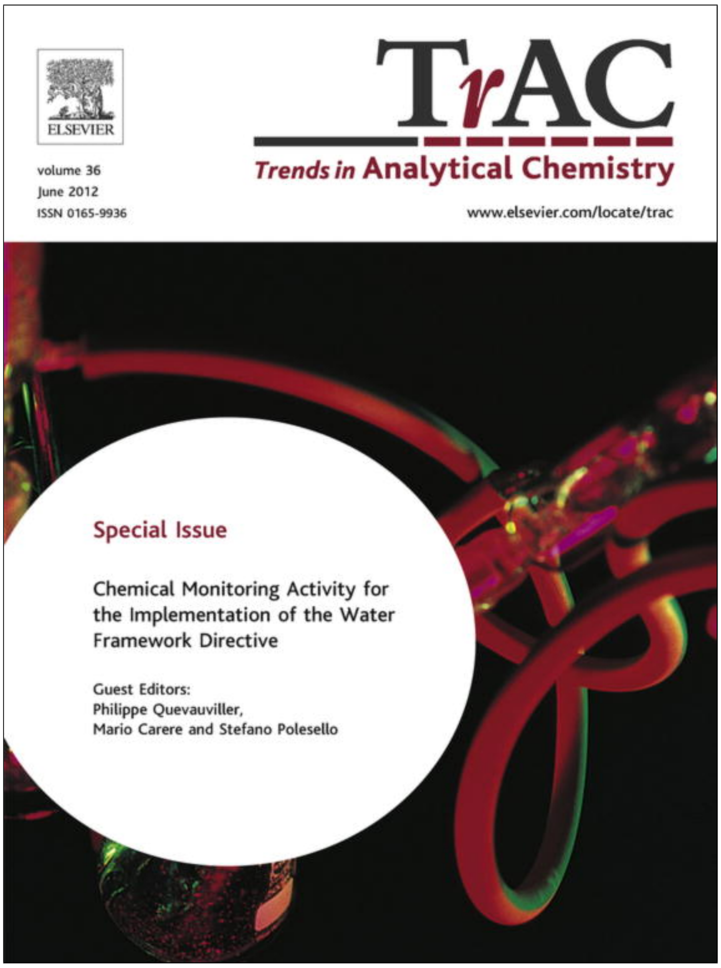
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Biomonitoring of lake sediments using benthic macroinvertebrates

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The Water Framework Directive (2000/60/EC) is an innovative piece of legislation aimed at protecting the quality of all continental and coastal waters in Europe through an ecological evaluation of the ecosystems. Since it is widely acknowledged that the greater the ecological realism the greater the difficulty of its definition, we describe the different uses of benthic organisms as a tool for assessing the quality of sediment in lakes. We review the responses from single species to the community. We focus on studies in the laboratory and in the field, and we also critically consider the use of predictive models for these evaluations.

Our discussion of the information collected underlines the importance of the relation between sensitivity of single species and contaminants. Moreover, the recent approach in developing mechanistic models to predict the response of natural communities seems to be particularly powerful for community ecology, and we strongly recommend more effort along these lines.

Keywords: Benthic organisms; Biomarker; Biotic indices; Chemical contamination; Community structure; Laboratory tests; Field tests; Mesocosms; Predictive models; Species traits

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1. Introduction

The important innovation of the Water Framework Directive (WFD 2000/60/EC) [1], aimed at improving and protecting the quality of all waters (rivers, lakes, groundwater and coastal waters) in Europe, is undoubtedly the integration of chemical and biological approaches to monitor and to manage the aquatic environment. The Directive still requires the chemical monitoring of priority pollutants as standard practice by analytical chemistry procedures, but the new approach means that further attention is given to the ecological evaluation of ecosystems, taking into account the organisms inhabiting the water environments. The key goal of the WFD is precisely to ensure a ''good ecological and chemical status'' of water bodies through the integration of different elements (e.g., biological, physico-chemical and hydro-morphological).

From an ecotoxicological point of view, nowadays it is widely accepted that organisms can be valuable indicators of the status of contamination by micropollutants of a certain environmental compartment, and not only of the strong stresses caused by anoxia or high concentrations of ammonia due to organic matter pollution. In freshwater ecosystems, all the organisms (principally macroinvertebrates, fish, and zooplankton) can be used as bio-indicators of the presence of bioaccumulative or toxic substances, as they incorporate pollutants from the ingestion of food (through biomagnification) and from the water environment by the direct uptake of pollutants through skin or gills (bioconcentration) in strict relation to their trophic levels. Acute adverse effects are not necessarily always evident and hazards can be recognized only when organisms are chronically exposed or at the highest levels of the food-webs. However, even if no direct toxic effects are evident, bioaccumulation can be considered a prerequisite for negative consequences on the whole ecosystem [2].

Ecotoxicology currently provides useful tools for detecting the effects of single or mixed toxicants on single species (in the laboratory and directly in the field) and on specific populations (i.e. organisms of the same species colonizing the habitat). Screening tools using ecotoxicological tests to find out dangerous unknown organic chemicals have been successfully used [3,4], but the effects of contaminants at an ecosystem level, as at the end required by the WFD, still remain an open question.

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According to the new legislation, community (i.e. different populations colonizing the habitat) is the biological organization level that best represents ecosystem integrity, since it can integrate the overall effect of different anthropogenic stresses [5]. Communities are determined by the relationships between organisms and both abiotic (i.e. chemical and physical variables) and biotic (i.e. competition and trophic relations between species and individuals) factors, and the response of the organisms to stressors can be described in terms of alteration of their structure and/or functioning [6,7].

Of the different habitats, sediments undoubtedly play a crucial role, as they represent the major repository for persistent chemicals (organic and inorganic) introduced into surface waters. Concentrations of chemicals in sediments may be several orders of magnitude higher than in the overlying water, depending on many factors (e.g., aqueous solubility, pH, redox potential at the water-sediment interface, affinity for the organic carbon content, grain size, mineral constituents and presence of volatile acid sulfides).

Besides, sediments provide the habitat for a number of organisms, among which benthic macroinvertebrates represent an important ecological element, as they live in direct contact with the bottom littoral substrates and the profundal sediments, and they are an important link between primary producers, detrital deposits and higher trophic levels. Moreover, their presence and assemblage can reflect eventual environmental changes occurring in the ecosystem, integrating the information provided by the chemical characterization.

In order to provide direct, quantifiable evidence of biological consequences of single or known mixtures of contaminants in whole sediments, in the past 20 years, a number of standard methods have been developed to carry out bioassays in the laboratory under controlled conditions [8,9], using single species of benthic invertebrates (amphipods, non-biting midges, oligochaetes, mayflies or cladocerans).

A further successful evaluation of chemical or physical stress has come from analyses of biomarkers (i.e. known biochemical or physiological variations measured in a tissue or in a biological fluid evidencing exposure and/or the effect of one or more contaminants) [10]. Biomarkers can be used to assess the health status of single organisms, thereby anticipating changes at higher levels of the biological organization (i.e. population, community or ecosystem) [11].

