Characterisation of the impact of aqueous industrial waste in mesocosms: biological indicators and pilot streams

A. Bassères* and B. Tramier**

* ELF, Groupement de Recherches de Lacq, BP 34, 64170 Lacq, France

** ELF, Direction Environnement et Sécurité, Tour Elf, 92078 Paris la Defense Cedex, France

Abstract The use of exposure biomarkers in measuring the impact of aqueous waste holds out promise because such tools have short response times, are of flexible use and give an indication of the type of pollution. However, their ecological significance has not yet been demonstrated. During field studies focusing on aqueous industrial waste, the correlations obtained between several biocoenotic indicators and biomarkers measured in a fresh-water bivalve (*Corbicula fluminea*), demonstrate the need for work to be carried out under controlled conditions.

Working in partnership with the Adour Garonne Water Board, the ELF company has developed a pilot scheme incorporating such controlled conditions. This pilot is made up of 16 canals 40 m in length supplied by river water. The pilot scheme, which is currently at the validation stage, makes it possible to reconstitute an aquatic ecosystem which, once established, will be exposed to perfectly controlled pollution conditions. The responses provided by all the indicators – biocoenotic and microbiological indicators, biomarkers – will then be correlated in order to attribute an ecological significance to the biomarkers.

Keywords Artificial ecosystem; biological indicators; biomarkers ; fresh water ecosystem; impact; mesocosm

Water Science and Technology **Vol 44 No 2–3 pp 135–143** © IWA Publishing 2001

Introduction

In many cases, the impact of waste in aqueous environment is measured only by means of a physico-chemical approach whose increasing sophistication is beginning to lead to prohibitive costs. Moreover, the elements sought are not necessarily those which induce an impact on the biological component of the ecosystem. Alongside these physico-chemical systems of pollution surveillance, a biological *monitoring* approach making use of biological indicators of pollution is beginning to emerge. This is consistent with French and European regulations which increasingly incorporate this notion in their texts.

Biological monitoring is a relatively old technique (Nylander, 1866) which proposes several biological indicators. Irrespective of the ecosystem, these biological indicators may be classified by "degree of organisation", i.e. by moving from the biocoenosis to the cell: biocoenotic indicators (Water Boards, 1993), indicators based on the presence/absence of fish, benthic invertebrates (Verneaux, 1973), molluscs, oligochaeta (Lafont *et al*., 1991, Lafont 1984), chironomidae, diatoma and algae (Coste and Prygiel, 1993) towards biochemical, molecular indicators. In his bibliographical study, Melancon (1995) notes that while all the biochemical techniques have the merit of existing, they lack validation, particularly *in situ*. Generally speaking, it seems that these approaches are relatively well advanced in sea water (Narbonne *et al*., 1991, Garrigues *et al*., 1993), but less so in fresh water. There are, however, works showing the feasibility of such and such an approach on fresh-water organisms, fish and molluscs (Livingstone, 1993, Adams *et al*., 1989, Van der Oost *et al*., 1997, Cossu *et al*., 1997). One of the promising organisms for this kind of application is the fresh-water mollusc, *Corbicula fluminea* (Narbonne *et al*., 1999, Labrot *et al*., 1996, Milam and Farris, 1998).

It should be noted that biocoenotic indicators were among the first to be considered and that the current trend is towards the development of biochemical/molecular indicators or biomarkers. These early indicators of pollution have the following advantages : short response time, more appropriate response in function of the pollution and scope for extending their application to different ecosystems (air, soil and water). However, it very soon became apparent that the response of the bioindicators, of whatever sort, needed to be correlated to the degree of pollution. In other words, there is a need to establish grills demonstrating sensitivity to pollution. It is also necessary to establish the ecological significance of these biomarkers. Few studies provide a comparison between the biocoenotic and cellular approaches. Taking into account all the works conducted in this sphere, it now appears necessary to validate the biomarker approach in the measurement of impact.

