Review

A review on the application of microbial toxicity tests for deriving sediment quality guidelines

Patrick van Beelen *

RIVM, Laboratory for Ecotoxicology, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands

Abstract

The results of microbial toxicity tests are needed for the risk assessment of polluted sediments. In comparison with animals the anaerobic microorganisms are more tolerant to natural sediment conditions whereas they are more sensitive for a number of specific pollutants.

Microbial toxicity tests from a literature search were classified in seven categories. Category A, B and C use polluted sediments and are applied for sediment monitoring. In category D, a pure chemical is added and the organisms and the test conditions were derived from sediment. Therefore this category can be used for setting sediment quality guidelines which protect sediment functions for the toxic effects of chemicals. In category E, organisms from a polluted site are separated from the sediment and are tested with pure chemicals. Organisms from a more polluted site can be more tolerant to a local pollutant. This is called pollution-induced community tolerance and can be used as evidence for the occurrence of toxic effects in a specific sediment. In category F pure chemicals are tested with a pure culture of microorganisms under sediment conditions. The results of category F tests can be combined with single species tests with animals and plants to obtain sediment quality guidelines sufficient for species protection. This can be compared with the sediment quality guidelines which protect sediment functions. When one of these quality guidelines is exceeded for a compound at a specific location a category E test can be used to determine whether the compound shows toxic effects in that sediment.

Keywords: EC50; Processes; Sediment; PICT; Microtox

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* Tel.: +31-30-2743133; fax: +31-30-2744413.
E-mail address: p.van.beelen@rivm.nl (P. van Beelen).
1. Introduction

1.1. The ecological role of sediment bacteria in aquatic ecosystems

Sediment microorganisms are crucial for the biodegradation of organic matter and the cycling of nutrients while these microorganisms are susceptible to toxic pollutants (Eismann and Montuelle, 1999). The degradation of organic pollutants in aquatic ecosystems is mainly performed by bacteria (Pfaender and Bartholomew, 1982; Verrhiest et al., 2002). Most of the bacteria in an aquatic ecosystem are bound to sediment particles. For example 0.1 mm of a Dutch River sediment did contain as many bacteria as 10 m of water (Van Beelen and Fleuren-Kemilä, 1989). Anaerobic conditions are common at the bottoms of lakes and slow flowing rivers because the precipitation of organic material is high when the water current is low. The activity of the bacteria at the sediment surface rapidly degrade organic compounds and thereby generate an oxygen gradient. When oxygen is depleted sulphide can be formed which has a strong influence on the partitioning of metals and the degradation of organic compounds in the sediment. The depletion of oxygen in anaerobic sediments limits the occurrence of many animals and plants. The plants and animals which do occur in anaerobic sediments are specially adapted to obtain oxygen from the surface water or from the air. The effects of pollutants on the activity of bacteria at the sediment surface has not been studied in great detail, whereas it is vital for the health of the aquatic ecosystem. For the protection of the sediment ecosystem one needs information on the sensitivity of the microorganisms, plants and animals which are living in and on the surface of sediments. Therefore the results of toxicity tests with microorganisms, plants and animals must be combined in order to derive sediment quality guidelines.

1.2. The use of the equilibrium partitioning method to derive sediment quality guidelines from water quality guidelines

When there are no results of toxicity tests with microorganisms, plants or animals available it is very difficult to derive sediment quality guidelines. In the Netherlands these guidelines are derived from aquatic toxicity data using a sediment/water partitioning coefficient (Crommentuijn et al., 2000). The aquatic quality guideline (expressed in µg/l) is multiplied by the partitioning coefficient (expressed in l/kg) to obtain the sediment quality guideline (expressed in µg/kg). This procedure is unreliable when there is a large variation of partitioning coefficients for a single compound (Wang et al., 1999). Metals often show a large variation of sediment/water partitioning coefficients that depend on
many factors like pH, clay content, organic matter content, iron content, sulfur content and redox potential (Mahony et al., 1996). In the Netherlands there are a large number of aquatic sediments with a high clay content, a low redox potential and elevated metal concentrations (Van den Berg et al., 1998). When dredging of these sediments is necessary this can be a problem because they have to be treated as polluted sediments. The question remains open at which conditions the elevated metal concentrations in these sediments really pose an ecotoxicological risk.