It is only in recent times that the whole-community response to toxic contamination has been taken into account, developing effective indices of the effects of toxicity in freshwater ecosystems, using different approaches (e.g., methods based on community structure, protocols considering indicator organisms and regarding the ecological role of taxa) [12–14]. Riverine invertebrate communities have been extensively studied in response to gradients of contamination, producing effective toxicity indices [15–17]. By contrast, benthic communities in lakes have still been rather neglected, since the ecological status of lentic ecosystems has for long been assessed on the basis of only chemical and physical parameters.

Nevertheless theoretical approaches can be similar in both habitats, and the experience reached for riverine ecosystems might be used to develop effective assessment tools for lakes. Indeed, toxicants are mainly adsorbed by the finest fractions of the sediment (i.e. in the depositional zones of rivers and in lakes, where sediment-feeders organisms prevail, mainly belonging to oligochaete and chironomid groups). Other macroinvertebrates may be influenced indirectly by eventual contamination of sediments (i.e. for contamination of the

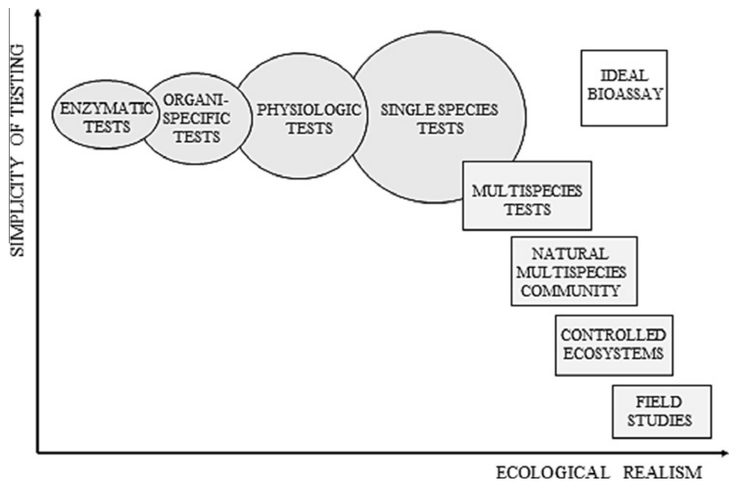

Figure 1. Schematic presentation of the inverse relationship between simplicity and ecological realism in different typologies of test systems (Modified from Calamari et al. [21]).

water and/or for alteration of local food-webs) [18]. Differences between river and lake ecosystems can more involve sediment composition and dynamics. Sediments are more heterogeneous and unstable in rivers, and contamination can follow unexpected routes of dissemination and mixing. Lakes are characterized by fine, stable sediments derived from all inlets, and thus are preferential targets of toxic contamination derived from the watershed [19,20].

In the present article, we critically review the use of macroinvertebrates as a tool for the assessment of the quality of lake sediments both in the laboratory and directly in the field, from single-species tests to community-structure evaluations. As already pointed out many years ago by Calamari et al. [21], it has to be expected that the higher the ecological realism, the greater the difficulty of its definition (Fig. 1).

2. Lake sediments and macroinvertebrates

2.1. Bioindicators in the laboratory

Ecotoxicity-test methods can be easily used to find out the effect concentrations (in terms of EC50, IC50 or LC50) of specific chemicals directly added in sediments (spiked sediments) or in the overlying water (spiked water) at increasing concentrations, and for monitoring environmental samples collected directly in the field (lake or river). A control sediment (a reconstructed formulated sediment or a ''natural'' unpolluted sediment) where organisms can develop without any disturbance is used as the reference.

Several criteria should be considered when selecting a test species. The ecology of the test animal is undoubtedly important, since the organism could be a sediment dweller or a sediment feeder [22], and the uptake route of the contaminants could therefore be directly from sediments, pore water and/or the overlying water. The relative importance of these three routes of exposure also depends on the physico-chemical characteristics of the contaminants [22]. As for the assessment of water quality, even for sediments, batteries of tests should therefore be preferred.

Moreover, as for water, negative effects (acute or chronic) on single organisms are evaluated by the lethal or sub-lethal responses of test animals (end-points) (e.g., effects on growth, reproduction, behavior, developmental or metabolic abnormalities). Survival of test organisms in quite short exposures (e.g., 10 days for *Chironomus riparius*) is also considered an end-point. In this case, only high concentrations of contaminants can be identified [23] and sub-lethal end-points are generally better estimates of responses of benthic populations to contaminants, particularly in the field [24].

Even in the case where all the chemical concentrations are known, the toxic effects on biota are not necessarily correlated, since bioavailability has to be taken into account [25,26]. The availability of chemicals to organisms depends on several factors (e.g., the nature of sediments), since contaminants exhibit different affinities for the various fractions of sediments [e.g., clay minerals, Fe and Mn oxides/hydroxides, carbonates, organic substances (e.g., humic acids) and biological materials (e.g., algae and bacteria)]. Quantifying the bioavailability of chemicals and relating the bioavailability to effects is therefore essential for assessing the impact of pollutants on biota. A useful measure of bioavailability of contaminants is the evaluation of bioaccumulation, with significant implications for the process of risk assessment.

2.2. From laboratory to field tests

Natural sediments collected in rivers and lakes are physically and chemically dynamic, being disturbed by many natural and anthropogenic processes (e.g., biota metabolism, bioturbation, currents, storms, waves, dredging and fishing). When the hazard of sediments in a lake has to be evaluated, problems related to the manipulation of sediments arise. The remobilization of sediment-associated contaminants during their collection, subsequent handling and storage procedures may alter the contaminant concentrations and their bioavailability by changing the physical, chemical and/or biological characteristics of sediments. Manipulations (e.g., mixing, homogenization and sieving) may temporarily disrupt the equilibrium of organic compounds in sediment, or similarly, the oxidation of anaerobic sediments can increase the availability of some metals [27]. For these reasons, laboratory toxicity tests, even if very useful, do not always give ecologically reliable information on the area of concern, mainly because of their obligatory manipulation.

An effective tool to obtain a holistic assessment of contaminants can be the use of *in-situ* bioassays, in which bred organisms are exposed directly *in situ* to environmental compartments that are potentially contaminated. *In-situ* bioassays reduce the artifacts related to sample handling and, at the same time, allow much more realistic exposure, bypassing the uncertainties that arise when attempting to extrapolate the results of laboratory bioassays to the field. Sibley et al. [28] summarized the advantages of *in-situ* sediment bioassays as follows:

(1) no need for sediment manipulation, excluding the possibility of altering the contaminant bioavailability (moreover contaminants that volatilize are taken into account) through sampling, sieving or other processes that are normally associated with laboratory sediment bioassays;
(2) the natural stratification of the sediments is preserved, providing more natural vertical contaminant gradients and hence a more realistic exposure regime;

(3) natural biotic and abiotic variables that influence toxicity are accounted for.

