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Biomarkers as tools for monitoring within the Water Framework Directive context: concept, opinions and advancement of expertise

Milinkovitch Thomas [1,*], Geffard Olivier [2], Geffard Alain [3], Mouneyrac Catherine [4], Chaumot Arnaud [2], Xuereb Benoit [5], Fisson Cedric [6], Minier Christophe [5], Auffret Michel [7], Perceval Olivier [8], Egea Emilie [1], Sanchez Wilfried [1]

[1] Fdn ROVALTAIN, F-26300 Alixan, France.
[2] Ctr Lyon Villeurbanne, Irstea, UR RiverLy, Lab Ecotoxicol, F-69625 Villeurbanne, France.
[3] URCA, UMR I 02, SEBIO Stress Environm & Biosurveillance Milieux A, Reims, France.
[4] Univ Catholique Ouest, Fac Sci, MMS, EA2160, 3 Pl Andre Leroy, F-49000 Angers, France.
[5] Univ Normandie, SCALE, CNRS, UMR I 02,SEBIO,FR 3730, F-76600 Le Havre, France.
[6] GIP Seine Aval, 115 Blvd Europe, F-76100 Rouen, France.
[7] Inst Univ Europeen Mer, LEMAR, UMR, Brest, France.
[8] Agence Francaise Biodiversite, F-94300 Paris, France.

* Corresponding author : Thomas Milinkovitch, email address : t.milinkovitch@hotmail.fr

Abstract :

The Water Framework Directive (WFD) currently supports chemical and ecological monitoring programmes in order to achieve the good water surface status. Although chemical and ecological assessments are necessary, they have some limitations. Chemical approaches focus on certain substances identified as priorities, but they do not take into account other potentially harmful substances and also ignore the hazards related to contaminant cocktails. On the other hand, while ecological approaches provide holistic information on the impairment of biological communities in ecosystems, they do not distinguish the role of contaminants in these alterations, and consequently do not allow the establishment of contaminant impact reduction plans. Consequently, ecotoxicologists suggest the use of effect-based tools such as biomarkers. Biomarkers highlight the effect of potentially harmful substances (or a cocktail), and their specificity towards the chemicals makes it possible to properly discriminate the role of toxicants within biological community impairments. Thus, the integration of such tools (besides existing chemical and ecological tools) in the WFD could considerably improve its biomonitoring strategy. The B n' B project (Biomarkers and Biodiversity) exposes key objectives that will allow to (i) establish an inventory of the biomarkers developed by French laboratories; (ii) determine their methodological advancement and limits and, on this basis, formulate recommendations for biomonitoring use and future research needs; (iii) discuss the biomarkers' ecological significance, specificity to contaminants and interpretation capacity; (iv) establish, in fine, a selection of valuable biomarkers to enter the WFD; and (iv) propose integrative tools to facilitate the decision-taking by stakeholders.

Keywords : Biomarkers, Ecotoxicology, Water Framework Directive, Biomonitoring strategy, Contaminants

1. Limitations of ecological and chemical approaches as a plea for an integration of effects-based-tools in the WFD strategy.

The water framework directive (WFD 2000/06/EC) has been established in order to achieve a good environmental status of coastal, continental and transitional waters. For this purpose, WFD promotes the set-up of biomonitoring programs aiming at evaluating, and if necessary, improving the chemical and ecological status of body waters. Briefly, the chemical status is considered good if concentrations of priority substances in biota and water column do not overpass calculated environmental quality standards. The ecological status is evaluated using ecological indicators such as the diversity and the abundance of plants and animals groups. Although these chemical and ecological analyses were of great interest for the first WFD management plan (achieved in 2015), the scientific community and decision makers highlighted several bottlenecks in these approaches. Chemical approaches focused on selected priority compounds that are highly suspected to pose a threat to ecological communities or human health, since screenings of all contaminants present in the environment is impossible for technical and economic reasons (as noticed by Amiard and Amiard Triquet, 2008). Thereby, chemical assessment ignore a corpus of potentially harmful substances, including emerging ones. In addition, the methodology only takes into account the toxic profiles of each compound separately. Such an approach ignores the “cocktail” effects and consequently the toxicity of contaminants mixtures formed in the field, to which organisms are exposed (Beyer et al., 2014). In parallel to these chemicals analyses, the ecological evaluation was proposed as a tool of interest to assess the impact of chemical and physical stress on ecosystems. However, such an *a posteriori* approach, *i.e.* determining the decline of population and/or community after contamination, is thought not to be precocious enough (Amiard-Triquet et al., 2015), so that only remediation plans can be set-up afterward while prevention plans are more cost-effective. Moreover, using ecological approach, discrimination of the effects of contaminants from the effects of other abiotic factors (*e.g.* temperature, salinity, food resources, eutrophication, habitat degradation) is complex since all these factors are likely to alter biodiversity and abundance indices (*e.g.* Schiedek et al., 2007; Trush et al., 2008; Moe et al., 2013).

