Methods for Identifying Causes of Toxicity in Whole-Sediments

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Certificate of Authorship of Thesis

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ABSTRACT

Whole-sediment toxicity identification and evaluation (WS-TIE) is a relatively new approach for assessing the cause of toxic effects to benthic organisms in sediments. Akin to aqueous TIE methods, the premise of the WS-TIE method is that the chemical toxicant(s) responsible for observed effects can be identified through a series of treatments that are designed to reduce the bioavailability and, thus, toxicity of key contaminant classes. While standardised WS-TIE methods have been developed for a range of contaminants (US EPA, 2007), many contaminated sediments exist for which the methods can not adequately identify the cause(s) of toxicity. Standard WS-TIE methods primarily manipulate the toxicity of dissolved contaminants, but do not address effects that may occur via dietary exposure to chemical contaminants.

The research presented herein, recognises that standard WS-TIE methods do not address all of the major contaminant exposure pathways for some benthic organisms. New WS-TIE methods to address toxic effects of those contaminants acting via dietary exposure routes were developed as a part of this research. The new methods were specifically designed for whole-sediment toxicity tests using the epibenthic amphipod *M. plumulosa*; a deposit feeding species that can display acute toxicity from dietary exposure to contaminated sediments (Simpson and King, 2005; Mann and Hyne, 2008; Spadaro et al., 2008). WS-TIE methods using the microalgae *Entomoneis cf. punctulata* were also developed to compliment results achieved using *Melita plumulosa* in sediment quality assessments.

New WS-TIE treatments were developed to modify the organism’s exposure to sediment contaminants by modifying the bioaccessibility of particulate-associated contaminants (PACs) to *M. plumulosa*. The two new techniques were principally employed to achieve this goal. Firstly, a mesh exposure chamber (MEC) was developed that effectively prevented *M. plumulosa* from ingesting sediments, but did not modify the exposure to dissolved contaminants in the overlying water. Secondly, resins deployed at the sediment surface, metal chelating resin-top (MCR-Top) or carbonaceous adsorbent resin-top (CAR-Top) to both remove from the dissolved phase, metals and organic contaminants, respectively. It was demonstrated that sediment nutrition had a large influence on the outcome of whole-sediments toxicity tests, and a food addition (FOOD) treatment was incorporated into the suite of WS-TIE treatments to help differentiate between the natural effects caused by nutritionally-poor sediments and the toxic effects of dietary exposure to contaminants.
For the amphipod *M. plumulosa*, the standard WS-TIE methods were demonstrated to be ineffective for reducing, or eliminating, the toxicity of a range of sediments. For ~80% of the sediments investigated, >50% of the toxicity to *M. plumulosa* was unaccounted for using standard WS-TIE methods. By applying the new WS-TIE treatments in combination with the standard WS-TIE methods, >90% of toxicity could be identified for each of the sediments. For almost half these sediments, PACs were considered to have significantly contributed to toxicity (50-100%). Additionally, new WS-TIE treatments were able to improve the identification of dissolved metals and hydrophobic organic contaminants (HOCs), when compared to standard WS-TIE methods alone. The application of both standard and new WS-TIE methods identified dissolved metals and HOCs as the chief causes of toxicity to *M. plumulosa* in ten sediments containing a wide range of contaminants.

Apparent toxicity due to inadequate sediment nutrition was demonstrated to confound the interpretation of the WS-TIE studies. It was found to be beneficial to apply a ‘minimal’ feeding regime, comprising approximately 0.06 mg fish-food per amphipod on days 3 and 7 of 10-d toxicity tests, to ensure that a lack of nutrition did not cause toxic effects in sediments containing low nutritional value (e.g. sandy sediments with low amount of organic matter). Sediments that contained ≥10% fine particles (silt, <63 µm) and >2% organic carbon were determined to have adequate nutrition and FOOD treatments were unnecessary. The addition of the FOOD treatment in WS-TIEs was generally a useful tool for discerning poor nutrition from toxicity to *M. plumulosa*. However, in applying a FOOD treatment to WS-TIEs, it was observed that selective feeding could mask the toxic effects of some contaminants, namely hydrophobic organic chemicals. Therefore, the FOOD treatment was used cautiously to discern poor nutrition from toxicity using additional lines-of evidence, such as knowledge of changes to the organism’s sensitivity to contaminants and contaminant concentration-effect relationships.

