

A two-step approach to sediment toxicity testing Proof-of-concept

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Introduction

According to the US EPA, there are at least 666 chemicals and chemical categories that are known environmental hazards. Many of these chemicals enter our waterways where they can have deleterious effects on marine organisms and ecosystems. Additionally, there exists the possibility of detrimental impacts resulting from synergistic interactions of otherwise benign chemicals. With so many potential pollutants in our waters, testing for the presence of individual pollutants can be prohibitively expensive and very labor intensive.

ORCA has developed an ecotoxicity monitoring program to quickly and inexpensively identify pollutants in marine environments. We use a two-step approach to survey and monitor marine pollution. First, a rapid, inexpensive, broad-spectrum bioassay identifies toxic "hot spots". This approach allows us to test a large number of sediment samples within the study region, thus minimizing the possibility of missing an area of high toxicity. Next, sites identified as toxic "hot spots" are analyzed further for specific pollutants by an EPA laboratory. This allows us to use budget dollars to test for a greater number of possible pollutants since we will only conduct the more expensive analyses for individual pollutants on a small number of samples with known toxicity.

ORCA tested the Microtox® solid-phase bioassay (SDIX, Newark, DE) as the broad-spectrum toxicity assay for step one of our ecotoxicity monitoring program. Microtox® has been used to test sediment toxicity in the US and abroad (Ringwood et al., 2002; Cotou et al., 2001; Beg and Alie, 2008).

The Benthic Ecology Laboratory at the Smithsonian Marine Station (SMS) in Fort Pierce has continually monitored the soft-sediment infaunal communities at 13 sites in the Indian River Lagoon since February 2005, with two additional sites added in April 2007 (figure 1). As proof-of-concept for our program, we collected sediment samples from the same 15 sites monitored by the SMS to identify the relationship between sediment toxicity as measured by Microtox® and SMS's benthic data.



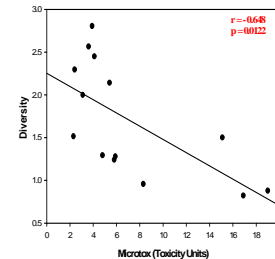
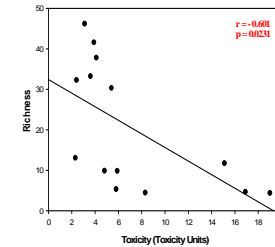
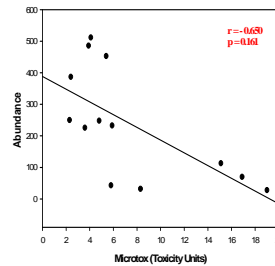
Materials and methods

In April 2009, the SMS sampled the 15 stations. At each station three 0.02m² Ponar grab samples were collected. Grab samples were processed in the Benthic Laboratory at SMS. Animals were sorted and identified to their lowest possible taxonomic level. Abundance defined as mean number of individual animals per 0.02 m², richness defined as mean number of Taxa per 0.02 m², and diversity were determined for each station.

In May 2009 ORCA collected sediment samples from the same 15 Smithsonian stations. Using a core sampler, five sediment samples were collected from each station. Each sediment sample consisted of the top 5 cm of the core. The five sediment samples from each station were homogenized and composited into one sample for toxicity analysis. Two 100 gram aliquots of each sample were used for grain size analysis and determination of dry weight. Toxicity analysis of the sediment samples were conducted following the standardized Microtox® solid-phase protocol in which *V. fischeri* colonies were exposed to serial dilutions of sediment solutions. The luminescence inhibition after 30 minutes was measured by the Microtox® 500 analyzer (SDIX, Newark, DE). The data obtained was used to calculate the EC₅₀, which is the median sample concentration that caused a 50% reduction in bacterial bioluminescence. Sediment dry weights were used to determine water-corrected EC50s, and toxicity units (TU) were calculated to standardized results with the formula TU = (100/EC₅₀₀) X 1000.

Results

As shown in the graphs below (Figures 2,3,4), the Microtox® EC₅₀ from the sediment samples tested were significantly inversely correlated with the benthic abundance, richness and diversity of samples collected from the same sites.



Conclusions

Ecologically relevant losses in the abundance and diversity of benthic infauna have been shown to correspond with acute sediment toxicity when measured by amphipod survival in laboratory tests (Long et al., 2001). Corresponding the Microtox® acute sediment toxicity bioassay with benthic infauna abundance, richness and diversity serves as our proof-of-concept for step one of our two-step approach to toxicity monitoring. These results indicate that the Microtox® solid-phase test is a reliable method to identify toxic "hot spots" in marine sediment. Further testing and validation is underway to verify the appropriateness of the Microtox® solid-phase test as the broad-spectrum bioassay for use in ORCA's ecotoxicity monitoring program.



Literature cited

- Beg, K.R., Ali, S. 2008. Microtox toxicity assay for the sediment quality assessment of Ganga River. *American Journal of Environmental Sciences* 4:467-472.
- Cotou, E., Papatthanassiou, E., Tsangaris, C. 2002. Assessing the quality of marine coastal environments: comparison of scope for growth and Microtox® bioassay results of pollution gradient area in eastern Mediterranean (Greece). *Environmental Pollution* 119:141-149.
- Long, E.R., Hong, C.B., Severn, C.G. 2001. Relationships between acute sediment toxicity in laboratory tests and abundance and diversity of benthic infauna in marine sediment: a review. *Environmental Toxicology and Chemistry* 20:46-60.
- Ringwood, A.H., DeLorenzo, M.E., Ross, P.E., Holland, A.F. 1997. Interpretation of Microtox® solid-phase toxicity tests: the effects of sediment composition. *Environmental Toxicology and Chemistry* 16:1135-1140.

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Figure 2. Pearson Product Moment Correlation between benthic abundance (mean number of individual animals per 0.02 m²) and toxicity (Microtox® EC50s).

Figure 3. Pearson Product Moment Correlation between benthic richness (mean number of Taxa per 0.02 m²) and toxicity (Microtox® EC50s).

Figure 4. Pearson Product Moment Correlation between Shannon (H1og) species diversity and toxicity (Microtox® EC50s).