1.3. The use of sediment toxicity tests to derive sediment quality guidelines

Sediment quality guidelines might also be derived from sediment toxicity tests with animals, plants or microorganisms that live in the sediment. The procedure would be similar to the derivation of soil quality guidelines and can be explained as follows: The concentration effect relation is summarized with a no observed effect concentration (NOEC), and an EC10 or EC50. (The NOEC is the highest toxicant concentration that produces no significant difference with the control. The EC10 and EC50 are the toxicant concentrations that give 10% or 50% inhibition.) Many processes or enzymatic reactions can be monitored in sediment samples and can be used to obtain concentration effect relations. When the EC10 values of a specific toxicant for different processes and enzymatic reactions are collected, a microbial sensitivity distribution is obtained. The lowest EC10 value of this distribution may be taken to derive a safe concentration that can be used to set a sediment quality guideline. In practice however it is better to statistically derive (Aldenberg and Jaworska, 2000) the concentration that is safe for 95% of the processes and enzymatic reactions (Van Beelen and Doelman, 1997). This avoids excessively low values for the compounds for which many test results are available and relatively high values for the compounds for which only a few tests are available in the literature. Since the organisms are tested together with the sediment it is not possible to attribute differences in sensitivity to the sediment properties or the properties of the microorganisms. It is always a combination of sediment properties and properties of microorganisms that determines sensitivity. Therefore, Van Beelen and Doelman (1997) suggested that each separate test should be used independently for the setting of sediment quality guidelines instead of grouping tests with the same process or function together as if it were tests with the same species (Van Beelen and Doelman, 1997). They also suggested that no sediment type correction should be performed since the microbial species and the sediment are tested together. This makes it impossible to separate species sensitivity and the bioavailability in the sediment. A similar procedure was described to derive sediment quality guidelines from toxicity tests with sediment microorganisms (Van Beelen and Doelman, 1997). It was shown that for 1,2-dichloroethane, chloroform and zinc the quality guidelines derived from microbial toxicity tests in sediment were orders of magnitude lower than the quality guidelines derived from aquatic toxicity tests using the equilibrium partitioning method. For zinc this might be attributed to a high sensitivity of microbial toxicity tests or to uncertainties in the equilibrium partitioning constant. For 1,2-dichloroethane and chloroform this was caused by the high sensitivity of the anaerobic microbial processes (Van Beelen and Doelman, 1997).

1.4. Sediment properties can decrease toxic effects

Sediments contain clay particles, organic matter, iron oxides, sulfides and other compounds that can bind the toxicant and mitigate toxicity. The toxicity depends also on the pH and the presence of dissolved inorganic and organic compounds (Mahony et al., 1996). This mitigation also occurs in soils. For the setting of the Dutch soil quality guidelines however, the soil pH is not taken into account although it has a pronounced influence on the toxicity of metals (Janssen et al., 1997). The situation in sediments is even more complicated since also the amount of sulfide and the redox potential plays an important role (Di Toro et al., 1990). These factors make it difficult to compare the toxicity of a compound in different sediments. Therefore a scientifically underpinned sediment type correction will be difficult to obtain.

1.5. The conditions in unpolluted anaerobic sediments can be toxic for aerobic organisms

While the presence of sediments can decrease toxic effects of pollutants, some of the naturally occurring compounds in anaerobic sediments can cause inhibitory effects. Animals and plants need oxygen for their metabolism that is obtained from the water or in the case of plants even from the air (Adam, 1990). The low oxygen concentrations and high sulfur and ammonia concentrations which naturally occur at the surface of anaerobic sediments can be inhibitory for plants or animals (Cote et al., 1998; Pardo et al., 1999). The microorganisms that live in anaerobic sediments are well adapted to these concentrations. In some cases these microorganisms are so well adapted to anaerobic conditions that they are not able to survive in the presence of molecular oxygen (Balch et al., 1979). This illustrates the general principle that toxicity is not a substance property only, but it is the combination of the substance, the organisms, the conditions and the exposure duration that can cause toxic effects.
1.6. The use of pollution induced community tolerance (PICT) for the determination of sediment quality guidelines