Sibley et al. [28] also proposed the use of a simple, inexpensive bioassay chamber for testing sediment toxicity (through measurement of survival and growth) and bioaccumulation under field conditions, using the midge *Chironomus tentans* and the oligochaete *Lumbriculus variegatus.*

Castro et al. [29] even developed an *in-situ* sediment-bioassay chamber, suitable for performing toxicity bioassays with benthic invertebrates, using the midge *C. riparius*. Their study aimed to evaluate the ecological relevance of the standardized 10-day larval growth-and-survival-test protocol in estimating the toxicity of sediments, through comparing laboratory and *in-situ* results. Standard laboratory toxicity testing did not detect any significant alteration in any of the end-points assessed, but toxic effects were found in the field. Differences of responses in laboratory and in the field may be due to the reduction of sediment toxicity during transport and storage and to the fluctuating environmental conditions in the field, which enhanced the toxic effect of the contaminated sediments. In the case of deep great lakes, *in-situ* tests seem to be really difficult to perform and this can represent an obstacle for the determination of the ecotoxicity, as foreseen by Calamari et al. [21] (Fig. 1).

In-situ bioassays achieve realistic exposure, even considering the toxicity of the surface water. Robertson and Liber [30] evaluated the toxicity of sediments and water to *H. azteca* in order to make stronger comparisons between the two potential exposure pathways in two Canadian lakes. Results of the *in-situ* bioassays revealed significant mortality, relative to the respective reference site, with no significant differences between survival in surface water and sediment-exposure chambers, suggesting that surface water contributed to the *in-situ* mortality of *H. azteca.*

2.3. Early warning in the field: biomarkers

The integrated environmental risk assessment required by the WFD can also comprise the use of biomarkers, early-warning sensitive signals of contaminants exposure evaluated as biochemical, cellular, physiological or behavioral variations in tissues or in body-fluid samples [31,32]. This approach has been successfully used for the protection of the marine environment since the 1990s {[33,34] and references therein}, which can be followed even for freshwater environments. In some situations, standard toxic end-points of ecotoxicity tests (e.g., reproduction, growth and mortality) may not be sensitive enough, and biomarkers could represent an effective way to point out stress of a toxic substance on a population [35,36]. Biomarker responses seem to be in relation to specific classes of compounds [37], so the use of a battery of sensitive biomarkers is recommended to establish a weight-of-evidence relationship between environmental stressors and ecological effects [33,38].

Biomarkers for the environmental risk assessment of freshwater environments (rivers and lakes) were initially focused on vertebrates, particularly fish [32,33,39]. Subsequently, they have also been developed for benthic invertebrates, and, given their diversity, abundance and key ecological role, invertebrate biomarkers have an important role in environmental assessment.

Two commonly studied biochemical biomarkers in invertebrates are cholinesterase (ChE) and glutathione-*S*-transferase (GST) activity. Inhibition of ChE activity has been proposed as an effective tool for monitoring the exposure of organisms to organophosphorus and carbamate pesticides, whereas induction of GST can be suggestive of exposure to organochlorine compounds. McLoughiln et al. [40] have used these biomarkers, together with mortality and feeding inhibition, to assess the potential risk of major classes of toxic chemicals (i.e. metals, surfactants, organochlorine, organophosphorus, and pyrethroid pesticides) to amphipod *Gammarus pulex*. Both biomarkers were more sensitive than traditional end-points, providing a rapid, sensitive indicator of toxicant exposure.

Berra et al. [41] have evaluated the basal-level activities of three enzymes (catalase, acetylcholinesterase and GST) in order to investigate potential biomarkers of exposure to pollutants in different species of macroinvertebrates [Insecta (Diptera, Plecoptera, Odonata, Ephemeroptera and Trichoptera); Crustacea (Gammaridae); and, Oligochaeta (Lumbricidae)] in two Italian rivers. Results showed that the responsiveness of the enzymes differed among taxa of the same community, and from taxon to taxon from different river systems.

The aquatic larvae of non-biting midges (Diptera *chironomidae*), sensitive to many pollutants and used to assess the acute and sub-lethal toxicities of contaminated sediments and water [42], possess a hemoglobin-like eme complex, which is a potential, sensitive biomarker for lake monitoring [43–46]. In *C. riparius* larvae, Fisher et al. [47] measured the cytochrome P450 activity as a response to xenobiotics, indicating its importance in the detection of freshwater pollution.

2.4. From population to community level: mesocosms

A direct method for studying the response of a community to stressors is to use mesocosms, where different species are tested together as communities in order to simulate simplified ecosystems. Mesocosms can be set in the laboratory or directly in the field as enclosures, and can give information on single-species effects in natural conditions or even whole communities.

In the laboratory, they can be used to analyze possible interferences of biological factors on toxicity test results.

For example, Reynoldson et al. [48] showed that the presence of oligochaetes in sediment, when conducting toxicity tests with *C. riparius*, *H. azteca*, and *Hexagenia limbata*, did not affect the survival but did reduce the growth of the test species, so biotic interrelations between species may be taken into account when evaluating the toxic effects of contamination on communities.

Clément et al. [49] verified the suitability of a microcosm protocol previously set up with sediment formulated for testing natural sediment in the laboratory, using a number of different organisms, including *C. riparius* and *H. azteca*.

In-situ mesocosms may provide a more realistic picture of the effects of a given xenobiotic contamination and may underline better the effects of the abiotic factors in determining the final responses of an ecosystem to anthropogenic stress. Currie et al. [50] analyzed Cd distribution and bioaccumulation in nutrient-enriched enclosures in one of the experimental lakes in Northwestern Ontario, Canada. Nutrient levels varied the accumulation rate of Cd by different macroinvertebrate species (chironomids, mussels, crayfish) according to the metal speciation in pore water and sediment, and the biomass dilution.

Giller et al. [51] reviewed the use of experimental cosms to analyze the effects of single and multiple stressors on species interactions in artificial communities, after the manipulation of the abundance and the composition in species. They proposed the use of experimental ponds to study the effects of the loss of non-random species on communities and to test the additivity of ecosystem functioning between habitats (Fig. 2). As a result, toxic contamination may cause a change in community structure as a direct effect, with the elimination of sensitive species and springing up of tolerant taxa [12,52]. In any case, ecosystem functioning may be altered with a shift in food-web interactions. Nonetheless, indirect effects may imply unexpected responses (e.g., replacement of tolerant species with other taxa with similar functional role, thus maintaining ecosystem functioning) [53]. The overall effect then closely depends on the specific sensitivity and the resilience of single populations. These characteristics are often scarcely known for native organisms, so assessing the effects of toxic stress at a higher level of organization and the potential for recovery is rather complicated.

Though mesocosms are very useful to detect unexpected effects of toxic contamination in simplified ecosystems, experiments are still rare, their repeatability is untested and finding cause-effect relations or mechanistic processes is unreliable at present [53].

