The state of the aquatic environment as deducted from bioassays-quantitated ecosystem risks

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Abstract: - Bioassays represent powerful tools for the assessment of environmental quality. This report focuses on the measurement of the toxicity of surface water samples towards a battery of several differing aquatic organisms. The toxicity is determined by isolating toxic organic chemicals from the surface water by use of a concentration procedure, and by exposing the organisms to the concentrates thus obtained. Subsequently this method evaluates the results statistically, to yield a measure for the degree to which the water represents a risk for the aquatic ecosystem. This risk is expressed in terms of 'toxic potency' which represents the fraction of the ecosystem that is affected in any way by the environmental conditions.

Key-Words: - surface water monitoring ecotoxicology risk assessment

1 Introduction

The EU Water Framework Directive wants the European member states to report on the state of the aquatic environment on a regular basis. Surface waters must have both a 'good ecological quality' and a 'good chemical quality'. For the latter, member states must have a fair view of the degree to which chemical concentrations exceed the standards. However, any regular evaluation of the surface water quality by means of measuring the chemicals present is impracticable, because of the countless number of substances that may occur in water samples. Furthermore, analytical methods for the measurement of many chemicals are lacking, whereas in many instances standards have not (yet) been derived. An evaluation as to whether chemicals are present in acceptable concentrations or not is therefore practically impossible. In addition, individual measurements of substances do not account for any possible additive effect of chemicals that are present concomitantly in the same sample.

Ecological observations on aquatic systems do give information on the impact of the water quality on flora and fauna, but unfortunately under many circumstances such observations may prove rather insensitive, due to a relatively long period of time that may elapse between the actual moment of exposure to the chemicals and the moment at which the effect can be observed.

Bioassays render the opportunity to measure the total effect of the presence of all chemicals in a water sample and to deduce from such measurements the potential effect, *i.e.,* the ecological risk of the water quality towards the aqueous system.

2 Methods

The procedure of sampling and of preparation of the samples has been described earlier [1]. In short, it is based on a selective concentration of organic compounds from surface water samples, upon which the toxic effect of these compounds is determined with bioassays. An evaluation of the toxic end-points thus obtained yields a quantitative approximation of the ecosystem risks.

2.1 Sampling of surface water

Samples of surface water (60 litres) were taken at regular monitoring sites at regular intervals during a year. The samples were taken immediately to the laboratory by cooled transport.

2.2 Preparation of samples

Immediately upon arrival of the samples at the laboratory a 1:1 mixture of well-purified XAD-4 and XAD-8 resins was added, after which the organic compounds in the samples were allowed to adsorb onto the resin under continuous agitation during 48 h at room temperature. Thereafter the loaded resin was isolated by sieving, and dried subsequently overnight at room temperature. The organic compounds were transferred from the resin to acetone by elution, which yielded a 1000-fold concentrate (60 ml).

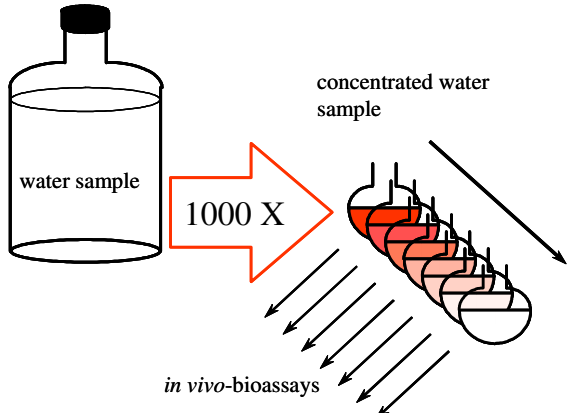

Fig 1. Schematic representation of the concentration procedure by which surface water samples are prepared for use in bioassay tests.

