DEVELOPMENT OF A WHOLE-SEDIMENT TOXICITY TESTING USING A BENTHIC MARINE MICROALGA

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Abstract—An acute whole-sediment toxicity test with a benthic marine microalga was developed and optimized using flow cytometry to distinguish algae (based on their chlorophyll a autofluorescence) from sediment particles. Of seven benthic marine algae screened, the diatom Entomoneis cf punctata was most suitable because of its tolerance of a wide range of water and sediment physico-chemical parameters, including salinity, pH, ammonia, and sulfide. A whole-sediment and water-only toxicity test based on inhibition of esterase activity in this species was developed. Enzyme activity rather than growth was used as the test endpoint, as nutrient release from sediments has previously been found to stimulate algal growth, potentially masking contaminant toxicity. The sensitivity of the bioassay to a range of metals (copper, zinc, cadmium, lead, arsenic, manganese) and phenol in water-only exposures was compared to the standard 72-h growth rate inhibition test. The esterase enzyme inhibition test was sensitive to copper, with a 3-h inhibitory concentration to cause a 50% (IC50) reduction in a fluorescein diacetate fluorescence value of 97 ± 39 μCuL. A concentration-dependent response was also observed in the presence of sediment particles (copper tailings), with and without dilution, using a control clean sediment. The primary route of exposure to copper was via pore water rather than by direct contact with tailings particles. This is the first whole-sediment bioassay developed with a benthic alga suitable for sediment quality assessment in marine/estuarine systems, and its advantages and limitations are discussed.

Keywords—Sediment Algae Toxicity Flow cytometry Fluorescein diacetate

INTRODUCTION

The development and application of microbial sediment tests to assess sediment quality has lagged behind the use of sediment-dwelling invertebrates such as amphipods, polychaete worms, and chironomids. This is surprising given that microorganisms play an important role in nutrient cycling and in degrading organic matter in sediments [1]. Even though microorganisms do not ingest sediment particles, they are in intimate contact with sediments, and this has been shown to enhance sediment toxicity to bacteria [2]. Bacterial sediment toxicity tests (e.g., Microtox® [Strategic Diagnostics, Newark, DE, USA] and ToxiChromoPad® and SOS-Chromotest® [Environmental Biodetection Products, Brampton, ON, Canada]) are an increasingly popular means for testing a large number of samples and enable the rapid identification of areas of high contaminant concentration for further investigation [3,4]. However, microbial tests such as the Microtox solid-phase test may give false-positive results, particularly in fine sediments, where light-emitting bacteria may be lost by adsorption to sediment particles removed in the filtration procedure. The measured decrease in light emission may be due to this loss rather than direct toxicity, and the problem is difficult to overcome unless reference sediments of similar particle size to test sediments are used.

Benthic algae (microphytobenthos) play an important role in stabilizing sediments, providing habitat, modulating chemical transformations, and remobilizing metals at the sediment–water interface [5,6]. The development of useful whole-sediment tests with algae has been limited by the ability to distinguish and count algae in the presence of sediment particles.

For this reason, chronic tests based on growth inhibition (where cells are counted daily) have rarely been attempted. Moreno-Garrido et al. [7] developed a growth inhibition test with the diatom Cylindrotheca closterium in which cells were exposed to metal-spiked sediments and counted daily using either a light or a fluorescence microscope. Unfortunately, neither pH nor pore-water metal concentrations were measured in the spiked sediments, making interpretation of the test sensitivity to metals difficult. Moreover, this test is suitable only for coarse sediments, as sediments with >15% silt significantly reduced algal growth. Given that most contaminated marine sediments have a high proportion of fines, this test requires further development before routine application to assessing the toxicity of contaminated sediments is possible.

Because of these difficulties, other endpoints apart from growth inhibition, such as algal photosynthesis and enzyme activity, have been used in the development of freshwater sediment tests [8,9]. Munawar and Munawar [8] used inhibition of carbon assimilation rate (photosynthesis) in the freshwater alga Chlorella vulgaris as a measure of toxicity over a 194-h exposure. However, carbon assimilation rates in test sediments were often stimulated compared to controls, presumably because of the release of nutrients such as ammonia, and this stimulation may have masked toxicity of the test sediment. Similar stimulation problems have been encountered when testing sediment elutriates [10].

One technique that can potentially separate the algal signal/response from test sediment is flow cytometry, a rapid method for the measurement of the light-scattering and fluorescence properties of cells in a moving fluid. Microalgae are ideal for flow cytometric analysis, as they are single-celled organisms containing photosynthetic pigments, such as chlorophyll a, that...
autofluorescence. Live and dead cells and other particles, such as sediments, can easily be distinguished on the basis of this chlorophyll a fluorescence [11]. Flow cytometry can be used to count cells and measure cell size and chlorophyll fluorescence, or it can be used in conjunction with fluorescent probes to detect metabolic changes in cells (e.g., enzyme inhibition or cell membrane permeability). A number of aquatic toxicity tests with marine and freshwater algae using growth (chronic) or enzyme activity (acute) as the response variables have been developed with flow cytometry as the detector [12,13]. For example, Blaise and Ménard [9] developed a whole-sediment toxicity test with the freshwater alga Selenastrum capricornutum (now called Pseudokirchneriella subcapitata). Toxicity was measured as a decrease in algal esterase activity compared to a control after exposure to whole sediment for 4 to 24 h.

In this paper, we describe the development and optimization of a whole-sediment toxicity test with a marine benthic diatom Entomoneis cf. punctulata. A range of benthic algal species were initially screened for suitability as toxicity test species. A whole-sediment toxicity test and a pore-water toxicity test were developed with E. cf. punctulata using esterase activity as the test endpoint. Esterases are a group of enzymes, including phosphodiesterase and acetylcholinesterase, that are involved in lipid turnover in cell membranes. Fluorescein diacetate (FDA), a lipophilic dye that readily penetrates the cell membrane, is cleaved by esterases inside live viable cells to yield fluorescent fluorescein, a hydrophobic anion that is retained within viable cells. When excited by blue light, fluorescein fluoresces in the green region of the spectrum, and this can be detected by flow cytometry. Toxicants may either decrease FDA uptake or decrease esterase activity, both of which have been regarded as indicators of cell viability [14]. Plant cell viability, as determined by FDA, has been found to correlate to standard cell viability tests with the membrane integrity dye trypan blue [15]. Enzyme inhibition over 3 to 24 h is considered to be an acute response, that is, a sublethal effect over a small part of the organism’s life span. The tolerance of the enzyme test to a range of sediment physico-chemical parameters was determined. The sensitivity of the test to metals (copper, zinc, cadmium, lead, arsenic, manganese) and phenol (in water-only exposures) and copper (whole-sediment exposure) was also determined.