PICT can be caused by the following chain of events: The organisms in polluted sediments are exposed to elevated concentrations of pollutants. When the pollution exceeds a critical level the most sensitive organisms become inhibited by toxic effects. This cause a decreased fitness in these organisms which can then be outcompeted by other more tolerant organisms. Therefore the absence of sensitive species can be used as an indicator for the toxic effects of a certain pollutant (Blanck, 2002). The occurrence of PICT is often accompanied by a loss of species diversity (Boivin et al., 2002). The tolerance of the organisms extracted from the sediment is measured under controlled laboratory conditions without sediment and is commonly expressed as the EC50 (in mg/l) (Rutgers et al., 1998). For the comparison of the tolerance of the organisms it is necessary to separate the organisms from the sediment in order to distinguish microbial tolerance from sorption to the sediment. The difference in tolerance between the microorganisms from a control site and a polluted site can give information about the percentage of the original microflora that has been affected at the polluted site (Van Beelen et al., 2001).

1.7. Is a separate approach for microbial toxicity tests necessary for ecotoxicological risk assessment of polluted sediments?

Microorganisms do not form a separate taxonomic group like vertebrates or angiosperms since they are only defined as creatures which are too small to be seen by the naked eye (Stanier et al., 1980). There are however taxonomic groups like the gram-positive bacteria or the cyanobacteria that contain only microbial species (Woese, 1987). Microorganisms do not grow more rapidly than plants or animals. The predominant microorganisms in soil, sediments and surface water do not grow rapidly. They have doubling times in the order of magnitude of weeks (McLaren, 1973; Poindexter, 1981; Baath, 1998; Baath et al., 1998). Therefore there is no need to treat microorganisms differently from animals or plants, when performing ecotoxicological risk assessment. Accordingly, the microbial tests with Vibrio fischeri or single species of algae were used together with the tests with fish or invertebrates to derive quality guidelines for aquatic ecosystems (Crommentuijn et al., 2000).

There is however a large number of microbial toxicity tests which focus on the functions and processes that these microorganisms support. These tests are quite different from single species tests and are therefore treated in a separate way. These functional tests are often combined to form a sensitivity distribution which is different from the sensitivity distribution of single species tests. This functional sensitivity distribution is subsequently used in a risk assessment which leads to a separate ecotoxicological risk level for microbial functions. Subsequently the lowest risk level of either the single species tests or the functional tests are used to determine the ecotoxicological quality guidelines (Van Beelen and Doelman, 1997; De Bruijn et al., 1999).

1.8. How can different types of microbial toxicity tests be used to derive sediment quality guidelines?

The answer to this question strongly depends on the type of microbial toxicity tests. Therefore the different types of microbial tests must first be categorized before this question can be answered separately for each category. This is not an easy task since there are very different types of microbial toxicity tests used for the assessment of contaminant effects in sediments (Eismann and Montuelle, 1999). All these different toxicity tests use either microorganisms (1) or conditions (2) or toxicants (3) taken from sediment. These three criteria give $2^3 = 8$ categories of tests. Table 1 shows the eight different categories of tests. The first seven tests can be designated as sediment tests because either the toxicants or the organisms or the conditions are taken from sediments. In category A toxic sediments are tested with sediment organisms under sediment conditions. Category C are aquatic toxicity tests with sediment as toxicant. Category A, B, C and G are tests with polluted sediments as toxicants. These can be used for location specific risk assessment of polluted sediments. Category D, E, F and H use specific chemicals or mixtures as toxicants and can be used for the generic risk assessment of a specific chemical or mixture.

In the following paragraphs the application of each of these categories of microbial toxicity tests for the risk assessment of polluted sediments will be discussed. The discussion deals with the pro and cons of these categories for either the determination of environmental risk limits of specific pollutants or location specific risk assessment of polluted sediments.

2. Methods

A literature search was performed in May 2003 using the current contents database (starting at 1996) with the following search statement: toxic and sediment and (microb or bacteri or procex or microflora or mineral). The is a wild-card used to find a number of different words with the same starting letters like toxic, toxicant, toxicological, toxicity, etc. These publications were used to obtain a database on sediment toxicity tests.
using microorganisms. A selection was made based on
the title and abstract of these publications. The reference
lists of the relevant publications were scanned for rele-
vant older literature. The tests were classified into the
categories A–G (see Table 1), according to the microbial
test involved. For example when a publication describes
experiments where polluted sediments are tested with
sediment animals but only an extraction of the sedi-
ments is tested with a pure culture of marine microor-
ganisms, the publication is categorized as “C”, elutriate
test.

