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REVIEW

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Assessing chemical pollution with biomonitoring approaches in streams and rivers: a critical review

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Abstract

Many rivers and streams are affected by chemical pollution, yet current chemical monitoring methods are limited for technical and economic reasons. Biomonitoring has been increasingly used as a means of assessing the impacts of chemical pollution and indirectly monitoring river contamination. To provide an overview of the wide variety of biomonitoring approaches, we conducted a systematic review of the literature investigating the relationship between chemical pollution and biological responses. We distinguished five main approaches for the biomonitoring of chemical pollution: (i) monitoring of native communities; (ii) laboratory bioassays; (iii) in situ bioassays; (iv) mesocosms; and (v) monitoring of wild populations. Although each of them covers a wide range of methods and endpoints, we have highlighted their main advantages and limitations. Because native communities are exposed to a wide range of stressors, isolating the effects of chemical pollution alone is often limited. Most of the existing community indices cannot depict the full extent of the impact of pollutants on communities, but rather provide information on either general degradation of water and/or sediment quality. Effect-based methods (EBMs), including ecotoxicological bioassays and biomarkers, can better isolate the effects of pollution, and, to some extent, of specific types of pollutants. The experimental design of EBMs must be adapted to the research question and the context of the study, so that the test organisms, exposure scenarios and endpoints accurately reflect the contamination. In this context, a comparison with theoretically non-stressful situations with either a dilution series of the exposure solution in the laboratory or a comparison of laboratory and field treatment is relevant. The main difficulties encountered in the approaches investigated in the present review are the comparability of sampling strategies, non-linear concentration–response relationships, extrapolation from laboratory to field exposure, the highly variable sensitivity of organisms and the geographical specificities. Overall, a combination of different EBMs can integrate the effects of exposure to specific contaminants at both spatial and temporal scales while accounting for confounding factors. The establishment of thresholds and guidelines would facilitate the integration of EBMs into regular monitoring programmes. This in turn will greatly facilitate the assessment of chemical impairment.

Keywords Biomonitoring, Chemical pollution, Bioassays, Effect-based methods, Community monitoring, Water framework directive, Screening tools

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Background

Ecosystems worldwide face a complex threat from human activities. Changes in land use, exploitation, climate change, pollution, and the presence of invasive alien species are considered to be the main reasons for the massive decline in biodiversity [1–3]. Particularly wetlands are affected, as 87% of these habitats worldwide have been destroyed by human activities since 1700 [4]. The intactness of rivers and floodplains is crucial for survival of aquatic and terrestrial organisms associated with these ecosystems. Relative to their surface area, they harbour remarkably rich biota that contribute to key ecological processes [5, 6]. In addition, they provide ecosystem services essential to functioning of human societies, such as clean water for drinking, agriculture, and industry. Floodplains act as natural buffers, reducing flood risks and safeguarding communities [7, 8]. Biodiversity-rich rivers and floodplains support fisheries, recreation, and tourism, enhancing local economies [9]. Preserving their integrity ensures a sustainable balance between human needs and environmental health. It also maintains resilience against climate-related challenges [10]. Thus, effective management and conservation efforts should address stressors holistically to halt anthropogenic-driven biodiversity loss and ensure the health of these vital ecosystems.

In practice, this holistic view of river ecosystems is not currently widespread. Rather, an attempt is made to identify the most obvious stressors and mitigate them first. In Europe, the Water Framework Directive (WFD) aims to achieve a good ecological and chemical status of water bodies of all member states [11]. In this context, many measures have been implemented over the last few years to reach a good ecological status focused on improving the morphological situation [12, 13]. However, morphological restoration alone does not necessarily lead to an improvement in the ecological condition of aquatic biota [14, 15]. This indicates the relevance of other stressors, such as water and sediment quality, which may impede the potential positive effects of such a restoration [16, 17]. Excessive discharge of nutrients and chemical pollutants, such as pesticides or pharmaceuticals play a dominant role in the impairment of lotic systems [18] on large scales [19]. Yet, the monitoring programs are not able to realistically depict this burden, partly because of the limitations of the current monitoring and assessment methods [20, 21]. Monitoring of contaminants and their effects on lotic biota is challenging because of two fundamental aspects: (i) restriction of quantitative measurement in regular monitoring programs; and (ii) their interaction in a multiple-stressor context.

The measurement of compound concentrations is limited by the overwhelming number of compounds, the dynamic of their exposure in the ecosystem, and the methodology employed to conduct the chemical analysis. Indeed, if more than 350,000 chemicals and mixtures had been registered for commercial use worldwide as of 2020 [22], the cost and manpower associated with their analysis lead to selecting only a restricted number in regular monitoring programs, such as monitoring in frame of the WFD. Until now, only 45 substances and substance groups were included in their list of priority substances [23], and 68 in an updated list within the new Commission proposal for the revision of the WFD [24], which are required to be analysed in grab samples at least once per year [25]. Environmental quality standards (EQS) based on ecotoxicological effect data to determine chemical status and potential effects on aquatic organisms are established only for these substances along with a handful of other parameters. However, even the analysis of a compelling list does not guarantee the detection of the main compounds a system is exposed to. Overall concentrations can be underestimated, because chemicals are unevenly bound across the different compartments of the ecosystem depending on their individual properties and external environmental conditions [26–28]. Moreover, diffuse and point sources of entry vary through time, with periodical contamination that grab samples might not record [29, 30]. As a result, most monitoring programs fail to provide a comprehensive quantitative assessment due to the small number of contaminants measured and the low frequency of sampling [31, 32]. Finally, technical restrictions can also undermine the quantification of specific substances; for example, short-lived compounds gradually degrade during sample storage [33]. In addition, analytical limits of detections are partially higher than the EQS levels (e.g., for the steroidal estrogens, 17β-estradiol and 17α-ethinyl estradiol [34]) or acceptable concentrations of the most toxic chemicals, and thus fail to detect substances already impacting a system [20].

Chemical substances not only interact with other stressors, but also among themselves. They rarely occur alone, and most come in complex mixtures fluctuating through time [35]. Compounds present below their individual effect levels might contribute to a mixture toxicity (additive effects), and the cocktail effect could even greatly enhance their toxicity (synergistic effects) [36]. However, routine monitoring programs and a large number of studies still focus on a limited number of compounds analysed due to limitations previously cited and do not acknowledge other stressors. Detrimental effects are notably increased in synergy with other stressors, such as water scarcity [37], remobilization of historical pollutants during flood events [38], or increase in temperature [39, 40]. Therefore, the extensive assessment of chemical exposure needs to be accompanied by the evaluation of the impacts of flow modification and habitat alteration changes.

The last few decades have seen the emergence and development of biomonitoring methods as an indirect means to monitor pollution. Here, we define pollution as the artificial introduction of compounds in a system, leading to an alteration of its functioning. Biological quality components, such as algae, macrophytes, macroinvertebrates, and fish, as defined by the WFD, also provide information about the deficits in the streams and rivers. The responses of these organism groups also largely depend on the ecological levels at which they are studied. Higher levels, such as communities, have a slower reaction to a sudden perturbation than individual organisms or the sub-organismic level [41]. By monitoring these components at different levels of biological organisation, scientists can theoretically pinpoint stressor sources and intensity. This aspect also makes valuable tools for practitioners and policy makers. For example, the Per- and polyfluoroalkyl substances (PFAS) were pointed out as substances of possible concern through bioassays that demonstrated the PFAS' effects on the biota, and thus on the aquatic ecosystems health [42, 43]. This supported the proposal of the European Commission to include 24 PFAS in the list of priority substances [44, 45]. Yet, if biomonitoring methods are integrated into regulatory frameworks of EU member states, it is not systematically the case of biological assessments specific to toxic pollution [46].

Against this background, the relevance of having an overview of the approaches currently used to assess the impact of chemical pollution on aquatic communities becomes evident. The aim of this study is to (i) overview biomonitoring approaches commonly applied to assess chemical contamination effects in freshwater communities; (ii) compare their weaknesses and strengths in light of the research question's context; and (iii) discuss the integration of further approaches in legislation to better fulfil the demands of the WFD, namely, the achievement of good ecological and chemical status. To this end, we conducted a systematic review of existing literature focusing on river and stream pollution. We have identified different types of biomonitoring approaches and highlighted the circumstances in which their use can successfully evaluate chemical contamination. Through this review, we will describe existing gaps and give indications for both future research directions and routine monitoring implementation.

Methods

The literature was selected through a systematic approach to ensure impartiality and reproducibility. We aimed to overview the most commonly applied methods with compelling chemical assessment without prior opinion or knowledge.