For the purposes of this validation, it is not enough to adopt an *in situ* approach which, although representing a real situation, is not controlled. We therefore proposed to set up an experimental mesocosm in which control would be exercised over contamination and the exposure times of living organisms. A description of this pilot study, consisting of 16 streams, is given below. The experiment is based on works cited in the literature (Belanger, 1997, Kosinski, 1989, Rodgers *et al*., 1996, Pusey *et al*., 1994).

Experimentations in situ

The impact of aqueous industrial waste in surface waters was measured by means of a multi-indicator approach. The biocoenotic indicators were selected on the strength of their ability to represent the ecosystem but also for their ease of use, their cost and their previous validation. We added *Corbicula fluminea* to our list of biocoenotic indicators since we wished to validate the biomarker approach in this sentry organism. The experimentations focused on the effluents from industrial sites, the reference station being located upstream of the effluent outlet.

Material and methods

The biological indicators adopted are presented below. These biological approaches were complemented by the physico-chemical characterisations of the water and sediment of the receptor environment (results are not included in this communication).

The Normalised Global Biological Index (Indice Biologique Global Normalisé – IBGN) is based on the analysis of the macro fauna (>500 µm) (diversity and indicator group) and has been developed since the 1960s (Verneaux, 1973). It is used to evaluate the general quality of a water course, in France. It was used in accordance with AFNOR standard NF T90-350.

The other widely used organisms include algae and in particular diatoms (brown algae of 10 to 500 µm), considered to be among the best bioindicators (Coste and Prygiel, 1993). Unlike macro-invertebrates, diatoms are more sensitive to the quality of the water than to that of the habitat. The ***Diatomic Biological Index (Indice Biologique Diatomée – IBD)***, now in the process of standardisation, was used. The diatoms are collected on natural substrates (stones) and/or artificial substrates (white earthenware plates surrounded by a plastic grill for protection against shocks).

Measurement of ***oligochaete indicators*** : Index of Coarse Sediment Biological Quality (*Indice de Qualité Biologique des Sédiments Grossiers – IOSG*) and Index of Fine Sediment Biological Quality (*Indice de Qualité Biologique des Sédiments Fins – IOBS*). This measurement was carried out in conformity with the methodology developed by Lafont (1984), Lafont *et al.* (1991). The IOBS is a descriptor of the organic pollution of the water and of sediment contamination by heavy metals.

The microbiological indicators were addressed by measuring bacterial toxicity and by bacterial count. Total heterotrophic bacteria and hydrocarbon specific bacteria were counted by the Most Probable Number method (MPN). Total bacteria were directly counted with the microscope after fixture of a fluorochrome on the DNA. Bacterial toxicity in the water and sediment of the receptor environment is calculated by the standardised **Microtox** test (French Standard NF T90-320).

Biochemical indicators: biomarkers in Corbicula fluminea. Exposure indicators, in many cases biochemical indicators corresponding to an enzymatic induction (reparation) in the presence of a pollutant, were measured in the fresh-water bivalve *Corbicula fluminea*. This organism was exposed in small cage in the receptor environment for 5 days. The organisms were then retrieved, opened and the crystalline style removed; they were then frozen in liquid nitrogen in accordance with a methodology developed by the Laboratoire de Physico-Toxicochimie des Systèmes (LPTC) (Narbonne et al., 1999, Narbonne et al., 1991, Labrot et al., 1996).

The measurement of the biomarkers under consideration is described by Vidal *et al*., (2001). These biomarkers are catalase inhibitors or activators, propionylcholinesterase (PChE) and peroxidation of lipids (malondialdehyde product (MDA)).

Results

The results obtained are presented by means of “radar” diagrams featuring the values of the reference station (upstream station) expressed at 100%, those of the near downstream station expressed as a percentage of the upstream station, and those of the far downstream station also expressed as a percentage of the upstream values. All the biological responses are recorded on each axis.

It emerges from Figure 1 that the near downstream situation (100 m after the waste of site A) shows a definite impact compared to the upstream situation. The situation has globally recovered at the far downstream station, situated 6 km further downstream.