3. Results

3.1. Category A: location specific risk assessment of
polluted sediments using sediment organisms

In this category of tests the microorganisms present
in polluted sediments are tested under conditions as
close as possible to the natural conditions in the sedi-
ment (see Table 1). Many sediments are anaerobic and
therefore it is often necessary to maintain anaerobic
conditions. The experimental setup is close to the nat-
ural situation and therefore the ecological relevance
is high. In a marine experiment, the concentration of
heavy metals in sediments was correlated with the
number, biomass and the percentage of dividing bacteria
(Fabiano et al., 1994). A negative correlation between
cadmium concentration and bacterial biomass was
found. The conclusions about field inventories of this
kind have to be cautious because of the lack of a proper
control that is identical to the polluted sites in all aspects
but the pollutant concentration (Nugent et al., 1980;
Long et al., 2002; Petanen et al., 2003). In experiments
with sediment animals one can easily introduce a single
species into a number of different polluted sediments,
avoid predation during the test and count the animals at
the end of the test (Burton et al., 1996). This is not
possible with a whole community of sediment bacteria
and therefore it was difficult to compare the sensitivity
of the sediment microbial communities with the sensi-
tivity of toxicity tests with sediment animals (Burton
et al., 1996). The natural spatial variance of microbial
activities in sediments also makes it difficult to obtain
significant correlations between microbial activities like
the enzymatic reactions phosphatase, galactosidase or
glucosidase with the toxicity for aquatic invertebrates
(Stemmer et al., 1990).

In summary, this category of microbial toxicity tests
often only yields correlations between pollution and
microbial activity. These tests can only be used for de-
ring sediment quality guidelines when proper control
sites are available which are identical to the test sites in
all aspects but the sediment pollutants.

3.2. Category B: location specific risk assessment of
polluted sediments using pure cultures of microorganisms

In this category of tests polluted sediments are tested
with pure cultures of added bacteria that are introduced
into the sediment (see Table 1). The sediments are often
anaerobic. The experimental setup is similar to a single
species toxicity test with sediment animals like for ex-
ample the Chironomus riparius midge larvae. Therefore
these experiments can suffer from the same drawbacks.
The introduced organisms are generally not adapted for
anaerobic sediments and therefore need molecular oxy-
gen for respiration and are sensitive for hydrogen sulfide
or ammonia which are naturally occurring toxicants in
anaerobic sediments (Cheung et al., 1997; Cote et al.,
1998; Pardos et al., 1999; Wegener et al., 2002). One of
the most popular tests is the solid phase tests with V.
fisheri. In this test the sediment is amended with sodium
chloride to obtain favorable conditions for the marine
bacterium V. fisheri. The bacteria are added to the

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<table>
<thead>
<tr>
<th>\text{Category}</th>
<th>\text{Toxicants}</th>
<th>\text{Organisms}</th>
<th>\text{Conditions}</th>
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<tbody>
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<td>A</td>
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<td>B</td>
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<tr>
<td>H</td>
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</table>

(+) Means taken from sediment, (−) means not taken from sediment.

Table 1
The eight different categories of sediment or aquatic toxicity tests
sediment suspension, incubated for 30 min and then removed by filtration. The surviving bacteria in the filtrate were tested using the natural bioluminescence of these bacteria. Often a V. fisheri test is combined with animal tests using Chironomus riparius, Lumbriculus variegatus, Hyalella azteca or Tubifex tubifex (Day et al., 1995; Mattiasson et al., 2000). The combined effects of pollutants and naturally occurring toxins make it difficult to discriminate between clean and polluted sediments (Van Urk et al., 1993). Normalization of the bioluminescence due to adsorption of the bacteria to the clay particles is necessary (Quintino et al., 1995; Ringwood et al., 1997). This solid phase Microtox test was found to be more sensitive for sediment polluted with sawmill effluent than tests with C. riparius or the worm L. variegatus (Lyytikainen et al., 2001). The solid phase Microtox test correlated also with enzymatic tests and biomass in sediment (Kwan and Dutka, 1995; Suominen et al., 1999) and with the concentrations of metals or polycyclic aromatic hydrocarbons (Mowat and Bundy, 2001). An interlaboratory precision study of the solid phase Microtox test showed that the method has an acceptable level of precision and can be developed as a standardized method (Ross et al., 1999). There are a few other tests that introduce a single microbial species into sediment for toxicity testing. For example there is a test that uses a special strain of Escherichia coli (a gut bacterium) with a inducible enzyme β-galactosidase. The enzyme can generate a color reaction which is used to monitor the activity of the bacteria (Day et al., 1995). Sensor genes in genetically modified bacteria can be used to measure the bioavailability of metals. When the sensor gene is coupled to a reporter protein like luciferase the amount of light produced increases with the amount of metal present (Lappalainen et al., 2000). This response should not be confused with a toxic response where the amount of light produced decreases with the amount of metal present. In general the microbial toxicity tests are much easier to perform than toxicity tests with animals. This makes microbial tests to relatively cheap and useful for methods screening.