For the microalgae, *E. cf punctulata*, for which the only significant contaminant exposure pathway is believed to be the passive diffusion of dissolved contaminants or their free ions across the cell surface, a rapid WS-TIE method based on FDA fluorescence inhibition in *E. cf punctulata* using flow cytometry was developed for dissolved metals (metal chelating resin, MCR, treatment), organic contaminants (carbonaceous adsorbent resin, CAR, treatment), and ammonia (zeolite, ZEO,
treatment). These treatments were effective for reducing the toxicity of contaminants in both natural-contaminated and spiked-sediments.

The identification of all the chemical toxicants within contaminated sediments was generally found to be very difficult. The variability between replicates was usually high, ranging from 0-19% (amphipod survival). The use of a multiple lines-of-evidence approach with WS-TIE is recommended for sediments containing a wide variety of chemical contaminants. The lines-of-evidence used to enhance the understanding of toxicants in the sediments of this study included: (i) chemical analyses of the test waters, (ii) analyses of physical/chemical properties of the sediment (i.e. total particulate metals and acid extractable metals, organic contaminants, sediment fractionation, total organic carbon, etc), (iii) understanding of the organisms contaminant-exposure pathways, (iv) quantifying the role of nutrition to sediment toxicity (i.e. silt and TOC content), (v) measuring the effects of added food on organism sensitivity, contaminant bioavailability, feeding behaviour or physiology (i.e. depuration and detoxification processes), and (vi) understanding the physiology and behaviour of the organism, and the factors that may have significant effects.

In order to strengthen the lines-of-evidence for interpreting WS-TIE data, contaminant concentration-effect relationships were determined for contaminants often observed in sediments. For *M. plumulosa*, the 10-d EC$_{50}$ for dissolved un-ionised ammonia, copper, and zinc were 980, 75, and 220 µg/L, respectively. The IC$_{50}$ values for dissolved copper and zinc to *E. cf. punctulata* were 13 µg/L and 1,500 µg/L, respectively. The IC$_{x}$ and EC$_{x}$ values were used to determine the specific contaminant(s) causing toxicity and/or the contribution of these contaminants to observed toxic effects in sediments containing a mixture of contaminants.

The effect of contaminants on the growth of *M. plumulosa*, and the ability of WS-TIE methods to determine which stressors were the causes of any growth effects, was assessed. Results from the natural-contaminated sediments and the chemical-spiked sediments demonstrated that the growth, measured as amphipod size (area), was a more sensitive indicator of toxicity than acute survival. However, WS-TIE methods were far less effective for identifying toxicity using amphipod growth than survival. Difficulties with applying a growth toxicity indicator using *M. plumulosa* arose from the significant influence of sediments physico-chemical properties, storage and handling of sediment and feeding regime during whole-sediment toxicity tests.
New WS-TIE methods and additional lines-of-evidence described in this research provide a more comprehensive approach for sediment quality assessment, specifically for those organisms exposed to sediment-associated contaminants via their diet. However, further research is required to enhance techniques for delineating toxicity due to dissolved and particulate-associated toxicity, and identifying specific classes of PACs. Due to the variability associated with whole-sediment toxicity tests, the use of multiple lines-of-evidence is essential for WS-TIE procedures.
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LIST OF ABBREVIATIONS

*AEM*: acid extractable metals

*CAR*: carbonaceous adsorbant resin

*CC*: coconut charcoal

*EDTA*: ethylenediaminetetraacetic acid

*FDA*: flouroscein diacetate

*GCC*: granular coconut charcoal

*HOC*: hydrophobic organic contaminant

*ISQG*: interim sediment quality guidelines

*LOEC*: lowest observed effect concentration

*MCR*: metals chelating resin

*MEC*: mesh exposure chambers

*NOEC*: no observed effect concentration

*PAC*: particulate-associated contaminants

*PAH*: polycyclic aromatic hydrocarbon

*PCC*: powdered coconut charcoal

*TBT*: tributyl tin

*TIE*: toxicity identification evaluation

*TOC*: total organic carbon

*TPM*: total particulate metals

*WS-TIE*: whole-sediment toxicity identification evaluation

*ZEO*: zeolite