Sediment Toxicity on the Mainland Shelf of the Southern California Bight in 1994

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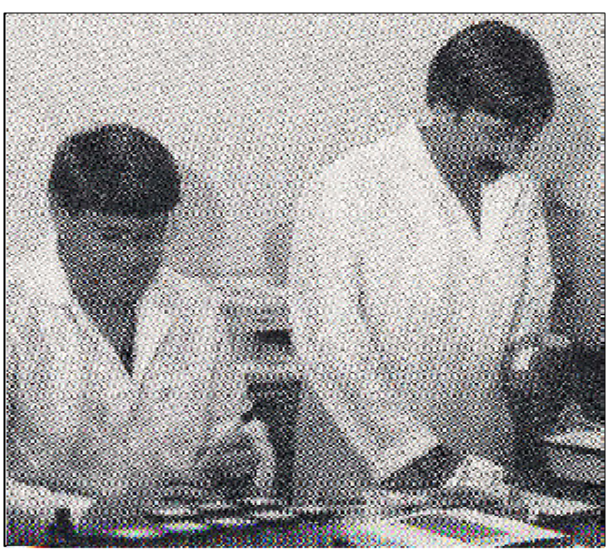
A pilot regional sampling program, the Southern California Bight Pilot Project (SCBPP), was designed and conducted as a cooperative effort by a group of local, state, and federal agencies concerned with understanding the effects of contaminants on the marine environment (Bergen *et al*. 1995). The SCBPP was based on the probability-based sampling design used by the United States Environmental Protection Agency (USEPA) Environmental Monitoring and Assessment Program (EMAP; Overton *et al*. 1990).

The benthic environment was the principal focus of SCBPP sampling and measurements. Most chemical contaminants entering the marine environment have an affinity for particles and tend to accumulate to elevated concentrations in sediments. Contaminated sediments have the potential to cause toxicity to benthic or demersal organisms. Therefore, a major concern of the SCBPP was the evaluation of various indicators of sediment quality, such as sediment toxicity, that could be used to evaluate the health of benthic environments.

Most sediment toxicity tests involve the exposure of a single species in the laboratory to sediments collected from the area of interest. The entire sediment sample may be tested (whole sediment toxicity) or various fractions may be evaluated, such as interstitial water or solvent extracts of the sediment. A wide variety of species and endpoints are used, such as survival, growth, and reproduction (Lamberson *et al*. 1992).



Two different sediment toxicity tests were selected for use with SCBPP samples in an effort to balance the advantages and limitations of this assessment tool. The first method measured whole sediment toxicity to the tube-dwelling amphipod, *Ampelisca abdita*. Amphipods are among the first species to disappear from benthic communities impacted by pollution (Swartz *et al*. 1982). Sediment toxicity tests with amphipods are widely used in a variety of monitoring programs and standardized methods have been developed to improve data reliability (ASTM 1991). Survival of amphipods following a 10-day exposure period is the endpoint for this test. This bioassay minimizes many of the limitations of sediment toxicity tests by measuring a clearly adverse response (death), requiring relatively little manipulation of the sample, and using a species representative of an ecologically important group.

An interstitial water toxicity test using Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryos was selected to provide greater sensitivity (and thus better discrimination between sites). In this test, developing sea urchin embryos are exposed to several dilutions of interstitial water for three days and then examined for abnormalities in development. Sea urchin embryos are often more sensitive to contaminants than the adult life stages of fish or crustaceans (Bay *et al*. 1993). This test provides an evaluation of a sediment compartment (interstitial water) considered very important for contaminant bioavailability and toxicity.

This report presents the results of sediment toxicity tests conducted on one quarter of the SCBPP sediment samples. Sediment toxicity tests are not widely used for monitoring the coastal environment of Southern California; these tests were included in the SCBPP survey to demonstrate their feasibility. Our results illustrate that sediment tests are feasible for regional monitoring; they identify method improvements for future sampling efforts.

