#### CHARACTERISTICS AND EFFECTS OF CONTAMINATED SEDIMENTS FROM SOUTHERN CALIFORNIA\* SCCWRP #0223

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A multidisciplinary study of sediments from ten coastal sites in southern California was conducted in summer and fall of 1987. These sites included areas of high contamination near sewage outfalls and within harbors. Measurements were made of metal, chlorinated hydrocarbon, polynuclear aromatic hydrocarbon, and dissolved sulfide concentrations in the sediment surface layer. These sediment samples were also examined for toxicity using three different test methods (Microtox, amphipod survival, and sea urchin growth). Sediment samples were also taken for examination of the benthic macrofauna resident at each site (Fig. 1).

High concentrations of chlorinated hydrocarbons, polynuclear aromatic hydrocarbons, and trace metals were found in sediments from most stations. At open sea sites contamination was highest at the Santa Monica Bay sludge outfall (SMS) and the Palos Verdes outfall (PV). The Orange County outfall (OC) was relatively similar in contamination to our reference site at San Mateo Point (SMP). As expected from previous analyses, the harbor sites contained significant amounts of polynuclear aromatic hydrocarbons and metals. Tributyltin was found in sediments from the harbor stations and organotins of a different composition pattern were detected at the outfall sites. Based on the relative concentrations of contaminant classes, macrofaunal communities, and toxicity for amphipods and urchins, the open sea and protected station groups can be ranked from most to least affected (Table 1).

While it is not surprising that the sludge outfall in Santa Monica Bay and the outfall at Palos Verdes are more impacted than the Orange County outfall and the reference station, it is gratifying that all analyses agree. Therefore, at the open sea sites, the two most contaminated outfall sites (SMS and PV) clearly separate themselves from the cleaner outfall at OC and the reference station. There is an obvious need to include more offshore stations in this type of ranking to aid in evaluating the sediment factors responsible for changes in macrofauna and toxicity in the laboratory.

At protected stations there was a better range of sediment types, leading to some differences in the ranking of stations. In most cases (4 of 6), the sediment from the Seventh Street channel in San Diego Bay (SD7) was the most affected. As described previously (2), this location is in 6 meters of water near naval fuel dock facilities and could also be receiving input from storm runoff. Evidence of the latter is the high proportion of lead and



Figure 1. Locations of test stations.

<sup>\*</sup>This paper is taken from a report by these authors to the California State Water Resources Control Board (1).

	S	ediment	s		Tox	icity
	Metals	PAH	CHC	Macro- fauna	Amphi- pod	Urchin
<u>Open sea</u>						
Most affected	SMS PV OC	SMS PV OC	PV SMS OC	SMS PV OC	SMS PV OC	SMS PV SMP
Least affected	SMP	ŠMP	SMP	SMP	SMP	ÖC
Protected						
Most affected	SD7 SDN LAR SDC LAH	SD7 LAR SDC LAH SDN	SD7 LAR LAH SDN SDC	LAR LAH SD7 SDC DPM	SDN SD7 LAH DPM SDC	SD7 LAH SDC SDN DPM
Least affected	DPM	DPM	DPM	SDN	LAR	LAR

Table 1. Ranking of open sea and protected station groups from most to least affected based on concentrations of contaminant classes, macrofaunal communities, and toxicity for amphipods and urchins.

zinc at this station, as at the Los Angeles River mouth (LAR), compared to the other metals (Fig. 2). The Los Angeles River mouth ranked high in impact for three of the six parameters measured. These sediments were high in all contaminants and they produced a different macrofaunal assemblage. However, they were not toxic to amphipods and urchins unlike the Seventh Street station.

The sediments from Los Angeles Harbor (LAH) and the two other stations in San Diego Bay (SDN and SDC) ranked next in relative effects. It is interesting that these three stations formed a group when lead and zinc content was plotted against other metals in sediments (Fig. 2). This may not necessarily indicate that lead and zinc are the significant toxic agents, but that these stations receive input from similar sources of contamination. These stations may represent locations receiving multiple inputs from storm runoff and numerous small spills. The types of aromatic hydrocarbons found at these three sites are also quite similar (Fig. 3). At protected sites, toxicity was the greatest at the four harbor (L.A. plus three San Diego) stations.

While sediments from the Los Angeles River mouth showed high concentrations of sediment contaminants and effects on infaunal populations, these samples were the least toxic for both amphipods and sea urchins. For unknown reasons, even the sediments from the Dana Point Marina reference station (DPM) were more toxic than the Los Angeles River sediments. However, the contaminants measured were lowest in sediments from the Dana Point Marina, indicating factors not analyzed are responsible for the toxicity.



Figure 2. Plot of PCA factor scores for metals data.

Multivariate analysis (principal components analysis) was used to identify distribution patterns in the chemistry data. This method identified two principal groups (each having independent distribution patterns) within each of three major categories of contaminants. One of the two major PAH groups identified was composed primarily of low molecular weight compounds, while high molecular weight compounds predominated in the other; these groups represented hydrocarbons characteristic of petroleum and fossil fuel combustion, respectively. The remaining four contaminant groups identified were characteristic of PCB, DDT, lead and zinc, and other metals.