In summary, this category of microbial toxicity tests can be used for rapid screening of polluted sediments.

3.3. Category C: location specific risk assessment of polluted sediments using elutriates of these sediments together with aquatic toxicity tests

Historically this is the oldest category of sediment toxicity tests. It was developed in the time that only aquatic toxicity tests were available. The maintenance of anaerobic conditions is not necessary in this category of tests. The available aquatic toxicity tests were used to evaluate the toxicity of elutriates extracted from sediments. There are several extraction methods available: The simplest one is mixing sediment with water, centrifuge and use the supernatant for toxicity testing (Wong et al., 1995). A more refined method uses the porewater from the sediment that is extracted from the sediment by centrifugation and filtration (Gupta and Karuppiah, 1996). The sediment porewater can contain high amounts of ammonia (2–4 mM/l) that can inhibit V. fisheri bacteria, daphnia, algae and other organisms which are not adapted to high concentrations of ammonia (Gupta and Karuppiah, 1996; Cheung et al., 1997; Stronkhorst et al., 2003). Even more elaborate extractions can be used to elutriate specific pollutants from sediments. This can be used to indicate groups of pollutants causing the toxic effects in the sediment (Guzzella et al., 1996; Stronkhorst et al., 2003). In general these elutriate tests are less sensitive than the corresponding solid phase tests (Cheung et al., 1997; Kemble et al., 2000). In many cases whole sediment tests correlated much better with sediment pollution compared to elutriate and porewater tests (Cote et al., 1998; Matthiessen et al., 1998). Toxicity tests with sediment elutriates can give information about the possible contamination of surface water by polluted sediments (Guzzella, 1998; Hyotylainen and Oikari, 1999a). Sediment extraction with organic solvents can extract and concentrate organic pollutants from the sediment giving a concentrated solution that can be subjected to aquatic tests (Johnson and Long, 1998). This has the additional advantage that confounding factors such as interference due to toxicity caused by ammonia is avoided (Cote et al., 1998).

The V. fisheri test (Microtox) is one of the more common microbial tests used for sediment elutriates. A V. fisheri test using porewater can be more sensitive than an Hyalella azteca test (McGee et al., 1995) or a Daphnia magna test (Hyotylainen and Oikari, 1999b). A V. fisheri test using porewater can even be more sensitive than a Hyalella azteca test with whole sediment (Steevens et al., 1998). Another microbial single species test used for sediment elutriates is the Metplate test utilizing β-galactosidase of E. coli (Bitton and Koopman, 1992; Bitton et al., 1992). This test is especially sensitive for metals (Kwon and Lee, 1998; Huang et al., 1999).

In summary, this category of microbial toxicity tests might be used to unravel which classes of compounds cause the toxicity in polluted sediments. The extraction procedures are very critical because these procedures might not concentrate certain classes of pollutants which do cause effects in the sediments on one hand, while on the other hand these procedures might liberate pollutants from the sediment which are bound and do not cause toxic effects in the sediments.
3.4. Category D: compound specific risk assessment of pollutants in sediment

In these tests a mixed community of sediment microorganisms in an unpolluted sediment are exposed to different concentrations of a specific chemical or mixture of chemicals in the laboratory. In this category D the toxicants are not taken from the sediment while the organisms and the test conditions are derived from sediment (see Table 1). In this manner a dose effect relation is obtained. These tests can be used to derive sediment quality guidelines in a manner analogous to the derivation of terrestrial quality guidelines. In a number of older experiments it was not possible to derive a dose effect relation expressed in mg/kg because the data were reported in mg/l (Mills and Colwell, 1977; Lee et al., 1988).