MATERIALS AND METHODS

Field Sampling

Sediment samples were collected from 72 stations during the SCBPP survey of July-August 1994. Station locations ranged from Point Conception, California to the United States-Mexico border at depths of 10-200 m (Figure 1). These stations represented a subset of SCBPP sites selected for the benthic chemistry analysis. The stations analyzed represented the geographic and depth range of the entire SCBPP. Samples were also distributed between areas near and away from municipal wastewater outfalls. The distinction between outfall and nonoutfall stations was somewhat arbitrary, as it was based primarily on the boundary of the sampling grid currently used to monitor each outfall (see *The Southern California Bight Pilot Project: Sampling Design*, this annual report). Sediments were collected using a 0.1 m^2 Van Veen grab. A minimum of three liters of surface (top 2 cm) sediment was collected from each station with a polyethylene scoop and placed in one-liter polyethylene jars.

Sediment Handling

Sediment samples were stored under refrigeration (3-5 °C) in the dark for up to four weeks before testing. The contents of the storage jars for a single station were combined and homogenized by hand to prepare a composite sample for toxicity tests.

Whole Sediment Toxicity

Solid phase toxicity tests were conducted using a 10-day amphipod survival test. Approximately half of the samples were tested by the Southern California Coastal Water Research Project (SCCWRP). The remaining samples were analyzed by Science Applications International Corp. (SAIC; Narragansett, Rhode Island). A total of 10 experiments were conducted to test all of the samples.

Both laboratories used essentially the same test procedures (ASTM 1991). All sediment samples were passed through a 1 mm (2 mm for SAIC) mesh stainless steel screen (without adding water) to remove large debris and potential predators. The sediment passing through the screen was used in the test. A 2 cm layer of sediment was placed in the bottom of a quart-sized glass canning jar and covered with 600 mL of laboratory seawater having a salinity of 30 g/kg (ppt). A glass aeration tube was then added to ensure that all samples had acceptable dissolved oxygen concentrations. Sediment addition occurred one day before the start of the test. Five replicate jars were set up for each sediment sample.

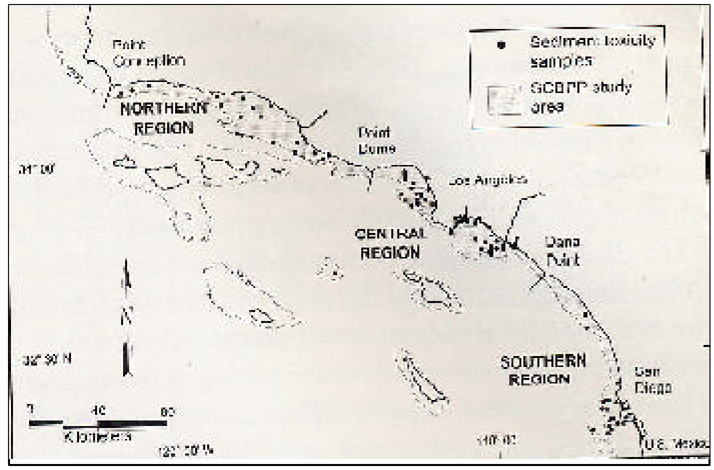


FIGURE 1. Location of stations for collection of sediment toxicity samples, Southern California Bight Pilot Project, July-August, 1994.

The toxicity test was started by adding twenty individuals of the tube-dwelling amphipod, *Ampelisca abdita*, to each jar. Amphipods were collected from tidal flats in the Pettaquamscutt (Narrow) river, a small estuary flowing into Narragansett Bay, Rhode Island. The exposure was conducted at 20°C under constant light for 10 d. Surviving amphipods were screened from the sediment at the end of the exposure and counted to determine the percent survival. Negative controls (collection site or nontoxic reference sediment) and reference toxicants (sodium dodecyl sulfate or cadmium chloride) were tested concurrently for quality assurance purposes.

Interstitial Water Toxicity

Interstitial (pore) water was obtained from each sediment sample and tested for toxicity using a sea urchin embryo development test. The sea urchin embryo development test is based on widely-used procedures refined at SCCWRP (USEPA 1995). All interstitial water tests were conducted by SCCWRP. Five experiments were required to test all samples.

Interstitial water was obtained by centrifuging approximately 400 mL of sediment at 3,000 x g for 30 min. The supernatant was carefully removed with a pipette and transferred to a glass jar. The salinity and pH of the interstitial water sample was measured before the sample was diluted and tested. Samples with low pH ($\leq$7.5) were adjusted to 7.6-8.0. Three concentrations of interstitial water (100, 50, and 25%) were prepared if sufficient sample volume was available. Laboratory seawater was used to prepare dilutions. Three replicates of each concentration were prepared and 10 mL were placed into 22 mL glass scintillation vials.