Determination of initial pool of publications

Information on biomonitoring approaches to assess the water contamination of streams and rivers as published in national and international peer-reviewed literature was assembled from Web of Science using predefined sets of search terms. Only articles with a core text written in English were considered. We chose several sets instead of a single one to ensure that a diversity of approaches would be included in the articles found. The sets included (i) a common core of search terms related to lotic system and chemical pollution; (ii) one out of four biological quality components (i.e., macrophyte, diatom, benthic invertebrate, and fish) and; (iii) one out of three ecological organisation levels (i.e., community, population, and species) (Fig. 1). Several choices of ecological levels were selected to ensure a wider diversity of biomonitoring approaches. We focused on the four biological quality components defined by the WFD to ensure a sufficient number of studies. We obtained 12 different sets of search terms, each being a specific combination of the common core, one quality component and one ecological level. All the 12 combinations were searched across keywords, abstracts and titles of research articles published between 1980 and February 2023. The result of this first step was 6456 articles (Fig. 2).

Selection process

Once the initial pool of literature was obtained, in a second step, 373 articles were excluded, because they mentioned words, such as "birds", "human health", "fish consumption" in their title and/or keywords, as it was assumed that they would focus on aspects not relevant to our research question.

The extent to which the papers selected so far addressed chemical pollution in addition to the above-defined search terms was examined according to the method developed by Grames et al. [47]. The littersearch package in R (R core version 4.2.1) identified the most common combinations of two to three words which occurred within the titles, keywords, and abstracts of at least 0.5% of articles. This threshold was chosen through preliminary analysis as a trade-off between an over abundance of terms unrelated to chemical pollution and an outcome with too few articles. Only the resulting terms related to pollution types were considered and later assigned to categories (Table 1). These consisted in either a source of pollution (e.g., mining activities and wastewater) or a group of chemical parameters (e.g., nutrient, pesticide, other organic pollution, and heavy metal). One last category was created for terms indicating an interest in

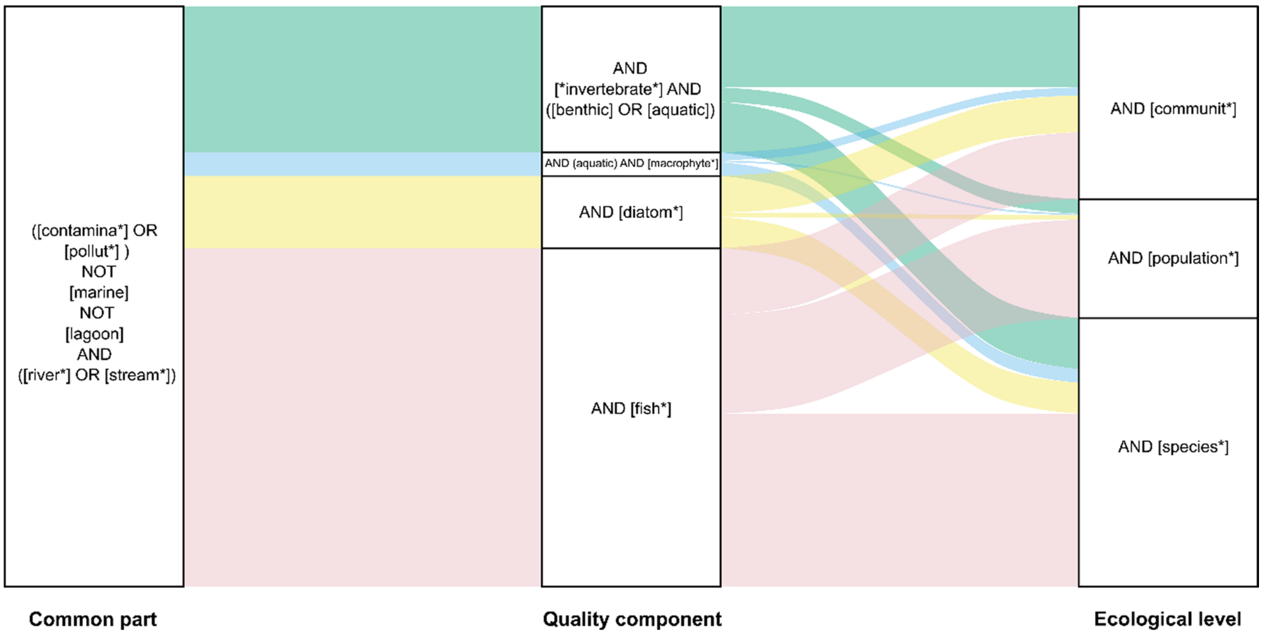

Fig. 1 Combination of search words used on Web of Science to obtain the initial literature. The thickness of the lines is proportional to the share of articles found with the set search word combinations. Green = benthic macroinvertebrates, blue = aquatic macrophytes, yellow = diatoms and pink = fishes

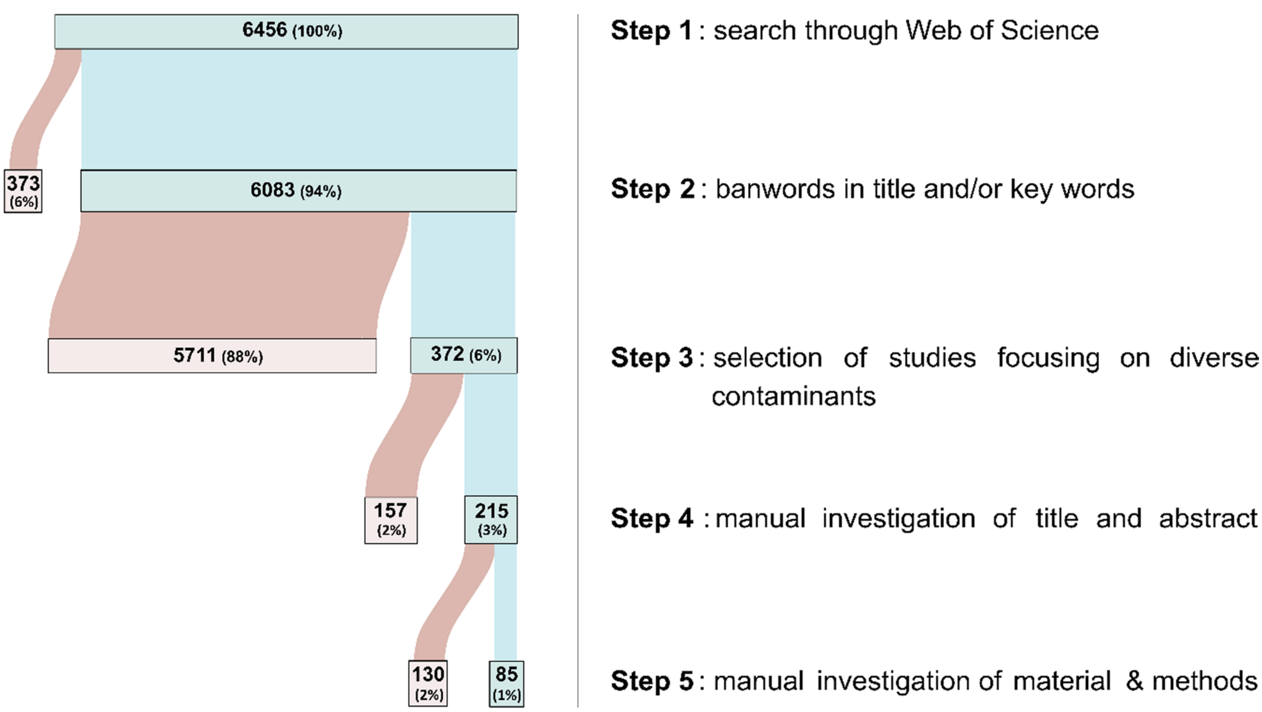

Fig. 2 Schematic overview of the step-by-step data selection process with the number of articles excluded (red) and kept (blue)

pollution though unspecific (e.g., "contaminant concentration"). A few words were manually incorporated into the list when considered to be important by the authors (e.g., "pharmaceutical" and "pesticides"). For each study, we then determined the number of terms cited at least once in either the title, abstract or keywords. Since our

Table 1 Categories attributed to combinations of two to three words which occurred within the titles, keywords and abstracts of at least 0.5% articles

Pollution types	Categories	Example of words combination
Group of chemical parameters	Organic compound	"Persistent organic pollutants"
	Nutrients	"Nutrient enrichment"
	Pesticides	"Organochlorine pesticides"
	Pharmaceuticals	"Pharmaceutics"
	General physico-chemistry	"Electrical conductivity"
Pollution source	Wastewater	"Municipal wastewater"
	Mining industry	"Acid mine drainage"
Other	Unspecific terms related to pollution	"Contaminant concentrations"

goal was to prioritise multiple-stressor studies focusing on different types of pollution, we selected articles containing terms from three or more different categories, resulting in a list of 372 articles.

