BIO-INDICATORS AND CHEMICAL POLLUTION OF SURFACE WATERS*

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Abstract. Attention is given to two different approaches to determine the water quality in relation to toxic stress.

The first approach is based on direct observations on the state of health of the biota naturally occurring in the environment to be judged. In the original concept of biological indicators of water pollution the existence of differences in stress-susceptibility of different species is assumed. However, toxicity studies indicated that there are no species whose absence or presence gives information on the degree of toxic pollution. When attention is solely directed to health aspects of one species, a higher specificity and response-rate is obtained, but water quality indexing is not possible. Examples of effects of chemical pollution on the health of fish from the river Rhine are presented.

The second approach is based on indirect observations, determining the water quality by examination of the state of health of organisms experimentally exposed to the water under controlled conditions. To save testing time it is useful to concentrate the toxic compounds prior to testing. An example of these methods is given, describing the water quality of the rivers Rhine and Meuse in terms of toxicity and mutagenicity.

As both approaches are complementary with respect to ecological significance and specificity, it is recommended to apply them simultaneously to obtain appropriate information on environmental quality and stress factors.

1. Introduction

The strength of biological methods for environmental judgement is the acquisition of information on the total effect of perturbations in a rather unique and direct way. However, this is also a weakness. Although each stress contributes to a loss of energy (Odum, 1967), the ecosystem response to a stressor depends on the point of attack of the stressor on the system (Lugo, 1978), as well as on the properties of the ecosystem. Therefore a proper quality assessment based on biological characteristics needs a thorough knowledge of the relationships between the type and extension of the stress and the response of the system. As to pertubations by toxic chemicals, this knowledge is insufficient and therefore environmental qualification is seriously hampered. In case of toxic substances biological monitoring should be based on the measurement of (1) deleterious effects specific for toxicity, (2) which are of ecological significance, (3) can be measured accurately, (4) with a high signal to noise ratio, (5) with a high response rate, (6) which are perceptible over a wide range of stress intensity, (7) which are reversible, (8) can be expressed in indices and (9) can be assessed at relativeley moderate costs (UNESCO, 1980). In practice, some of these criteria are not consistent with each other since their fullfilment is directed by different levels of biological integration. In this

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paper examples are given to emphasize the need to use different biological methods to indicate the stress caused by toxic chemicals in the aquatic environment.

2. Stress Quantification Based on Field Observations

2.1. Community studies

The existence of 'keystone' species for chemical stress was already recognized at the turn of this century. Kolkwitz and Marsson (1909) introduced a system, in which species are classified according to their tolerance to biodegradable organics. Although extended by many authors, this so-called saprobian system has not been essentially altered (Sládeček, 1973; Persoone, 1979; Hawkes, 1982).

As for toxicants, it is known that there are large differences in the susceptibility between species (McKee and Wolf, 1963). Differences in the susceptibility of species occupying crucial positions in the food-web may have far-reaching consequences for the structure and functioning of an ecosystem. To indicate whether or not there is some proof for the existence of 'keystone' species relative to toxic compounds, results of some comparitive toxicological studies are evaluated.

The results of a study on the (sub)acute toxicity of 15 chemicals to 22 different species are summarized in Table I and II. (Slooff *et al.*, 1983a) Table I shows that the sensitivity to the toxicants is highly variable amongst the species depending on the chemical tested. This leads to the conclusion that compound-specific indicator species may be recognized. However, when the relative susceptibility of a species based on the individual

TABLE I

Maximum difference in the susceptibility of 22 fresh water species for toxic compounds based on (sub)acute toxicity (Slooff *et al.*, 1983a)

Test compounds	Factorial difference in susceptibility
Allylamine	8970
Aniline	8000
Cadmiumnitrate	2800
Mercury (II)chloride	1000
Pyridine	364
Acetone	278
Pentachlorophenol	185
Ethylpropionate	87
n-Propanol	68
Benzene	58
o-Cresol	46
Trichloroethylene	40
Salicylaldehyde	33
Ethylacetate	32
n-Heptanol	30

TABLE II

Relative susceptibility of 22 fresh water species for 15 chemicals based on (sub)acute toxicity (Slooff *et al.*, 1983a)

Species	Taxonomical group	Relative susceptibility
Pseudomonas putida	Bacteria	4.0
Microcystis aeruginosa		1.2
Chlorella pyrenoidosa		4.2
Scenedesmus pannonicus	Algae	4.2
Selenastrum capricornutum		3.5
Entosiphon sulcatum		2.0
Uronema parduczi	Protozoa	5.3
Chilomonas paramacium		3.2
Daphnia magna		1.4
Daphnia pulex	Crustaceans	1.0
Daphnia cucullata		1.2
Aedes aegypti	Insects	4.6
Culex pipiens		6.3
Lymnaea stagnalis	Molluscs	2.9
Hydra oligactis	Coelenterates	4.6
Leuciscus idus		3.5
Salmo gairdneri		1.7
Poecilia reticulata	Fish	5.3
Oryzias latipes		5.3
Pimephales promelas		1.8
Xenopus laevis	Amphibians	3.7
Ambystoma mexicanum		2.7

toxicity data on all compounds are taken into account (Table II), the differences are much smaller. Therefore the existence of indicator species for gross chemical pollution can be seriously doubted.