Toxicity tests were initiated within eight hours of interstitial water collection. Pacific purple sea urchin embryos were used in all experiments. Gametes were obtained by injecting sea urchins with potassium chloride; the eggs were fertilized, and then 250 embryos added to each vial. The exposures were conducted at 15°C for 72 h under a 12 h light:12 h dark photoperiod. The salinity was approximately 33 g/kg.

The preserved samples were examined at a magnification of 100 x and approximately 100 embryos from each sample were evaluated for abnormal development. Abnormal development is usually expressed as a delay in developmental rate or the presence of pathological conditions (e.g., dead cells, abnormal gut development, irregular cell division).

Reference toxicant (copper chloride) and negative control samples were tested concurrently for quality assurance purposes. The control consisted of laboratory seawater that had been passed through the centrifugation process.

Water Quality

Initial and final water quality measurements were made during each toxicity test. These measurements consisted of temperature, dissolved oxygen (DO), pH, salinity, and total ammonia. Un-ionized ammonia concentration was calculated using information obtained from Whitfield (1974) and Hampson (1977).

Data Analysis

Toxicity data were summarized for each station by calculating the mean and standard deviation of percent amphipod survival or percent normal embryo development among replicates. Data were normalized by dividing the sample mean by the respective control mean for the experiment to express the response (survival or normal development) as a percentage of the control response.

Statistically significant test responses were determined by calculating t-tests between each sample and the control ($p \leq 0.05$). Samples were not identified as toxic based solely on t-test results. Rather, a threshold of a 20% response relative to the control also had to be attained to identify reliably toxic samples (i.e., only samples having ≤ 80% of the control value were identified as toxic). These criteria have been established by USEPA for other EMAP projects (USEPA 1994). Analysis of prior *A. abdita* data by SAIC shows that the use of these criteria results in about a 90% power to detect a 20% difference in survival (SAIC 1994). Analysis of SCCWRP data for SCBPP samples indicated that an 80% response was also an appropriate threshold for the sea urchin embryo development test.

Logistic regression of the urchin embryo percent normal and ammonia concentration data was performed using the SigmaStat software package (Jandel Scientific 1994).

RESULTS

Whole Sediment Toxicity

Amphipod survival tests were successfully conducted on 71 of the 72 samples collected. Mean control survival was 91% for all tests (range: 84-97%) and water quality parameters (DO, pH, salinity, and temperature) were usually within acceptable limits. The overlying water ammonia concentration was elevated for many samples, with most being greater than 1 mg/L of total ammonia. Typical seawater concentrations are usually less than 0.1 mg/L.

Amphipod survival was high in all samples tested, ranging from 80-98%. Statistically significant reductions in survival were identified for six samples. However, none of the 72 samples were identified as toxic, as amphipod survival was >80% of the control for all samples (Figure 2).

Few trends are evident when the normalized data are examined by region or proximity to outfalls. Survival values in the northern region (no large sewage discharges)