Next, titles and abstracts were manually investigated to ensure whether the studies were actually fitting our purpose. We excluded several studies from our selection either, because they were measuring concentration of pollutants in fish tissues only, because the sampling site was not in a freshwater lotic system or because the article studied a low number of chemical substances (e.g., only basic physico-chemical parameters and a few nutrients). We afterwards enumerated and identified chemical compounds in the material and methods sections of the remaining 215 articles. To once more ensure that the articles selected would investigate a diverse set of chemicals, we selected articles with either more than two groups of chemical pollution type, or a total number of compounds superior to 20. We distinguished nine groups that consisted of basic physico-chemical parameters (e.g., water temperature and conductivity), nutrients, major ions, pesticides and biocides, metals and metalloids, industrial chemicals (e.g., fire retardant and plasticizers), pharmaceuticals (e.g., drugs, antiseptics, hormones, and narcotics), home and personal care products (e.g., fragrances and hygiene products) and miscellaneous (e.g., food additives and sweetener). Studies that were descriptive and did not effectively link the biological and chemical components through either their study design or statistical analysis were not considered in the further process. This whole process resulted in a final amount of 85 papers (Table S1). All of them address the impact of chemical exposure on at least one of the four biological quality components mentioned above.

Data extraction

We extracted the main information of the resulting publications and organized it in a database on Microsoft Excel. This document lists the title, authors, publication year, journal and the set of search terms used to find each article. During the reviewing process, we confirmed whether the information suggested by the set of search terms was correct or not and corrected the quality component(s) investigated and the level of ecological organisation if necessary. In addition, we extracted the exact biological organisation level, target taxa, the groups of contaminants (as previously defined in Sect. "Selection process"), the exact name of compounds, the compartment in which the compounds were measured (i.e., water, sediment, and tissues) and the statistical analysis conducted. Further categories were created according to the biological organisation and exposure scenarios. We then listed all the endpoints measured in each of the articles.

Results

The number of studies that consider exposure to multiple types of chemicals as stressors to aquatic species and communities is not high; we found only 85 papers (1.32% of the 6456 papers initially selected) that met our criteria. A non neglectable number of potential studies had to be dismissed, because they did not address both chemical and biological aspects together, but rather focused exclusively on one. Basic physico-chemical parameters (e.g., water temperature, turbidity, and dissolved oxygen) and nutrients were the most studied stressors (Fig. 3). Such parameters were measured in more than 70% of the articles selected, followed by metals and pesticides, occurring, respectively, in 64% and 61% of the papers. In comparison, household products and components indicators of waste water were under-represented.

The biological quality components were unequally studied within our selection (Fig. 4). Studies on benthic macroinvertebrates were preponderant, followed by fishes and diatoms. Macrophytes were severely neglected, only two papers conducted part of their study on them. About a quarter of our selection focused solely on a

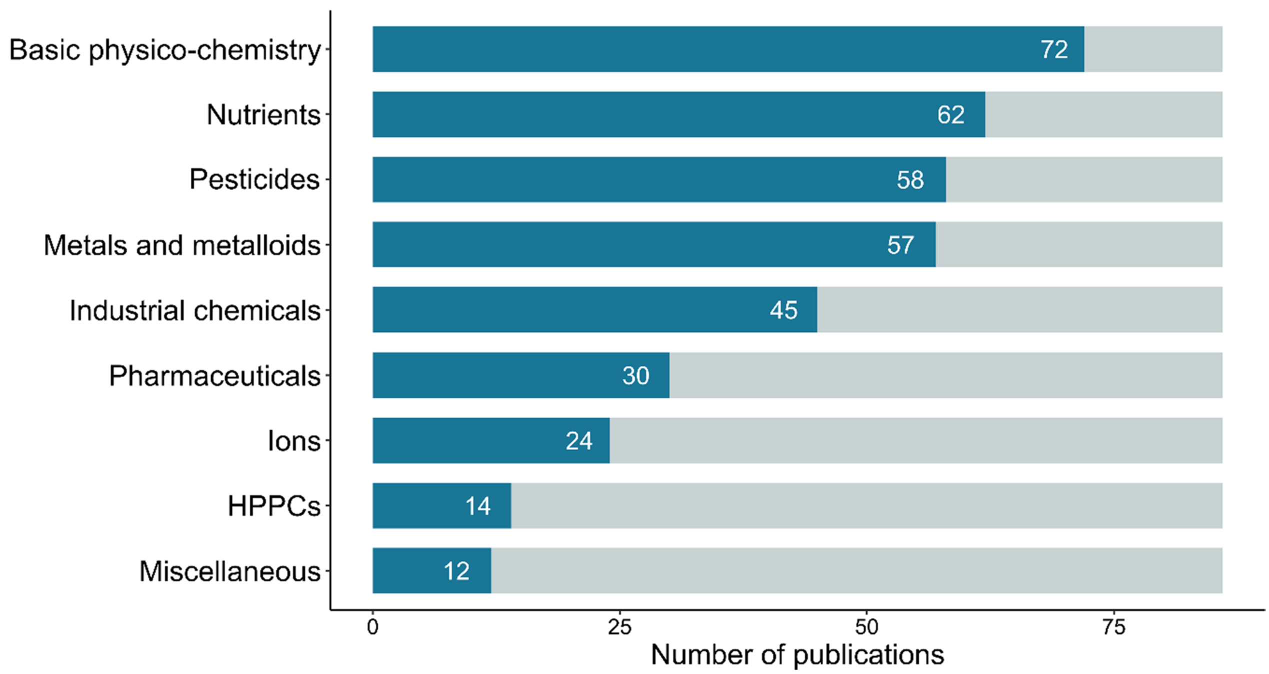

Fig. 3 Number of publications in which at least one compound or parameter of the different type of chemical pollution occurred. HPPCs, home and personal care products

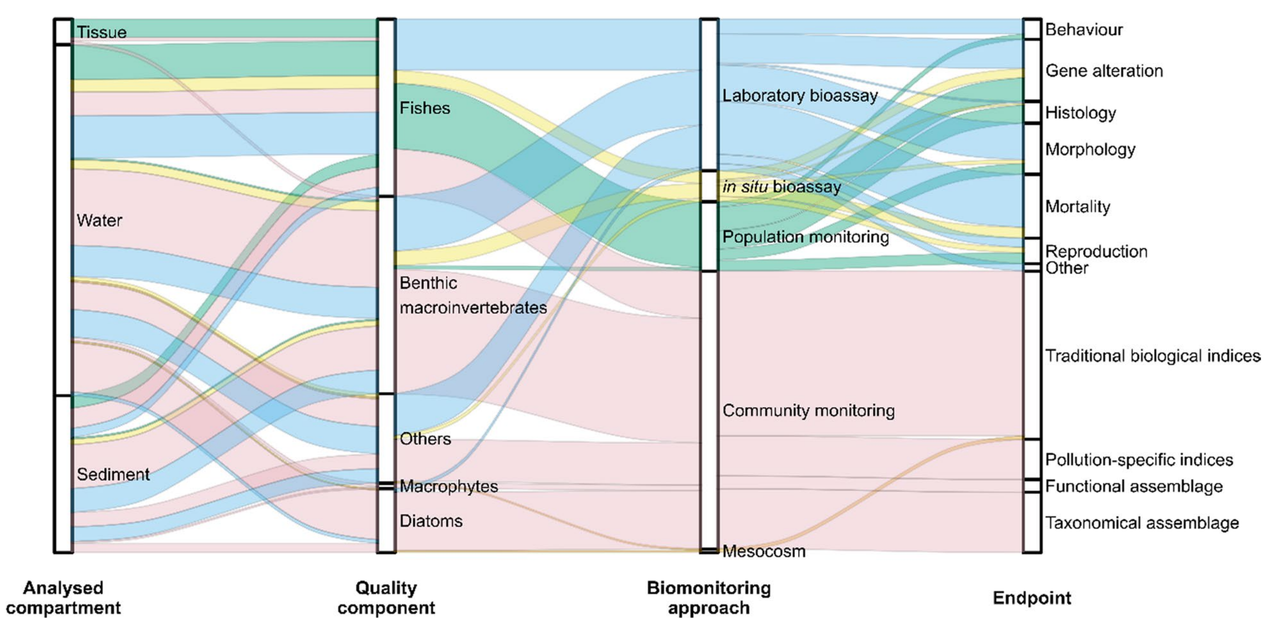

Fig. 4 Approaches and methods employed in the 85 articles selected. The thickness of the lines is proportional to the frequency of the approaches' combination. The colours indicate the different biomonitoring approaches: blue = laboratory bioassay, yellow = in situ bioassay, green = population monitoring, pink = community monitoring, and orange = mesocosm

single quality component without considering either taxa belonging to another component or other unqualified taxa. Indeed, a large proportion of our selection included taxa not defined as a quality component by the WFD, such as phytoplankton other than diatoms, zooplankton (e.g., *Daphnia magna*), or bacteria (e.g., *Vibrio fischeri*).