The analysis of the biological indicator campaigns carried out on different sites brings to light several facts:

The biological indicators may be broken down into three subgroups:

- the biocoenotic indicators – benthic invertebrates (IBGN), oligochaeta (IOBS) and diatoms (IDB);
- the microbiological indicators consisting of bacterial counts and the metabolic activity of the microorganisms;
- the biomarkers analysed in *Corbicula fluminea* : inhibition of acetylcholinesterase or propionylcholinesterase (AchE or PchE), the evolution of catalase and malonedialdehyde (MDA) levels.

The microbiological indicators clearly reveal that the waste contains a quantity of organic matter inducing the increase of the bacterial microflora liable to cause the biological degradation of the biodegradable substances. At the far downstream station, the microbiological indicators recovered their reference values.

This situation, more marked in 1996 (Figure 1) in the water compartment, is repeated in 1997 (Figure 2), particularly in the sediment compartment. The analysis, that year, of hydrocarbon specific bacteria suggests the presence of hydrocarbons in the waste and the sediment.

The biodegradable nature of the polluting source allows the ecosystem to recover a few kilometres downstream.

The biocoenotic indicators incorporated in the sediment: IBGN and oligochaeta also clearly indicate an impact at the near downstream station in 1996 and 1997, then recovery at the far downstream station (not complete in the case of the oligochaeta).

The diatomic indicators, for their part, are less sensitive to this type of pollution. Measurements were taken on rocky samples and artificial substrate : there is little change in the faunistic list between these two sampling methods. Observation of the fresh state also showed that the diatoms observed are living at the time of identification.

As for the biomarkers, these develop in 1996 (Figure 1) in the same way as the biocoenotic indicators, that is to say that they show an impact at the near downstream station and a recovery at the far downstream station. Thus, catalase which is highly activated at the near downstream station returns to the reference value further downstream. Although MDA follows the same trend, its development is slight. The propionylcholinesterase inhibition observed at the near downstream station drops noticeably to recover the reference value at the far downstream station.

In 1997 (Figure 2) the evolution of the biomarkers is different. Catalase is activated at the near downstream station, but this time catalase inhibition is observed at the far downstream station. This change is difficult to interpret. A physico-chemical analysis (not presented here) in both the water and the sediment fails to reveal a particular chemical pollution which would explain this phenomenon. PChE inhibition follows much the same pattern in 1996 and 1997. On the other hand, the quantity of MDA differs totally from one year to the other. The observed presence of MDA suggests a lipid peroxidation of the tissues of *Corbicula fluminea*, with a tendency towards recovery at the far downstream station. Attention should, however, be drawn to the difficulty of measuring the highly elusive MDA.

The analysis of site A in 1996 brings out a correlation of the biomarker response with the biocoenotic response : it would seem that the biomarker response measured in *Corbicula fluminea* has an ecological significance.

Correlation for the same site in 1997 appears less clear-cut. It is difficult to make confident interpretations on the basis of the (insufficiently controlled) ecosystem.

With regard to Site B: It may be noted that the marked impact at the near downstream station (100 m below the waste) is maintained at the far downstream station (5 km downstream) (Figure 3). It is interesting to note that the microbiological indicators do not indicate that the waste is biodegradable; however, it does not induce toxicity on the