Table 2 shows the EC50 values of available sediment toxicity tests using pure chemicals or a mixture of chemicals. The experiments performed with Cr, tributyl tin (TBT), chlorpyrifos and a mixture of phenanthrene, fluoranthene and benzo(k)fluoranthene are listed in the before last column in order to save space in the table.

The mercury methylation, chloroform mineralization and methanogenesis are sometimes listed more than once for the same author. In these instances the same process was studied in several sediments. It is clear that there can be large differences (more than four orders of magnitude) between the EC50 values of the same process in different sediments. Since the sediment microorganisms are tested together with the sediment there is no way to differentiate between the sensitivity of the microflora and the ability of the sediment to bind and mitigate toxicity. This is especially important in long-term experiments (more than one day) which allow slow sorption and biodegradation to decrease toxicity whereas at the same time the exposed microflora might obtain tolerance.

In summary, this category of microbial toxicity tests can be used for setting sediment quality guidelines protecting sediment functions. These tests can be extremely sensitive compared to the Dutch quality criterion for unpolluted sediment the “Maximum Permissible Concentration”, but the same test shows large variations in sensitivity between different sediments. The tests can be influenced by the method by which the chemicals are added since, for example, the toxicity of metals and TBT that are already bound to the sediment is much smaller than the toxicity of freshly added metals or TBT.

3.5. Category E: pollution induced community tolerance (PICT) of microbial communities in polluted sediments

Table 3 shows an overview of PICT experiments with sediment microorganisms. These tests were performed with aerobic microorganisms even when the deeper layers of the studied sediments might be anaerobic. The sediment microorganisms that are able to grow on the artificial medium in the PICT test are just a relatively small fraction of the total microflora. This is however not a problem when the sensitivity of this fraction is comparable to the sensitivity of the microflora as a whole (Blanck, 2002). In this category E of tests, only the organisms are taken from the sediment while the toxicants and the experimental conditions are not derived from sediment (see Table 1). The tolerance of the microflora is expressed as the EC50 measured in mg compound/l medium. The microorganisms were extracted from the sediment and a specific activity was measured in the laboratory under controlled and artificial conditions. Therefore it is possible to compare the EC50 of clean reference sediment with the EC50 of polluted sediment since both EC50 values are measured in the same medium. The medium can have a pronounced influence on the EC50. The different authors in Table 3 used different media and therefore it is not advisable to compare the EC50 from one author with the EC50 from another author.

In the experiments of Dean Ross (see Table 3), the EC50 values were measured in medium with nalidixic acid and the growing enlarged cells were counted microscopically (Dean Ross and Rahimi, 1995). Nalidixic acid inhibits cell division but not cell growth. Only the total phenol content of the sediment was measured and therefore the concentrations of the individual phenolic compounds in the sediment are not known.

The Cu tolerant bacteria were measured in a medium that contained 0.1 M hydrous ferric oxide which binds the Cu. Therefore the copper concentrations in Table 3 are expressed relative to the iron concentration.

For some pollutants it is difficult to detect PICT (Van Beelen et al., 2001). No difference in TBT tolerance was observed between the microflora originating from polluted sites and the microflora from clean sites (Jude et al., 1996). TBT did show toxic effects on the nitrogen transformations in sediment (Dahllof et al., 2001) and tolerance to TBT was coupled with metal tolerance (Pain and Cooney, 1998). This might explain the difficulty of finding PICT with TBT. Sites with low concentrations of TBT can have high concentrations of other metals that might evoke cross resistance to TBT.

Some older publications report the percentage of resistant colonies on agar plates with one specific metal concentration (Mills and Colwell, 1977; Montuelle et al., 1994). This has the disadvantage that tolerance is not observed when the metal concentration in the agar plates is not optimal.

In conclusion, PICT can be used as evidence for the occurrence of toxic effects of organic compounds and metals in sediments. It is however possible that cross resistance occurs.
Table 2
The EC50 values (mg/kg) of microbial sediment tests