**METHODOLOGY**

**Collection and analysis of sediments**

Sediments were collected from both pristine coastal lakes (for use as sediment controls) and urban/industrial-contaminated rivers, including Cooks River, Haslams Creek, and Hornibush Bay (Sydney, Australia), to optimize the bioassay protocol. A grab sample of approximately 2 kg of the top sediment layer (0–3 cm) was collected from each site using an acid-washed plastic trowel and was stored in 2-L polycarbonate containers (Nalgene®, Nalge Nunc International, Rochester, NY, USA). Samples were transported to the laboratory on ice. Leaf material, large rocks, and shells in the samples were removed by hand sorting. All samples were stored at 4°C.

Preliminary chemical screening of the sediments showed that two sediments (from Durras Lake [35°44′S, 150°20′E] and Termeil Lake [35°29′S, 150°22′E]) were suitable for use as controls for marine and estuarine samples, respectively. Durras Lake and Termeil Lake sediment contained 2.4 and 2.3% total organic carbon, respectively; 58 and 30% fines (<63-μm fraction) respectively; excess acid-volatile sulfide compared to simultaneously extractable metals; and very low total particulate metals concentrations (4–33-fold less than sediment quality guidelines) [16]. Pore-water and whole-sediment tests showed that both these sediments were nontoxic to E. cf punctulata esterase activity, with fluorescence intensity >85% of seawater controls after a 3- or 24-h exposure.

**Algal cultures**

The unicellular benthic marine alga Entomoneis sp. (strain CS-426) was obtained from Commonwealth Scientific and Industrial Research Organisation, Marine Research, Hobart (Tasmania, Australia). This species was originally isolated from Little Swanport (Tasmania, Australia) (42°20′S, 147°56′E), on the east coast of Tasmania by Richard Knuckey in 1995 and identified as Entomoneis cf punctulata Osada et Kobayashi (CS-426) (Gustaff Hallegraeff, University of Tasmania, Hobart, Australia, personal communication).

The alga was cultured in a modified half-strength f medium [17], with the iron and trace element concentrations halved. Seawater used for the culture medium was collected 0 to 1 km offshore from Port Hacking (NSW, Australia) immediately filtered through a 0.45-μm acid-washed cellulose-acetate membrane capsule filter with 0.65-μm prefilter (Sartorius, Goettingen, Germany) and stored at 4°C. Cultures were maintained on a 12:12-h light:dark cycle (Philips, Danvers, MA, USA; TL 40-W fluorescent daylight, 60 μmol photons/s/m²) at 21°C.

**FDA fluorescence inhibition bioassays**

Nutrients (nitrate and phosphate only) were added to all test vials for water exposure experiments (both enzyme and growth bioassays). For whole-sediment tests, the addition of nutrients was unnecessary, as sufficient nutrients were available from the sediments for the short (3 or 24 h) test duration.

_Whole-sediment exposure—Final protocol._ Sediment samples (each in quadruplicate) were prepared by weighing 1 or 2 g of wet sediment into 30-ml polycarbonate vials and adding either 9 or 8 ml of filtered (0.45 μm) seawater to give 10 and 20% (w/v), respectively. Seawater was salinity matched to the overlying water salinity (±5%) at each test site, then gently added to minimize sediment disturbance. All tests included clean sediment controls (10 and 20% w/v) with a similar particle size and salinity to the test sediment and seawater controls (10 ml of seawater supplemented with 15 mg NO₃⁻/L and 1.5 mg PO₄³⁻/L) in quadruplicate. Two vials were prepared for each replicate, one vial for 3-h analysis and one vial for 24-h analysis. Algal preparation and test exposure conditions were the same as that used in standard marine algal growth inhibition tests [18]. Exponentially growing cells of _E. cf punctulata_ (5 d old) were washed and centrifuged three times with seawater to remove culture medium prior to use in the algal bioassays. Each vial was carefully inoculated just above the sediment surface with 5 to 9 × 10⁴ cells/ml of this prewashed algal suspension, and the vials were capped loosely. Samples were incubated at 21°C on a 12:12-h light:dark cycle at 150 μmol photons/s/m² without disturbance for 3 or 24 h.

Cell esterase activity after a 3- or 24-h exposure was determined using the fluorogenic substrate, fluorescein diacetate (FDA, F-7378, Sigma, St. Louis, MO, USA). Fluorescein diacetate is cleaved only by live viable cells, whereas dead cells (e.g., heat treated) do not take up or hydrolyze FDA. One-millimolar FDA stock solutions were prepared volumetrically in acetone (reagent grade, BDH Laboratory Supplies, Poole,
UK) and stored at −4°C for up to one month to minimize hydrolysis. Working stock solutions were kept on ice during flow cytometric analysis and discarded after 60 min to prevent FDA precipitation.

After a 3- or 24-h exposure, vials were shaken for 2 s to resuspend the algae and allowed to settle for 30 s to remove large sediment particles. To limit the possibility of sediment particles blocking the flow cytometer, the settling time was optimized to achieve maximum algal recovery with minimum sediment. A 5-ml subsample of the supernatant was homogenized in a handheld tissue grinder to break up sediment clumps and dislodge algae from sediment particles. Preliminary experiments showed that this process did not disrupt the cells. The FDA (final concentration of 25 μM) was added to a subsample of homogenate and incubated for 5 min. Immediately prior to flow cytometric analysis, a small fraction was filtered through a Microtox solid-phase filter column (~50-μm particle size filter, prerinsed with seawater). The FDA fluorescence was determined as described in the following discussion. Unstained and FDA-stained deactivated (4% formalin) cells were included as negative controls.

A sediment was defined as toxic if it was more than 20% different from the reference (control) sediment (FDA fluorescence intensity was less than 80% of the control sediment). This criterion was based on test variability during the test development period and was in agreement with published protocols for amphipod acute toxicity tests using whole sediments [19].