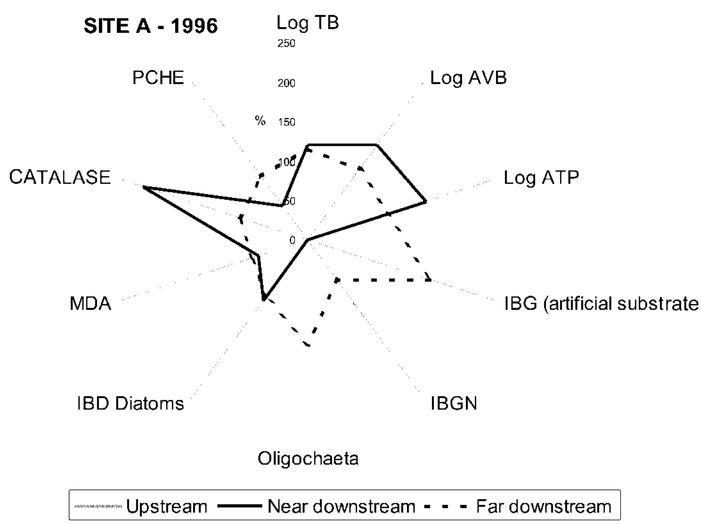

Figure 1 Biological test between stations situated upstream and downstream (near and far) of an industrial site (SITE A). Test carried out in 1996. *TB: Total Bacteria; AVB: Aerobic Viable Bacteria; ATP: Metabolic activity*

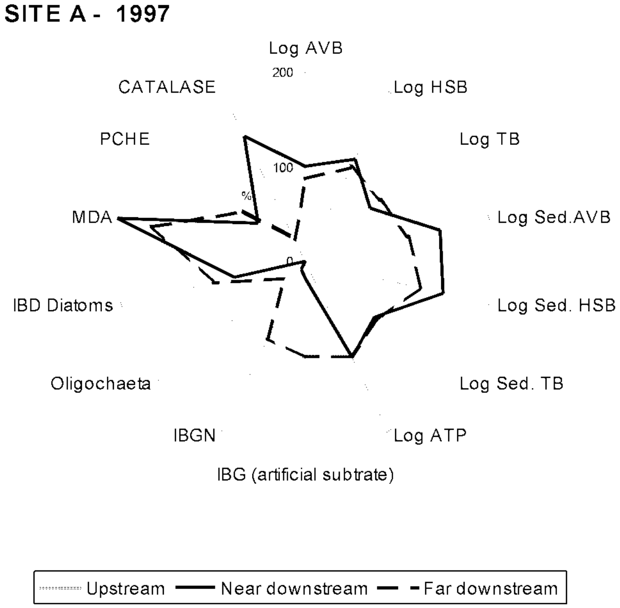

Figure 2 Biological test between stations situated upstream and downstream (near and far) of an industrial site (SITE A). Test carried out in 1997. *TB: Total Bacteria; AVB: Aerobic Viable Bacteria; HSB: Hydrocarbon Specific Bacteria; ATP: Metabolic activity*

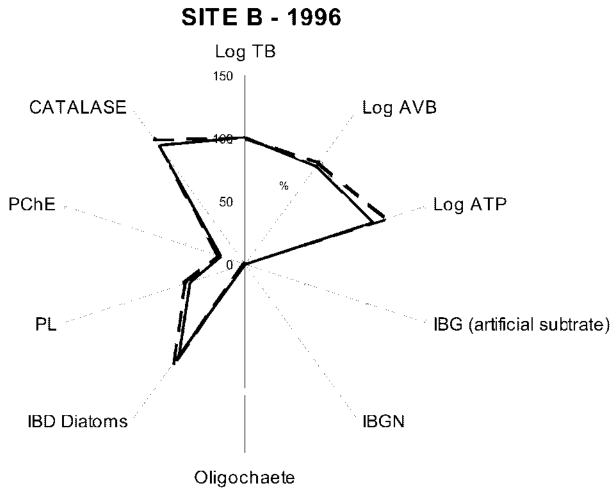

Figure 3 Biological test between stations situated upstream and downstream (near and far) of an industrial site (SITE B). Test carried out in 1996. *TB: Total Bacteria; AVB: Aerobic Viable Bacteria; ATP: Metabolic activity*

microbial compartment. The pollution is therefore exported along the water course. The biocoenotic indicators incorporated in the sediment are totally affected by the waste whereas the diatoms are insensitive to this type of pollution.