2.5. Predictive models

When a good level of knowledge of macroinvertebrate communities has been reached, a statistical approach (e.g., multivariate analysis) can be applied to develop

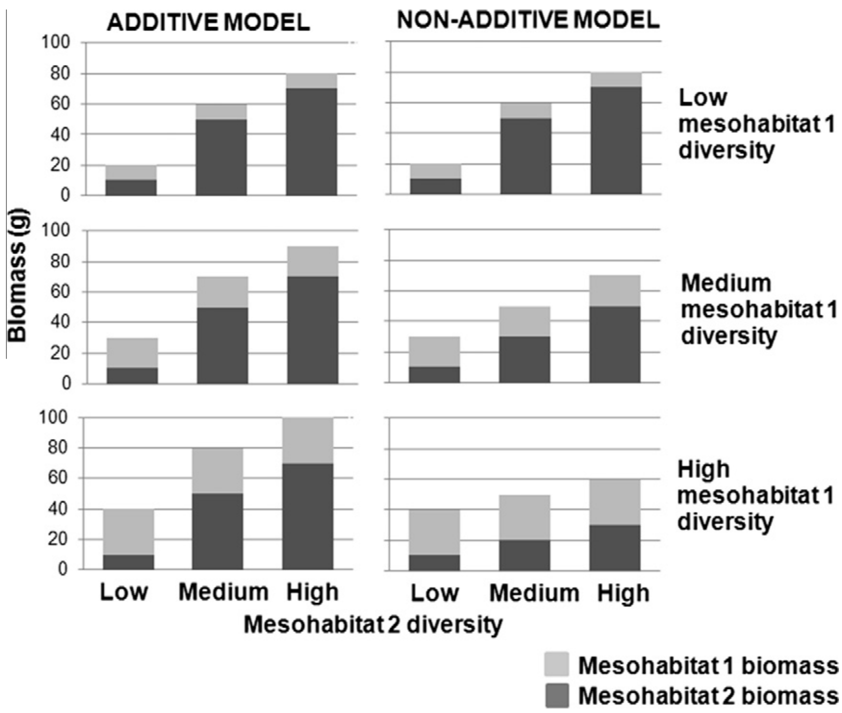

Figure 2. Effects of a shift in biodiversity of a hypothetical mesohabitat 1 on biodiversity of a connected mesohabitat 2 according to the additive and non-additive models. In the additive case, biodiversity of mesohabitat 2 is not affected by mesohabitat 1; in the non-additive case, increasing biomass of mesohabitat 1 leads to a decrease biomass of mesohabitat 2, thus differently affecting ecosystem functioning. Mod. from: Giller et al. [51].

empirical models for prediction of the composition of the communities in relation to the natural features of the sites [54]. The communities observed can be compared with those expected at a site in order to measure the deviations from the natural conditions (i.e. the so-called reference conditions). This goal was first achieved for rivers in the UK, with the development of the RIVPACS model (River Invertebrate Prediction and Classification System) [55], which can predict benthic community compositions in running waters. The model was developed using a large database on slightly impacted macroinvertebrate communities in British rivers and streams, along with information on the geographical, morphometrical, physical and chemical characteristics of the sites.

A similar approach was developed by the BEAST (BEnthic Assessment of SedimentT) model [56,57] for the North American Great Lakes, as an attempt to develop biological guidelines for the lacustrine sediment assessment using macroinvertebrate community structure and laboratory responses in terms of survival, growth and reproduction of the different species. Invertebrates were sampled in fine-grain, unimpaired sediments of a number of lakes, and six expected communities were defined in relation to sediment characters (i.e. % particle-size composition, major chemical elements, nutrients and metals). Moreover, laboratory tests with sediments collected in reference sites identified 10 end-points for four test species (*C. riparius*, *H. azteca*, *Hexagenia* spp., and *Tubifex tubifex*).

The biological responses of the organisms exposed to a test sediment were then compared to their responses in a reference sediment, and three ecotoxicological categories were found by statistical analysis – non-toxic, potentially toxic and toxic. The categories were found by calculating the population mean and the standard deviation (SD) for a certain end-point in a reference sediment [57]. The ''non-toxic'' condition was arbitrarily set at 2 SD above and below the mean (i.e. 95% confidence limit for the response); the ''toxic'' category at 3 SD above and below the mean (i.e. 99.7% confidence limit for the response); and, the ''potentially toxic'' was the condition between 2 and 3 SD.

Based on different test species (*C. riparius*, *H. azteca*, *Hexagenia* spp., *T. tubifex*), this approach could be conceptually compared to Species Sensitivity Distribution (SSD) curves [i.e. curves that represent in probabilistic terms the percentage of species at risk in a community for a certain exposure (Hazardous Concentrations, HCs)]. After defining the sensitivity of some test species towards a given toxicant, the curve of the sensitivity of all species of the community can be defined by a statistical approach following a normal distribution [58]. Moreover, comparison between community tolerance of reference and impaired sites can somehow resemble the recent PICT approach (Pollution Induced Community Tolerance). In this case, the increased tolerance of an impaired site community, compared to a reference community, is considered a direct measure of the contamination level [52].

Both RIVPACS and BEAST models were developed on empirical data collected in limited geographical areas and cannot be applied elsewhere [54]. Moreover, they make an *a posteriori* prediction, after the calculation of the main ecological gradients. Besides, being based on reference-site data, the models can be used to measure the overall effect of pressures, while they cannot be used to identify the effects of single stressors.

A more useful approach may be provided by mechanistic models, which are based on *a priori* predictions of the effects of contaminants on communities and ecosystems. For example, the AQUATOX model [59] was developed to predict the fate and the effects of pollutants in aquatic lentic and lotic ecosystems, mimicking the changes in animal communities (e.g., biomass variation). Even if the model takes into account a mechanistic relationship (e.g., reduced ingestion caused by sublethal toxicant effects, suspended sediments, and habitat preferences), it cannot predict the shift in community composition after impairment.

So far, a full understanding of the ecological processes driving direct effects into indirect effects is still lacking and much has still to be done to improve the predictive power of models.

2.6. Community structure: biotic indices

A common approach for assessing the ecological quality of water bodies is based on biotic indices (i.e. numerical adimensional values expressing the general status of ecosystems). Biotic metrics describe different aspects of the structure and the sensitivity of communities (diversity, abundance, tolerance) and are based on taxonomic identification of organisms, from family to species level [54]. These indices have been included in many monitoring programs, since they are easy to calculate and they can be combined in multimetric indices in order to quantify with a single value the overall effect of stressors on communities at specific sites. Nonetheless, they cannot be used to discriminate different anthropogenic impacts, as single metrics may respond similarly to different pressures (e.g. organic enrichment, morphological impairment, eutrophication, toxic contamination, and general degradation of sites) [54,60].

With respect to the response to the presence of toxic substances, biotic indices show contrasting results. For example, the relationships between commonly-used metrics based on aquatic invertebrates (e.g., BMWP, ASPT, Shannon's index, equitability, number of families, and total abundance) and the water concentration of toxic compounds were tested in Spanish streams, emphasizing strong correlation only at high levels of contamination [61]. At lower concentrations, metrics were more responsive to other stressors (e.g., organic content).

Similar results were obtained for lakes. For example, benthic communities were analyzed in Clear Lake, California, USA [62], impaired by Hg. A decreasing gradient of total Hg in sediments from the mine hot-spot pollution point allowed evaluation of the impact on population-level and community-level parameters. Shannon-Wiener diversity and Pielou's evenness showed no significant trends with distance from the mine, as well as most invertebrate taxa. Only chironomids exhibited lower densities closer to the mine. Benthic invertebrate communities in some areas of Lake Ontario appeared to be limited by both contaminants (PAHs and metals) and by the oxygen depletion due to the high organic content [63,64]. Similarly, macroinvertebrate growth and survival in two floodplain lakes in The Netherlands were related more to seston food quality than to the presence of micropollutants in sediments [65,66].