2.3 Bioassays

Bioassays were performed on diluted samples of the 1000-fold concentrate. For this, a dilution series was made (Fig. 1) and test organisms were exposed to the dilutions using standard ecotoxicological protocols. Inherent to this dilution method, high toxicity of water samples was found when effects were found in the relatively less concentrated samples, whereas a toxic effect of 'clean' water only was observed in the more concentrated samples. Endpoints of the toxicity tests were expressed as EC_{50}^{f} or LC_{50}^{f}, where the suffix 'f' stands for the use of concentration factors instead of the usual substance concentrations. The bioassays were chosen such, that (i) only a small volume of the original concentrated sample is sufficient to obtain results of the tests, (ii) results can be obtained in a relatively short period of time, and (iii) the technique by which the bioassay is done is relatively simple (e.g., because it is commercially available).

2.3.1 Microtox [3]

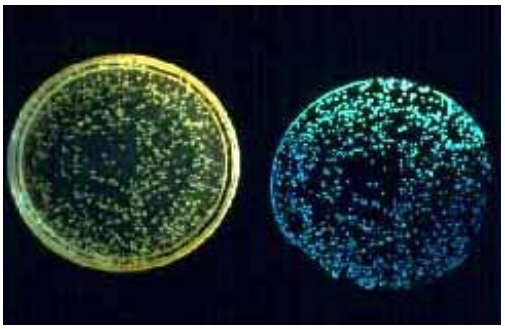

Fig 2. Agar plates with luminescent V. fischeri *colonies.*

Luminescence of the bacterium *Vibrio fischeri* (Fig. 2) is a measure of the energy state of the organism. It is determined in a luminometer after exposure of the bacteria during 5 or 15 minutes to dilutions of the sample. The EC_{50}^{f} is taken as the concentration factor that decreases light emission by 50%.

2.3.2 Algae (PAM [4])

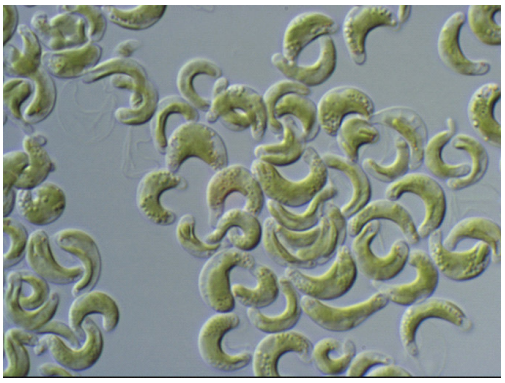

Fig 3. Pseudokirchneriella subcapitata *under the microscope.*

Changes in chlorophyll fluorescence by *Pseudokirchneriella subcapitata* (Fig. 3) in reponse to pulses of light are a measure of the efficiency of photosynthesis by this alga. It is measured in a fluorescence spectrophotometer (Water-PAM, Heinz Walz GmbH, Effeltrich, Germany) after 4,5 hours of exposure to dilutions of the sample. The EC_{50}^{f} is taken as the concentration factor that decreases fluorescence yield by 50%.

2.3.3 Daphnia IQ [5]

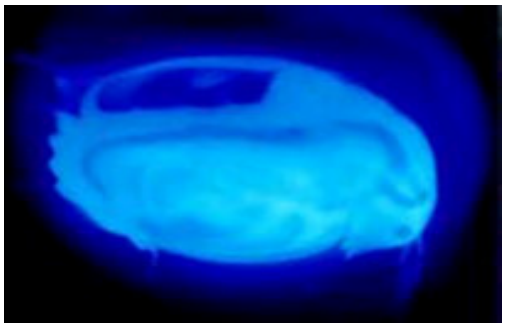

Fig 4. Active fluorescing Daphnia magna *as observed in the IQ test.*

The water flea *Daphnia* (Fig. 4) expresses its β-galactosidase enzyme activity by cleaving 4-methyl-umbelliferyl β-D-galactoside which yields the fluorescing umbelliferyl determinant. Fluorescence of the daphnids upon irradiance with ultraviolet light was observed by eye. The EC_{50}^{f} is taken as the concentration leading to 50% inhibition of fluorescing daphnids after 1,25 hour exposure.

