Influence of Sediment Type on Phenanthrene Toxicity

High concentrations of polynuclear aromatic hydrocarbons (PAH) have been found in sediments throughout the Southern California Bight, especially in harbors and around sewage outfalls (Anderson and Gossett 1987). The toxicity of PAH and other contaminants is greatly modified by the nature of the sediments to which they are bound. For example, sediments with an elevated total organic carbon (TOC) content bind some contaminants more tightly than do lower TOC sediments, producing reductions in contaminant concentrations in interstitial water, bioavailability, and toxicity (Swartz et al. 1988).

The effects of sediment characteristics on phenanthrene bioavailability/toxicity to the amphipod *Grandidier-ella japonica* were investigated by Steven Bay and Darrin Greenstein of SCCWRP under contract from the California State Water Resources Control Board. *G. japonica* is an abundant species that is tolerant of variations in temperature, salinity, and sediment grain size, making it a good candidate for testing with various types of contaminated sediments.

Previous sediment toxicity studies with this species have measured the effects resulting from exposure to contaminated field sediments (Nipper et al. 1989). Statistical analysis of these data indicated correlations between many contaminants, including PAHs and biological effects (Anderson et al. 1988). However, the complex nature of sediment contamination in southern California prevented a definitive determination of toxicity for specific contaminants.

Methods

In this study clean sediment was spiked with a single contaminant, the PAH phenanthrene, to determine the effective levels of this compound when bound to different types of sediment. Phenanthrene was selected for this work for two reasons: (1) its molecular structure indicated that it would be moderate in both binding affinity for sediments and bioavailability, and (2) it was relatively abundant at sites contaminated with PAHs.

Two types of sediment were used in these laboratory experiments. The sediments, which were collected from Newport Bay and San Mateo Point, varied greatly in TOC and grain size (Table 1). Previous studies have shown these sites to be relatively uncontaminated. Known quantities of phenanthrene, including a small amount of ^{14}C-phenanthrene, were added to the sediment to produce nominal concentrations of 10, 30, and 90 mg/kg dry wt (ppm). Amphipods were exposed to a 2-cm layer of the spiked sediments for 14 d, under flowing seawater, at 20°C.

Sediment and interstitial water samples were collected for chemical analysis after 0, 4, 10, and 14 d of exposure. Amphipod samples were taken during the same exposure periods for survival, growth, and bioaccumulation measurements.

The ^{14}C-phenanthrene added to the sediment was

Table 1. Characteristics of sediments used in amphipod exposures to phenanthrene.

Collection Site	Depth (m)	% TOC	% Sand	% Silt	% Clay
Upper Newport Bay	0	0.1	96.7	1.5	1.8
San Mateo Point	60	1.0	5.0	85.4	9.6

used as a tracer to calculate the total phenanthrene concentration in the exposure system and amphipods. Sediment and amphipod samples were first extracted with methanol, then back-extracted with hexane after adding distilled water. This procedure was used to isolate the nonpolar, radioactive compounds, which were presumed to be primarily phenanthrene, into the hexane phase. The water samples were first acidified to drive off CO_2 and carbonates and then extracted with hexane.

The radioactivity in each of the resulting fractions was measured by liquid scintillation counting (LSC). The total concentration of phenanthrene and/or metabolites in each fraction was then calculated from the LSC data. The hexane phase of selected samples was analyzed by gas chromatography/mass spectrometry (GC/MS) for verification of the calculated phenanthrene concentrations.

Results

Analysis of sediment samples at the start of the experiment (after 2 d of sediment dosing) indicated that approximately 55% of the phenanthrene added to the sediment had been degraded to other unidentified forms. Consequently, the actual sediment concentrations obtained in these experiments were much lower than the nominal levels calculated from the amount of phenanthrene added. The water samples contained $^{14}CO_2$, indicating that the phenanthrene degradation was due to metabolism by microorganisms present in
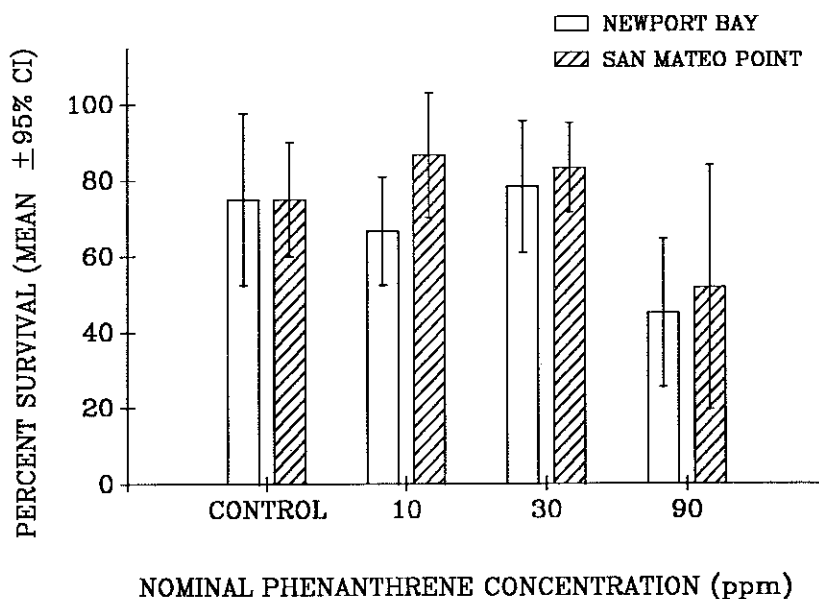