Water-only exposure—Final protocol. The FDA fluorescence inhibition bioassays were carried out on pore waters or overlying waters using the same test conditions (e.g., washed inoculum, incubation conditions) as that used for the whole-sediment bioassay. Samples of unfiltered pore water (10 ml), each with three to four replicates, were dispensed into 30-ml polycarbonate vials. Salinity- and pH-matched seawater controls were also prepared in triplicate. Each vial was supplemented with 15 mg NO₃/L and 1.5 mg PO₄³⁻/L as nutrients and inoculated with 2 to 4 × 10⁶ cells/ml of a prewashed algal suspension. Staining with FDA and subsequent analysis followed the same procedure as the sediment-exposure bioassay, except that samples were not left to settle for 30 s, homogenized, or filtered through Microtox columns.

Growth inhibition bioassays (water-only exposure)

Growth rate inhibition bioassays on waters were carried out using a modified method [18]. Samples (6 ml), each in triplicate, were dispensed into 20-ml glass scintillation vials coated with a silinizing solution (Coatasil, Ajax Chemicals, Auburn, NSW, Australia) to reduce adsorption of metals to the vial walls. Salinity- and pH-matched seawater controls were also prepared in triplicate. Each vial was supplemented with 15 mg NO₃/L and 1.5 mg PO₄³⁻/L as nutrients and inoculated with 2 to 4 × 10⁶ cells/ml of a washed algal suspension. Cell densities were determined daily over 72 h using flow cytometry (see the following discussion). A regression line was fitted to a plot of log₁₀ (cell density) versus time (h) for each vial and growth rate (cell division rate, µ) determined from the slope. Growth rates (µ) were calculated as doublings per day (2.303 ÷ In 2 × µ × 24) and compared to salinity- and pH-matched seawater controls. The pH of the test solutions was monitored throughout the tests.

Flow cytometric analysis

Algal cells were analyzed using a Bryte HS Flow Cytometer (Bio-Rad, Richmond, CA, USA) with a xenon excitation lamp and FITC-520 filter block (excitation wavelength 470–490 nm). The flow cytometer is equipped with a microsyringe for volumetric sample injection into a fluid stream flow (0.22-μm filtered Milli-Q water, Millipore®, Bedford, MA, USA), enabling cells to be counted directly without the need for internal calibration beads. Operating conditions of the flow cytometer were flow rate 20 μl/min, pressure 0.7 bar, and counting time 120 s.

Two light scatter detectors were used to identify the morphology of the cell. The forward angle light scatter (LS1, <15°) detector provided information on cell size, while the side angle light scatter (LS2, 15–85°) detector provided information on the cell size/shape/granulality. Fluorescence was collected at a range of wavelengths by three-color photomultiplier tubes (PMTs) with fluorescence emission filters (FL1, 515–565 nm, green fluorescence; FL2, 565–605 nm, orange fluorescence; FL3 650–720 nm, red fluorescence).

Because algae and some sediment particles were of similar size, they were separated initially on the basis of chlorophyll a fluorescence by setting an acquisition threshold on FL3 (positioned to the left of the distribution of healthy control cells). Size was used in further gating procedures to help identify algae after removal of sediment particles in the analysis. Data were collected and displayed in one-dimensional histograms (256 channels) based on cell number versus LS1, LS2, FL1, FL2, and FL3 and two-dimensional cytograms (64 × 64 channels) based on a combination of fluorescence and light scatter signals. Total cell counts were obtained directly from histograms as the area under the curve (cell number vs FL3).

Shifts in algal FDA fluorescence were determined by using the histogram of cell number versus FDA fluorescence intensity (FL1 fluorescence). Region S2 (normal/healthy FDA fluorescence) was defined manually around the normal distribution of control (healthy) cell FDA fluorescence to incorporate greater than 85% of the cells. The percentage of cells falling into regions S1 (decreased FDA fluorescence), S2 (normal/healthy FDA fluorescence), and S3 (enhanced FDA fluorescence) were recorded and expressed as a percentage decrease in S2/S3 compared to a control according to the following equation:

\[(100 - %S1) / (100 - %S1) \times 100\]

where %S1 is the percentage of treated cells in S1 and %S1 is the percentage of control (untreated) cells in S1.

Samples containing sediments, where an acquisition threshold on FL3 was not sufficient to exclude all sediment particles, required further gating based on cell size to definitively separate algae from the sediment. A combination of LS1 and FL3 histograms and cytograms were plotted, and FL1 fluorescence histograms were replotted with the gated algal population.

Test optimization

Water-only exposures. The FDA fluorescence of E. cf puniculata exposed to natural seawater for 24 h was determined after a variety of exposure conditions and preinoculum treatments, using the same procedure described previously. Six treatments, in triplicate, were investigated: standard (150 μmol photons/s/m² light), low light (40 μmol photons/s/m²), + nutrients (addition of 15 mg NO₃/L and 1.5 mg PO₄³⁻/L and incubated at 40 μmol photons/s/m²), unwashed cells (cells taken directly from the stock culture and incubated at 40 μmol
photons/s/m²), diluted inoculum (washed cells diluted to the original culture cell density prior to use as an inoculum and incubated at 40 μmol photons/s/m²), and time to use (3 h) (test vials inoculated 3 h after algal inoculum was prepared and incubated at 40 μmol photons/s/m²).

The deactivation of esterase activity in algal cells was optimized to achieve reproducible negative controls. Esterase activity of *E. cf punctulata* (2–4 × 10⁶ cells/ml) in seawater was deactivated by heat treatment (100°C for 10 min or microwaved for 3 min at 100% power) or by the addition of formalin (4%) for a minimum of 1 h. The FDA fluorescence intensity was measured by flow cytometry before and after enzyme deactivation using the standard protocol.

**Whole-sediment exposures.** The effect on algal FDA fluorescence intensity after filtration of controls through a Microtox solid-phase filter column was investigated. The FDA fluorescence intensity after a 24-h exposure was measured by flow cytometry before and after filtration through a Microtox solid-phase filter column with and without preceding three times with seawater.

The recovery of algal cells in seawater-only and 10% w/v sediment exposures was investigated before and after filtration through a Microtox solid-phase filter column. The cell density of a prewashed algal suspension (5 × 10⁶ cells/ml) was measured using a Coulter Multisizer II particle analyzer with a 70-μm aperture (Coulter, Toronto, ON, Canada), and volumes of cells were added to give nominal cell densities of 1, 5, 10, 50, and 100 × 10⁶ cells/ml. Each cell density was then measured by flow cytometry.