In a similar study the acute toxicity of the same 15 chemicals to 13 different macro invertebrate species was determined (Slooff, 1983a). The test organisms used were representatives of 'keystone' groups for the whole range in the saprobian systems. From the results presented in Table III it can be derived that the differences in relative susceptibility of the invertebrate species are rather small. Table IV, showing the toxicity of an organic mixture concentrated from water of the river Rhine, confirms the assumption that 'keystone' species for toxic stress resulting from a variety of chemicals are not likely to be identified. Yet, this conclusion can be criticized for obvious reasons, taking into account the relatively short exposure time and the disregard of sublethal effects like influences on reproduction, development and growth.

However, a study on the long-term effects of toxicants on different fresh water species yielded results similar to those obtained from the (sub)acute tests (Slooff and Canton, 1983).

TABLE III

Relative succeptibility of macro invertebrates for 15 chemicals (see Table I) based on acute toxicity (Slooff, 1983a)

Species	Relative susceptibility
Tubificidae	3.2
Chironomus gr. thummi	2.3
Erpobdella octoculata	3.3
Asellus aquaticus	1.9
Lymnaea stagnalis	2.6
Dugesia cf lugubris	2.8
Hydra oligactis	2.7
Corixa punctata	3.4
Gammarus pulex	1.0
Ischnura elegans	1.5
Nemoura cinera	2.6
Cloëon dipterum	4.9

TABLE IV

The tolerance of benthic invertebrates to mixtures of organic compounds concentrated from water of the river Rhine (Slooff, 1983a)

Species	Concentration factor which results in 50% mortality in 48 h (LCF50)
Oligochaeta	
Tubificidae	159 ×
Diptera	
Chironomus gr. thummi	144 ×
Isopoda	
Asellus aquaticus	158 ×
Amphidopa	
Gammarus pulex	192 ×
Trichoptera	
Athripsodes cinereus	124 ×
Ephemeroptera	
Cloëon dipterum	184 ×
Plecoptera	
Nemoura cinera	214 ×

These examples stress the warning of Sládeček (1973) that traditional biological water classification in which the water quality is expressed in terms of the presence or relative abundance of certain species is not applicable to toxic pollution. Only when a surface water is polluted by a few known toxicants, certain biotic indices may be workable (Winget and Magnum, 1979; Lawrence and Harris, 1979). Loading with a variety of toxic chemicals will result in a non-specific decrease of species richness and population size.

Therefore, the measurement of parameters describing the structure or functioning of ecosystems seems to be of little value in estimating water quality explicitly related to toxicity.

2.2. Single species studies

Laboratory studies on individuals have revealed much about possible toxic effects of all kinds of chemicals. The knowledge gained from this expertise can contribute to the recognition of biochemical, physiological and morphological effects possibly observable in the field related to toxicity. In this way a study on the health aspects of bream (*Abramis brama*) inhabiting surface waters of a different degree of chemical pollution (the rivers Rhine and Meuse, and Lake Braassem as a reference) was performed. The results of this study are summarized in Table V. Bream from the river Rhine areas showed a somewhat higher prevalence of tumors than fish from the river Meuse (hepatocellular carcinoma),

TABLE V

Some parameters on the state of health of bream (*Abramis brama*) from the rivers Rhine and Meuse. The parameters are expressed relative to reference values obtained from Lake Braassem fish.

Biological parameter	Meuse	Rhine	Waal	Lek
Prevalence of tumors	≥ 1	$\longrightarrow$	≥ 1.7	$\longleftarrow$
Prevalence of deformed fins	1.5	3.6	3.8	5.8
Prevalence of pugheadedness	1.7	9.0	8.7	9.7
Relative liver weight	2.0	2.3	2.1	1.7
Hepatic enzyme activity	1.8	2.2	-	-
Fecundity	1.0	1.3	1.0	1.2
Annual mortality (♀♀)	1.5	2.1	1.7	1.4
Annual mortality (♂♂)	1.5	1.9	1.9	1.7