Pinel-Alloul et al. [67] sampled macroinvertebrates in Lake Saint-François (Québec, Canada) and derived the Invertebrate Community Index (ICI-SL). Variation in community composition and ICI-SL index was mainly due to chemical (conductivity and TP concentration) and morphological (macrophytes, sediment grain size) factors. Some metals and organic micropollutants measured in water (Fe, Cr, Pb, Mn, Zn) and sediments (Mn, Pb, Se, total PAHs) were also significant factors. However, a large amount of variance remained unexplained by environmental factors, and Pinel-Alloul et al. underlined the limitations of the use of the indices of macroinvertebrate community structure in the absence of a strong contrasting pollution gradient.

Similar results were found also by:

- Canfield et al. [68] in the Great Lakes, Indiana, USA;
- Watzin et al. [69] in Lake Champlain, Vermont, USA;
- Borgmann et al. [70] in Rouyn-Noranda Lakes, Quebec, Canada; and,
- Van Griethuysen et al. [71] in some floodplain lakes in The Netherlands.

Another constraint in the use of biotic indices is that the reference values may be missing, especially for lowland lakes located in heavily urbanized areas. In these cases, paleolimnology can provide useful techniques for reconstructing original communities in different time periods (e.g., before and after impairment). Meriläinen et al. [72] analyzed chironomid paleo-communities in Lake Päijänne in Southern Finland from the 1800s up to date, in order to study the effects of paper and pulp mills on biological communities. Chironomid communities were also compared using the Benthic Quality Index (BQI) [73], a trophic index used for lakes. Community composition had a much stricter relation with trophic status than with toxic impairment.

Miskimmin and Schindler [74] analyzed sediment cores collected in two toxaphene-treated basins, finding that manipulation of stocked fish was a confounding factor that accounted most for macroinvertebrate community changes.

These results showed that relationships between macroinvertebrate community composition and sediment toxicity in lakes are biased by different factors, which may act directly or indirectly in determining the final response [52].

Jackson and Harvey [75] analyzed the composition of macrobenthic fauna in relation to environmental parameters in 40 lakes in Ontario. They found a strong relation with pH, which in turn determined the concentration and the bioavailability of toxic metals (e.g., aluminum). However, indirect effects of toxic contamination may also contribute strongly to altering the structure of the community (e.g., large predators, such as crayfish, are generally reduced or eliminated in acidic lakes, thus determining the springing up of more acid-tolerant pray taxa) [75]. To discriminate among the effects of multiple stressors acting simultaneously on ecosystems, a different approach should therefore be adopted, considering also the ecological role of different taxa within the ecosystems.

2.7. Community functioning: species traits

A recent development in invertebrate ecology is the characterization of communities according to their functional composition through identifying species traits. Each species is characterized by biological (e.g., life cycle, respiration mode, reproduction, and body size) and ecological (e.g., feeding habits, habitat preference, and tolerance to stressors) traits, which are selected by evolution as strategies to cope with environmental stress (habitat templet theory) [76]. The resistance and the resilience characteristics adopted by taxa therefore determine the response of the community to disturbance events. The advantage of using functional traits instead of taxonomic composition of communities is bound to the *a priori* predictable response of traits to individual stressors [16,54,77]. Each trait is supposed to respond independently to a given pressure and each pressure affects different traits. Moreover, the response can be predicted following a mechanistic approach (i.e. considering the functional role of the trait in the organism and in the ecosystem).

This approach was adopted to study the effects of toxic contamination on invertebrate communities in running waters [15–17]. It was shown that tolerant taxa are characterized by resistance and resilience traits [e.g., high mobility (which permits avoidance of exposition and dispersion) and short life cycles (which allow rapid re-colonization after disturbance)]. Moreover, according to the insurance hypothesis [12,78], communities comprising many species and functional groups may be more resistant to changes in ecosystem functioning, since the loss of sensitive species may be compensated for by tolerant taxa with a similar ecological role [12,18,51,79].

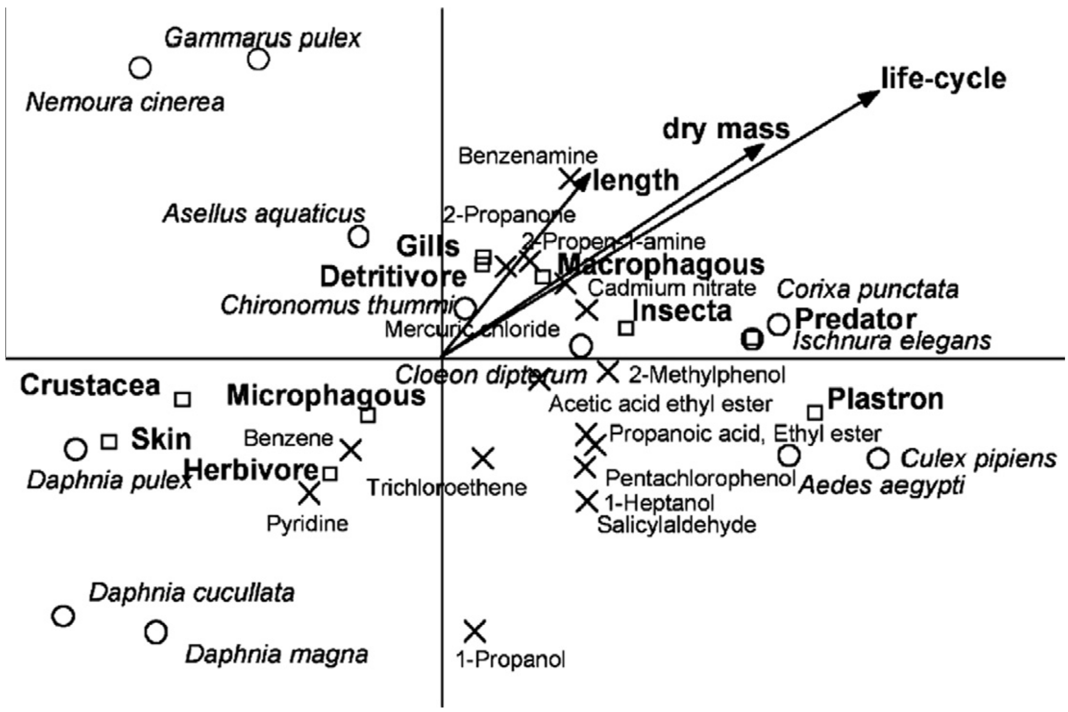

Figure 3. A PCA correlation tri-plot showing the association between substance toxicity and biological traits of aquatic arthropod species. Of all the variation in toxicity, 91% was explained by these traits, of which 49% is displayed on the first axis and another 16% on the second axis. Of all the variance, 45% is displayed on the first axis and another 16% on the second axis. All traits together explained a significant part of the variation in toxicity between the compounds ($P < 0.05$, Monte Carlo permutation test) [80].