**Tolerance of *E. cf punctulata* bioassays to sediment physicochemical characteristics (water-only exposures)**

The tolerance of the FDA inhibition bioassay to a range of sediment parameters, including salinity (5–35%), pH (6.0–8.5), ammonia (0.42–86 mg NH₃-N/L, added as ammonium chloride, analytical reagent [AR] grade, May and Baker, Dagenham, UK), and sulfide (0.13–80 mg S²⁻/L) in water-only exposures was determined and compared to the 72-h growth bioassays. The pH was monitored throughout the test duration and readjusted if necessary. Ammonia at each test concentration was measured using the photometric test kit Spectroquant® (Merck, Darmstadt, Germany). Hydrogen sulfide was tested at two pH values, 7.0 and 8.1. Seawater was spiked with the appropriate volume of approximately 500 mM sodium sulfide (Na₂S·9H₂O, AR grade, Ajax Chemicals) prepared in deoxygenated Milli-Q water and the pH adjusted to either 8.1 or 7.0. The total sulfide concentration was measured daily using the method of Cline [20]. The pH was also measured daily and readjusted to 7.0 or 8.1 if necessary. Toxicity test endpoints were calculated on the basis of measured rather than nominal ammonia and sulfide concentrations.

**Sensitivity of *E. cf punctulata* bioassays to metals and phenol (water-only exposures)**

The toxicity of arsenic, cadmium, copper, lead, manganese, zinc, and phenol to *E. cf punctulata* (in water-only exposures) was determined individually using both the esterase and the growth inhibition bioassays. Copper stock solutions (5 and 100 mg Cu/L) and zinc and cadmium stock solutions (100 mg/L) were prepared from their respective sulfate salts (AR grade, Ajax Chemicals) in Milli-Q water and acidified by the addition of 10 ml HCl (Suprapur grade, Merck) per liter. Manganese (II) (250 mg/L) and arsenic (III) (30 mg/L) stock solutions were prepared from MnCl₂·4H₂O (AR grade, Ajax Chemicals) and NaAsO₂ (laboratory reagent, BDH Laboratory Supplies, Poole, England), respectively. Stock solutions of lead (1 mg/ml) were purchased as aqueous solutions in dilute HNO₃ (AR grade, BDH Laboratory Supplies). A phenol stock solution of 10 g/L was prepared from phenol (AR grade, BDH Laboratory Supplies). Subsamples from each metal concentration (5 μg/L–100 mg/L) and the controls were acidified for determination of metal concentration by inductively coupled plasma atomic emission spectrometry (Spectroflame EOP, Littleton, MA, USA). Subsamples from each phenol concentration were acidified with H₂SO₄ and analyzed by Amdel Laboratories (New South Wales, Australia) for total phenols using gas chromatography. All toxicity results were expressed on the basis of measured metal and phenol concentrations.

**Sensitivity to copper-contaminated particles**

In order to establish a concentration–response relationship in the presence of contaminated particles, whole-sediment bioassays were carried out using copper tailings from a mine in Southeast Asia. Five concentrations (0.1, 0.3, 1.1, 3.3, and 10% w/v) were tested by adding the appropriate amount of tailings and seawater to 30-ml polycarbonate vials. To achieve a constant total sediment concentration of 10% w/v, toxicity tests with tailings diluted in a clean sediment (from Durras Lake, Australia) were also conducted. Particle size of the clean control sediment was matched to the tailings particle size distribution. One higher concentration (20% w/v) was also prepared. Sediment blanks (no algae) were prepared for each tailings concentration tested, and overlying water from these vials was syringe filtered through an acid-washed 0.45-μm filter (Sartorius, Goettingen, Germany) for analysis of dissolved copper by inductively coupled plasma atomic emission spectrometry. Tailings pore water was also tested for toxicity to *E. cf punctulata* using the pore-water FDA fluorescence inhibition bioassay described previously. Pore water was extracted by centrifugation (Sorvell, Asheville, NC, USA) for 15 min at 12,000 rpm at a constant temperature of 18°C. Pore water was filtered through a 0.45 μm filter and carefully transferred with acid-washed pipette tips into polyethylene containers and stored at 4°C. Pore-water toxicity tests were initiated within 24 h of pore-water extraction.

**Statistical analysis**

Toxicity was expressed as an IC₅₀ value, that is, the inhibitory concentration to affect 50% of the cells. This effect was either a decrease in esterase activity in 50% of the cells or a 50% reduction in growth rate compared to controls. The IC₅₀ values were calculated using linear interpolation with bootstrapping (ToxCalc Ver 5.0.23.C, Tidepool Software, McKingleyville, CA, USA). After testing the data for normality and homogeneity of variance, Dunnett’s multiple comparison test or Bonferroni t test was used to determine which treatments were significantly different from the controls. The no-observable-effect concentration was the highest concentration tested at which no statistically significant effect was observed on esterase activity or growth rate. The lowest-observable-effect concentration was the lowest concentration tested to cause a statistically significant effect on the esterase activity or growth rate. Tests for significance between single test concentrations and controls were determined using the homoscedastic or heteroscedastic t test. Significance levels were tested at the *p* ≤ 0.05 level.
Whole-sediment algal toxicity test


Fig. 1. Time course of intracellular fluorescein accumulation in Entomoneis cf. punctulata (μM represents μM of fluorescein diacetate).

RESULTS

Esterase inhibition test optimization—Water-only exposures

Preliminary screening of seven benthic marine microalgae showed that E. cf. punctulata best fulfilled the following criteria: ecologically relevant (a widely distributed benthic species), acceptable growth rates in minimal nutrient media, esterase activity (FDA fluorescence), sensitivity to a reference toxicant (copper), slow settling rates for ease of separation from sediment particles, and amenable to automated counting.

