Verh. Internat. Verein. Limnol.	24	2313–2321	Stuttgart, September 1991

Bioassessment of the toxicity of freshwater sediment

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There are a number of toxic chemicals, potentially associated with sediments, which are not routinely monitored for and for which there is little or no toxicological information. Therefore, it is difficult to set chemically-based sediment quality criteria for these compounds. It is also difficult to conduct chemical analyses if the types of chemicals are unknown. It has been estimated that there are approximately 63,000 chemicals in common use (HUNTER 1987) and over 8.5 million chemicals have been documented in the American Chemical Society's Chemical Abstract Service (CAS) as of April 1989. In addition, other chemicals may form as side-reactions or due to chemical reactions in the environment. Therefore, many chemicals, potentially associated with sediments, are not part of routine monitoring protocols.

The toxicity toward aquatic organisms of only a few chemicals, other than pesticides, have been determined. It has been estimated that only 5 to 10 % of the known chemicals have been tested for their toxic properties and less than 1 % of the 50,000 or so compounds manufactured in the United States have been tested for their toxicity to aquatic organisms (MARTELL et al. 1988). The situation is complicated by the fact that organisms are exposed, simultaneously, to a number of contaminants in sediments (GIESY et al. 1990) and bioassays can often classify sediments as toxic even when the concentrations of standard contaminants are small (ANKLEY et al. 1989).

Aquatic sediments can become contaminated with both inorganic and organic chemicals, which are sorbed to particulate matter or in solution in sediment pore water (KNIGHT 1984, CAIRNS et al. 1984, SALOMONS et al. 1987, TESSIER & CAMPBELL 1987, CHAPMAN 1987). These contaminants can be accumulated by and directly affect benthic organisms (CIBOROWSKI & CORKUM 1988, GIESY et al. 1988 a, 1989, SCHLOESSER 1988) or affect other aquatic organisms by becoming bioavailable through resuspension or leaching (LEE et al. 1978, JONES & LEE 1978, MALUEG et al. 1983, NEBEKER et al. 1983).

In some locations, the use of chemical analyses to determine potential adverse effects and map the distribution of the contamination can be done by chemical analyses because there is primarily one dominant contaminant, such as PCBs in Waukegan Harbor, WI, USA, or the Hudson River, NY, USA. However, the presence of specific contaminants or exceedence of established criteria for concentrations of chemicals in sediments is not always the primary concern relative to sediment contaminants. In Puget Sound, Washington State, USA, the observed biological effects were a greater concern than were concentrations of typical sediment contaminants (Anon. 1988).

An alternative approach to chemical criteria would be to assign numerical criteria to biological response variables. These could be agreed upon and applied in a uniform numerical chemically-based criteria, but would account for the incomplete chemical information available for most locations and integrate interactions among contaminants. This approach could also include assays to measure bioconcentration and mutagenic potential as well as lethality.

A number of biological methods have been suggested to assess the toxicity of sediment. Each of the proposed assays has positive and negative attributes. Here we propose a battery of rapid, simple, reproducible bioassays to screen for the toxicity of sediments. The assays recommended for inclusion in the screening battery for evaluation of sediment toxicity are: 1) the bioluminescence inhibition test with *Photobacterium phosphoreum* (Microtox^{R}), 2) an algal assay using *Selenastrum capricornutum* PRINZ, 3) a benthic invertebrate chronic growth test with *Chironomus tentans*, and 4) an acute lethality test with the cladoceran *Daphnia magna*. This battery of tests is proposed to be used as an initial phase of establishing site-specific, biology-based sediment quality criteria. These assays can also be used in conjunction with toxicity identification and evaluation (TIE) procedures, which use selective fractionations and chemical and physical adjustments of sediments and sediment pore waters to isolate and identify the primary toxic agents in sediments.