2.3.4 Rotox [6]

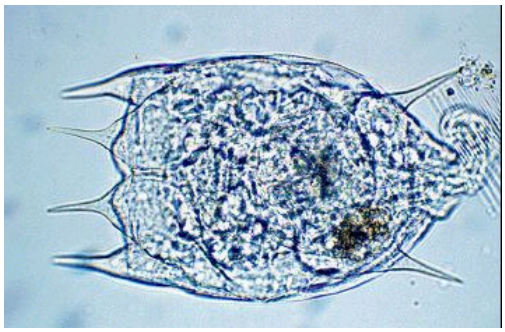

Fig 5. Brachionus calyciflorus *under the microscope.*

Mortality of the rotifer *Brachionus calyciflorus* (Fig. 5) in response to the samples was expressed as LC_{50}^{f}, the concentration factor leading to 50% mortality after a 24 hour exposure.

2.3.5 Thamnotox [7]

Fig 6. Brachionus calyciflorus *under the microscope.*

Mortality of the crustacean *Thamnocephalus platyurus* (Fig. 6) in response to the samples was expressed as LC_{50}^{f}, the concentration leading to 50% mortality after a 24 hour exposure.

2.4 Risk assessment

Since the bioassays represent different types of species in morphology and trophic level, it was assumed that from their endpoints a species sensitivity distribution could be deducted, which was specific for the types of toxic substances in the samples and for the aquatic ecosystem. Classically, species sensitivity distributions are valuable tools in risk assessment, because they represent the number of species that may be affected at a given toxicant concentration. When dealing with toxicant mixtures, such a distribution can be set up comparably from concentration factors instead of substance concentrations.

In order to obtain Fig. 7, the acute endpoints from the bioassays were fitted onto a distribution curve. The resulting cumulative species sensitivity distribution curve was extrapolated to a chronic no-effect one by assuming an average acute-to-chronic ratio of 10 [1]. The potentially affected fraction in the original water sample was inferred from the value of this curve when the concentration factor = 1. This parameter was defined as the toxic potency of the surface water sample. Mathematical and statistical descriptions of the method, as well as a discussion on its uncertainties, have been presented elsewhere [1].

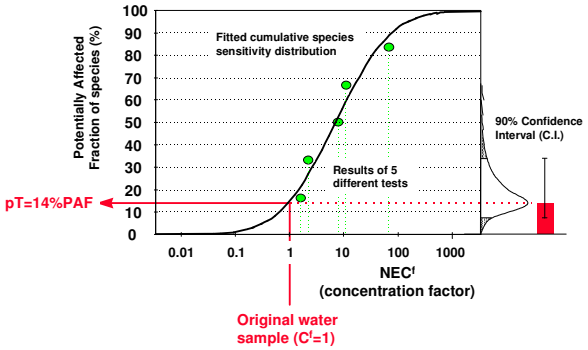

Fig 7. Typical example of a cumulative species sensitivity distribution curve for the end-points of five bioassay tests, and its extrapolation for the ecological risk in the not concentrated sample.

3 Results and Discussion

During the year, surface water samples were taken in bimonthly intervals. This was done at several regular monitoring sites in The Netherlands, in the catchment areas of the Rhine, Meuse and Scheldt rivers. In many instances the surface waters appeared to be more toxic in summer than in winter. Fig 8 shows that in January the sample had to be concentrated 80-fold in order to obtain the EC_{50}^{f} toxic level in the algae PAM test, whereas in July a mere 4-fold concentration gave this toxic endpoint. This course in time of the toxicity is typical for the effect on algae, but not necessarily for the other organisms we examined in our bioassays. The cause of this phenomenon is not known. Chemical analysis of the surface water shows, of course, fluctuations in substance concentrations due to differing loads of water over the seasons. However the occurrence of specific toxic compounds in summer, for instance pesticides, cannot be excluded.