The FDA concentration and incubation time were optimized for this alga according to the method of Franklin et al. [13]. Uptake of FDA was linear over the first 5 min, with maximum fluorescence occurring after 7 to 10 min of incubation with FDA (25 and 45 μM) (Fig. 1). Longer incubation times resulted in decreased FDA fluorescence intensity. To avoid possible limitation of FDA uptake, an incubation time of 5 min was chosen from the linear portion of the curve. The optimal FDA concentration was 25 μM, as good separation occurred between the FDA fluorescence intensity of the control and deactivated (4% formalin) FDA-exposed cells (Fig. 2, control). Absolute fluorescence of the control varied between tests, but within-test variability was <5% because of the use of an exact
incubation time of 300 ± 2 s. All results were expressed as a percentage of control.

Optimizing the control response. To ensure a reproducible FDA fluorescence intensity response in healthy E. cf punctulata control cells (seawater only), a number of preinoculum and exposure conditions were investigated. Bioassay test conditions included low light (40 μmol photons/s/m²) versus high light (150 μmol photons/s/m²) and the addition of nutrients (15 mg NO₃/L and 1.5 mg PO₄/L), neither of which altered FDA fluorescence intensity. Pretreatment of the algae prior to use in the test inoculum did, however, have a significant effect on algal FDA fluorescence. Cells taken directly from the culture medium (unwashed cells), cells diluted to their original cell density after removing the culture medium (diluted inoculum), and cells washed and left to stand for up to 3 h prior to use, had significantly reduced FDA fluorescence intensity (50, 60, and 38% of controls, respectively) compared to the standard inoculum pretreatment (data not shown). Maximum FDA fluorescence intensity was obtained by using the standard procedure for preparing algal cells for toxicity tests [18]. Algal cells had to be inoculated into the test vials as soon as the inoculum was prepared to minimize the time to use and to prevent reduced FDA fluorescence. The addition of nitrate and phosphate was incorporated into the standard bioassay exposure conditions for pore-water-only test samples and controls to ensure that nutrient limitation would not occur during the 24-h test duration. Standard light conditions of 150 μmol photons/s/m² used for algal growth inhibition tests [18] were also selected for the FDA fluorescence intensity inhibition bioassays.

Inclusion of negative controls. For quality assurance purposes, negative controls were included in the bioassay protocol. Cells deactivated by boiling in a water bath for 10 min showed high variability in FDA fluorescence intensity, indicating that on some occasions the heat treatment was not sufficient to completely deactivate esterase activity and kill algal cells. Cells microwaved for 3 min underwent complete lysis and were therefore not able to be analyzed by flow cytometry. Cells fixed with 4% formalin for a minimum of 1 h remained intact and when stained with FDA consistently gave low fluorescence, similar to the fluorescence for unstained cells. All negative controls were therefore prepared by the addition of formalin for 1 h. The method of deactivation had the added advantage that the algal chlorophyll (red) fluorescence was not affected for up to 24 h after the addition of formalin. This was important, as separation of the algal esterase signal from the sediment particles relied on a high chlorophyll fluorescence intensity (a chlorophyll a threshold) for subsequent FDA fluorescence determination.

Esterase inhibition test optimization—Whole-sediment exposures

Filtration. Analysis of cellular FDA fluorescence intensity by flow cytometry required a compromise between having a sufficient number of cells for analysis and reducing the amount of sediment (which may block the flow cytometer) in the final samples. All samples were briefly shaken and then subsampled after a 30-s settling time. For some sediments that blocked the flow cytometer’s sampling nozzle, filtration through a Microtox solid-phase filter column (pre rinsed with seawater) prior to analysis was used. In algal-sediment mixtures, no algal cells were recovered in unfiltered samples; however, when the sample was filtered, recovery of algal cells was 60%, the same as the recovery of cells in the absence of sediment. A recovery of 60% of algal cells was acceptable for the enzyme (esterase) bioassay, as, unlike growth inhibition tests, it does not rely on absolute cell counts. The initial cell density used in the sediment bioassays was increased to 5 to 9 × 10⁴ cells/ml compared to water-only bioassays (2–4 × 10⁴ cells/ml) to compensate for the loss of cells analyzed by flow cytometry when sediment particles were present.

Tolerance of the FDA fluorescence inhibition bioassay to typical sediment–water physicochemical characteristics

The tolerance of the E. cf punctulata enzyme bioassay to a variety of salinities, pH values, and ammonia and sulfide concentrations, typical of natural sediments, is shown in Figure 3. Control FDA fluorescence intensity remained constant at salinities of 5 to 34% (Fig. 3A). The FDA fluorescence intensity in cells exposed to copper also showed similar sensitivity at salinities ranging from 15 to 34%, with 60% of cells having reduced FDA fluorescence at 25 μg Cu/L for a 24-h exposure. At salinities below 15%, sensitivity to copper was significantly reduced, with only 10% of cells showing a reduction in FDA fluorescence. The bioassay is therefore suitable for use over the salinity range 15 to 35%, similar to the 72-h growth inhibition bioassay with this species.

The effect of pH (6.0–8.5) on FDA fluorescence intensity in E. cf punctulata was compared to the FDA response at pH 8.0 (Fig. 3B). No significant inhibition or stimulation of FDA fluorescence was observed after a 3-h exposure (data not shown); however, significant (p ≤ 0.05) stimulation of FDA fluorescence (with cells shifting from S2 to S3) was observed at pH 6.0 after a 24-h exposure. This suggests that an optimal pH range for this bioassay was pH 6.5 to 8.5.

Concentration–response curves, comparing the effect of ammonia on 3- and 24-h FDA fluorescence intensity with 72-h growth rate, are shown in Figure 3C. As the measured concentration of total ammonia increased, the FDA fluorescence intensity decreased. After a 3-h exposure, FDA fluorescence intensity was significantly (p ≤ 0.05) inhibited relative to the controls in all ammonia treatments ≥8.5 mg N/L (Table 1). After a 24-h exposure, FDA fluorescence intensity was significantly inhibited in ammonia treatments of ≥13 mg N/L (Table 1). The FDA fluorescence intensity was less sensitive to ammonia than the 72-h growth inhibition bioassay. Algal growth rate was sensitive to ammonia, with complete inhibition (100%) after exposure to 20 mg N/L (Fig. 3C). The concentration of ammonia to cause a 50% reduction in 72-h algal growth rate (IC50) was 12 mg N/L, with concentrations ≥2.1 mg/L significantly inhibiting algal growth (Table 1).