We can distinguish five main types of approaches: (i) the monitoring of native communities; (ii) laboratory bioassays, using organisms exposed to native water or sediment samples in controlled laboratory conditions; (iii) in situ bioassays consisting of field exposure of individuals either artificially bred or originating from other locations; (iv) mesocosm experiments whether they *in* or ex situ; and (v) the monitoring of wild populations, in other words the monitoring of physical condition/characteristic of native individuals from populations across the studied rivers systems (Fig. 4). Ecological and biological levels of organisation studied in relation to pollution were diverse, ranging from the community, with the evaluation of diversity indices or the study of community assemblages, to the organism, down to the sub-organism level, including mechanism-specific approaches, such as gene alteration in exposed individuals. The large majority of the articles conducted a single biomonitoring approach, with only 19 articles that combined two approaches and only one that considered three approaches at once.

	Single approach	Combination of two approaches	Combination of three approaches
Number of articles	66	18	1
Frequency (%)	77.65%	21.18%	1.18%

Community monitoring was the most commonly applied approach by nearly three quarters of the articles selected. However, they were not necessarily the sole focus, as community monitoring was occasionally combined with either bioassay experiments or, more rarely, with local population monitoring. We found one study monitoring communities in a mesocosm experiment and not through a field campaign. Either way, a wide range of community metrics have been employed, from common diversity indices to metrics designed to assess the sensitivity of a system to a specific type of pollutant. While less common, approximately 50% of the articles using the community approach conducted multivariate analysis of both taxonomical and functional assemblages, accompanied by the calculation of at least one community metric.

Discussion

The aim of this study was to overview biomonitoring approaches with regard to biological quality components and ecological levels of organisation to assess chemical contamination in streams and rivers. In addition, the study intended to show which of the approaches used are best suited to indicate chemical exposure and differentiate it from other types of stressors.

The majority of studies that have examined the effects of chemical contamination have done so at the level of species communities through field investigation. Bioassay experiments were found to be less commonly conducted, yet they displayed a higher diversity of endpoints.

Biomonitoring of communities

Community sampling is widely implemented in monitoring programs to assess the ecological health of lotic systems [48], whereas other approaches cited here are rarely part of routine monitoring programs. More than 400 biological assessment systems were developed in recent years for the implementation of the WFD in Europe [46]. However, there is a lack of assessment systems that are sensitive enough to distinguish between specific stressors, such as hydromorphological changes and toxic contamination.

Over our selection, already described and conventional trends have emerged as for the relation between the quality components and stressors. Benthic macroinvertebrate communities have been found to be more strongly associated with pollution than diatoms and fish communities [49]. Diatoms exacerbate the effects of nutrients and heavy metals compared to other quality components [49, 50], resulting, inter alia, in the mitigation of other contamination types. Altogether, fish community assemblages tend to display a lower response to water or sediment quality stressors [49, 51] and seem more associated with physical stressors [52]. It should be noted that these generalities should nonetheless not be taken as blank statements that the biological quality components are clear indicators for certain stressors. The unambiguous indication of a single stressor only occurs in systems in which this stressor is dominant and has a limiting effect on its own. However, this is not the case in most water bodies. Moreover, the effects of a single stressor can be underestimated if it is considered in isolation without estimating combined effects, as stressors influences can overlap and interact strongly [53, 54].

When analysing communities, seasonal variation should also be taken into account, especially for macroinvertebrates [55]. For instance, species that emerge early in the year may be under-represented or absent in benthic invertebrate samples collected during summer or autumn. This absence does not necessarily reflect the species' absence from the stream or terrestrial ecosystem as adults but is likely due to the early developmental stages being insufficiently captured by the sampling methods. Because of this phenomenon, samples from different time-periods within a year should be compared with care, if not considered entirely separately.

Traditional biological and ecological metrics

Rivers can be assessed by calculating so-called traditional biological and ecological metrics. These indices were not developed in relation to a specific type of pressure or stressor and are used in a wider context. They can provide information about the system characteristics and/or overall health disregarding the responsible pressure. They are, of course, more or less linked to certain types of stressors, but they do not aim to focus solely on them and to identify them. We include in this category, for example, the taxonomic richness, total abundance, or several diversity metrics, such as the Shannon index or the Pielou index.

Among common indices are the abundance, richness and/or proportion of the Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa. In this review's selection, metrics related to the EPT were calculated in 17 articles, representing more than a third of the articles studying benthic macroinvertebrates communities. If EPT are such common indicators, it is because they encompass numerous taxa sensitive to pollution [56]. This broad panel of species within the EPT creates a discordance; first, a few species are comparatively more tolerant to contamination than others, such as individual species within the family Hydropsychidae, leading to adjustments in the metrics calculation with the removal of such taxa to make the index more sensitive [49]. Second, each of the taxa has a specific response depending on the pollution type [57], meaning that the EPT metrics as a whole cannot, by definition, be indicative of a specific pollution type but rather of general pollution trends. In addition, EPT taxa have been shown to be sensitive not only to oxygen depletion which is often co-occurring with organic contamination [58], but also to other confounding stressors [18]. This underlines a prominent characteristic of traditional indices: they are reacting to environmental pressure in general and are not specific to contamination per se. The detection of contamination effects can thus be mitigated by other stressors acting in concert [52, 59, 60], explaining why diversity metrics do not necessarily concord with water and/or sediment quality [61].

As for assemblages, traditional indices require a strong gradient of pressure to anchor a comparison point. However, even with a strong gradient, the reliability of such metrics is questioned by the numerous contradicting results. Although some studies found significant relations with contamination [62, 63], many found little to no direct effects of pollutants on traditional metrics [52, 64, 65] even when other approaches detected effects of contamination [51, 66]. This can partly be explained by the nature of many of these indices; if the commonly calculated alpha-diversity (e.g., number of taxa, biomass, and abundance) describes the community as a whole, it overlooks the immigration and extinction of species that underlie the dynamics of a community [67]. Compensatory processes can be investigated with indices linked to beta diversity, as they account for the loss and gain of new species [68, 69]. Nevertheless, these indices give all species equal weight in their calculation, disregarding their sensitivity and tolerance to certain stressors. Consequently, if beta diversity indices have a more refined response to changing chemical pressure, they are likely to also react to other stressors.

Still, it is possible to identify general trends using several indices in parallel at a spatial scale large enough to include different pollution types and intensities of pollution, but not so large as to create significant taxonomic disparity between sites [54]. To further improve the accuracy of multimetric indices, their calculation should be river type-specific or at the very least to the eco-region [70]. Thus, traditional biological and ecological metrics are limited tools. As these indices were not created to be specifically sensitive to chemical contamination, they cannot isolate the effect of pollution alone and may respond to multiple stressors. However, as mentioned above, with a sufficient number of indices the identification of general trends is possible.

Community assemblages

The communities' taxonomical assemblages have been frequently investigated with various ordination techniques in the collected studies. Overall, they provide general trends in a multiple stressors' context. They highlight sensitive and tolerant taxa in relation to groups of stressors [56, 71–73] and make it possible to point out potential confounding factors, such as biotic and abiotic stressors, that may influence the effects of contaminants [53, 74, 75].

To better isolate and assess the effects of contamination, this approach must be accompanied by a comprehensive chemical screening, including both chemical and biological samples across a contamination gradient. If not, unraveling patterns within communities and identifying tolerant or sensitive taxa requires extensive pre-existing knowledge. The sampling strategy should be adapted to the type of pollution being investigated; for example, a study of the effects of point source pollutants may only require sampling communities up- and downstream of sources (e.g., [76, 77]), whereas the effects of diffuse or multiple source pollutants may require multiple sampling sites along a large pollution gradient, for example, along a longitudinal gradient (e.g., [53]), across several rivers (e.g., [56]), or both (e.g., [78]). Without such a gradient, species turnover due to toxic pressure can easily be overlooked, and since thresholds for assemblages cannot possibly be calculated, we end up with a mash of information with no conclusions to be drawn. Accordingly, a sufficient number of sampling sites should be considered to unravel the communities' spatial pattern associated with pollution [78]. Conversely, a study must be carried out on a relatively small geographical scale and on similar river types to avoid taxonomical disparity due to natural processes [79]. This constraint can be compensated by plotting taxa at a higher taxonomic level, subsequently reducing the influence of rare species that might exacerbate the assemblages' dissimilarity [80].

Investigation of community assemblages is also limited by the frequency and timing of the sampling strategy. Indeed, if exposure to point-source pollution is relatively constant in time, exposure to pollutants from diffuse sources frequently occurs as events and not as trends, as stated in the introduction. Such pulses are often not recorded in regular monitoring within the WFD due to inadequate monitoring frequency and type, resulting in an underestimation of the effects of pollutants on species communities [31]. Communities can bear the marks of long-term exposure to contaminants, but they can also record events on a lower temporal resolution, e.g., if they are affected by a strong peak event [32]. Because of the communities' resilience, effects of short-term peak events might be observable only during a restricted time-frame before the recolonization and regeneration of the system. Only frequent or extreme peak pollution events or chronic exposure that prevent recovery of the community might lead to longer lasting consequences observable in a routine sample over time. To detect these short-term toxic pollution events, monitoring campaigns should include event-based samples (e.g., after a rain event), which is evidently not the case within the WFD. Adjustments of temporal resolution of routine monitoring are recommended to capture both chronic and episodic pollution events. In this regard, sampling strategies would benefit from the inclusion of sampling periods relevant to expected peak exposure and higher sampling frequency to make up inter alia for seasonal variations. Not accounting for this temporal limit hinders the ability of these correlative analyses and might underestimate the relevance of chemical pollution relative to other stressors.