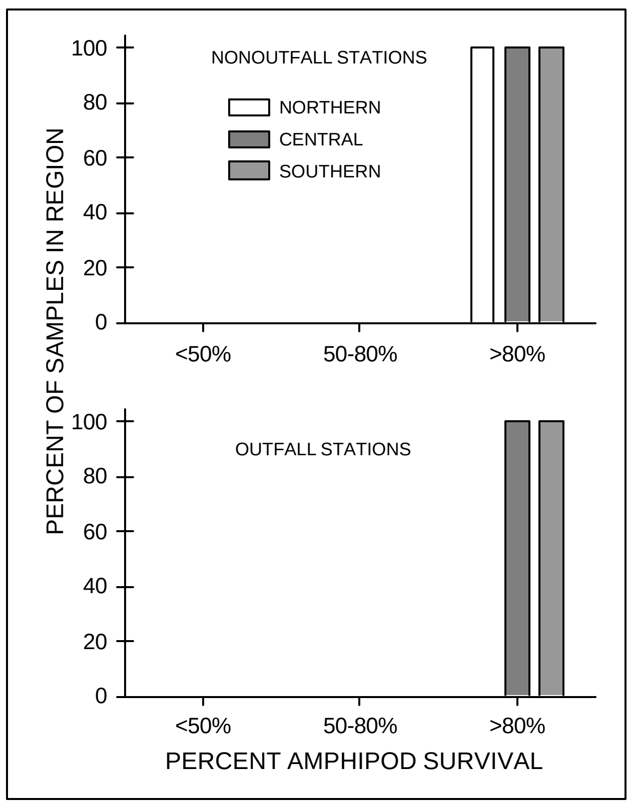


FIGURE 2. Summary of 10-day amphipod (*Ampelisca abdita*) toxicity test results for whole sediment, from Southern California Bight Pilot Project Survey. The results are grouped in categories corresponding to no toxicity (>80% survival), moderate toxicity (50-80% survival), and high toxicity (<50% survival).

were similar to those in the more highly urbanized central and southern regions. Sediment from sites located near sewage outfalls generally produced similar survival percentages compared to other locations.

Interstitial Water Toxicity

Sea urchin embryo development tests were conducted on 50% interstitial water from all SCBPP sediment toxicity samples collected. An insufficient interstitial water volume was obtained from a few samples to permit testing at the other two concentrations; however, 69 and 71 stations were tested at 100% and 25%, respectively. All experiments had acceptable control development (80-89%, mean=85%), temperature, salinity, and DO values. Many of the samples had a relatively low pH (≤ 7.5) immediately after centrifugation; these were adjusted to pH 7.6-8.0 with a dilute sodium hydroxide solution before testing. Total ammonia concentration was elevated in many of the samples. No adjustments to the ammonia concentrations were made before testing.

In contrast to the amphipod survival test data, sea urchin embryo development was affected by interstitial water from many sediment samples. Most of the 100% and 50% samples produced less than 80% of the control development in embryos, with many samples producing less than 5% normal development. Even a majority of the samples diluted to 25% interstitial water produced substantial effects on embryo development.

Effects on embryo development were evident in samples from all three geographic regions and did not appear to have any relationship to sewage outfall proximity (Figure 3).

Many of the interstitial water samples contained elevated ammonia concentrations, even at the 25% concentration (Figure 4). There appeared to be a correspondence between ammonia concentration and embryo toxicity for many of the samples. Strong effects on embryo development were consistently present whenever total ammonia concentration exceeded about 3 mg/L. A similar relationship was found when embryo toxicity was compared to the concentration of un-ionized ammonia (NH_3), which is generally thought to be the form toxic to marine life. Embryo development was always greatly reduced in samples containing >0.1 mg/L of un-ionized ammonia. Subsequent analyses of the interstitial water toxicity data were limited to the 25% samples in an effort to minimize the influence of ammonia and provide better discrimination between stations.

Additional toxicity tests were conducted to verify the toxicity of ammonia to Pacific purple sea urchin embryos (see *Toxicity of Ammonia to Pacific Purple Sea Urchin*