The biomarkers respond in exactly the same way as the biocoenotic indicators incorporated in the sediment : the response is identical between the near downstream station and the far downstream station. The catalase presents a (fairly moderate) activation, PChE is strongly inhibited and the quantity of MDA is low. In this example, the biomarker approach appears to be correlated to the other approaches and would also seem to have an ecological significance. However, it should be noted that the degree of the responses, e.g. catalase

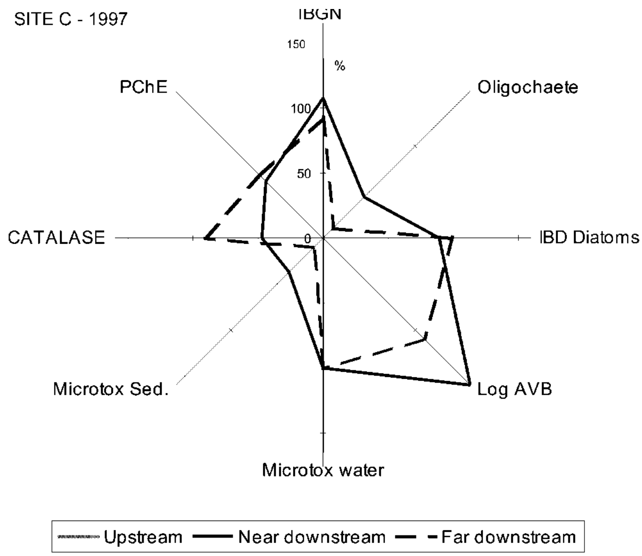

Figure 4 Biological test between stations situated upstream and downstream (near and far) of an industrial site (SITE C). Test carried out in 1997. *AVB: Aerobic Viable Bacteria*

activation, is low compared to what was observed on site A. Once again, lack of comparative control makes it difficult to advance a detailed interpretation of the biomarker response. In many cases, the waste is in fact a mixture of several types of product : the biomarker response is modulated in function of the products to which the organism is exposed. It is not a global approach – unlike the biocoenotic approaches. Nevertheless, a multi-indicator approach could have an ecological significance and provide information on the nature of the pollution.

With regard to Site C: The ecosystem seems to respond differently for the water compartment compared to the sediment compartment (Figure 4).

Taking the diatoms (IBD), the bacteria counted in the water (aerobic heterotrophic bacteria), the water microtox analysis and the biomarker response as representing the water compartment, we note an impact at the near downstream station (100 m downstream from the waste) with a tendency towards recovery at the far downstream station (2 km downstream from the waste). By way of contrast, attributing the oligochaeta and the sediment microtox response to the sediment compartment, we note an alteration in the ecosystem : impact at the near downstream station but more pronounced impact at the far downstream station. This situation may be explained by a history of pollution already present in the sediment while the quality of the effluent itself has actually improved. Moreover, the far downstream station is influenced by a phreatic rise supplying this part of the water course.

In this example, as far as the quality of the water is concerned, we once again observe that the biomarker response appears to be correlated with biocoenotic indicators. We also note that certain biomarkers such as the catalase reveal a development which is opposite to that observed in the previous examples (in this example the catalase is inhibited). This raises the question of the validity of measuring a biomarker response if the pollutants give rise to opposite responses for this biomarker. There is also the question of pollutant interactions. These questions have to be seen in the context of the promising results provided by these easy-to-use and inexpensive biomarkers. If the biomarker approach is validated and its significance approximated, this tool will prove extremely useful in estimating the degree of impact on an ecosystem.

It is for this reason that it was decided to create an artificial ecosystem pilot scheme as a reliable experimental tool for working in controlled conditions.

Pilot streams

In the light of the results obtained on the sites, the pilot (Figure 5) described below was constructed for the purposes of validating the approach by means of a perfectly controlled experimental system.

Situation

The artificial outdoor streams are situated at a distance of 30 km from Pau, in south-western France, next to the Elf Aquitaine plant of Lacq.