An alternative to the taxonomic approach is the study of functional assemblages; this involves defining organisms by their biological characteristics (i.e., traits) rather than by their taxonomy, therefore, considering a community as an assemblage of traits. Similar to the taxonomic approach, for reasons cited above, a large gradient of concentrations is necessary to better link functional responses to diffuse pollution [53, 81]. However, the modalities of biological traits do not change according to the geographical region considered, and while their frequency might be affected by large spatial case processes [82], they partially overcome geographical limitations [83, 84]. Functional assemblages are also less variable through seasons and ensure a higher intra- and inter-annual stability [85]. The relatively small number of modalities (i.e., traits) has allowed to successfully point out traits associated with the tolerance or sensitivity to pollution [81, 86, 87], which in addition give insights on the mechanisms underlying the changes in the community. Although these traits seem to be more indicative of general contamination rather than of toxicant-specific effects [53], they have been used to help develop biological indices relative to the said contaminant groups [88, 89]. It should be noted that certain traits exhibit a high sensitivity, reacting within a short range of toxic pressure; as such, these traits are unlikely to manifest subtle changes in pollution that extend beyond this narrow range [90].

Biological indices specific to pollution sensitivity

In addition to the traditional indices, the past few years have seen the development of indices that are specifically designed to evaluate the effects of pollution on communities and that respond primarily to pollution. Such metrics are expected to have a clear link to chemical contamination and should reflect it accordingly. In our selection, the majority of the indices relative to pollution were not specific to a single type of contamination, but rather indicators for more general water quality degradation. Such is the case, for example, of the Specific Polluosensitivity Index (SPI) (e.g., [91]) or the Eutrophication and/or pollution index-diatom (EPI-D) (e.g., [70]).

If pollution sensitive indices have repeatedly shown to be associated with stream and river contamination [92], even more so than traditional metrics [66, 93] and punctually more than bioassays [94], this finding is not systematic [91, 95]. This can be a result of several limitations. Just like for assemblages, their efficiency can be hindered by the geographical context of a study; indices that are developed or calibrated for certain geographical regions may be less responsive when applied elsewhere [95] because of the interference of natural factors [96]. Moreover, indicators of general water or sediment degradation are regularly used to assess a specific type of chemicals, which they are unsuited for [91] as they respond to multiple contaminations and environmental factors [97]. This in turn signifies that if only a handful of indices are to be used, they need to be coherent with the studies context otherwise the research question may not be answered.

In this context, the SPEcies At Risk (SPEAR) approach [88] provides pertinent tools. This approach is based on a division of the local benthic macroinvertebrate community into sensitive and non-sensitive species. The subdivision is based on the species-specific characteristics with regard to their physiological sensitivity to insecticides, their life cycle or their behaviour. The SPEAR index is calculated from the abundance of sensitive species, as the proportion of sensitive species (species at risk) decreases with high levels of pollution in a watercourse. This index was first developed for pesticides, and has since been derived for various contaminants, such as the ecological assessment of increased salinity [98], organic pollutants [89], bitumen-derived contaminants [93] and even for other type of stressors, such as habitat degradation [99]. It can also be applied for a compartment-specific quality assessment by developing indices based on taxa that are more closely linked to a compartment, e.g., sediment [100]. While based on the same taxonomic data as traditional biological and ecological indices for macroinvertebrates, the SPEAR approach defines ecological classes that retain significant explanatory power at relatively high taxonomic resolution. It not only provides a better balance between effort and results, but is also aligned with the taxonomic resolution set in the monitoring routines of different countries [101, 102].

The indices derived from the SPEAR approach have certain restrictions. First of all, their establishment is based on a considerable amount of data, while information about sensitivity and tolerance characteristics of species is still relatively scarce [88]. As a result, current calculations are based only upon already available resources. In addition, their efficiency might still be mitigated by confounding factors possibly interacting with the pollution effects [97, 103, 104], and, most of all, prior exposure to pollutants may induce better tolerance [68, 105, 106] through the progressive selection of the most resistant individuals.

This demonstrates that a single index alone is often insufficient and needs to be combined with other biomonitoring methods or further screening of abiotic parameters and chemical concentrations to accurately depict the full extent of pollutants' effects on the communities while still taking into account confounding factors [66, 107–110]. The above-described indices, traditional ones as well as specific ones, and community assemblages are thus complementary [108, 111], especially since their combination does not result in supplementary sampling efforts.

Effect-based methods and population monitoring

Definition, status within the WFD, and generalities

Over the last few years, effect-based methods (EBMs), including ecotoxicological bioassays and biomarkers, have been progressively getting more attention. Unlike traditional chemical monitoring, which measures the presence and concentration of individual, known substances, EBMs detect biological responses to the chemical burden, including unknown or unmonitored compounds, thus providing a more integrative picture of potential ecological risks. To link chemicals to the ecological status, a combination of chemical and effect-based monitoring using in vitro, in vivo, and in situ EBMs is strongly recommended [112] and proposed for integration into the WFD [113]. In the European subgroup Chemical Monitoring and Emerging Pollutants (CMEP) within the Common Implementation Strategy (CIS) for the WFD, a specific task was established for the development of a technical report on EBMs. According to the mandate of the CMEP, the aim of the technical report of the European Commission was to identify potential EBMs that could be used in the context of the different monitoring programs (surveillance, operational and investigative) linking chemical and ecological status assessments, and to be used as a proposal for the revision of the WFD [114]. Based on a joint activity of the collaborative EU project SOLUTIONS and the NORMAN network on emerging pollutants, a common battery of bioassays has been suggested that covers major toxicological endpoints [115–118]. The recommended bioassay battery was detailed in a Policy brief of the SOLUTIONS project [117] and included as an Annex V of a technical proposal for Effect-based Monitoring and Assessment under the WFD by the CIS Working Group Chemicals of the European Commission [119]. It is suggested to complement in vitro assays with in vivo bioassays representing at least the aquatic triad, i.e., fish, invertebrates and algae also considered as biological quality elements for pelagic communities in the WFD. Of the mechanism-specific in vitro assays, priority should be given to endocrine disruption and mutagenicity, but this can be tailored to the specific contamination expected [120–122]. As summarized by Backhaus [24], EBMs are now included in the proposal for the legal text of the WFD. However, the Commission proposal limits EBMs to explorative studies and does not include the setting of EQS values based on EBM methods, as suggested by the CIS Report [119].

EBMs detect, identify, and measure the effect of a single compound or a mixture of compounds using the response of a living target organism (in vivo) or cells (in vitro) to its exposure. They cover a wide range of endpoints at different levels of biological organisation, from organismal to sub-organismal levels (effects on organs, cells, enzymes, molecules and the genome). In the publications selected for our review, bioassays were often conducted on individual or multiple organisms/whole populations. Endpoints included lethal parameters (e.g., mortality) as well as sub-lethal parameters (e.g., frequency of deformities, behavioural changes), but also biotic population parameters (e.g., mean body length and sex ratio). Because of this tight relationship between bioassays and population monitoring, we address them together as a whole in this section.

We distinguish five major groups among the most common endpoints within our selected literature: (i) mortality; (ii) behaviour; (iii) reproduction; (iv) morphology; (v) histology; and (vi) gene alteration (i.e., production of enzymes or proteins, transcriptional changes), which can be evaluated through biomarkers and/or in in vitro bioassays. For each group, we can identify main trends partially determined by the biological level of organisation and their respective velocity of response. The higher the level, the slower the response to pollutant exposure is [123, 124]. While higher concentrations are required to elicit acute effects, e.g., on the organismic level compared to the sub-organismic levels, long-term exposure to low concentrations of pollutants can lead to chronic effects on organisms and populations. Consequently, endpoints at higher biological levels might not record recent or subtle changes in the systems, whereas endpoints at lower levels, such as blood parameters and molecular changes, may detect short-term peak exposure [125]. Lower levels can serve as warning systems before significant damages occur in the most ecologically relevant levels (i.e., decline in population abundance, changes in community assemblages). As each of the major groups of endpoints encompass several ecological levels at once, we can observe strong disparities in terms of sensitivity within a single group. This is the case for morphological alterations: whereas biometric measurements (e.g., length and weight) have a weak association to contamination exposure [126] and occur at higher levels of chemical pollution [127], developmental anomalies (e.g., mouthpart deformities) have a stronger association to contamination [127, 128] and have even been found to detect toxicity when contaminant concentrations are below regulatory thresholds [129]. It should be noted that the inertia of response also varies at lower biological levels; the delay is determined, inter alia, by the contaminated compartment within the river system and the tissue concerned. Responses will be induced faster in tissues directly in contact with contaminated compartments (e.g., gills) than in tissues exposed to contamination only after bioaccumulation of the substances (e.g., muscles) [125].