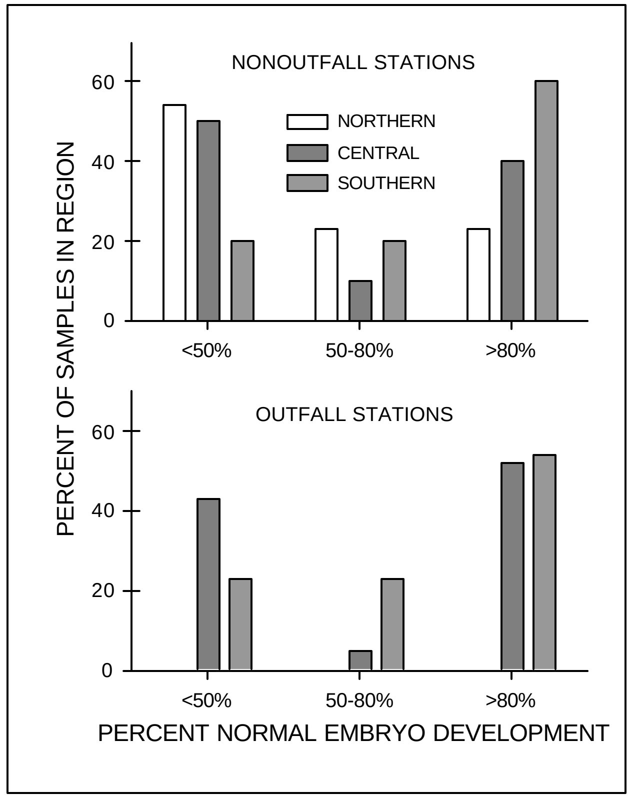


FIGURE 3. Summary of 72-h Pacific purple sea urchin embryo (*Strongylocentrotus purpuratus*) toxicity test results for 25% interstitial water, for the Southern California Bight Pilot Project Survey. The results are grouped in categories corresponding to no toxicity (>80% normal development), moderate toxicity (50-80% normal development), and high toxicity (<50% normal development).

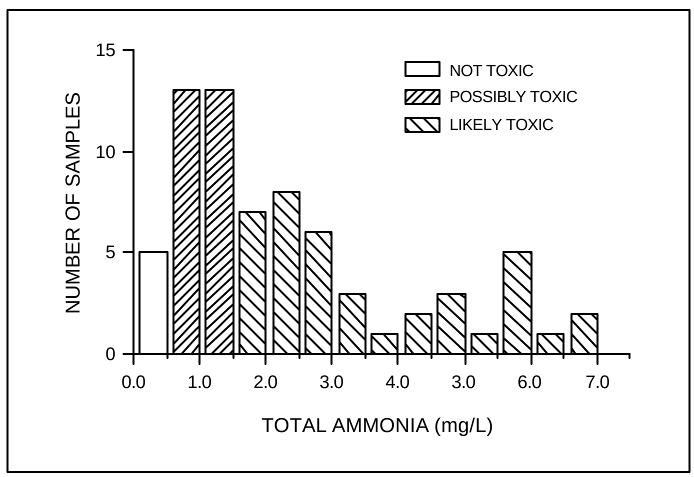


FIGURE 4. Average total ammonia concentration of 25% interstitial water during toxicity tests, for the Southern California Bight Pilot Project Survey. The shaded bars indicate total ammonia concentrations that occasionally (possibly toxic) and almost always (likely toxic) result in un-ionized ammonia concentrations above the toxic threshold of 0.04 mg/L. Un-ionized ammonia concentrations can vary for the same concentration of total ammonia due to differences in pH, temperature, and salinity.

(Strongylocentroutus purpuratus) *Embryos*, this annual report). The results show that sea urchin embryos are adversely affected by ammonia at concentrations measured in many SCBPP interstitial water samples.

In an attempt to separate the effects of ammonia toxicity from other possible sources of toxicity, the following procedure was used. The percent normal development at the 25% concentration was plotted against the mean of the initial and final un-ionized ammonia concentration for that sample. A logit regression with 99% confidence intervals was then fitted to the data. Next, the percent normal development for each sample was compared to the value predicted by the regression (lower bound of 99% CI) for the corresponding un-ionized ammonia concentration. Finally, the results of the comparison were used to classify the 25% interstitial water samples into one of three groups.

> *Indeterminate*: samples with ≥0.08 mg/L un-ionized ammonia and <10% normal development. Toxicity from factors other than ammonia could not be evaluated because ammonia concentrations were high enough to mask any additional response.
>
> *Nontoxic*: samples with <0.08 mg/L un-ionized ammonia and a percent normal development at least as high as that predicted from the lower bound of th 99% CI or samples with ≥0.08 mg/L and ≥10% normal development. All of the toxic ity in these samples could be attributed to the presence of ammonia.
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> *Toxic*: samples with <0.08 mg/L ammonia and a percent normal development below the 99% CI of the ammonia regression. These samples showed an effect greater than that expected solely from the presence of ammonia.

This procedure was able to evaluate (as toxic or nontoxic) 52 of 71 samples tested at 25% (Figure 5). The remaining samples were classified as indeterminate because of high ammonia concentration.

Fifteen samples were classified as toxic. The measured percent normal for these samples usually differed from the predicted value by at least 10% (Figure 5). Most of the samples classified as nontoxic had percent normal values within the 99% confidence interval based on ammonia concentration. There were a few samples with percent normal development values greater than predicted from the un-ionized ammonia effect (Figure 5).