The dose-response relationship characterizes the toxicity of a mixture of contaminants in a sediment and permits quantification of the threshold

0368-0770/91/0024-2313 $ 2.25

concentrations of contaminants in sediments for adverse effects on organisms (GIESY et al. 1988 a, 1990). To determine the dose-response relationship for a sediment or pore waters (interstitial water), one must perform dilutions.

Due to the complications of making dilutions of whole sediment, the use of pore water extracts and elutriates has been proposed (Anon. 1977, BAHNICK et al. 1980, MUNAWAR et al. 1983, CAIRNS et al. 1984, CHAPMAN & FINK 1984, NEBEKER et al. 1984, GIESY et al. 1988 a, b, 1990). Aqueous solutions of the contaminants associated with sediments can be made by: 1) extracting the pore water (BATLEY & GILES 1980, BELLAR et al. 1980, JENNE et al. 1980); 2) making an elutriate (Anon. 1977, HOKE & PRATER 1980); or 3) making differential extractions to selectively remove particular classes of toxic substances (SAMOILOFF et al. 1983). A number of parameters, such as organic carbon content and particle size distribution, can affect the availability of both metals and organic toxicants to benthic organisms (BABICH & STOTZKY 1977, WARD & YOUNG 1984, LAXEN 1985, OLIVER 1985).

The use of pore waters or elutriates as toxicant solutions has facilitated the testing of standard non-benthic bioassay organisms, such as *Daphnia magna* (BAHNICK et al. 1980, SAMOILOFF et al. 1983, MALUEG et al. 1984 a, b, SCHUYTEMA et al. 1984, CAIRNS et al. 1984, ZIEGENFUSS & ADAMS 1985, ZIEGENFUSS et al. 1986, GIESY et al. 1988 a, b, 1990), *Ceriodaphnia dubia*, *Pimephales promelas* (HOKE et al. 1989) and the bacterium *Photobacterium phosphoreum* (GIESY et al. 1988 a, b, 1990). The use of these organisms has facilitated the comparison of sediment extract or elutriate results with established surface water criteria. In addition, these techniques eliminate many of the problems associated with sediment dilutions and allow for use of an appropriate control treatment in a defined medium (NEBEKER et al. 1984). The relationship between the results of pore water or elutriate tests and those conducted with whole sediments is unclear (BAHNICK et al. 1980, CAIRNS et al. 1984, NEBEKER et al. 1984) because the partitioning of toxicants from the solid phase into the pore water or elutriate is dependent on many chemical and physical processes (SINEX et al. 1980, KNEZOVICH et al. 1987) which are not well understood. Nevertheless, these assays are effective screening tests.

Benthic organisms can be exposed to toxicants in either of two-ways; directly from sediment-bound contaminants or via the pore water. The simplification of sediment toxicity testing by pore water exposure, rather than whole sediment exposure, is based on the assumption that organisms receive most of their exposure through contact with the pore water (NEBEKER et al. 1984). The concentrations of toxic materials in the pore water are in a dynamic equilibrium with the solid phase and its associated contaminants. Most contaminants associated with sediments have limited solubility in water (HALL et al. 1986, KNEZOVICH et al. 1987) and it is possible to have a considerable excess of contaminant associated with the solid phase of a sediment such that observed toxicity may not be well correlated with the concentrations of toxic materials in the bulk sediment (PATRICK et al. 1977, SHANER & KNIGHT 1985, KOSALWAT & KNIGHT 1987 a, DITORO et al. 1990). Thus, pore waters and concentrations of their associated contaminants have been suggested as a more proximate measure of sediment toxicity (JONES & LEE 1978, LEE & JONES 1982).

