetation, and other obstructions near the surface. Gill nets can be set over any substrate but when the substrate is very uneven, catch efficiency is reduced. Net color, light, and turbidity also affect catch efficiency (Jester, 1973; Portt et al., 2006). Physical injury during retention and removal can also occur. While mortality is typically high, it varies with species and how the fish are wedged to the net. While all types of fish sampling gear are generally considered selective and their use depends on the sampling time and other environmental variables such as temperature and environmental features (Mcinerny and Cross, 2000), electrofishing has become the preferred method for collecting stream fishes, especially for IBI purposes.

Sampling should be done quantitatively and should also be standardized to reduce sampling error (Masese et al., 2013). Fish samples are laboratory frozen in dry ice and stored in a freezer until prepared for analysis (Schmitt et al., 1990). Species identification can be done in the field or laboratory with the help of relevant regional taxonomic guides such as Skelton (2012) and Bell-Cross and Minshull (1988) in the case of Zimbabwe.

2.5.3 Fish indices

Ecological indicators for fish were historically based on parameters associated with individual species. As a result, they did not fully represent the entire biological community of organisms present (Niemi and McDonald, 2004). Karr (1981) described a multimetric index- IBI based on fish assemblages. It was after the formation of this multimetric index, that similar indices were been developed for benthic macroinvertebrates and periphyton (Hering et al., 2006b).

Several studies have shown that the Index of Biotic Integrity (IBI) is widely used to assess stream health based on fish and can be modified to suit different ecological regions and fish communities in the world (Harris, 1995; Ganasan and Hughes, 1998; Zhu and Chang, 2008). Many IBIs have therefore been developed and are currently available for different regions and river basins. The IBI uses a combination of key metrics that have demonstrated a response to human influence. Each metric is scored in comparison to the values of the regions' least impaired streams (Karr, 1981). It is based on 12 criteria, including such metrics as the number of native species, the relative abundance of tolerant and intolerant species, and the percent of the fish in the sample that show evidence of disease or parasites. The sample site is given a rating of very poor, poor, fair, good, or excellent based on the IBI score (Karr, 1986).

2.5.4 Other fish biomonitoring techniques

Tissue samples of flesh and other parts such as the liver and kidney of fish can also be taken for analysis for trace metals (Lee et al., 2019). Fish dissection can either be carried out in the field or laboratory. Tissue samples are freezedried, weighed and microwave acid digested, and metal concentrations are determined using an atomic absorption spectrophotometer (Swales et al., 1998). The presence of microplastics in water systems can also be assessed using fish as they enter fish using different pathways such as ingestion and/or through the gill and skin and cause negative effects that include intestinal damage and inflammation. The whole fish or parts of it such as the gills or guts are collected and used to assess the intake of microplastics by fish (Su et al., 2019).

Real-time monitoring of water quality can also be done using fish by observing their behavioral and physiological responses (Kuklina et al., 2013). Acoustic monitoring (Conti et al., 2006), Vision-based real-time monitoring (Chew et al., 2009), and Ethovision (Noldus et al., 2001) are some of the methods used to observe aquatic organisms and evaluating the environment in which they exist (Kuklina et al., 2013).

2.6 Recommendations

While biomonitoring has increased in importance because of the human-induced deterioration in water quality, a lot of challenges still need to be addressed particularly in developing countries as highlighted in this chapter. The deficiency in taxonomic identification skills and later on the taxonomic keys themselves is undoubtedly a big challenge in developing countries. There is a need to develop local identification guides, especially for diatoms and macroinvertebrates using species assemblages of the region. This necessitates sampling surveys of local assemblages, identifying them at the genus and species level and establishing their sensitivity and response to various stressors. Also, there have been advances in the use of molecular genetics in taxonomy and in identifying species all over the world (Ochieng et al., 2019; Mezgebu, 2020). This makes identification faster and more accurate. Furthermore, any life stage or remains of the organism can be used in the process (Li et al., 2010). Developing countries should also consider applying these tools in enhancing taxonomic resolutions.

Developed countries usually have legal and policy framework which clearly articulates aquatic ecosystem monitoring and diligently enforce their environmental laws (Mezgebu, 2020). While environmental regulations are increasingly being codified into law in developing countries, the problem remains that of enforcement. International funding agencies and aid organizations should assist by increasing pressure to require enforcement as was done in West African countries (Burkina Faso, Ghana, Ivory Coast, Guinea, Senegal, Niger, Mali, Benin, Togo, Sierra Leone, and Guinea Bissau) in a biomonitoring program that sought to address possible impacts of insecticide application in the control of Onchocerciasis (River blindness) (Resh, 2007).