A majority of the samples identified as toxic were located in the northern region (Figure 6a). The two

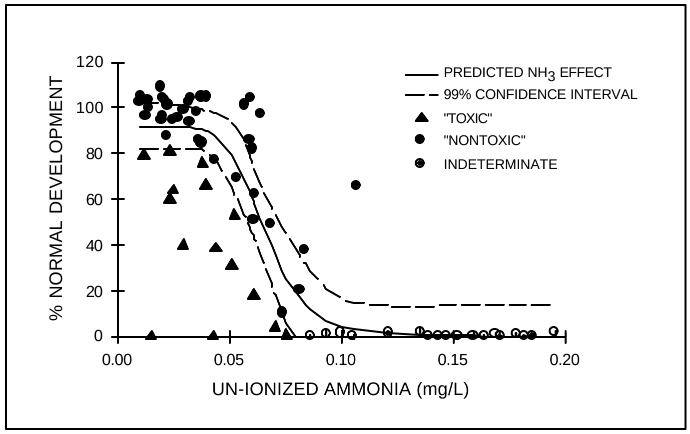

FIGURE 5. Results of logit regression on interstitial water ammonia and Pacific purple sea urchin (*Strongylocentrotus purpuratus*) embryo development results, for the Southern California Bight Pilot Project Survey. Samples falling below the 99% confidence interval for the regression were classified as "toxic" since reductions in normal embryo development were greater than could be attributed to ammonia toxicity alone. Samples containing >0.08 mg/L un-ionized ammonia and <10% normal development were classified as indeterminate.

samples with the greatest interstitial water effect from factors other than ammonia were located in the northern region. Of the seven sites identified as toxic in the central and southern regions, five were located relatively close to the City of Los Angeles, Hyperion Treatment Plant outfall in Santa Monica Bay (Figure 6b) and one was located near the City of San Diego Point Loma Wastewater Treatment Plant outfall (Figure 6c).

Examination of SCBPP measurements of sediment grain size, total organic carbon (TOC), and total nitrogen (TN) did not reveal obvious relationships with the pattern of interstitial water effects (data not shown). Information on sediment contaminant concentrations was not available for comparison at the time of report preparation.

DISCUSSION

SCB Sediment Quality

Whole sediment. Toxicity tests using survival of the amphipod, *Ampelisca abdita* provide the most reliable measure of SCBPP sediment quality since the test is standardized, uses an ecologically relevant exposure method, and measures a clearly adverse response (mortality). The amphipod test results indicated good sediment quality at all sites tested. While sediment from some sites caused a statistically significant increase in amphipod mortality relative to the control, none of the effects were sufficiently large to meet the criterion established for toxicity (≥20% mortality).

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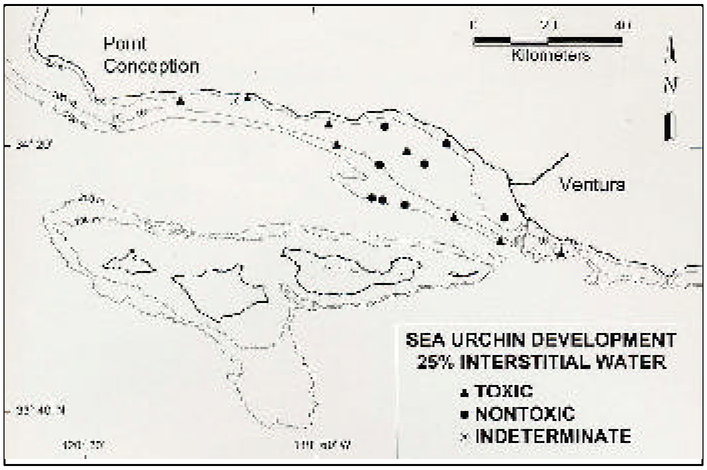