This signifies that while general trends may exist, each individual endpoint within these groups exhibits its specific relationships with substances, substance groups and other environmental parameters. The same contaminant may greatly affect a restricted set of endpoints, while others are irresponsive or respond to a lesser extent [129, 130]. This phenomenon allows to distinguish narrow indicative (specific) endpoints to some that reveal a toxicity in a more general sense (non-specific). For example, while it has been proven difficult to find a clear link between histopathological endpoints and specific types of compounds [131], the tight relation of behaviour with neuronal and endocrine systems makes many behavioural change indicators of compounds that result in neurodevelopmental deficit or hormonal disbalance [132]. Likewise, biochemical biomarker responses and responses measured in in vitro bioassays (e.g., reporter gene assays for the measurement of estrogenic or androgenic activity or the activation of pollutant metabolism) are more tightly connected to chemicals that have similar modes of action; they grant more depth in the characterization of the effects [127, 133, 134]. Considering several endpoints known for being responsive to different components might depict the pollution accurately.

Experimental design is a crucial yet complex aspect of bioassays, as the outcome of a bioassay heavily relies on the test organism considered and the conditions under which it is exposed to contaminants. The same bioassay with different taxa may lead to disparities among the responses, some species being more sensitive than others to specific or overall chemical pressure [109, 135, 136]. Pollutants are bound to different compartments within the system, leading the organisms to take up contaminants via different primary routes. For example, species that feed on bioaccumulators of certain substances may retain a greater load of these substances than other species from the same river but with different feeding habits [137]. Similarly, acute toxicity may be detected by bioassays conducted with sediment, when assays with surface water do not detect any toxic effects, simply because the pollutants present in the system are bound to this compartment [127]. An organism's sensitivity to specific components also varies according to the pollutant's mode of action, meaning that the intensity of the response to similar burden may differ according to the physiological characteristics of the taxa considered [127, 138]. This emphasizes the need for prior knowledge of the site's history and type of contamination expected, so that the target taxa can be chosen according to the type of toxic pressure exerted in the study area when assays are conducted with no standard representative species [139, 140]. To minimise the risk of false negatives and to better overview the complex mixture of contaminants, more than one species can be selected and safety factors can be applied [127, 137, 140]. By combining multiple single-species toxicity tests, species sensitivity distribution (SSD) probability models can be developed, an important tool in hazard and risk assessment [141]. From the SDD, community-level thresholds are derived to evaluate toxic pressure (i.e., predicted no effect concentrations), whose robustness yet again depends on the species selected for toxicity testing and other factors related to the study design [141, 142].

It should be noted additionally that some substances, such as endocrine disruptors (e.g., estrogenic or androgenic toxicants), can affect characteristics specific to either females or males [139, 143], with distinct symptoms and more or less pronounced responses. Therefore, according to the contaminants expected, the endpoints studied, and the research question, males and females are to be considered separately.

The origin of the target organism must also be determined, i.e., whether the individuals originate from wild populations or have been artificially bred in laboratories. This aspect is closely linked to the exposure scenario, which is determined by the environment in which the organisms are exposed to the contaminants (i.e., in the laboratory or in the field), the duration, and the life stages of the organism. With these aspects in mind, we can distinguish four main categories of experimental designs for the population monitoring and bioassays: (i) laboratory bioassays; (ii) in situ assays; (iii) mesocosms assays; and (iv) wild populations' monitoring. Some of them provide a comparison mode to theoretically non-stressful situations with either a dilution gradient of the exposure solution in the laboratory, or with a comparison of laboratory and field treatment.

Whether it is for laboratory or field experiments, bioassays need to be run for a sufficient period of time depending on the type of bioassay/organism and the endpoints chosen to be able to record a response (acute or chronic). However, there is also a risk of missing a response [109, 130, 135] due to interactions between stressors, influences of confounding factors, such as nutrient availability, masking toxicity, potential non-linearity of the concentration–response relationship, and other parameters. For example, taxa bound to the water column may exhibit a delayed response in whole sediment exposure tests, simply because substances progressively spread out into the aqueous phase over time; therefore, an acute assay might not record the highest concentration of contaminants into the water, while a chronic one does [135]. The reliability and variability of bioassays can differ significantly across species and environmental contexts, and factors such as seasonal changes or episodic pollution events may further influence the outcomes of biomonitoring studies. Short-term studies can be sufficient if associated with sub-organismic in vitro-based approaches (e.g., receptor interactions), if they evaluate many different endpoints [144], and if the contamination in the environmental sample is relatively high.

Laboratory bioassays

Laboratory bioassays are conducted in a controlled environment, where the effects of single compounds, mixtures of compounds and multiple stressors on single or multiple aquatic organisms can be assessed under defined conditions. Guidelines (e.g., ISO [145], OECD [146]) and standardised protocols are available for many of the methods. They are important tools for the environmental risk assessment of pollutants and for the evaluation of environmental samples. They allow the assessment of a range of different conditions through a dilution gradient of exposure solutions to establish a concentration–response relationship. This is particularly valuable, because bioassays are, in some cases, also confronted with nonlinear responses with more U-shaped concentration–response curves [128, 147].

An advantage of these methods with respect to the assessment of mixtures of chemical compounds and other stressors is that the contribution of each stressor can be determined by targeted experiments in which the effects of individual compounds/stressors and their combinations are assessed [148]. With respect to environmental samples, this is more difficult as only the whole mixture can be tested, but with methods like effect-directed analysis an evaluation of the individual contributing fractions is still possible and eventually compounds responsible for the effects can be identified [21, 149, 150].

Another aspect to consider is that laboratory bioassays alone do not simulate natural conditions and the changes to which organisms are exposed in the field [130]. These environmental factors, such as water temperature, pH, and humic substances can influence the toxicity of contaminants [151]. Consequently, extrapolating findings from simplified laboratory conditions to field conditions comes with uncertainties [91], even for assays theoretically narrow indicative [121, 152]. Thus, for the environmental risk assessment of chemicals present in water samples and to ensure a precautionary approach, safety or uncertainty factors (also called assessment factors) are applied to the bioassay results; they account for laboratory-to-field extrapolations or for inter- and intra-species variabilities [153, 154].

EQS for individual compounds are derived from results of laboratory bioassays and/or mesocosm approaches, taking into account the corresponding assessment factors, and compared with the measured environmental concentrations in an environmental sample to derive a risk quotient indicating potential risks to aquatic organisms [154].

When directly evaluating environmental samples, laboratory bioassays can be considered as first screening tools that need further assessment as part of a refined risk assessment process. Because they provide an understanding of the type of chemical pressure exerted on a system, investigations can greatly benefit from combining several bioassays in a bioassay battery, targeting organisms of different trophic levels and specific effects tailored to the expected contaminant profile in the sample, and ideally including chemical measurements and field surveys [21, 117, 120]. Furthermore, the ecological relevance of lab-based experiments can be verified by EBMs at population level or from organisms exposed in situ [121, 152, 155] in so-called triad or weight-of-evidence approach.

An important aspect for laboratory bioassays with environmental samples is the sampling strategy of the contaminated compartment. In contrast to both wild populations monitoring and in situ measures, where organisms are continuously exposed to the compartments under investigation, samples for laboratory bioassays need to be brought to the laboratory. The collection of samples that represent the actual exposure in the environment is essential for a successful and realistic assessment of the ecotoxicological risks of environmental samples. If it does not reflect the dynamics of the pollution or a chronic exposure, the EBMs may not properly predict the reality of the pollution pressure and may underestimate it. Out of the 21 publications with laboratory bioassays selected in the present review, 14 supplied their experiments with one single water or sediment grab sample, which risks either not recording punctual peak events but merely grasping the background pollution or sampling precisely a peak alone, leading to an overestimation of the contamination. Nonetheless, several methods are now able to register episodic events and/or chronic contamination, although they require considerable resources and/or greater sampling efforts. Peak concentrations of many substances, in particular pesticides, can efficiently be captured with rain event-based samples [156], yet it might still overlook punctual direct inputs, i.e., from industry. Passive samplers or composite sampling offer solid alternatives that only requires a more frequent collection of samples [157–159]. Furthermore, sample preparation in the laboratory could affect the bioavailability of the substances. For all the sampling techniques cited above, the outcome of the biotests may differ with native samples and extracted samples. For example, porewater may under-represent the bioavailable fraction of the pollutants and depict a comparatively lower toxicity compared to whole-sediment tests [160]. The extraction method chosen also emphasizes the focus on specific types of substances (i.e., extraction of hydrophobic substances only) [161].