<table>
<thead>
<tr>
<th>Process or enzyme</th>
<th>Cd</th>
<th>Cu</th>
<th>Hg</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
<th>Benzene</th>
<th>Chloroform</th>
<th>DCE</th>
<th>PCP</th>
<th>Simazine</th>
<th>Compounds</th>
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<tr>
<td>Acetate</td>
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<td></td>
<td></td>
<td>60</td>
<td>60</td>
<td>2</td>
<td></td>
<td></td>
<td>Barnhart and Vestal (1983)</td>
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<tr>
<td>Glucosidase</td>
<td>100</td>
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<td>23</td>
<td>68</td>
<td>60</td>
<td></td>
<td>100</td>
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<td>Glucose uptake</td>
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<td>Thymididine uptake</td>
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<td>Methanogenesis</td>
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<td>&gt;1000</td>
<td>1000</td>
<td>≤1000</td>
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<td>Capone et al. (1983)</td>
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<td>Nitrogen cycle</td>
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<td>Crude oil degradation</td>
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<td>&gt;2500</td>
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The underlined EC50 values are below the target value which indicates a clean sediment. The italic EC50 values are below the intervention value which indicates a polluted sediment (VROM, 1999).
3.6. Category F: compound specific risk assessment using pure cultures of microorganisms added to sediment

In this category F of tests pure chemicals or toxic mixtures are tested with pure cultures of added microorganisms that are introduced into the sediment. This means that only the conditions of taken from sediment (see Table 1). Sometimes even artificial sediment is used in order to obtain a controlled and reproducible test. The experimental setup is similar to the one in category B. Pure chemicals were used to develop an automated color correction method for the *V. fisheri* solid phase toxicity test (Lappalainen et al., 2001).

Category F is often used together with category B as a control with a pure toxic chemical instead of a polluted sediment. This can then be used to study the influence of the speciation of a specific chemical between the sediment, porewater and surface water on the toxicity. For example, a combination of whole sediment, porewater and elutriate testing with artificial sediment amended with *Bacillus cereus* and five different chemicals yielded the following conclusion: Sediment quality guidelines based on the distribution of chemicals between sediment and aqueous phase and assessment of toxicity of a sediment with water quality guidelines were not confirmed (Liss and Ahlf, 1997). This was in accordance with elutriate testing of cadmium and zinc within the test algae *Selenastrum capricornutum* (Pardos et al., 1998). The addition of a fresh toxicant to a sediment can give a much higher bioavailability compared to aged sediment where the toxicant might be more tightly bound (Besser et al., 1996; Looser et al., 1998). When the influence of speciation on toxicity is known, it is possible to compare similar toxicity tests performed in different sediments.

The results of category F can be combined with the results of animal toxicity tests in sediment to derive sediment quality guidelines protecting individual species. A pure culture of microorganisms can be regarded as a single species. In aquatic ecotoxicology the tests with pure cultures of microorganisms like *V. fisheri* or single species of algae are used together with the tests with fish or invertebrates in the risk assessment for aquatic ecosystems (De Bruijn et al., 1999). Sediment toxicity tests with animals are performed with a single species in a standard unpolluted sediment that is then amended with increasing concentrations of a toxicant in the laboratory (Verrhiest et al., 2002; Wegener et al., 2002). A microbial test with a single species and a pure compound in sediment would categorize as F. In this category only very few microbial tests are available which can join the single species animal toxicity tests for a combined risk assessment.

In conclusion, category F is used to study the influence of speciation of added chemicals on the toxicity. This category of experiments are needed to derive a sediment type correction between toxicity experiments performed in different sediments. The results of category F tests can be combined with results of animal toxicity tests to derive sediment quality guidelines protecting individual species.

3.7. Category G and H

Category G experiments were not found in the literature search. It requires sediment organisms to be tested with pollutants from sediment under artificially controlled conditions (see Table 1). This was not performed because it is impractical. When a sediment pollution is tested using sediment organisms it is very practical to perform the test under sediment conditions. Category H tests are not discussed here because these are pure aquatic tests and have nothing to do with sediment.