Sulfide toxicity to E. cf punctulata was measured at two pH values: pH 8.1 (typical of seawater) and pH 7.0 (typical of pore waters). The effect of sulfide at pH 8.1 on 3- and 24-h FDA fluorescence inhibition is shown in Figure 3D. After a 3-h exposure, FDA fluorescence intensity for all sulfide treatments was similar or slightly stimulated compared to the controls. As the sulfide concentration increased, 24-h FDA fluorescence intensity decreased, with complete inhibition in FDA fluorescence after exposure to 80 mg S²/L. Algal FDA fluorescence intensity was significantly inhibited after exposure to 11 mg S²/L or greater, with an IC50 value of 22 mg S²/L. Sulfide concentrations of 2.0 mg/L or less did not affect algal FDA fluorescence intensity compared to controls (Table 1). Concentration–response curves of sulfide at pH 7.0 after 3- and 24-h exposures (data not shown) gave a similar but more variable response.
Fig. 3. Tolerance of *Entomoneis cf. punctulata* to salinity, pH, ammonia, and sulfide in water-only exposures. (A) The effect of salinity on shifts in fluorescein diacetate (FDA) fluorescence intensity (healthy control region S2) of *E. cf. punctulata* after a 24-h exposure. (B) The effect of pH on shifts in FDA fluorescence intensity of *E. cf. punctulata* after a 24-h exposure. * indicates FDA fluorescence is significantly different to the control at *p* ≤ 0.05. (C) The effect of ammonia on 3- and 24-h FDA fluorescence inhibition and 72-h cell division rate of *E. cf. punctulata*. (D) The effect of sulfide on 3- and 24-h FDA fluorescence inhibition and 72-h cell division rate of *E. cf. punctulata* at pH 8.1. Error bars represent one standard deviation.

Algal 72-h growth rate was more sensitive to sulfide than esterase activity. As the measured concentration of sulfide increased, algal growth rate decreased. Based on measured initial sulfide concentrations, complete inhibition of algal growth rate occurred at 80 mg S²⁻/L, with a 50% effect (IC50 value) at 3.0 mg S²⁻/L (Table 1). After a 48-h exposure, algal growth rate was five times more sensitive to sulfide with an IC50 value of 0.60 mg S²⁻/L. However, sulfide toxicity to algal growth was confounded by the rapid loss of sulfide in solution over the 48- and 72-h test durations. Stirring of the test solutions each day, particularly when pH adjustments were made to pH 7 solutions, enhanced the loss of volatile H₂S, contributing to the reduction in aqueous sulfide concentrations. No detectable sulfide was present after 72 h at any concentration tested, except for 80 mg S²⁻/L at pH 8.1, where only 12% of the initial sulfide was present in solution after 72 h. It was therefore very difficult to accurately determine sulfide toxicity to algal growth over 48- and 72-h exposures. The advantage of the FDA fluorescence inhibition bioassay was that sulfide losses were minimal over short exposure times, and enzyme
activity was more tolerant of sulfide concentrations typically found in marine sediments (micromolar to millimolar concentrations) [21].

Sensitivity of the FDA fluorescence inhibition bioassay to metals and phenol in water-only exposures

The toxicity of six metals (copper, zinc, lead, cadmium, manganese, and arsenic) and phenol to FDA fluorescence in *E. cf punctulata* after 3- and 24-h exposures was compared to 72-h growth inhibition in Table 2. Typical FDA fluorescence intensity histograms obtained after 3- and 24-h exposures to copper are shown in Figure 2. As the copper concentration increased, FDA fluorescence intensity decreased compared to the control. Of the metals tested, copper was the most toxic with an IC50 value of 97 ± 39 μg Cu/L and 260 ± 80 μg Cu/L for 3- and 24-h exposures, respectively. Acute toxicity of copper (100 μg/L) to enzyme activity generally decreased with increasing exposure time, from 35% inhibition at 3 to 6 h to 14% inhibition at 48 h. After 3- and 24-h exposures, the lowest-observed-effect concentration values for copper were 42 and 72 μg Cu/L. The FDA fluorescence was less sensitive to copper than cell division rate. Copper had an inhibitory effect on the cell division rate of *E. cf punctulata* after 48- and 72-h exposures, with IC50 values of 13 ± 1 μg/L and 22 ± 4 μg/L, respectively (Table 2).

For the metals cadmium, lead, manganese, and zinc, FDA fluorescence in *E. cf punctulata* was less sensitive than for copper with IC50 values greater than 1 mg/L. Algal growth was a slightly more sensitive endpoint for cadmium, zinc, and lead. Arsenic (III) concentrations of up to 75 μg/L did not affect algal FDA fluorescence compared to controls. Enzyme inhibition in *E. cf punctulata* showed similar sensitivity to phenol (IC50 value of 21–25 mg/L) as the marine bacterium *Vibrio fischeri* (Microtox) and was more sensitive than algal growth (IC50 value of 74–76 mg/L).

Application of the FDA fluorescence whole-sediment bioassay to assess the toxicity of copper tailings

Because of problems in maintaining neutral pH in sediment artificially spiked with copper [22], copper tailings were used as a surrogate for a copper-contaminated sediment. Mine tailings were tested for toxicity to *E. cf punctulata* FDA fluorescence intensity with and without dilution with Durras Lake control sediment. The mine tailings had a moisture content of 26% and a size fractionation of 42% silt (<63 μm), 32% sand (63–180 μm), and 26% coarse (>180 μm) particles. The total

<table>
<thead>
<tr>
<th>Bioassay</th>
<th>IC50a</th>
<th>LOECb</th>
<th>NOECc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia mg NH₃-N/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-h FDA inhibition</td>
<td>&gt;86</td>
<td>8.5</td>
<td>4.2</td>
</tr>
<tr>
<td>24-h FDA inhibition</td>
<td>&gt;86</td>
<td>13</td>
<td>8.5</td>
</tr>
<tr>
<td>48-h growth rate inhibition</td>
<td>11 (9.8–12)d</td>
<td>2.1</td>
<td>0.91</td>
</tr>
<tr>
<td>72-h growth rate inhibition</td>
<td>12 (11–14)</td>
<td>2.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Sulfide mg S²⁻/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-h FDA inhibition</td>
<td>&gt;80</td>
<td>&gt;80</td>
<td>80</td>
</tr>
<tr>
<td>24-h FDA inhibition</td>
<td>22</td>
<td>11</td>
<td>2.0</td>
</tr>
<tr>
<td>48-h growth rate inhibition</td>
<td>0.60 (0.30–1.0)</td>
<td>—</td>
<td>0.61</td>
</tr>
<tr>
<td>72-h growth rate inhibition</td>
<td>3.0 (1.6–8.5)</td>
<td>2.0</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*IC50 = inhibitory concentration to affect 50% of the cells.
*LOEC = lowest-observable-effect concentration.
*NOEC = no-observable-effect concentration.
*FDA = fluorescein diacetate.
*95% confidence limits.