In situ bioassays

We define in situ bioassays here as EBMs based on experiments set up directly in the field, also called active biomonitoring. They include a variety of methodologies; while some experiments are conducted directly in the river system, others are conducted in a semi-controlled environment fed by water continuously extracted from the system, for example, using flow-through systems [162, 163] or mini-mobile environmental monitoring units [121, 164, 165]. Because the organisms are continuously exposed, these bioassays can capture not only permanent but also transient effects, and thus account for pollution dynamics on a relatively short temporal scale, limited only by the duration and timing of the experiments. This aspect allows for the integration of fluctuating environmental conditions and episodic pollution events, thereby offering a more realistic assessment of ecological impacts.

In our selected publications, this approach was relatively uncommon as it is included in only six articles. Two of them had their experiments set up on-site next to the systems [163, 164] and four conducted transplantation assays [130, 166–168]. The latter consists of transferring organisms from either the laboratory or from another site (e.g., further upstream or from another river) to the studied ecosystem. Individuals are usually caged and are exposed for a fixed period of time. Their responses are recorded at the end of the experiment or periodically throughout the experiment.

Without the combination with theoretically non-stressful conditions, e.g., evaluation of a reference site upstream of the site to be investigated, in situ bioassays need a sufficient pollutant gradient or the measurement of narrow indicative endpoints [169], similar to community and wild population monitoring (see Sections. "Biomonitoring of communities" and "Wild populations' monitoring"). However, an in situ bioassay can be conducted with fewer geographical limitations compared to these approaches. Test organisms can be artificially bred so that their natural distribution is no longer a limiting factor. Results tend to be more comparable from one experiment to another, facilitating wide-scale implementation of effect thresholds.

Transplantation assays and other in situ EBMs are to be used with great caution for biosafety reasons. The accidental release of transplanted individuals and their interaction with native populations may entail risks similar to the one observed in species reintroduction, namely, the disbalance of the local genetic pool, the spread of pathogens and the introduction of invasive species [170]. Preventing measures are to be taken so that risks are minimized. Most importantly, the material should ensure that no subject can escape from the experiment if caging is used. To preserve the integrity of local populations even in case of an outbreak, individuals that are native to the studied river can be used for transplantation (e.g., from upstream sites) rather than those from breeding facilities or distant river systems. This also once more results in geographical limitations and prevents interproject comparisons. Overall, the potential impact of transplantation tests on the local environment needs to be recognized and further investigated. International consensus needs to be reached for the best practices and standardized measures must be derived as, for example, on the type of enclosure and condition of exposure.

Although not included in the articles selected, this approach is particularly useful in the context of biological early warnings systems (BEWS). By recording responses of bioindicators, such as algae, water fleas or amphipods, BEWS monitor pollution levels in surface water, drinking water and wastewater in real time [162, 171]. Detection systems in BEWS mostly rely on behavioural reactions, some also evaluate other parameters, such as effects on algae chlorophyll fluorescence, to peak pollution inputs, from which threshold values are derived. Behavioural changes are preferred to mortality rates and other sublethal responses, because they bridge the gap between individual and population relevance, and are indicators of significant impacts of chemical contamination on a population before more severe consequences (i.e., decline in population abundance) [2, 172]. As an indicator of pollution peaks, an alarm system is triggered when detection thresholds are exceeded. With regard to surface water monitoring, BEWS have been employed for several decades, for example, to monitor the water quality of the Rhine [173]. Since their implementation in the river Rhine, BEWS have successfully detected spills of harmful substances, allowing to take early protection measures and to initiate investigation for the sources of pollution.

Mesocosms

A mesocosm study was implemented in only a single article within our selection pool [68]. This can be a result of our selection procedure, as no key words explicitly related to mesocosms were included. Despite this prerequisite, it is most likely that mesocosms are merely underrepresented compared to other approaches. Mesocosms can be implemented in or ex situ and thus have characteristics of either or both laboratory and in situ bioassay depending on the experimental design. Their particularity is that they can record biological responses at a wider range of levels of biological organisation, including at the community level.

The goal of this type of experiment is to reproduce field conditions in a controlled or semi-controlled environment, so that biological responses to individual chemicals or chemical mixtures can be monitored under several exposure scenarios while still accounting for other stressors potentially interacting with them. The multiplicity of exposure scenarios allows a concentration–effect relationship to be studied at the community level, including the assessment of tolerance development at the community scale [68]. In addition, they are also particularly useful to investigate in detail the interactions between specific physical and chemical stressors, and thus identifying which conditions may have stronger environmental impact [174, 175].

One notable limitation of mesocosms is their inability to allow for the colonization or establishment of biotic communities identical to the ones found in the wild. If the study aims to set up a mesocosm experiment with assemblage's representative of a wild ecosystem, it needs to employ an adequate biotic sampling strategy. Despite sampling efforts, biotic communities established often differ from native communities *in natura* due the impossibility to reproduce natural conditions. The acclimatisation period leads to the loss of the most sensitive species, leaving among the most ubiquitous or tolerant taxa.

Wild populations' monitoring

This approach consists in the collection of individuals from a wild population native to the system of interest, also called passive biomonitoring. Measures are taken on whole individuals in situ (e.g., biometric characteristics), on parts of the individuals with or without removing them from the system (e.g., blood samples), or on whole individuals in the laboratory for further screening (e.g., assessment of gills' deformities). In the literature reviewed here, this approach was almost exclusively conducted on fish populations, except for two publications that studied benthic macroinvertebrates, namely, *Chironomus riparius* and *Gammarus fossarum*.

When using endpoints that are not yet well-characterised or not narrowly indicative, wide gradients allow effects of one type of contamination to be better isolated from other stressors. Under field conditions, the effects of chemical pollution cannot be considered in isolation, as there may be interactions with other stressors (e.g., temperature) that may have additional effects on the toxicity of the mixture as well as on the endpoints under investigation. Even though field monitoring and experiments provide the most realistic exposure scenario, they are not only costly and time-consuming but also do not guarantee the identification of the stress-driving aspect (e.g., chemical exposure and abiotic effects) as, for example, laboratory bioassays do (4.2.4).

The core difference of this approach with the previously cited bioassays lies in its temporal aspect; wild population monitoring can provide information on the status of a population at a precise moment, whereas in situ approaches, mesocosm experiments, or laboratory bioassays evaluate organisms' responses and/or a population's dynamic during its exposure to the potentially contaminated compartment. Endpoints for population monitoring measured at the organism or lower biological levels can be the same as with laboratory bioassays, but wild population studies do not allow to measure the expected changes at the population level through a specific duration, such as mortality rate, behavioural changes, biometric changes and reproduction rate. On the other hand, this approach registers contamination on a larger temporal scale. Because the individuals collected were native to the system and constantly exposed to it through all of their life stages, they bare evidence of pollution's dynamic within months, days or hours, [125, 126]. Therefore, field monitoring investigates not only the consequences of prolonged exposure to chemical mixtures, but also long-lasting impacts of episodic contamination events. Relatively low organisation level biomarkers and behavioural changes may also record episodic peak inputs with fast response, but trace of such events might fade over time as the organism recovers from transient exposures [125, 132]. Conversely, if some taxa may record temporary exposure with permanent consequences, others can recover with sufficient time after the end of the event. This highlights once more the importance of the choice of endpoints and target taxa, and how a combination of short-term and long-term endpoints along with multi-species monitoring enables a more compelling assessment of toxic pressures.

The choice of species should be based not only on the type of contamination (see Section "Definition, status within the WFD, and generalities"), but also on the distribution and history of potential target organisms. If the environment is unfavourable for aquatic organisms due to too high pressures, populations may adopt an avoidance strategy, which is manifested by their absence from such sites [176]. If this response is indicative of already severe ecological consequences, it prevents collecting organisms and thus from population monitoring in the most constrained sites. It also limits the assessment of a potentially wider gradient within the study, with heavily polluted and unpolluted sites, to a narrower range.

As the choice of taxa depends on the natural distribution of species, the study of wild populations is highly geographically restrained, which prevents the establishment of thresholds or standards on a large scale. In addition, native species have been found to be more sensitive to chemical pollution in comparison with exotic species when the same biomarker was considered [166]. Therefore, selecting the target taxa must consider the species natural distribution, its sensitivity to pollution types, and the expected intensity of pressure.

Although studies on behaviour of fish populations in situ have been rapidly expanding over the last decades with the development of new technologies, we found only a single publication linking behaviour *in natura* with water contamination [137]. This gap between toxicology and behaviour science in wild populations may be due to the greater effort and resources required to set up such a study compared to more controlled environments, such as in BEWS. This might explain why behavioural endpoints were recorded more frequently in the selected articles that implemented laboratory experimentations [135, 144, 167, 177]. Considering that behaviour studies in laboratory conditions can often predict in field behaviour [172], laboratory studies and BEWS appear to be a less resourceful yet reliable alternative.