The simple assays used can be of three types. These include: 1) assays with populations of endemic or surrogate populations to determine the direct toxicity of the sediments to bacteria, algae, invertebrates or vertebrates; 2) assays with populations of organisms to determine the potential of contaminants in the sediments to be bioaccumulated or cause long-term, adverse effects such as mutagenesis or teratogenesis, and 3) assays, which use biochemical endpoints to integrate the impact of mixtures of chemicals with the same mode of toxic action. Examples of these types of classes of compounds could be transition metals, planar, chlorinated hydrocarbons such as PCBs, chlorinated, dibenzo-p-dioxins and furans, herbicides, organo-phosphorus insecticides, surfactants and neutral, chlorinated solvents, which act as narcotics. It is difficult to use endemic species in toxicity bioassays. This is due, in part to the lack of consistency of species among geographic regions and lack of background toxicological information and methods for culturing and dosing of benthic organisms in sediments. For this reason biomonitoring with simple surrogate assays is advocated to develop site-specific sediment quality criteria (Anon. 1985). In addition to their use in screening sediments for toxicity, simple, biologically-based assays can be used to direct the allocation of resources for quantification of chemical toxicants. The differential responses of assays in the battery of tests can give insight into the causes of sediment toxicity due to knowledge of the differential sensitivities of the test species. Also, simple bioassays can be combined with chemical and physical fractionation techniques to determine the most

Table 1. Characteristics of ideal sediment bioassays.

– rapid
– simple
– replicable
– inexpensive
– standardized
– sensitive
– discriminatory
– ecologically relevant
– relatable to field effects
– useful in developing, and relatable to, regulatory standards

probable causes of observed toxicity of sediments. A battery of simple bioassays can be used to provide rapid, relative toxicity values, which account for bioavailability of and interactions among toxicants (GIESY et al. 1988 a, b, 1990). Assays also can provide dose-response toxicity for contaminated sediment above or below a threshold for adverse effects. Such information could be used to calculate the probability of effect in simulation models of effects. Because bioassays are a direct measure of functional responses, they should have more impact on the decision making process than criteria based on concentrations of chemicals.

Ideally, sediment toxicity bioassays and assay organisms should have the following characteristics (GIESY & HOKE 1989): 1) Test organisms should be easy to culture and maintain in the laboratory and be available for tests at any time. 2) The responses of control organisms should be predictable and constant. 3) The assay organisms should respond similarly to many classes of toxicants or organisms with sufficiently different responses should be included in a battery of tests (CHAPMAN et al. 1987). 4) The results of the assays should be related to ecologically relevant processes under field conditions. 5) The results of bioassays should be related to sediment or water quality standards and criteria. 6) The bioassays should be applicable to a number of sediment types and environments. 7) The assays and dilution methods should be chosen to provide information, which is correlated with observed adverse effects on organisms under field conditions (Anon. 1977, SWARTZ et al. 1982, MALUEG et al. 1984 a, b, NEBEKER et al. 1984). 8) The assays should be rapid, replicable, inexpensive and easily implemented so that large areas of concern can be surveyed rapidly with good resolution. 9) The assays should be standardized to facilitate widespread use. 10) They should be sensitive enough to identify potential problem sediments yet discriminatory enough to permit ranking of the relative toxicity of many samples (Table 1).

The advantages of standardized protocols for effluent and pure compound toxicity tests as presented by DAVIS (1977) are as follows: 1) use of uniform test protocols among laboratories, 2) increased data accuracy, 3) facilitation of test replication, and 4) increased comparative value of test data. These concepts also apply to sediment toxicity tests. The use of standard protocols has greater regulatory and legal impact by virtue of the verification process to which such protocols are subjected and the publication of the protocols themselves in both the open and "gray" literature. Standardization does, however, increase the risk that creative new approaches to problems may be ignored, particularly if these new approaches are at odds with existing regulatory agency policy (DAVIS 1977). Typical standard methods also may not address such concerns as delayed toxicity (BUIKEMA & BENFIELD 1979) or potentiation of contaminant effects as a result of the stress of a standard test environment (BUIKEMA et al. 1982). The potential also exists for inadequate characterization of contaminant effects in a changing natural environment as a result of the optimization of all conditions in a standard test (GECKLER et al. 1976). By increasing comparability, and reliability of test data, standard methods will facilitate investigations of the relative toxicity of different sediments and the sensitivity of different assays.