Table 2. Toxicity of metals and phenol to *Entomoneis cf punctulata* in water-only exposures

<table>
<thead>
<tr>
<th>Toxicant</th>
<th>Fluorescence intensity inhibition</th>
<th>Division inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-h FDAb</td>
<td>24-h FDAa</td>
</tr>
<tr>
<td>Arsenic (III)</td>
<td>&gt;0.075c</td>
<td>&gt;0.075c</td>
</tr>
<tr>
<td>Cadmium (II)</td>
<td>2.8 ± 0.2e</td>
<td>3.7 ± 0.7e</td>
</tr>
<tr>
<td>Copper (II)</td>
<td>0.097 ± 0.039f</td>
<td>0.260 ± 0.080h</td>
</tr>
<tr>
<td>Lead (II)</td>
<td>&gt;1.0f</td>
<td>&gt;1.0f</td>
</tr>
<tr>
<td>Manganese (II)</td>
<td>&gt;2.4c</td>
<td>&gt;2.4c</td>
</tr>
<tr>
<td>Zinc (II)</td>
<td>8.6 (6.5–11)j</td>
<td>1.7 (0.2–3.0)j</td>
</tr>
<tr>
<td>Phenol</td>
<td>21 ± 13c</td>
<td>25 ± 8c</td>
</tr>
</tbody>
</table>

*Mean ± one standard deviation. IC50 = mean inhibitory concentration.
*FDA = fluorescein diacetate.
*ND = not determined.
*n = 1.
*10 = 2.
*n = 5.
*95% confidence limits.
concentration of particulate copper in the tailings was 1,800 ± 200 mg Cu/kg (dry wt).

Dilution with control sediment. Toxicity of the copper tailings/sediment mixture to algal FDA fluorescence intensity after 3- and 24-h exposures is shown in Table 3. Copper concentrations measured in the overlying water in the Durras Lake sediment controls were <2 μg Cu/L. Copper concentrations in the overlying water of the diluted tailings (0.3–3.3%) were also low (<13 μg Cu/L), and no toxicity was detectable. In contrast, high concentrations of copper (87–103 μg Cu/L) were measured in the overlying water at high tailings concentrations, with copper concentrations decreasing after a 24-h exposure. The tailings (10 and 20%) were toxic to the algae, with 37 to 72% inhibition after a 3-h exposure, and toxicity decreased after a 24-h exposure, in agreement with the measured copper concentrations.

Without dilution with control sediment. Toxicity of the copper mine tailings (without dilution with control sediment) and associated pore water after a 3-h exposure are shown in Figure 4. A concentration–response relationship was observed after algal exposure to mine tailings particles. As the tailings concentration increased, copper released from the tailings into the clean overlying water increased (up to 120 μg Cu/L). As the tailings concentration increased and subsequent overlying water copper concentrations increased, algal FDA fluorescence intensity decreased. At the highest tailings concentration, about 40% inhibition of FDA fluorescence was observed.

The toxicity of isolated pore waters from the copper tailings to algal FDA fluorescence intensity was also determined after a 3-h exposure. When FDA fluorescence intensity was plotted versus measured dissolved pore-water copper concentrations, the toxicity of pore water was similar to the toxicity of the tailings particles. This suggests that the primary route of exposure was via copper in solution (either pore water or overlying water) and was not due to direct contact with the copper-tailings particles.

The toxicity of the pore water and tailings particles was less than that predicted by the toxicity of copper alone (as copper sulfate) in a water-only exposure (Fig. 4). In this experiment, the 3-h IC50 for copper alone was 50 μg Cu/L, compared to an IC50 of 111 μg Cu/L for pore water. This suggests that copper in the tailings and tailings pore water was less bioavailable than ionic copper in the water-only exposure.

DISCUSSION

A pore-water test and a whole-sediment toxicity test with the estuarine benthic microalga *E. cf. punctulata* was successfully developed using flow cytometry to separate the algal enzyme response from sediment particles. The acute bioassay, based on inhibition of esterase activity (FDA fluorescence), was tolerant to a wide range of pH, salinity, ammonia, and sulfide concentrations typical of estuarine and marine sediments, suggesting the test’s potential as a widely applicable whole-sediment bioassay.

Prior to flow cytometry, FDA fluorescence detected by fluorimetry has been used to evaluate the metabolic activity and cell viability of microalgae in aquatic tests [23–25]. Inhibition of FDA fluorescence intensity may indicate either inhibition of FDA uptake into the cell or inhibition of cellular esterase activity. Using flow cytometry and propidium iodide as an indicator of altered FDA uptake, we previously showed that reduced uptake of FDA is unlikely to contribute to the decrease in FDA fluorescence observed in *E. cf. punctulata* [13]; that is, the decrease in FDA fluorescence is due to a real decrease in esterase activity.

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**Table 3.** Toxicity of copper tailing diluted with a clean sediment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu (μg/L)</th>
<th>Cu (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fluorescence intensity (%)</td>
<td>fluorescence intensity (%)</td>
</tr>
<tr>
<td>10% sediment control (Durras Lake)</td>
<td>&lt;2 ± 1</td>
<td>&lt;2 ± 1</td>
</tr>
<tr>
<td>20% sediment control (Durras Lake)</td>
<td>&lt;2 ± 1</td>
<td>&lt;2 ± 1</td>
</tr>
<tr>
<td>0.3% tailings</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>1.1% tailings</td>
<td>3 ± 2</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>3.3% tailings</td>
<td>8 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>10% tailings</td>
<td>87 ± 5</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>20% tailings</td>
<td>103 ± 10</td>
<td>25 ± 13</td>
</tr>
</tbody>
</table>

*a* Copper concentrations in overlying water in each sediment test vial.

*b* FDA = fluorescein diacetate.

*c* One standard deviation.

*d* Samples do not contain diluent clean sediment.