4. Discussion

4.1. Are microbial toxicity tests needed for the risk assessment of polluted sediments?

Microbial tests are often used in first-tier testing because they are relatively cheap. A German study for
example recommended to use the *V. fisheri* test along with algae, bivalves, sea-urchins and amphipods in the first-tier of sediment testing (Nendza, 2002). Microbial tests are also used because they are more sensitive than tests with animals or plants for a number of compounds. Chloroform for example is very toxic for mineralization processes and methane formation in anaerobic sediments whereas it is much less toxic for fish and invertebrates (Van Beelen et al., 1994). In the Netherlands the Maximum Permissible Concentration (MPC) is used as a quality guideline. When the pollutant concentration is below the MPC, a sediment is considered to be clean. When the concentration is above the intervention value the sediment is considered to be polluted (VROM, 1999). For copper, zinc, chromium and TBT there are a number of EC50 values that are below the MPC (see Table 2). The MPCs of copper, zinc and chromium were set equal to the rural background concentrations in Dutch soils of 36, 100 and 140 respectively (VROM, 1999). The sediments used by Van Beelen and Van Vlaardingen (1994) contained an ambient concentration of 800 mg Zn/kg sediment dry weight. This is above the intervention value for sediment of 720 mg Zn/kg (VROM, 1999). A small addition of 21 mg Zn/kg to the 800 mg Zn/kg caused a 50% inhibition of the chlorophenol degradation (Van Beelen et al., 1994). Apparently there is a large difference between the toxicity of the zinc present in the sediment compared to the zinc added to the sediment. There also was a large difference between the TBT present in the sediment (0.03 mg/kg) (Dahllof et al., 1999) and the added amounts of 0.00008 or 0.0007 mg/kg that caused effects on the nitrogen cycle (see Table 2). The results of the sediment toxicity tests of category D can be combined to derive a sediment quality value that aims at the protection of the sediment functions (Van Beelen and Doelman, 1997). In summary, microbial processes are not always sufficiently protected by quality guidelines derived from animal toxicity tests.

4.2. A comparison of the test categories A, B, and C which can be used to to assess the risks of polluted sediments

In principle the categories A, B, C, and G can be used for a site-specific risk assessment because these tests utilize polluted sediment as toxicant. Category A however needs a control site that is identical to the polluted sites in all aspects but the pollution. This is not often available. Category G is not present. This leaves category B and C as practically available options for a site-specific risk assessment. These tests can also detect the toxic effects of compounds that are not measured by chemical analysis. Category C tests use elutriates of sediments together with aquatic toxicity tests. The extraction method of the elutriates can be specific enough to avoid the concentration of natural toxicants like hydrogen sulfide or ammonia (Cote et al., 1998). This specificity is also the major drawback of elutriate testing since the extraction and concentration method of the toxicants from the sediments determine the toxic effects. On the one hand, toxic compounds that are tightly bound to the sediment and therefore do not pose an ecotoxicological risk might be extracted. Whereas on the other hand, toxic compounds that do pose an ecotoxicological risk might not be concentrated. Separate extraction methods are needed for hydrophobic compounds, volatile compounds, metals or hydrophilic compounds. In category D and F the addition of a fresh toxicant to sediment can give a much higher bioavailability compared to aged sediment (Besser et al., 1996; Looser et al., 1998). These categories D and F can therefore give an overestimation of ecotoxicological risks. The outcome of these tests can therefore be used to make a worst-case estimate of ecotoxicological risk limits like the MPC and the intervention value assuming a large bioavailability (Crommentuijn et al., 2000). When the intervention value is exceeded at certain locations a site-specific risk assessment is needed to show that the pollutants are really bioavailable at that site.

Category E uses pollution induced community tolerance to detect effects of certain chemicals at a specific site. This method is very useful for site-specific risk assessments at the locations where the risk limits derived from category D tests are exceeded. In that case the pollutants that exceed the risk limits are known. The PICT method can determine which of these pollutants did cause toxic effects at the site.

5. Conclusions

Bacteria at the sediment surface play a vital role in aquatic ecosystems. Microbial toxicity tests are needed for the risk assessment of polluted sediments because sediment quality guidelines derived from animal toxicity data are not always low enough to protect sediment microorganisms. Anaerobic microorganisms are adapted to survive in the presence of naturally occurring toxicants like ammonia or hydrogen sulfide that inhibit the animals used in sediment toxicity testing. Many microbial tests are cheap, rapid and sensitive compared to toxicity tests with animals in sediment. The category B, and C tests can be used for site-specific monitoring of polluted sediments. The category D can be used for setting sediment quality guidelines protecting sediment functions. The category E tests can be used as evidence for the occurrence of toxic effects of a specific compound in a specific sediment. The results of category F tests can be combined with single species tests to obtain sediment quality guidelines sufficient for species protection.
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References


Patrick van Beelen was trained as a biochemist and wrote a PhD on coenzymes in methane forming archeabacteria. His current scientific fields of interest are microbiology, ecology, ecotoxicology and environmental chemistry.