Toxicity assays provide information on the effects of contaminants on the test species and are envisioned as a tool for delimiting the extent of further investigations. Prioritization of further investigations can then be based on the results of the screening assays and chemical analyses utilized to determine probable contaminants and their concentrations responsible for the observed biological effects. The discriminatory ability of a particular assay is also an important consideration in assay selection. Some assays exhibit quantal "all or none" type responses, resulting in the classification of a particular sediment sample as either toxic or non-toxic. For comparative evaluation of effects, it is useful to be able to rank toxic sediment samples relative to one another. The discriminatory power of an assay is the principal factor involved in the ranking of "toxic" responses and is dependent on the measured response variable. The use of a continuous response variable such as weight gain, as in the *Chironomus tentans* 10-day growth reduction assay (GIESY et al. 1988 a), rath-

er than a quantal response variable such as lethality increases the discriminatory power of an assay.

Ecological relevance and correlation to field effects and regulatory standards are characteristics less likely to be found in screening assays but are important for definitive assays or assays designed to address site-specific questions using indigenous organisms. Definitive assays generally are used to develop dose-response relationships and estimate the proportion of a population affected by a given contaminant level. Although mortality of an organism would prohibit further reproductive contributions to the population, gross effects such as mortality may not be sensitive enough to determine subtle ecological effects. Growth and reproduction effects are generally more sensitive test endpoints and may be indicative of ecological changes which result in altered species distribution and community structure in field situations. Assays using surrogate species, such as *D. magna* or *C. dubia*, and assays using important indigenous organisms such as *Chironomus* sp. are valuable tools in sediment toxicity investigations. The use of surrogate species facilitates assessment of reproductive impairment using sediment extracts which eliminate the physical problems encountered in the development of dose-response relationships using whole sediments (Giesy et al. 1990). The use of sediment extracts also enables comparisons to be made between contaminant concentrations in the extracts and existing water quality criteria.

Many assay procedures have been developed in an attempt to standardize results so that sediment toxicity bioassays are more reproducible, have greater applicability among classes of chemicals (APHA 1985, Chapman et al. 1987, Depinto et al. 1987, Giesy et al. 1988) and are more simple and more readily interpreted (Nebeker et al. 1984). While a number of organisms have been used in bioassays of sediment toxicity, a few, such as the midge, *C. tentans*, and water flea, *D. magna*, have been demonstrated to be particularly useful (Nebeker et al. 1984 a, b, Giesy et al. 1988 a, b, 1990). The response of one test organism to single compounds or mixtures is often correlated with that of other species but seldom is the correlation perfect (Giesy et al. 1988 a). Therefore, no single bioassay can be expected to be adequate for the detection of potential adverse effects of complex mixtures of contaminants (Chapman 1987, Giesy et al. 1990) due to the relative sensitivities and natural history characteristics of different bioassay organisms. For this reason, it is suggested that a battery of several test organisms be used to screen for the toxic potency of sediments. The organisms in the battery of tests need not be the same tests as those suggested here, but should meet the criteria for sediment toxicity bioassays and have sufficiently different sensitivities to classes of toxicants to give sufficient predictive power. Based on previous work by our laboratory (Giesy et al. 1988 a, b, 1990) and others (Nebeker et al. 1984 a, b) several organisms meet these criteria (Giesy & Hoke 1989). It is suggested that a battery of sediment toxicity tests should include, as a minimum, 1) the *Daphnia magna* acute lethality test or some alternative sensitive crustacean or insect, 2) the *Photobacterium phosphoreum* bioluminescence reduction assay (Microtox^{R}), 3) the *Selenastrum capricornutum* carbon fixation assay or other as appropriate algal assay. In addition, assays such fish embryo-larval tests may be appropriate. When an assessment of sediment pore water is not appropriate or practical and surrogate species cannot be used it is suggested that the *Chironomus tentans*, chronic, survival and growth assay be used (Giesy et al. 1988 a).