The trait approach has also provided a successful framework for deriving species sensitivity to toxicants [80,81]. As an example, it was shown that few species traits (i.e. skin/gill respiration, insect/crustacean, and life-cycle duration) explained most of the variability in sensitivity to toxicants within a group of 12 macroinvertebrate species exposed to 15 chemicals (Fig. 3).

While the method has been successfully used to develop effective indices for running waters responding to individual pressures {e.g., eutrophication, morphological alteration, climate change and toxic contamination [77]}, works on invertebrate community traits in lakes have not been published yet.

3. Future research directions

To predict the effects of toxicants on ecosystems and the shift in community composition, it is necessary to understand the relation between species sensitivity and contaminants. To this end, single-species tests and specific biomarkers can be useful in defining the sensitivity of specific taxa to single contaminants. However, while much information about standard test organisms is available, knowledge about native species and communities is still rather scanty (e.g., database AQUIRE) [80,82,83].

As a consequence, sensitivity is generally derived by statistical approaches {e.g., SSD curves are used to predict the fraction of species affected by a specific toxicant, but cannot provide information on which taxa are supposed to be lost or reduced [80]}. The QICAR method (Quantitative Inter-specific Chemical Activity Relationships) combines information about the mode of action of specific contaminants and the inter-specific relationships between organisms to establish sensitivity ratios between species (i.e. test and native taxa). Nevertheless, different taxonomical levels are generally compared (e.g., *Daphnia* and fish) and the response refers to a class of chemicals, lacking in precision [84].

The recent trait approach was successfully used to develop mechanistic models based on a solid theoretical basis to predict the response of natural communities, considering both structural and functional aspects [13,77,80,82]. To understand how multiple stressors may affect trait composition, microcosms and mesocosms can be used to find *a priori* models explaining direct and indirect effects on communities. In particular, the loss of non-random species, the additivity of ecosystem functioning between habitats and the impact of local processes should be clarified [50]. Moreover, it is necessary to disentangle the effects of multiple stressors into single impacts with direct effects on community [77].

Results indicate that the trait approach may offer a powerful framework for community ecology, but effort is needed to fill the gaps in species-trait information. In particular, for lacustrine communities, mainly comprising chironomids and oligochaetes, the taxonomic, biological and ecological characteristics of species should be better described, in order to find the ecological role of single taxa [54,68].

Moreover, the assignment of species to functional groups requires objective methods. At present, fuzzy coding analysis has been successfully used to match species with individual traits [16,77], but other approaches may be more powerful {e.g., Giller et al. [51] suggested the allocation of species into groups based on several relevant traits}.

However, we need to take into consideration that so far no biotic index (single or multimetric) can represent the overall structure and functioning of a given ecosystem [51]. This is particularly true for lacustrine ecosystems, since knowledge of biotic communities is still fragmentary and requires intensive study before we can understand the ecological effects induced by toxic contamination or other specific pressures; so, besides functional analysis of communities, an integrated study comprising different and complementary approaches must be adopted.

4. Effect-based monitoring and WFD

There is a need for new monitoring tools that help to understand the link between the chemical status and the ecological status, as also stated in the Common Implementation Strategy Guidances on surface-water monitoring [85] and sediment and biota monitoring [19].

Combined biological and chemical-analytical approaches are making significant progress towards identifying those toxicants that are relevant for site-specific risks and towards estimating the proportion of an effect that can be explained by the chemicals analyzed. Chemical analysis of pre-selected sets of toxicants (e.g., priority pollutants) is often unable to explain the ecotoxicological effects of complex environmental samples. Risk assessment based on concentrations (e.g., priority pollutants in sediments or water) obviously does not reflect the risk of the mixture of contaminants, but only the risk of those pre-selected toxicants. We therefore recommend bioassays, biomarkers and other ecotoxicological tests to improve definition of the response of species to toxicants at sub-lethal levels (i.e. to define exposure/sensitivity traits of species) and make them a particularly useful tool in surveillance and investigative monitoring programs.

Combining chemical, ecotoxicological and ecological assessment methods improves understanding of the

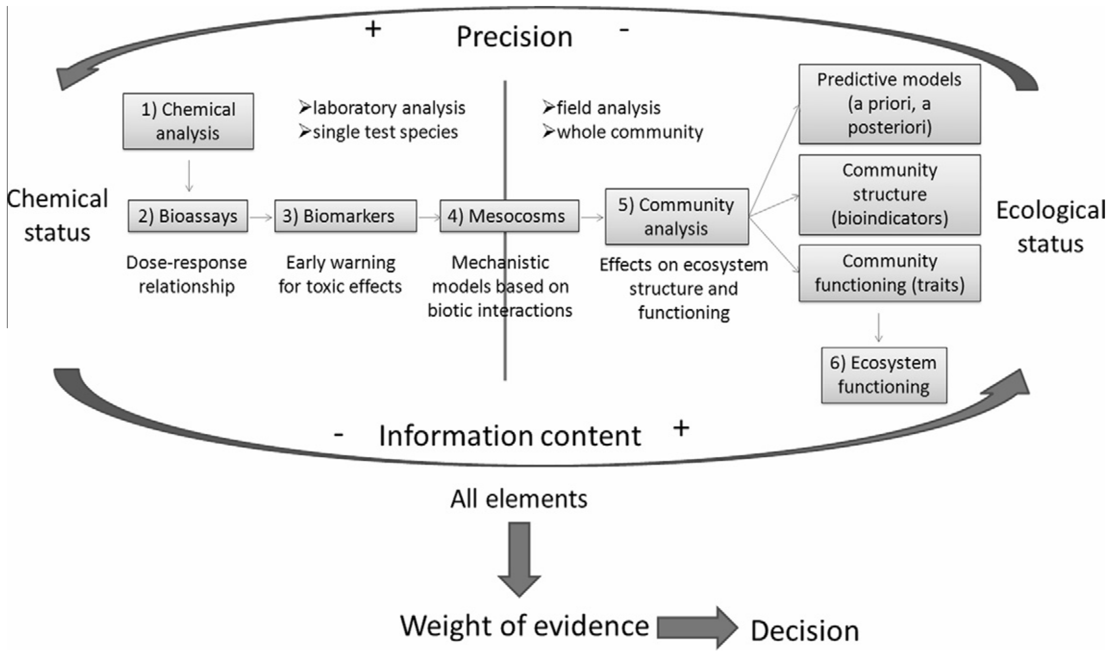

Figure 4. Conceptual framework of methods and approaches linking chemical and ecological status assessment. Chemical analysis (1) and laboratory tests (2; 3) allow high precision in detecting toxicants but lack ''realism'' to translate results in terms of the quality of the ecosystem (6); by contrast, ecological status based on *in-situ* analysis of populations (4) and communities (5) provides a high information content to derive the status of the ecosystem (6) but lacks precision to characterize the causes of impairment. All ecological quality elements, from chemical analysis (1) to more complicated biological analysis [from single laboratory tests (2; 3) to *in-situ* community analysis (4; 5)], may together provide, according to a weight-of-evidence approach, the information needed to allow decision making.

effects caused by certain levels of contamination at specific sites, as required by the WFD. For example, the TRIAD approach combines measurements of sediment chemistry, sediment toxicity, and benthic community composition to evaluate sediment quality and provides a useful tool for finding relationships between biological response and toxic contamination of sediments [86]. This method was applied to lake ecosystems by Canfield et al. [68], who related biological metrics based on invertebrates to metal and organic compound contamination of sediments. This approach showed power in minimizing both the incidence of false-negative error (e.g., low chemical concentrations, non-toxic response in laboratory tests, and benthos adversely impacted) and false-positive error (e.g., high chemical concentrations, toxic response in laboratory tests, and benthos not adversely impacted) by the weight-of-evidence. Future applications of TRIAD should consider more elements, adding power to the evidence approach, so assessing both exposure and effects. According to Chapman and Hollert [87], TRIAD will become a tetrad, a pentad, or even a hexad, in order to provide complete information for decision making (Fig. 4).