whereas no neoplasms were found in Lake Braassem fish (Slooff, 1983b). This accordates with the observation that bile fluid of Rhine fish is relatively highly mutagenic (Van Kreijl *et al.*, 1982) and that liver homogenates of Rhine fish livers were able to activate pre-mutagens in the Ames-test (Slooff and Van Kreijl, 1982). Besides the livers of Rhine fish were significantly enlarged (hypertrophy; Slooff *et al.*, 1983b). Liver enlargement often results from exposure to xenobiotic compounds and is usually associated with induction or stimulation of hepatic enzyme activities. Based on differences both in time and place of liver mono oxygenase activity and of degree of metabolic activation of pre-mutagens such an association was established (Slooff and Van Kreijl, 1982; Slooff *et al.*, 1983b). Furthermore, several skeletal anomalies were observed such as pugheadedness, deformed fins, absence of fins and girdles, asymmetric crania, shortened operculae, fusions of vertebrae and spinal curvatures (Slooff, 1982). The highest frequency of occurrence was found in the river Rhine areas, the lowest in Lake Braassem. Based on the age-frequency distribution of representative samples of the local populations, the annual mortality proved to be the highest amongst fish from the Rhine areas (Slooff and De Zwart, 1983). This higher mortality rate can not be explained by a higher density of parasites or predators, nor by a more intensive fishing. Rhine fish displayed also a higher fecundity which may be a compensatory response to the enhanced mortality. This hypothesis is strongly supported by (1) the observations on other fish species that showed a significant increase in fecundity when the size of the population was reduced by fishing (e.g. Jensen, 1971), and (2) laboratory observations on neutralization of toxic effects at low pollutant concentrations by antagonistic responses of regulatory mechanisms (e.g. Stebbing, 1981). Besides information on the water quality in terms of toxic stress, the observations also indicated that the load of the river Rhine with toxicants has been reduced during the last years. The first indication is that the frequency of occurrence of the most prevalent skeletal anomalies decreased for succeeding year classes (Slooff, 1982), which corresponds with a decrease of concentrations of possible causative chemicals in the last decade (Rijkswaterstaat, 1980; De Kruijf, 1983). Also the back-calculated length of 5-yr old bream of succeeding spawning years (1966–1976) increased (Slooff and De Zwart, 1983). A third indication of improved water quality is that in the period of 1979–1982 the liver size of Rhine fish decreased to normal proportions, accompanied by diminishing of enzyme activities to levels that do not deviate significantly from those of Lake Braassem fish (Slooff *et al.*, 1983b).

From this study it can be concluded that field observations on one species can be used to indicate temporal and geographic differences in toxic stress in surface waters. Although it can be assumed that, in principle, every species and parameter is suitable for this purpose, this approach has several drawbacks. Leaving aside the rather high research costs and the problems related to whether or not standardization of species and parameters is desirable, most problems are related to the transformation of the obtained data into an acceptable water quality index. Definitely, an indicator is not an index. To reach that state, it has to be compared to a standard (Inhaber, 1976). If reliable reference c.q. standard values are available, this will be no problem for the parameters separately. In the presented studies the data on Lake Braassem fish can be considered as reliable references since both chemical and biological studies revealed that the water quality of Lake Braassem is rather good (Anonymous, 1979; Hovenkamp-Obbema *et al.*, 1982). However, to arrive at a single index for comparison purposes, the indices on the separate parameters should be combined, taken into account the relative ecological significance of the parameters measured. In practice, this process evokes severe problems. For most parameters knowledge is lacking which value is indicative for 'the worst' possible quality. In most cases it is not possible to mark the parameter value within an index range varying from e.g. 0–10. For example, if a frequency of occurrence of skeletal anomalies of 100% is considered to be the maximum, Rhine water should be qualified as rather good as 'only' 20% of the fish showed skeletal anomalies. It is even more complicated when the maximum deviation from normal is not known (e.g. relative liver weights). Also there is insufficient knowledge to express the importance according to the ecological value of the impaired properties and to determine whether or not the properties are mutually independent. Therefore, it can be concluded that observations on the health aspects of one species will never lead to a uniform water quality index; their strength is solely directed to give evidence of toxic stress and the possible causative agents.