In order to deal with these issues, scientists promote the use of diversified effect-based tools. Wernerson et al. (2014) described two categories of effects-based tools besides ecological indicators: (i) bioassays which measure the toxicity, at cellular and individual levels, of environmental samples under defined laboratory conditions; (ii) biomarkers *i.e.* biological responses at sub-individual or individual level, observed in field exposed organisms. Concerning biomarkers, these tools, if correctly and judiciously used, could adequately supplement chemical and ecological approaches in order to solve the main issues cited above. Indeed, the use of a set of biomarkers with a selective responsiveness for certain families of contaminants will inform on the presence of harmful and/or emerging substances in the ecosystems and consequently serving as first non-targeted chemical screening steps before defining the orientation of further necessary chemical measurements (Depledge et al., 1995). Moreover, pollutants responsive biomarkers allow to evaluate the biological effects of mixtures of chemicals as well as to discriminate the effect of contaminants from the effects of others factors such as temperature, oxygen levels, seasonality. Finally, since biomarkers are measured at the sub-individual or individual levels they are claimed to be more precocious than ecological indicators (Amiard-Triquet et al., 2015), measured at the population or community level. They could therefore give first insight on the future potential alteration of the health status of ecosystems and thus allow the set-up of cost effective prevention plans by decision makers instead of expensive remediation plans.

However, in response to this different stakes, several issues remain concerning the use of biomarkers for biomonitoring program. Forbes et al. (2006) point out that biomarkers modulation, instead of providing early warning, could be false alarms. The authors argue that certain biomarkers responses observed on the field could be the results of confounding factors rather than exposure to chemicals. To address this criticism, several authors recently investigated, integrated and/or corrected confounding factors in their methodologies. They defined reference and statistical threshold values which integrate the confounding (biotic and abiotic) factors and ensure that the biomarkers modulations observed are due to contaminants (*e.g.* Coulaud et al., 2011; Charron et al., 2013 ; Erraud et al., 2018). Another criticism to the use of biomarker was the “complicated time or dose-dependent responses” (Forbes et al., 2006) of biomarkers since these indicators already showed some transient responses which greatly reduce while the chemical stress was still present or even more intense. In this line, kinetic and dose-responses of biomarkers have been studied in order to improve the interpretation of the biomarkers modulations observed on the field (*e.g.* Zhang et al., 2008; Nahrgang et al., 2009; Pathiratne and Hemachandra, 2010). Finally, since biomarkers are measured at the sub-individual or individual level, Forbes et al. (2006) raises the question to know how such modulation could affect population, then community and finally the whole ecosystem structure. Initially, the main goal of multi-biomarkers studies was to provide an early warning of a potential alteration of ecosystem health status without quantifying the repercussions between sub-individual and higher levels. More recently, mechanistic pathways have been investigated in order to highlight and quantify these repercussions (e.g. Durou et al., 2008; Xuereb et al., 2009; Lacaze et al., 2011; Coulaud et al., 2015) and consequently obtain better information about the alteration of ecosystem health status.