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References

[1] European Council, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy, Offic. J. Eur. Union L327/1 (2000) 72.
[2] C. Franke, G. Studinger, G. Berger, S. Böhiling, U. Brukmann, D. Cohors-Fresenborg, U. Jöhncke, Chemosphere 29 (1994) 1501.
[3] S. Galassi, L. Guzzella, V. Croce, Chemosphere 54 (2004) 1619.
[4] W. Brak, H.J.C. Klamer, M.L. de Alda, D. Barceló, Environ. Sci. Pollut. Res. 14 (2007) 30.
[5] E.D. Parker, V.E. Forber, S.L. Nielsen, C. Ritter, C. Barata, D.J. Baird, W. Admiraal, L. Levin, V. Loecschcke, P. Lyytikäinen-Saarenmaa, H. Høgh-Jensen, P. Calow, Oikos 86 (1999) 179.
[6] D.M. Rosenberg, V.H. Resh, Freshwater Biomonitoring and Benthic Macroinvertebrates, Chapman & Hall, London, UK, 1993 p. 488.
[7] D. Chapman (Editor), Water Quality Assessments – A Guide to Use of Biota, Sediments and Water in Environmental Monitoring, Second Edition, E&FN Spon, University Press, Cambridge, UK, 1996.
[8] Environmental Protection Agency (EPA), Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, Second Edition, EPA 600/R-99/064, EPA, Washington DC, USA, 2000.
[9] Organization for Economic Cooperation and Development (OECD), OECD Guidelines for the Testing of Chemicals. Proposal for a New Guideline 218. Sediment-Water Chironomid Toxicity Test Using Spiked Sediment, OECD, Paris, France, 2001.
[10] M.H. Depledge, M.C. Fossi, Ecotoxicology 3 (1994) 161.
[11] M.L. Martin-Diaz, J. Blasco, D. Sales, T.A. DelValls, Trends Anal. Chem. 23 (2004) 807.
[12] M.H. Medina, J.A. Correa, C. Barata, Chemosphere 67 (2007) 2105.
[13] H.J. De Lange, J. Lahr, J.J.C. Van Der Pol, J.H. Faber, Environ. Toxicol. Chem. 29 (2010) 2875.
[14] P.J. Van den Brink, A.C. Alexander, M. Desrosiers, W. Goedkoop, P.L.M. Goethals, M. Liess, S.D. Dyer, Integrated Environ. Assess. Manage. 7 (2010) 198.
[15] M. Liess, P.C. Von der Ohe, Environ. Toxicol. Chem. 24 (2005) 954.
[16] V. Archaimbault, P. Usseglio-polatera, J. Garric, J.G. Wasson, M. Babut, Freshwater Biol. 55 (2010) 1430.
[17] M. Leiss, M. Beketov, Ecotoxicology 20 (2011) 1328.
[18] V.H. Clements, J.R. Rohr, Environ. Toxicol. Chem. 28 (2009) 1789.
[19] European Commission. WFD-CIS Guidance Document No. 25 Guidance on chemical monitoring of sediment and biota under the Water Framework Directive, Office for Official Publications of the European Communities, Luxembourg, 2010, p. 74.
[20] R. Reuther, in: H.I. Inyang, J.L. Daniels (Editors), Environmental Monitoring, Vol. I, The University of the North Carolina, Charlotte, North Carolina, USA, 2003, p.120.
[21] D. Calamari, G. Chiaudani, M. Vighi, in: B. Vouk, G.C. Butler, D.G. Hoel, D.B. Peacol (Editors), Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems, John Wiley and Sons, Chichester, West Sussex, UK, 1985, pp. 549–571.
[22] F. Wang, R.R. Goulet, P.M. Chapman, Chemosphere 57 (2004) 1713.
[23] C. Ingersoll, E.L. Brunson, F.J. Dwyer, D.K. Hardesty, N.E. Kemble, Environ. Toxicol. Chem. 17 (1998) 1508.
[24] N.E. Kemble, W.G. Brumbaugh, E.L. Brunson, F.J. Dwyer, C.G. Ingersoll, D.P. Monda, D.F. Woodward, Environ. Toxicol. Chem. 13 (1994) 1985.
[25] G.A. Burton, Environ. Toxicol. Chem. 10 (1991) 1585.
[26] A.J. Barlett, U. Borgmann, D.G. Dixon, S.P. Batchelor, R.J. Maguire, Can. J. Fish. Aquat. Sci. 62 (2005) 1243.
[27] D.M. Di Toro, Toxicol. Chem. 9 (1990) 1487.
[28] P.K. Sibley, D.A. Benoit, M.D. Balcer, G.L. Phipps, C.W. West, R.A. Hoke, G.T. Ankley, Environ. Toxicol. Chem. 18 (1999) 2325.
[29] B.B. Castro, L. Guilhermino, R. Ribeiro, Environ. Pollut. 125 (2003) 325.
[30] E.L. Robertson, K. Liber, Environ. Toxicol. Chem. 26 (2007) 2345.
[31] J.F. McCarthy, L.R. Shugart, in: J.F. McCarthy, L.R. Shugart (Editors), Biomarkers of Environmental Contamination, Lewis Publishers, Boca Raton, FL, USA, 1990, pp. 3–16.
[32] R. Van der Oost, J. Bayer, N.P.E. Vermeulen, Environ. Toxicol. Pharmacol. 13 (2003) 57.
[33] J.A. Hagger, M.B. Jones, D.R.P. Leonard, O. Richard, T.S. Galloway, Integrated Environ. Assess. Manage. 2 (2006) 312.
[34] J.E. Thain, A.D. Vethaak, K. Hylland, ICES J. Mar. Sci. 65 (2008) 1508.
[35] W.H. Clements, J. Aquat. Ecosyst. Stress Recovery 7 (2000) 113.
[36] C. Blasco, Y. Picó, Trends Anal. Chem. 28 (2009) 745.
[37] P. Vasseur, C. Cossu-Leguille, Environ. Int. 28 (2003) 711.
[38] T.S. Galloway, R.J. Brown, M.A. Browne, A. Dissanayake, D. Lowe, M.B. Jones, M.H. Depledge, Environ. Sci. Technol. 38 (2004) 1723.
[39] P.K.S. Lam, Ocean Coast. Manage. 52 (2009) 348.
[40] N. McLoughlin, D. Yin, L. Maltby, R.M. Wood, H. Yu, Environ. Toxicol. Chem. 19 (2000) 2085.
[41] E. Berra, M. Forcella, R. Giacchini, L. Marziali, B. Rossaro, P. Parenti, Ann. Limnol., Int. J. Limnol. 40 (2004) 169.