As discussed above, the responses of a number of assay organisms to the effects of a mixture of toxicants in sediments generally results in some degree of correlation among assays (Giesy et al. 1988 a, Dawson et al. 1988). However, it has been observed that there is not perfect correlation among tests because of differences in the relative sensitivities of test organisms among contaminants and the differences in relative concentrations of toxicants among locations (Giesy et al. 1988 a). For instance, the NOEL (no observed effect level) for toxicity of chemicals to *D. magna* has been found to be correlated with that to the fathead minnow for chemicals with a variety of chemical structures ($r = 0.79$) and highly correlated when only chemicals of similar structure are considered ($r = 0.98$) (Maki 1979). Therefore, it is probably unnecessary to include organisms of similar sensitivities in a battery of tests. *D. magna* is easier to culture and use in tests, so it is recommended that *D. magna* be included in a battery of tests. Also, the results of the *D. magna* and *C. dubia* assays may be similar but these species are typically used in acute and chronic assays, respectively. After sites exhibiting toxicity have been identified using the proposed battery of screening assays, the seven day *Ceriodaphnia dubia* and *Pimephales promelas* assays could be used to assess the potential chronic effects on reproduction and growth caused by contaminants from the less toxic sites.

When the responses of three assays including: the bacterium *Photobacterium phosphoreum*, embryos of the oyster and the marine amphipod (*Rhepoxynius abronius*) exposed to contaminated sediments were compared, there was "a high level of agreement among the three bioassays, however, individual correlations suggested considerable variation among the bioassays" (WILLIAMS et al. 1987). Those authors concluded that a range of toxicity tests should be used to obtain the maximum range of sediment toxicities resulting from different relative and absolute concentrations of contaminants.

Similarly, GIESY et al. (1988 a) compared the responses of a battery of three bioassays: *C. tentans*, *D. magna* and *P. phosphoreum* to sediments from the Detroit River, Michigan, USA, which are known to be contaminated by a variety of chemicals, including metals, petroleum hydrocarbons and synthetic, organic chemicals (GIESY et al. 1990). In general, sediments, which were found to be toxic in all three assays. For instance, sediment pore water from some locations caused maximum responses in both the *D. magna* and *P. phosphoreum* assays. However, while there was some correlation among the results of the three bioassays there was also variation in the response among assays at different locations. For instance, the response of the *D. magna* lethality assay was quantal, which resulted in an all or nothing type of response. Sediments from some locations caused almost complete inhibition of bioluminescence in the *P. phosphoreum* assay, while causing no lethality to *D. magna*. Thus, the *D. magna*, acute, lethality bioassay was less sensitive and less discriminatory than the *P. phosphoreum* assay.

Similarly, *C. tentans*, weight gain in whole sediment was more sensitive to and discriminatory among the effects of sediments from the Detroit River than was the *D. magna* assay. Sediments or pore water which caused no lethality of *D. magna* caused as much as a 50% reduction in the weight gain of *C. tentans*. The reduction in weight gain, which is a continuous response variable, exhibited more power to discriminate among the mixture of toxicants in sediments.

The correlations among the responses of the three assays suggests that any of the three assays could have belen used to identify the most toxic sediments. Eventhough the *D. magna*, aculte, lethality assay was less discriminatory than the other two assays, the LC_{50} value was equivalent to the toxicity which restricted colonization by a balanced macaroinvertebrate community of the sediments under field conditions (GIESY et al. 1988 a, FALLON & HORVATH 1985). It was also determined that the degree of toxicity of Detroit River sediments required to restrict colonization of the sediment by benthic invertebrates was equivalent to a 25% reduction in weight gain of *C. tentans* (GIESY et al. 1988 a). Similarly, a reduction of 25% in weight gain of *C. tentans* in laboratory bioassays, relative to that on reference sediments, was the threshold above which colonization of sediments in Toledo Harbor and western Lake Erie (GIESY & HOKE 1988). Thus, the results of a battery of simple tests can be calibrated to relevant, ecological effects.