Funding for biomonitoring programs is a problem in many developing countries. In many cases, aid agreements are often influenced by political policies. Developing countries must engage in multiple-country biomonitoring programs which can more easily receive funding from international agencies (Resh, 2007). They must take advantage of these multiple-country biomonitoring programs to develop ecoregion-based indices that are reliable and clearly show the status of ecosystem health in that ecoregion.

As indicated in this chapter, biomonitoring is a cheap and easy tool, easily reflecting the impact of pollution and other stressors on freshwater. Developing countries, therefore, stand to benefit if it is well developed in their regions. As a matter of course, developing countries should channel resources toward tackling these challenges particularly, training of skilled labor and acquiring and maintaining the necessary tools for biomonitoring. There is also a need to leverage citizen science networks. This is an attainable goal when taxa with societal and economic values (particularly fish) are used in biomonitoring. However, the key challenge will be on how to maintain continuity and more importantly quality control in these voluntary and unregulated bodies (Jackson et al., 2016). This falls under the section on monitoring techniques, current analytical approaches, and instrumental analyses with other chapters e.g., Kaserzon et al. (2022), Kaykhaii and Hashemi (2022), Kebede et al. (2021), Kumar et al. (2022) aims to understand how emerging freshwater pollutants are analyzed or detected within aquatic ecosystems.

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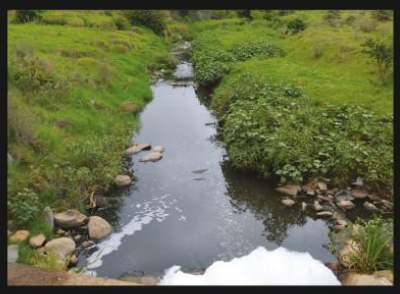

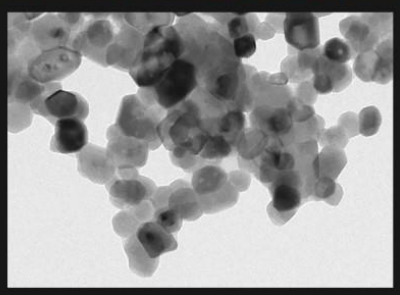

EMERGING FRESHWATER POLLUTANTS

ANALYSIS, FATE, AND REGULATIONS

Edited by **Tatenda Dalu and Nikita T. Tavengwa**

Emerging Freshwater Pollutants: Analysis, Fate, and Regulations comprises 20 chapters, all of which are written by leading experts. The book is written in the most practical terms and is easy to comprehend, with numerous helpful examples and case studies. The book is meant to be used as a practical guide and serves as an important educational tool to address issues concerning freshwater emerging pollutants. The book is organized in a way that exposes the reader in logical succession to the full range of complex scientific and management aspects of emerging freshwater pollutants in the developing world. The book recognizes that water chemistry, emerging freshwater pollutants, and management are interdependent disciplines. The book covers (i) the various monitoring techniques, current analytical approaches, and instrumental analyses; (ii) fate and occurrence of emerging pollutants in aquatic systems; and (iii) management policies and legislations on emerging pollutants. Thus, subsequent chapters elucidate chemicals with pollution potential, multi-detection approaches to the analysis of organic pollutants in water, the effects of microplastics, and photochemical transformation of emerging pollutants in freshwater systems. Whereas other chapters address the oxidation of organic compounds in aquatic systems, biomonitoring systems for the detection of toxic levels of water pollutants, and the health aspects of water recycling practices.

This book melds various perspectives on the subject of freshwater emerging pollutants and shows the interrelationships between the various professions that deal with water quality issues. Further, each chapter of the book deals with the various scientific and management aspects of the subject and its interrelation.

Key Features

- Includes case studies and practical examples in each chapter
- Presents a much-needed interdisciplinary approach, representing the overlap between water chemistry and emerging freshwater pollutants
- Provides a thorough introduction to tropical freshwater emerging pollutants that typically occur in these systems

About the Editors

Dr. Tatenda Dalu is Lecturer in the School of Biology and Environmental Sciences at the University of Mpumalanga, South Africa, and Research Associate at the South African Institute for Aquatic Biodiversity.

Dr. Nikita T. Tavengwa is Senior Lecturer in the Department of Chemistry at the University of Venda, South Africa.

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