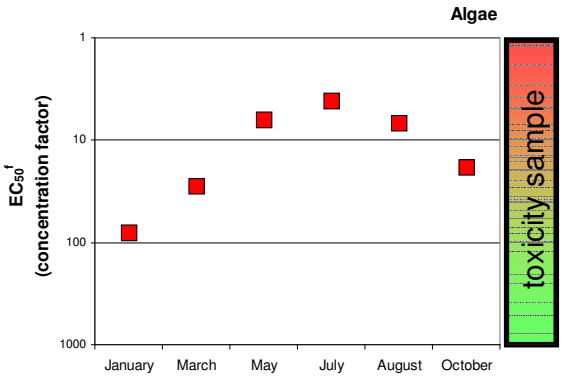

Fig 8. Course in time of the EC_{50}^{f} of Meuse (Eijsden site) samples from 2002 towards algae in the PAM test.

Fig 9 shows the course in time of the measured toxic effects towards the other test organisms. For daphnids a trend was found that was reverse to that of the algae, and for the other test organisms the outcome of the assay was rather indifferent towards seasonal changes.

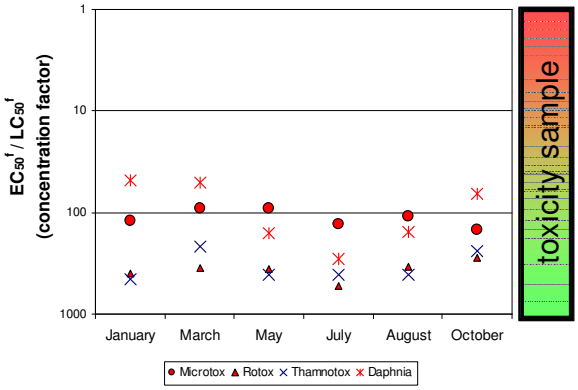

Fig 9. Course in time of the EC_{50}^{f} and LC_{50}^{f} of Meuse (Eijsden site) samples from 2002 towards the Microtox, Rotox, Thamnotox and Daphnia *IQ tests, respectively.*

With the sets of endpoints obtained from each bimonthly sample, risk assessment was done by fitting them to a sensitivity distribution curve, as described in the Methods section. From this calculation it appeared that in the summer season (May – August) the surface water was risky for approximately 10% of the aquatic species, whereas in the cold and wet winter season (October – March) the water was much less toxic for the aquatic organisms.

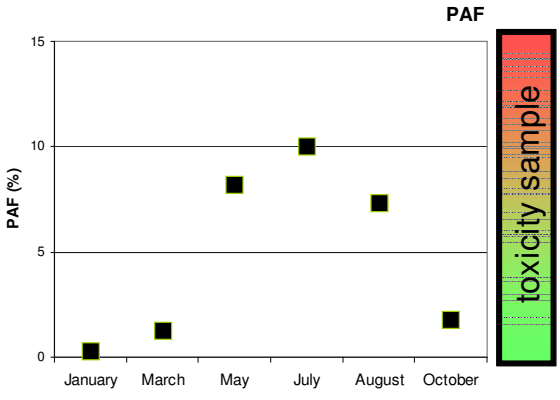

Fig 10. Ecological risk (potentially affected fraction of the ecosystem) of Meuse (Eijsden site) samples from 2002.

Since 1996 the state of the surface waters in The Netherlands has been monitored using the bioassays presented in this paper. By now their use has proved to be a solid method to assess the

ecological risk imposed by the chemicals concentrated from the surface water. Being integral, it is an attractive method for monitoring the chemical state of the environment and it is complementary to the chemical analysis of the individual substances.

Bioassays may play an important part within the monitoring system demanded by the Water Framework Directive. Under circumstances when a good ecological state is not observed in a surface water system, the results of the bioassay method presented here may indicate whether any (known or unknown) substances may cause a relevant effect on the ecosystem. Thus, bioassays are valuable tools helping water management authorities in focusing on measures to improve the water quality in order to attain a good ecological state.

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