Even when a battery of tests is used, one cannot expect to have perfect predictability. When the results of acute and chronic toxicity tests for a number of different species were compared, it was found that 25–30% of the test chemicals caused effects in at least one species which were not expected based on a battery of tests containing a standard algal, daphnid and fish species (SLOOFF 1985). However, when the data on a variety of chemicals were considered together, the difference among species of aquatic organisms was rather small. For this reason it has been suggested that a rather small number of test species would be necessary to screen for the effects of environmental contaminants. When a battery of tests is conducted the results of each test will contain some unique and some redundant information. A group of two or more bioassays can be used to classify the relative toxicity and relative similarities of the toxicity of three or more sediments. The goal of using multiple assays is to produce a canonical classification variable which maximizes the information about sediment toxicity while minimizing the probability of misclassifying the toxicity of a sediment. Multivariate techniques are available which facilitate the use of data from several assays to develop impirical descriptors (COOLEY & LOHNES 1971, MORRISON 1976). Using this approach, GIESY et al. (1988 a) developed dose-response relationships via probit analysis for three assays (Microtox^R, *Daphnia magna* and *Chironomus tentans*) of sediment toxicity in the Detroit River. Dose-response data from each assay were then combined in a multivariate principal components analysis. Microtox^R and *D. magna* assays produced quantal, "all or none", type responses to sediment contaminants while the response of *C. tentans* growth was a continuous variable. Sensitivity among the assays was approximately the same but the *C. tentans* growth assay was the most discriminatory. In

the principal components analysis, each assay considered separately accounted for approximately 60–70 % of the explained variance while any two assays in combination accounted for greater than 95 % of the explained variance. Therefore, one of the assays could have been deleted from the investigation with no appreciable loss of information deleted from the investigation with no appreciable loss of information and with a concurrent reduction in total study expense.

A centroid hierarchical cluster analysis can also be used to determine which locations are most similar, based on the variation explained using the non-redundant information provided by several toxicity assays. As with the principal component analysis, most of the variation among locations was explained by including the results of the *D. magna* and *P. phosphoreum* assays of the Detroit River sediment alone. GIESY et al. (1988 a) found that using only the *D. magna* and *P. phosphoreum* assays explained better than 99 % of the variation among locations.

Summary

Sediment quality criteria should be based, at least in part, on biological responses. One way to do this is the use of a battery of simple, site-specific, standard screening tests combined with multivariate statistical methods. The results of such tests can be used to classify sediments based on their inherent toxicity or compared to the results of tests with spiked sediments or water quality criteria. A combination of assays, which meets the criteria for effective sediment toxicity assessment tests are, 1) the *Daphnia magna* acute toxicity test, 2) the *Photobacterium phosphoreum* bioluminescence reduction assay, 3) the *Selenastrum capricornutum* carbon fixation test, and 4) the *Chironomus tentans* chronic survival and growth assay. The toxicity of sediments can be classified by the use of principal components and cluster analysis to provide good predictibility and discrimination among the suitability of sediments for colonization with benthic invertebrate organisms.

Acknowledgements

This manuscript was prepared with support from a cooperative agreement between the U.S. Environmental Protectiona Agency and Michigan State University (MSU) under agreement number DL 85-002-06 and the Michigan Agricultural Experiment Station, from which it is contribution No. 13126. Manuscript preparation was supported, in part by the Chair of Geochemistry and Ecological Chemistry of the University of Bayreuth, Bayreuth, West Germany.

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This is a reprint of

Verhandlungen – Proceedings – Travaux

of the International Association for Theoretical and Applied Limnology, Congress in Munich 1989

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