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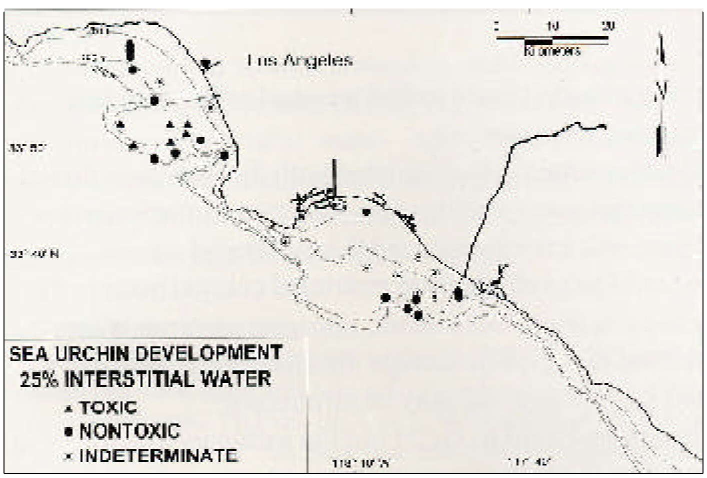

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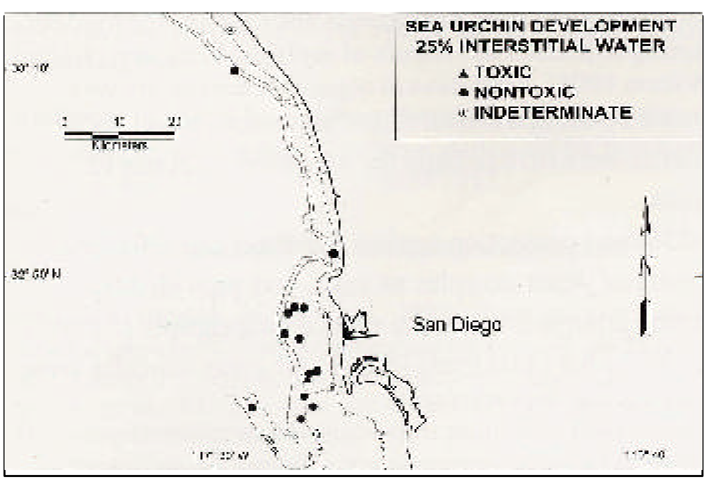

FIGURE 6. Location of 25% interstitial water samples classified as "toxic", in Southern California Bight Pilot Project Survey. a) northern region; b) central region; c) southern region stations.

Comparisons with previous data are difficult since most SCBPP stations were located in areas not previously tested for sediment toxicity. Prior studies have used different amphipod species and examined sediment only from stations near sewage outfalls off Orange County, the Palos Verdes Shelf, and in Santa Monica Bay (Anderson *et al.* 1988, SCCWRP 1992, Bay *et al.* 1994). No significant mortality was measured when the amphipod, *Rhepoxynius abronius* was exposed to sediment from five stations on the Palos Verdes Shelf (Bay *et al.* 1994). Exposure to sediment from a station nearest the County of Sanitation Districts of Los Angeles, White Point outfall reduced *R. abronius* survival by 15%, similar to the 10% reduction measured for a nearby SCBPP station. SCBPP results are also consistent with research by Swartz *et al.* (1986) that documented a reduction in Palos Verdes sediment toxicity between 1980 and 1983.

Prior amphipod toxicity tests conducted on sediments collected near the Orange County and Hyperion Treatment Plant outfall systems also agree with SCBPP results. Research conducted by SCCWRP using the amphipod, *Grandidierella japonica*, document an improvement in sediment quality in Santa Monica Bay following the termination of sewage sludge disposal in 1987 (SCCWRP 1992). Santa Monica Bay sediment samples collected in 1989 from several stations along the 100 m isobath did not produce statistically significant effects on *G. japonica* survival or growth. Similarly, no significant effect on *G. japonica* survival was produced by sediment collected near the Orange County Sanitation Districts outfall (Anderson *et al.* 1988).

Interstitial water. While sediment toxicity tests with amphipods provide ecologically relevant data, these tests do not measure sublethal effects. Interstitial water toxicity tests using sea urchin embryos were included in the SCBPP to provide a more sensitive measure of sediment quality. While adverse effects on sea urchin embryo development were produced by many interstitial water samples, most of the effects were produced by elevated ammonia concentrations.