---

**Fig. 4.** Fluorescein diacetate (FDA) fluorescence inhibition in *Entomoneis cf. punctulata* after a 3-h exposure to (○) copper sulfate (water only), (■) pore waters from copper tailings, and (▲) the tailings particles (sediment) versus measured dissolved copper concentrations. Error bars represent one standard deviation.
Blaise and Ménard [9] were the first to apply flow cytometric detection of esterase activity in the freshwater alga \textit{S. capricornutum} as the basis of a freshwater sediment toxicity test. In developing a marine test in this study, we considerably modified the protocol of Blaise and Ménard to improve the test’s environmental relevance. First, we screened a number of marine benthic algae for esterase activity and selected an appropriate benthic species rather than a planktonic species as used by Blaise and Ménard [9]. This necessitated additional pretreatment steps, including short settling times and coarse filtering of the sample, to maximize benthic algal recovery. Static exposures were also used to more closely resemble benthic algal exposure at the sediment–water interface. A comparison of sediment toxicity to \textit{E. cf. punctulata} under static and nonstatic exposure conditions (data not shown) showed that continuous shaking resulted in greater toxicity (66–82% inhibition) compared to that obtained under static exposure conditions (23–72% inhibition) for one test sediment. While Blaise and Ménard [9] found that many of their sediments were toxic to \textit{S. capricornutum}, their bioassay used exposure periods of up to 24 h of continuous shaking, effectively producing anoxic sediment elutriate that could lead to an overestimation of contaminant toxicity in sediment. Hormesis was also noted, indicating that at high sediment concentrations, additional factors influenced (masked) toxicity, similar to the stimulation problems encountered when testing elutriates [9]. In comparison, stimulation effects in the \textit{E. cf. punctulata} bioassay were rare.

The sensitivity of \textit{E. cf. punctulata} esterase activity to a range of metals (copper, arsenic, lead, manganese, cadmium, and zinc) in water-only exposures was determined. Copper was the most toxic metal to the alga, with 3- and 24-h IC50 values of 97 ± 39 µg Cu/L and 260 ± 80 µg Cu/L, respectively. After 3- and 24-h exposures, the lowest concentration of copper to cause toxicity to the algae was 42 and 72 µg/L, respectively. These concentrations are typically found in sediments of marine benthic algae for esterase activity and selected an enzyme test was of similar sensitivity to toxicity tests carried out with marine invertebrate species (LC50 0.2 to >50 mg S2-/L) [21].

Because of the difficulties associated with spiking sediments with copper, such as decreasing pH, high copper pore-water concentrations, and long equilibration times [22], copper mine tailings were used as a surrogate for a copper-contaminated sediment. A concentration-dependent response in the presence of particles was observed. The FDA fluorescence inhibition was similar when algal cells were exposed to tailings particles or pore water. No increase in toxicity was observed when tailings particles were present, indicating that the primary route of exposure to copper was via copper in solution (pore water/overlying water) and not from direct contact with copper adsorbed to tailings particles. The toxicity of the pore water and tailings particles was less than that predicted by the toxicity of copper alone in a water-only exposure run simultaneously, with IC50 values for ionic copper and pore-water copper of 50 and 111 µg Cu/L, respectively. This suggests that copper in the tailings and pore water was less bioavailable than ionic copper in the water-only exposure. Similarly, Bat et al. [28] found that the amphipod \textit{Corophium volutator} showed greater sensitivity to copper in water-only exposures (96-h LC50 value of 21 mg/L) compared to copper in water exposures with clean sediment present (96-h LC50 value of 38 mg/L), presumably because of copper adsorption to sediment particles. Algal esterase activity was of similar sensitivity to copper in water-only exposures (IC50 of 97 µg/L) as survival in the adult freshwater amphipod \textit{Corophium} sp. (96-h LC50 of 80–86 µg/L and 10-d LC50 of 99 µg/L) [29]. The authors concluded that evidence from these experiments suggests that the main route of exposure of this amphipod to copper was also via pore water [29]. Both algal esterase activity and survival in \textit{Corophium} sp. were more sensitive to copper than adult \textit{C. volutator} (96-h LC50 of 21 mg/L) and juvenile \textit{G. locusta} (96-h LC50 of 0.3 mg/L) [28,30].

One criticism of toxicity tests based on an enzyme endpoint is that they are less environmentally relevant compared to tests using standard endpoints such as survival, growth, and reproduction. Although less sensitive to metals than growth inhibition in water-only tests, a good correlation between the acute enzyme response (24-h exposure) and chronic growth rate inhibition was found in this study for copper, zinc, and cadmium (γ = 1.44x + 0.29, r2 = 0.997), suggesting that this acute test may be a useful surrogate for chronic growth tests of longer duration. Using the alga \textit{S. capricornutum}, Franklin et al. [13]...
showed excellent correlation between 3-h esterase activity tests and the standard growth inhibition test for a range of metals. Despite differing methodology, Snell and coworkers [25] also found a correlation between 1-h esterase activity and growth rate for a range of metals and organics using the same alga.

Evidence is increasing that sediments ranked as toxic using this algal enzyme test agree well with acute lethality tests with amphipods. In 15 polycyclic aromatic hydrocarbon–contaminated sediments tested using both 24-h algal enzyme inhibition and survival of juvenile amphipods (Melita plumulosa) over 10 d (M.S. Adams et al., unpublished data), a significant correlation ($r = 0.968$) existed between the percentage of viable cells determined by FDA fluorescence using flow cytometry and that determined by algal colony formation experiments. Further work is under way in our laboratory to definitively link the enzyme response with the ability of cells to survive and reproduce after sediment exposure to further improve the environmental relevance of the test.

With the recent introduction of sediment quality guidelines [16] in Australia and New Zealand, the need has increased for suitable whole-sediment and pore-water tests with local species. The benthic algal bioassay developed here is the first whole-sediment bioassay with marine algae to be developed and applied to testing contaminated sediments. This test will complement existing whole-sediment bioassays and add to the suite of organisms that can be used to assess sediment quality. The sensitivity of the bioassay to organic contaminants, particularly polycyclic aromatic hydrocarbons, is currently being assessed and compared to acute tests with amphipods and bi-valves. In addition, an in situ test based on the same algal enzyme response is being developed in order to avoid problems such as redox and metal speciation changes associated with sediment collection and transportation.

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