Figure 1. Survival of amphipods after 14 d exposure to phenanthrene-spiked sediment.

the test sediment. Metabolism of the phenanthrene spike continued at a slow rate during the experiments, resulting in declining exposure levels during the 14 d duration of each test.

Selected hexane extracts measured by GC/MS indicated that approximately one-half of the material detected by LSC was actually phenanthrene. The measured concentration values shown in the figures are based on the LSC data and are, therefore, overestimates of the actual amount of phenanthrene present.

The survival data indicated a trend toward reduced survival at the highest concentration in both sediment types (Figure 1). Survival results were variable, however, and did not represent statistically significant dif-ferences compared with the controls or between sediment types. These data indicate that the 14 d LC_{50} for phenanthrene was greater than 30 ppm (dry weight) when corrected for GC/MS results.

We unexpectedly found that the sublethal effects endpoint of amphipod growth (change in body length) was no more sensitive a measure of phenanthrene toxicity than was survival. Amphipod growth was not significantly altered at any of the phenanthrene concentrations tested and did not show a trend in response related to phenanthrene concentration.

The concentration of phenanthrene in the interstitial water varied greatly between the two sediment types. These results followed the pattern that would be predicted from the difference in
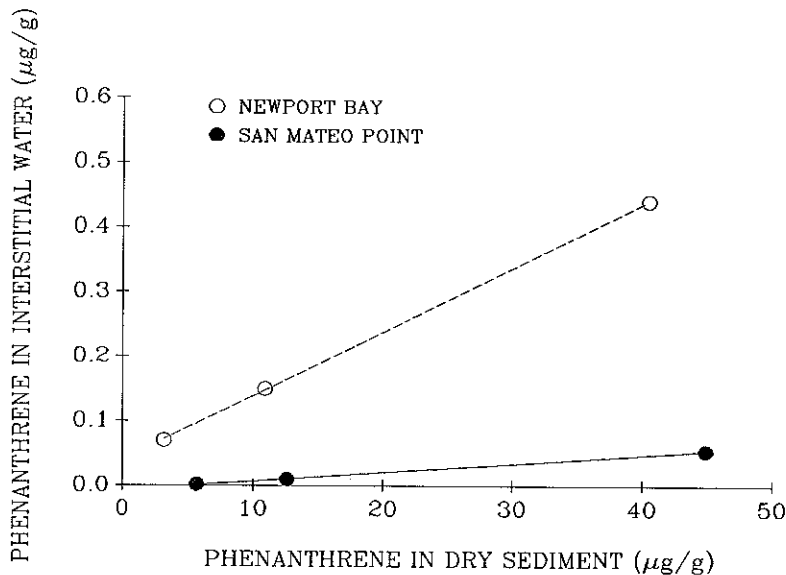
TOC between the sediments (Figure 2). At similar sediment phenanthrene levels, interstitial water phenanthrene concentration was always higher in Newport Bay sediment (low TOC), than in San Mateo Point sediment (higher TOC).

Radioactivity levels measured in the bodies of the surviving amphipods indicated that a large uptake of phenanthrene and its metabolites resulted from their exposure to the spiked sediment (Figure 3). The small size of the amphipods prevented GC/MS analysis of the tissues, but LSC analysis of the extraction fractions indicated that only a small portion of the accumulated material represented phenanthrene. Approximately 90% of the body burden consisted of polar and/or tightly bound phenanthrene metabolites that were present in the methanol extract or amphipod tissue residue fractions. About one-half of these metabolites was present in the non-extractable tissue residue fraction.