Water supply

The water comes from a tap on a pipe leading from a dam on the "Gave de Pau" river: the Abidos Dam. The quality of the water from this dam is rated at 15/20, which corresponds to good quality.

Structure

The structure is based on similar works found in the literature (Kosinski, 1989, Belanger, 1997, Pusey *et al*., 1994). It consists of a 50 m water supply pipe exiting into a flow adjustment trap. The flow is regulated by an "AVIO" valve and a width-adjustable sluice. This system provides all the flexibility required for managing the structure. The water flow thus controlled, the water travels along a water supply channel 100 m in length and 0.70 m in width. A layer of sediment, coming from a quarry and with known physico-chemical characterisation, is deposited in this channel. The channel constitutes a breeding-ground of benthic organisms and micro-algae and is kept supplied with water at all times. This ensures that the downstream channels are colonised more quickly.

The water is then directed to the structure via a third-circle shaped, perfectly isoplanar, concrete distribution structure. The water is distributed in the 16 experimental streams via 16 adjustable sluices. The pilot is made up of 16 parallel concrete channels (40 m long,

Figure 5 Pilot stream

0.5 m wide, 0.5 m deep). The channel outlet is also fitted with adjustable sluices for regulating the water height in each channel. In addition, sediments are deposited in the bottom of the channels, in accordance with the chosen experimental plan. Each stream is equipped with an on-line analysis of the following parameters : oxygen, pH, temperature and conductivity.

The waste is produced, either in the river in the case of the control streams and of concentrations not exceeding the thresholds specified by the French Water Law of 3rd January 1992 (with which the structure is in conformity), with the possibility of initial discharge in a macrophyte basin (reeds), or (for other cases) after treatment in the sewage treatment station of the Elf Aquitaine plant of Lacq.

The pilot study was carried out during the first six months of 1999. Flooding will take place in the course of the summer of 1999.

Objectives sought

In the first instance, this structure will be used for research purposes in partnership with the Adour Garonne Water Board.

The objectives sought are: the demonstration of the significance of the biomarkers response in the fresh-water mollusc *Corbicula fluminea*; establishing sensitivity-to-pollution grills (dose-response curve).

Conclusions

The impact measurement approaches carried out *in situ* have shown the interest of using biomarkers, but they have also drawn attention to the need to attribute an ecological significance to them. The “Pilot Streams” pilot study implemented by ELF and the Adour Garonne Water Board reconstitutes 16 controlled ecosystems. In addition to their value as a research tool, these “Pilot Streams” also make it possible to predict the impact of waste or products in surface waters.

References

Adams, S.M., Shepard, K.L., Greeley, M.S., Jimenez, B.D., Ryon, M.G., Shugart, L.R., McCarthy, J.F. and Himon, D.E., (1989). The use of bioindicators for assessing the effects of pollutant stress on fish. *Marine Environ. Research*, **28**, 459–464.

AFNOR (1992). Détermination de l’indice biologique global normalisé. Standard NF T90-350, 1–9.

Agences de l’eau (1993). Etude bibliographique des méthodes biologiques d’évaluation de la qualité des eaux de surface continentales. *Etude inter-agences* No **35**, 400 p.

Belanger, S.F., (1997). Literature review and analysis of biological complexity in model stream ecosystems; influence of size and experimental design. *Ecotoxicol. Environ. Saf.* (1997), **36**(1), 1–16.

Coste, M. and Prygiel, J. (1993).Utilisation des indices diatomiques pour la mesure de la qualité des eaux du bassin Artois-Picardie: bilan et perspectives – *Annales Limnologie*, **29**(3–4), 255–267.

Cossu, C., Doyotte, A., Jacquin, M.C., Babut, M., Exinger, A. and Vasseur, P., (1997). Glutathione reductase, selenium-dependent glutathione peroxidase, glutathione levels, and lipid peroxidation in freshwater bivalves, *Unio Lumidus*, as biomarkers of aquatic contamination in field studies. *Ecotoxicol. Environ. Saf.*, **38**(2), 122–131.