Ammonia toxicity was treated as an interference in this study for several reasons. First, interstitial water ammonia did not arise directly from discharges, but was the product of natural metabolic processes in the sediment. Second, sea urchin embryos are relatively sensitive to ammonia (Bay *et al.* 1993) and may not provide a meaningful assessment of sediment toxicity for benthic organisms that are more tolerant (Kohn *et al.* 1994). Finally, recent SCCWRP research indicates that storage and homogenization of SCBPP samples probably increased interstitial water ammonia concentrations, creating a toxicity artifact unrelated to actual sediment quality (see *Toxicity of Ammonia to Pacific Purple Sea Urchin* (Strongylocentroutus purpuratus) *Embryos*, this annual report).

Evidence of interstitial water toxicity was present in 29% of the samples (15 of 52 stations) that could be evaluated in the presence of interference by ammonia. The spatial distribution of toxic stations suggests the influence of regional factors and sewage discharges. Most of the toxic samples represented stations located in the northern region (Point Dume–Point Conception), an area lacking large sewage or industrial discharges (Figure 6). The factor(s) responsible for the toxicity in the northern region are unknown at this time; sites in this area may be influenced by natural oil seeps, oil drilling activities, runoff from the Santa Clara River, or other natural conditions. There are no prior interstitial water toxicity data from this area available for comparison.

Most toxic interstitial water samples from the central and southern regions were located relatively close to large sewage outfall systems in Santa Monica Bay or off Point Loma (Figure 6). Once again, no relevant prior interstitial water toxicity data are available for these specific stations. There is previous evidence of outfall-related interstitial water toxicity in Southern California sediments, however. Interstitial water toxicity was present in sediment samples collected within 2 km of the White Point outfall system and analyzed using a sea urchin fertilization test (SCCWRP 1994). The fertilization test results were not subject to the ammonia interference encountered during the SCBPP.

Sediment toxicity tests provide only a portion of the information needed to examine sediment quality in a given area. Until the results of the concurrent chemical and infaunal analyses are available from this project, it is not possible to determine whether the toxicity is related to outfalls, other sources of pollutants (e.g. dredged material disposal sites), or factors unrelated to contamination.

Data Reliability

All of the amphipod and sea urchin embryo toxicity test data reported are considered reliable measures of the toxicity of the samples analyzed. The tests were conducted according to widely used techniques and criteria for test acceptability were met.

Some uncertainty is associated with the interpretation of the interstitial water toxicity data, however. Interference from ammonia toxicity reduced the number of samples which could be evaluated and restricted comparisons to the 25% concentration. As a result, apparent patterns in the data related to region or sewage discharges are based on a reduced sample size and may be erroneous.

The method used to factor out the influence of ammonia assumes that the factors causing toxicity act in an additive manner and the sensitivity of sea urchins to ammonia is similar between experiments. The assumption of additivity cannot be proved, but is considered appropriate for models attempting to predict the effects of multiple toxicants (Logan and Wilson 1995). Variation in organism sensitivity was addressed by using a conservative approach (lower 99% confidence limit) to calculate the predicted response to ammonia.

Additional protection against incorrect classifications of interstitial water samples as toxic was provided by restricting comparisons to the most dilute sample (25%) tested. Only the most toxic interstitial water samples were identified using this approach.

It is possible that other important interstitial water constituents besides ammonia are affected by sediment handling. Variations in toxicity produced by the use of different interstitial water collection and storage methods have been reported by others (Ankley and Schubauer-Berigan 1994, Carr and Chapman 1995).

Sediment storage and other handling methods are also important variables in whole sediment toxicity tests. Sediment toxicity has been shown to vary unpredictably with differences in storage time and temperature (Malueg *et al*. 1986, Dillon *et al*. 1994, Becker and Ginn 1995).

CONCLUSIONS

Sediment toxicity testing was successfully carried out on 72 stations during the SCBPP, thus demonstrating the feasibility of performing these tests on a regional basis. No acute toxicity was found at any station based on the amphipod bioassay. Most interstitial water samples produced toxic effects in developing sea urchin embryos. However, the interference of ammonia in the interstitial water samples made identification of toxic stations difficult and possibly inaccurate. Correction of the data for ammonia effects resulted in the identification of 15 of 52 stations as toxic at a concentration of 25% interstitial water.

Variations in sediment storage and handling methods can influence the results of toxicity tests using bulk sediment or interstitial water. Additional research on sample handling methods are needed to improve toxicity data comparability. In future studies, the storage time of sediments should be reduced.

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