Perspectives

If current regulations demand only the target monitoring of individual substances, they might benefit from the addition of EBMs as part of an effect-based surveillance program [117], providing a biological context to chemical monitoring data. The case studies applied within the SOLUTIONS project demonstrated that in tandem with existing chemical monitoring, complementary in vitro and in vivo EBMs can help to identify mixtures that harm biological components [178, 178, 179]. Indeed, in combination with chemical target and non-target measurements, e.g., in the frame of an effect-directed analysis [180], EBMs are able to identify the drivers of toxicity and help prioritize river-specific pollutants, and develop solutions and toxicity reduction measures. More precisely, EBMs would first detect negative effects due to chemical pollution, narrow the spectrum of liable pollutants, and help to identify the ecological relevance of chemical monitoring. Followed by non-target screening and then, building on this, target monitoring of individual substances, practitioners could determine efficiently the pollutants impacting a water body and further monitor these with target screenings.

To facilitate the implementation of EBMs it is necessary to establish (i) clear guidelines supporting the choice of methods depending on the pressures expected or to be assessed [114, 117, 119] and (ii) effect-based thresholds defining quality classes according to the expected impact on biology [34, 181]. To this end, existing EQS and effect data can be derived into effect-based trigger (EBT) values, which are defined as values below which no adverse effects on organisms in the water (in terms of the measured effect) are to be expected [119, 182]. EBTs have already been developed for many assays, but as the robustness of EBTs is directly linked to the amount of data they are derived from, EBMs for which available data is relatively scarce are still subject to readjustments [182]. Thresholds would eventually allow to estimate the chemical pressure with a lesser need for substantial chemical analysis. Resources traditionally allocated to chemistry screening could thus be diverted for the conduction of EBMs. A current proposal for the revision of the WFD suggests the use of EBMs as screening tools, e.g., to assess the presence and total concentration of estrogenic compounds in water samples [25]; the responses in the bioassays are compared with EBT values to assess potential ecotoxicological risks in water bodies [25].

Most laboratory bioassays can be applied on broad scales, as commonly used model test organisms can be artificially bred. However, local species might be more sensitive test organisms than commonly used ones in widespread experiments, and even a link between tolerance and conservation status has been suggested [163, 166].

Several coherent and complementary endpoints, along with test organisms of different trophic levels, are to be included to provide an accurate understanding of the chemical pressure in a set system [168, 177] while minimizing risks of its underestimation [148]. The combination of multiple approaches is all the more important, because the methods chosen (field-based, in situ or laboratory evaluation) are associated with the environmental variables in different ways; therefore, comparing field and laboratory exposed individuals can identify false positive relationships between the same endpoints and contaminants [167]. This also raises an important point: the context in which bioassay subjects (i.e., target individuals or cells) are exposed can indeed greatly influence the response pattern. In other words, a lack of response does not necessarily mean that the endpoint or the biological components are not sensitive, but also that the exposure scenario might be improper, as suggested in [177]. Therefore, the experimental design needs to be carefully set up according to the research question. Compelling design may require significant resources, but it detects and quantifies toxicity more accurately. Overall, the complementarity of the endpoints and approaches with one another make them a beneficial addition to current monitoring practices. The integration of these three approaches along with emerging techniques in a multi-tiered framework would enable robust water quality assessments. This would in turn provide hints for the development of policies, with the establishment of new regulatory thresholds adapted to substances' effects.

AI-based ecotoxicological prediction models, such as quantitative structure–activity relationships and deep learning models [183, 184], offer powerful tools for risk assessment. If a manifold of studies has successfully linked measured compounds and detected effects in bioassays [185–187], strategies should combine both chemical analysis and information from EBMs to predict the substances' toxicity instead of relying merely on compound concentrations. Recent studies have demonstrated the potential of machine learning and deep neural networks to predict mixture toxicity and ecological risk across different taxa and environmental contexts [188, 189]. Despite their promise, they have yet to be exploited to evaluate the influence of toxic pressure on community structure and consequently ecosystem health. AI-based ecotoxicological models currently need to overcome limitations, including the need for large, high-quality training data sets, challenges in modelling mixture effects, and the interpretability of complex algorithms. Future research should focus on integrating big data from diverse sources, such as chemical monitoring, in situ bioassays, and ecological field data, to enhance predictive power. Another type of AI-based tools are image processing models, which enable accurate and reproducible assessment both in regards of species identification and for the assessment of toxic effects in bioassay [190]. Routine monitoring programs would benefit from their integration as they would optimize current practices in time and cost terms.

Over the last few years were developed new techniques that although employed in a single article of our systematic literature review [77], must be acknowledged and further explored, as, for example, environmental DNA (eDNA). This technique provides valuable information about the local community assemblages, a population's abundance and its genetic characteristics with non-invasive samples [191]. Tissue metabarcoding of communities is also receiving more attention, and while it is more invasive to local communities than eDNA, it provides a more accurate picture of the community structure [192]. These promising techniques offer a cost-effective add-on to traditional identification techniques and could eventually provide an alternative to facilitate the monitoring of communities in the future.

Despite not being included in our selection, there is a set of biological indicators that have hardly been used to date but have the potential to provide warning signals outside of monitoring programs. For example, fish kills, algal blooms, and mass occurrences of macrophytes and algae in water bodies are often the most easily observable indicators of extreme anthropogenic pressures on water bodies. Fish mass mortalities (i.e., die-offs, fish kills) are currently only inadequately recorded in official monitoring programmes of the WFD. As fish kills occur spontaneously and are difficult to predict, monitoring for investigative purposes can only be carried out after an extreme situation has already occurred, and it is often complex to determine the exact cause, as multiple causes are almost always possible, except in the case of accidents and disasters. In particular, the temporal (e.g., short-term events) and spatially limited occurrence of fish kills limits the possibilities of recording them as part of official monitoring. Nevertheless, the simple indicators mentioned above are easy to record through citizen science, citizen participation in water monitoring, and could support official monitoring programs of the administration. So far, however, there is a lack of corresponding internet portals that bring this information together nationally (e.g., a water protection app for cell phones and computers). A centralised, user-friendly platform or mobile application to collect and integrate citizen science observations could significantly enhance the early detection of, e.g., invasive species [193] and documentation of episodic pollution events, such as fish kills or algal blooms [194], and represents a promising direction for future development.

Conclusions

The evaluation of chemical contamination using biomonitoring approaches still receives comparatively little attention, especially when it is to be assessed in conjunction with other stressors. Most regular monitoring programs are currently based on the monitoring of chemical concentrations and native communities, but the resulting indices do not provide a detailed picture of the nature of the contaminants. They are implemented in a multiple stressors' context, thus the interactions with additional stressors may influence even pollutant-based indices. Consequently, monitoring of running water bodies under the WFD is currently too limited in terms of time and space and too unspecific to fully map pollutant effects. As a way to compensate for these limitations, future regulations should include intensification of the monitoring program, and/or suggest alternatives to the current costly solution. Laboratory bioassays with environmental samples, in situ bioassays, and population monitoring using biomarker approaches would be useful complements as they can more precisely identify the pollutants in a system and are less constrained by environmental conditions. Altogether, EBMs can isolate the effects of the chemical pressures, but their success relies on three fundamental aspects: (i) the choice of taxa and their life stages; (ii) the endpoints; and (iii) the exposure scenario. They must be coherent to the chemical pressure exerted in the studied system or to the type of contaminants studied. A combination of a diverse array of methods, including laboratory bioassays measuring specific effects and biomarkers, can integrate effects of exposure to rather specific contaminants and confounding factors on both a spatial and temporal scale. The definition of thresholds and guidelines would favor their integration in regular monitoring programs. This in turn will greatly facilitate the assessment of chemical impairment, especially because of their complementarity with other approaches, such as in the sediment quality triad [195].

To remain effective and future-oriented, monitoring frameworks must be adaptive—capable of incorporating emerging technologies and responding to newly identified contaminants and ecological threats in a timely and flexible manner. To ensure robust and future-ready water quality management, there is a pressing need for long-term, interdisciplinary research initiatives that integrate advanced chemical analysis, biological monitoring, and AI-based predictive modelling, enabling more dynamic, responsive, and ecologically relevant assessment frameworks.

Abbreviations

WFD	Water framework directive
EQS	Environmental quality standards
PFAS	Per- and polyfluoroalkyl substances
EPT	Ephemeroptera, plecoptera and trichoptera
SPI	Specific Polluosensitivity Index
EPI-D	Eutrophication and/or pollution index-diatom
SPEAR	SPEcies At Risk
EBM	Effect-based method
CMEP	Chemical monitoring and emerging pollutants
CIS	Common implementation strategy
SSD	Species sensitivity distribution
BEWS	Biological early warnings system
EBT	Effect-based trigger
eDNA	Environmental DNA

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