[42] R. Bettinetti, D. Cuccato, S. Galassi, A. Provini, Chemosphere 46 (2002) 201.
[43] P. Osmulski, W. Leyko, Comp. Biochem. Physiol. Biochem. 85 (1986) 701.
[44] J. Choi, H. Roche, Environ. Monit. Assess. 92 (2004) 229.
[45] S.M. Lee, S.B. Lee, C.H. Park, J. Choi, Chemosphere 65 (2006) 1074.
[46] M. Ha, J. Choi, Chemosphere 71 (2008) 1928.
[47] T. Fisher, M. Crane, A. Callaghan, Ecotoxicol. Environ. Safety 54 (2003) 1.
[48] T.B. Reynoldson, K.E. Day, C. Clarke, D. Milani, Environ. Toxicol. Chem. 13 (1994) 973.
[49] B. Clément, A. Devaux, Y. Perrodin, M. Danjean, M. Ghidini-Fatus, Ecotoxicology 12 (2004) 323.
[50] R.S. Currie, D.C.G. Muir, W.L. Fairchild, M.H. Holoka, R.E. Hecky, Environ. Toxicol. Chem. 17 (1998) 2435.
[51] P.S. Giller, H. Hillebrand, U.G. Berninger, M.O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, M.T. Monaghan, P.J. Morin, G. O'Mullan, Oikos 104 (2004) 423.
[52] W.H. Clements, J.R. Rohr, Environ. Toxicol. Chem. 28 (2005) 1789.
[53] P.J. Van den Brink, Environ. Sci. Technol. 42 (2008) 8999.
[54] N. Bonada, N. Prat, V.H. Resh, B. Statzner, Annu. Rev. Entomol. 51 (2006) 495.
[55] J.F. Wright, M.T. Furse, P.D. Armitage, D. Moss, Arch. Hydrobiol. 127 (1993) 319.
[56] T.B. Reynoldson, R.C. Bailey, K.E. Day, R.H. Norris, Aust. J. Ecol. 20 (1995) 198.
[57] T.B. Reynoldson, K.E. Day, T. Pascoe, in: J.F. Wright, D.W. Sutcliffe, M.T. Furse (Editors), Assessing the Biological Quality of Freshwater. RIVPACS and Other Techniques, Biological Freshwater Association, Ambleside, Cumbria, UK, 2000, pp. 165–180.
[58] T. Aldenberg, J.S. Jaworska, Ecotoxicol. Environ. Safety 46 (2000) 1.
[59] R.A. Park, J.S. Clough, M. Coombs Wellman, Ecol. Model. 213 (2008) 1.
[60] J. Böhmer, C. Rawer-Jost, A. Zenker, Hydrobiology 516 (2004) 215.
[61] S. Blanco, E. Bécares, Chemosphere 79 (2010) 18.
[62] T.H. Suchanek, C.A. Eagles-Smith, D.G. Slotton, E.J. Harner, D.P. Adam, A.E. Colwell, N.L. Anderson, D.L. Woodward, Ecol. Appl. 18 (2008) A158.
[63] G. Krantzberg, D. Boyd, Environ. Toxicol. Chem. 11 (1992) 1527.
[64] G. Krantzberg, Environ. Toxicol. Chem. 13 (1994) 1685.
[65] H.J. De Haas, I. Roessink, B. Verbree, A.A. Koelmans, M.H.S. Kraak, W. Admiraal, Environ. Toxicol. Chem. 24 (2005) 1133.
[66] H.J. De Lange, E.M. De Haas, H. Maas, E.T.H.M. Peeters, Chemosphere 61 (2005) 1700.
[67] B. Pinel-Alloul, G. Méthot, L. Lapierre, A. Willsie, Environ. Pollut. 91 (1996) 65.
[68] T.J. Canfield, F.J. Dwyer, J.F. Fairchild, P.S. Haverland, C.G. Ingersoll, N.E. Kemble, D.R. Mount, T.W. La Point, G. Allen Burton, M.C. Swift, J. Great Lakes Res. 22 (1996) 565.
[69] M.C. Watzin, A.W. McIntosh, E.A. Brown, R. Lacey, D.C. Lester, K.L. Newbrough, A.R. Williams, Environ. Toxicol. Chem. 16 (1997) 2125.
[70] U. Borgmann, M. Nowierski, L.C. Grapentine, D.G. Dixon, Environ. Pollut. 129 (2004) 39.
[71] C. Van Griethuysen, J. Van Baren, E.T.H.M. Peeters, A.A. Koelmans, Environ. Toxicol. Chem. 23 (2004) 668.
[72] J.J. Meriläinen, J. Hynynen, A. Palomäki, H. Veijola, A. Witick, K. Mäntykoski, K. Granberg, K. Lehtinen, J. Paleolimnol. 26 (2001) 11.
[73] T. Wiederholm, J. Water Pollut. Control Fed. 52 (1980) 537.
[74] B.M. Miskimmin, D.W. Schindler, Can. J. Fish. Aquat. Sci. 51 (1994) 923.
[75] D.A. Jackson, H.H. Harvey, Can. J. Fish. Aquat. Sci. 50 (1993) 2641.
[76] C.R. Townsend, A.G. Hildrew, Freshwater Biol. 31 (1994) 265.
[77] B. Statzner, L.A. Bêche, Freshwater Biol. 55 (2010) 80.
[78] S. Yachi, M. Loreau, Proc. Natl. Acad. Sci. USA 96 (1999) 1463.
[79] F. Ricciardi, C. Bonnineau, L. Faggiano, A. Geiszeinger, H. Guasch, J. Lopez-Doval, I. Muñoz, L. Proia, M. Ricart, A. Romaní, S. Sabater, Trends Anal. Chem. 28 (2009) 592.
[80] D.J. Baird, P.J. Van den Brink, Ecotoxicol. Environ. Safety 67 (2007) 296.
[81] M.N. Rubach, R. Ashauer, D.B. Buchwalter, H.J. De Lange, M. Hamer, T.G. Preuss, K. Töpke, S.J. Maund, Integrated Environ. Assess. Manage. 7 (2010) 172.
[82] P.C. Von der Ohe, M. Liess, Environ. Toxicol. Chem. 23 (2004) 150.
[83] D.J. Baird, C.J.O. Baker, R.B. Brua, M. Hajibabaei, K. McNicol, T.J. Pascoe, D. de Zwart, Integrated Environ. Assess. Manage. 7 (2010) 209.
[84] P. Tremolada, A. Finizio, S. Villa, C. Gaggi, M. Vighi, Aquat. Toxicol. 67 (2004) 87.
[85] European Commission, WFD-CIS Guidance Document No. 19 Guidance on Surface Water Chemical Monitoring under The Water Framework Directive, Office for Official Publications of the European Communities, Luxembourg, 2009, p. 130.
[86] P.M. Chapman, Sci. Total Environ. 97/98 (1990) 815.
[87] P.M. Chapman, H. Hollert, J. Soils Sediments 6 (2006) 4.