Garrigues, P., Narbonne, J.F., Lafaurie, M., Ribera, D., Lemaire, P., Raoux, C., Michel, X., Salaun, J.P., Monod, J.L and Romco, M. (1993). GICBEM experience (1986–1990). *The Science of Total Environm.*, **139/140**, pp. 225–236.

Kosinski, R.J. (1989). Artificial streams in ecotoxicological research. *In Aquatic ecotoxicology* : *fundamental concepts and methodologies*, vol I,. Boudou and Ribeyre Eds., CRC PressBoca Raton, F.L., pp. 297–316.

Labrot, F., Ribera, D., Saint-Denis, M. and Narbonne, J.F. (1996). *In vitro* and *in vivo* studies of potential biomarkers of lead and uranium contamination: lipid peroxydation, acetyl-cholinesterase, catalase et glutahion peroxydase activities in three non mamalian species. *Biomarkers*, **1**(1), 21–28.

Lafont, M., Juget, J. and Rofes, G. (1991). Un indice biologique lacustre basé sur l’examen des oligochètes. *Rev. Sciences de l’eau*, **4**, 253–268.

Lafont, M. (1984). Oligochaete communities as biological descriptors of pollution in the fine sediments of rivers. *Hydrobiologia* **115**, 127–129.

Livingstone, D.R. (1993). Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. *J. Chem. Tech. Biotechnol*. **57**, 195–211.

Melancon, M.J. (1995). Bioindicators used in aquatic and terrestrial monitoring. *Handb. Ecotoxicol. Hoffman D.J*., pp. 220–240.

Milam, C.D. and Farris, J.L., (1998). Risk identification associated withe iron-dominated mine discharges and their effect upon freshwater bivalves. *Environ. Toxicol. Chem*., **17**(8), 1611–1619.

Narbonne, J.F., Djomo, J.E., Ribera, D., Ferrier, V. and Garrigues, P. (1999). Accumulation kinetics of polycyclics aromatic hydrocarbons adsorbed to sediment by the mollusk *Corbicula fluminea. Ecotoxicol. Env. Saf*. **42**, 1–8.

Narbonne, J.F., Garrigues, P., Ribera, D., Raoux, C., Mathieu, A., Lemaire, P., Salaun, J.P. and Lafaurie, M. (1991). Mixed-function oxygenase enzymes as tools for pollution monotoring : field studies on the French coast of the mediterranean sea. *Comp. Biochem. Physiol*., **100C**(1/2), 37–42.

Nylander, W. (1866). Les lichens du jardin du Luxembourg, *Bull. Soc. Bot. Fr*., **13**, 364.

Pusey, B.J., Arthington, A.H. and Flanders, T.J. (1994). An outdoor replicated artificial stream system : design, operating conditions, and inital invertebrates colonization. *Ecotoxicology and environ. safety*. **27**, 177–191.

Rodgers, J.II., Crossland, F.R., Kline, E.R., Gillepie, W.B., Figueroa, R.A. and Dorn, P.B., (1996). Design and construction of model stream ecosystems. *Ecotox. Environ. Safety*, **33**, 30–37.

Van der Oost, R., Vindimian, E., Van den Brink, P.J., Satumalay, K., Heida, H. and P.E. Vermeulen, N. (1997). Biomonitoring aquatic pollution with feral eel (Anguilla anguilla). *Aquat. toxicol*. **39**(1), 45–75.

Verneaux, J. (1973). Cours d'eau de Franche Comté (massif du Jura). Recherche écologiques sur le réseau hydrographique du Doubs. Essai de biotypologie. *Ann. Sci. Univ. Besançon. Biol. Anim*., **3**(9), 260.

Vidal, M.L., Basseres, A. and Narbonne, J.F, (2001). Interest of a multibiomarkers approach in the assessment of freshwater ecosystems quality ; laboratory and field studies. *Wat. Sci. Tech.* **44**(2–3) 309–316 (this issue)