In the light of the advantages of biomarkers for monitoring strategy, scientists promote their insertion in the WFD revision text (soon to be revised, in 2019), besides ecological and chemical indicators (Lepom et al., 2009; Wernersson et al., 2014; Capela et al., 2016). However, taking into account biomarkers issues, but also recent advances in this field to solve these issues, a qualitative selection of valuable biomarkers seems necessary. To do so, the project B n’ B (Biomarkers and Biodiversity) will expose an inventory of the biomarkers used by the French laboratories and will propose a panel of relevant biomarkers likely to enter the WFD strategy.

2. B n’ B project: Participants, Objectives and Implementation

B n’ B project is funded by the French agency for the biodiversity (AFB) and led by the Rovaltain Foundation. It began on January 2018 and will end in November 2019. As a first step, the project identified members of the working group according to their expertise in the field of biomarkers use for biomonitoring purpose. In order to cope with the several issues presented above, concerning the use of biomarkers in biomonitoring strategy and to permit their entering in the revised text of the WFD, B n' B project has several objectives:

(i) To establish an inventory of the expert laboratories in the French territory and of their competence in term of biomarkers and sentinel species use for the biomonitoring of continental (*e.g.* rivers, lake, underground waters), transitional (*e.g.* estuaries) and marine coastal waters.

(ii) To evaluate, the maturity of these biomarkers in terms of methodological development for a use on the field, *i.e.* to determine which biomarkers could be used for large scale biomonitoring programs and which one needs further research development.

(iii) To discuss the ecological significance of the biomarkers and their specificity to contaminants.

(iv) To consider the limits and the potential of the biomarkers as tools of interest to fill the gap between ecological and chemical status evaluations in the framework of the WFD.

(v) To establish a critical inventory of the methodology (available at the international level) converting multi-markers results into an easily understandable evaluation of ecosystems health for decision makers.

(vi) To formulate recommendations and research needs for a better use of biomarkers in biomonitoring program and for the development of innovative tools.

To achieve these objectives, members of the consortium will firstly identify French laboratories experts in the field and an inventory will establish the know-how of each laboratory in terms of biomarkers and species use.

In a second time, the methodological maturity and the condition of application will be established for each biomarker/species couple (previously identified) thanks to a survey addressed to these laboratories. More precisely, the methodological maturity will be evaluated regarding the acquired knowledge of the laboratory concerning (i) the biomarker response profile (*e.g.* inhibition, activation), (ii) the biomarker kinetic and dose-response *i.e.* the biomarker modulation at several temporality and several intensity of contamination, (iii) the existence of reference and statistical threshold values which integrate the confounding factors and ensure that the modulation of the biomarkers is due to contaminants.

Regarding the condition of application, laboratories will give information on the acquisition time necessary to obtained results, the type of biomonitoring (active or passive) already performed with each biomarker/species couple, the level of expertise required and the ethical issues linked with the use of these biomarkers.

On the basis of these methodological criterions, the consortium will select valuable biomarkers to be use in large scale biomonitoring and biomarkers requiring slight development.

Thirdly, the ecological significance and the specificity to contaminants of the biomarkers will be determined by the consortium according to their expertise and also on the basis of literature. Finally the consortium will highlight the limits and the potential of the biomarkers inventoried on the French territory in order to formulate recommendations for their use and their improvement, in a regulatory monitoring context.
To complete, a bibliographic analysis will be conducted in order to make an inventory of the methodologies used to integrate multi-markers into an easily understandable tool for decision making. After categorizing these tools according to the computational method used to integrate biomarkers, the relevance of these methodologies will be evaluated by characterizing the pros and the cons of each method, by estimating their operability (needs of specific softwares, feasibility of the calculations), by discussing their significance (*e.g.* demonstration of statistical significance, numbers of species that could be integrated).

The B n' B project will highlight the French expertise on biomarkers in ecotoxicology and will contribute to promote the integration of these effect-based monitoring tools in the WFD strategy. Associated to others European research and expertise activities, the results of the project will contribute significantly to the update of the WFD planned in 2019 by the definition of an ecotoxicological status for European water bodies.

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