The pattern of phenanthrene/metabolite accumulation in *G. japonica* was influenced by the sediment type, with animals exposed in the lower TOC sediment (Newport Bay) having a higher body burden (Figure 3). This pattern was evident even though the total concentration of phenanthrene in the two sediment types was similar. Changes in the body burden of the animals closely followed the interstitial water concentration (Figure 4.) This relationship indicates that exposure to interstitial water is a significant mode of contaminant exposure for *G. japonica*,

Figure 2. Relationsip between bulk sediment and interstitial water phenanthrene/metabolite concentrations after a 14 d exposure period. Values were calculated from LSC data and include some phenanthrene metabolites.
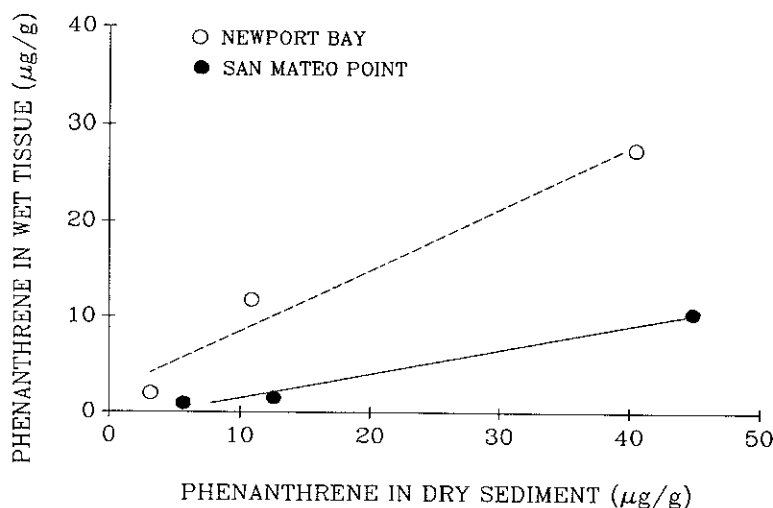

Figure 3. Accumulation of phenanthrene and nonpolar metabolites by amphipods after 14 d exposure to phenanthrene-spiked sediment.

even though this species lives in a burrow in the sediment. These data suggest that the wall of this amphipod's burrow does not greatly restrict the exchange of interstitial water.

Discussion

The results of this study show that long-term exposure of *G. japonica* to phenanthrene did not produce significant toxic effects at levels as high as 30 ppm (dry weight) in the sediment. This concentration greatly exceeds phenanthrene levels found in southern California, although phenanthrene concentrations as high as 2,000 ppm have been found in sediment from highly contaminated locations in Puget Sound, Washington (Swartz et al., 1989). Sediments contaminated with PAHs contain many other toxic compounds besides phenanthrene, producing total PAH levels of up to 20 ppm in southern California (Anderson et al., 1988).

The relatively low toxicity of phenanthrene to *G. japonica* (14-d LC_{50} > 30 ppm) contrasts with results of toxicity tests with another amphipod, *Rhepoxynius abronius*. Tests with this species indicate a greater toxicity for phenanthrene (10 d LC_{50} = 3.7 ppm) and also for a similar PAH, fluoranthene (LC_{50} = 4.2 ppm [Swartz et al., 1989]). Differences in sediment type and behavior of the test species may account for some of the disparity between these toxicity estimates.

Additional studies of sediment toxicity with other PAH compounds and marine species must be conducted before the significance of sediment contamination with
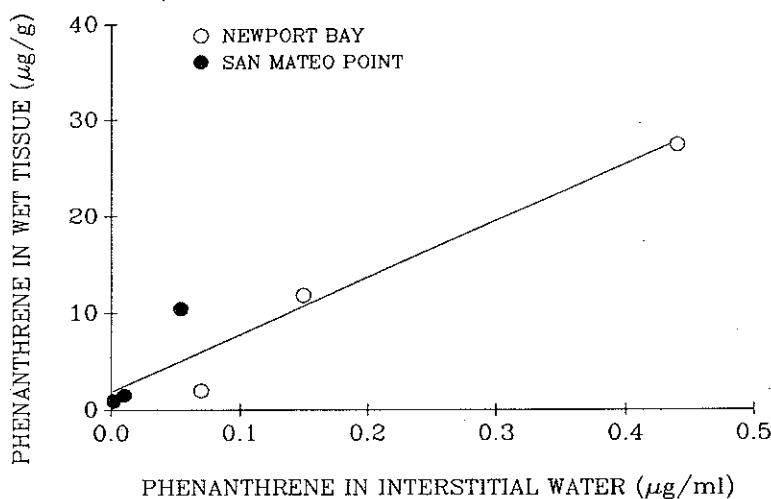

Figure 4. Relationship between 14 d bioaccumulation of phenanthrene/metabolites and interstitial water concentration.

PAH can be accurately assessed. Results from this study show that variations in sediment characteristics can greatly affect toxicant bioavailability and must be considered in assessing the impact of environmental contamination. Contaminants such as PAH in sediments from outfall areas with high organic content may be less bioavailable.

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