3. Stress Qualification Based on Laboratory Observations

Toxic stress can be determined also experimentally. Water quality can be assessed by exposing one group of test organisms to the water concerned and, subsequently, comparing their state of health with that of another group of organisms maintained in unpolluted water under strictly defined conditions (e.g. Van der Gaag *et al.*, 1983). Compared to field studies, this approach has the important advantage of a relatively high signal to noise ratio. However, laboratory experiments are often too time-consuming and too expensive to be performed on a routine base. This disadvantage, which is almost typical for biological methods, has led to the inferior role of biological methods compared to chemical measurements in assessing and controlling environmental quality. The only way to change the attitude towards biological methods is to cast them in the same mould as chemical techniques. Thus, the experimental design has to be modified in such a way that only sum-parameters are studied in simple and standardized tests giving results within a few days. A recently developed feature in this field is to apply a technique to concentrate toxic compounds from the water before testing to speed up the response rate. This also enables the testing of several concentrations in order to determine a concentration-response relationship. This method has been applied to assess the degree of hazardous chemical pollution of surface waters in The Netherlands (Kool *et al.*, 1981; Slooff and Van Kreijl, 1982; Slooff *et al.*, 1983c). Concentrates of the water were examined on mutagenic activity with the Ames *Salmonella*/microsome assay and on toxicity, using an acute (48 h) fish mortality test. Figure 1 shows that both mutagenic

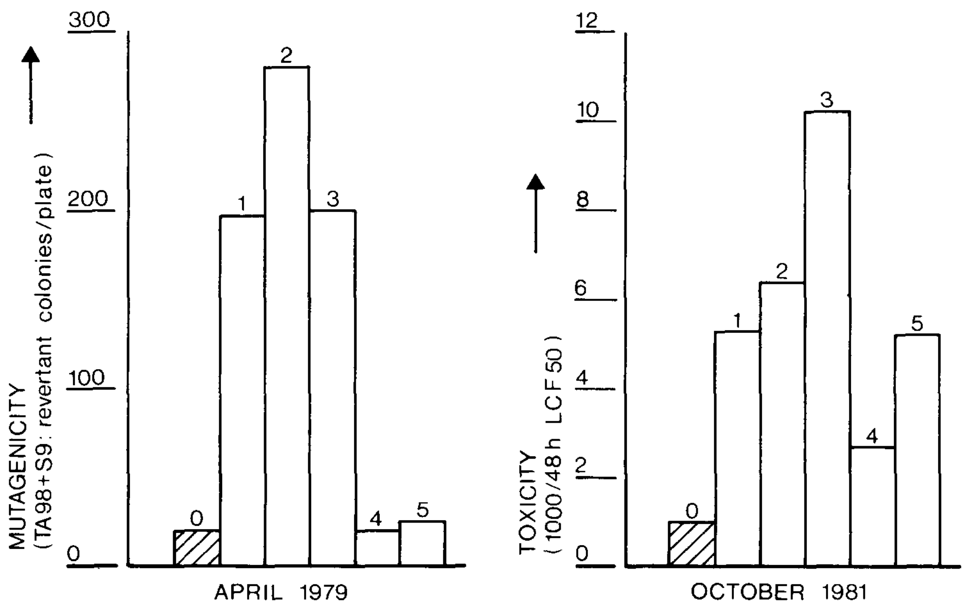

Fig. 1. Mutagenicity and toxicity of concentrates of water of the rivers Rhine and Meuse (0 = control; 1 = Rhine at Lobith; 2 = Waal at Gorinchem; 3 = Lek at Vreeswijk; 4 = Meuse at Eysden; 5 = Meuse at Keizersveer) (After Slooff and Van Kreijl, 1982; Slooff *et al.*, 1983c).

activity and toxicity of water of the river Rhine areas (Rhine, Waal, Lek) are generally higher than those of water of the river Meuse areas. Still the results of such studies have to be interpreted with some caution. Each concentration technique is specific for certain groups of chemicals and its application will result in an alteration of the chemical composition of the concentrate. However, the results give definitely an indication about the presence of hazardous chemicals in the aquatic environment. Therefore it is recommended to incorporate this kind of bioassay as a routine measurement of water quality in monitoring programmes.

TABLE VI

Advantages (+) and disadvantages (−) of the direct and indirect method for determining water quality related to toxic pollution.

Research method		Ecological relevance	Signal to noise ratio	Specificity	Response rate	Response range	Indexibility	Cost
Direct	community studies	+ +	− −	− −	+ +	+ +	+	−
	single species studies	+	±	±	±	±	− −	−
Indirect	longterm studies	±	+	±	±	±	− −	−
	short term studies	− −	+ +	+ +	− −	− −	+ +	+

Conclusions

In the present paper some examples were given of two principally different approaches in assessing water quality in relation to toxic stress: the direct (field studies) and indirect (laboratory studies) method. As summarized in Table VI both have their advantages and limitations. Major disadvantages of the direct method are the low specificity to toxic stress and the low signal to noise ratio, whereas the indirect method usually suffers from a poor ecological significance. Since both methods are complementary, it is recommended to apply them simultaneously to obtain information on the ecological state of surface waters in relation to possible causative factors, such as toxic pollution.

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