Further analysis of the contaminant data indicated that only four statistically independent patterns of contaminant distribution could be distinguished from the data. These patterns indicated associations between different groups of metal and hydrocarbon contaminants. One group represented the combined distribution pattern of PCB, petroleum PAH, most metals, and dissolved sulfide. This pattern was dominated by the contamination present at the sludge outfall (SMS) site. Fossil fuel PAH and lead and zinc were grouped together as having similar patterns, forming a group that characterized contamination patterns among most of the harbor stations. The remaining two groups represented contamination patterns that were characteristic of single stations. These stations were the Los Angeles County outfall, which had high DDT concentrations, and the Los Angeles River mouth, which had distinctive concentrations of several PAH.

Analysis of the benthic macrofauna data produced four groups of stations having similar species composition and abundance values. The sludge outfall site formed a group by itself and was most dissimilar in species composition and abundance when compared to the reference site. The Orange County outfall site was found to be most similar to the open water reference site. All but one of the harbor stations were grouped together with the protected reference site, even though these sites had high levels of some contaminants.

The Los Angeles River mouth site was unusual in that this station's benthos were different from the other protected sites and most similar to the L.A. County Outfall site. This result was unexpected because of large differences in depth, contamination, and laboratory toxicity between these two sites. These two stations did have similar organic carbon and dissolved sulfide contents, however. It appears that the river station represents a transition between an organically enriched site and a site having toxic levels of contamination in addition to enrichment.

Multivariate analysis of the macrofauna data identified differences between the open water sites that corresponded to a gradient of contamination and organic enrichment. Similar results were also found when indicator taxa abundance or measures of assemblage structure and diversity were compared statistically. With the exception of the L.A. River mouth site,



Figure 3. Plot of PCA factor scores for PAH data.

significant differences between the protected stations (including the protected reference site) were not found.

The laboratory toxicity tests usually identified more stations as being harmful than did macrofaunal analysis. There was generally good agreement among all three of the test methods in identifying the most toxic sites (SMS, PV, SD7). Evidence of chronic toxicity at the PV station contrasts with previous acute toxicity test results which indicated only slight toxicity at this station.

The sea urchin and amphipod test methods using moderate or long-term exposures to bulk sediment are well suited to future sediment assessment surveys in California. The results from this study indicate that each of the toxicity test methods used responded in a unique way to the sediment samples. This finding emphasizes the necessity of using multiple species and different test strategies in order to accurately assess sediment toxicity.

Data sets were combined and analyzed by a single principal components analysis (PCA) in order to identify additional associations between chemical and biological (field and laboratory) measurements. A strong association was found between a dominant macrofauna ordination axis, Microtox results, and many contaminants (PCB, most metals, petroleum PAH, and sulfide). Amphipod survival was associated with organotin, combustion PAH, lead, and zinc. The macrofaunal pattern in the protected sites was best correlated with sea urchin growth.

Our analyses have shown that patterns in sediment contamination, macrofauna, and toxicity correlate well in some cases, but not all. Most notably, DDT concentration did not correlate with short-term toxicity or macrofaunal patterns. We believe a better gradient of chlorinated hydrocarbon concentrations (particularly DDT) will be required to accurately evaluate threshold levels of these contaminants in sediments.

This report has emphasized data expressed on a dry weight basis. Organic content is certainly an important modifier of contaminant partitioning, but a better understanding of this process is needed before normalization of concentrations from field samples can be made with confidence. Inconsistent results were obtained with organic carbon normalization in this study and suggest that this method may not be as useful for organic material derived from sewage. It is also possible that the range in sediment organic carbon found in this study was too great for accurate normalization.

We have presented empirically determined response concentration estimates (concentration at or above which significant biological effects were always found) for benthic macrofauna and toxicity test results. Estimated concentrations for many individual contaminants were not calculated because our analysis showed that we could not separate patterns in greater detail than the contaminant groups listed. The small number of sites examined in this study was a major factor in preventing the determination of more specific and precise concentration estimates. Consequently, these values should be considered preliminary estimates and used with caution.

This study provided an initial evaluation of contaminated sediment effects in southern California. We were able to use these data to identify potential relationships between contaminant types and biological effects and also to estimate the range of effective concentrations of some sediment contaminants (Table 2). Additional studies are needed, however, in order to better understand the interrelationships between specific sediment contaminants and biological response.

Contaminant group	Group members	Response concentration (range, ppm)
Sulfide	dissolved sulfides	16-56
Organotin	tetra- and trialkyltin	0.06-0.19
DĎT	DDE, DDD, and DDT	0.03-0.20
PCB	Aroclors 1242 and 1254	0.06-0.66
PAH1	petroleum PAH	0.03-15.8
PAH2	combustion PAH	0.07-9.60
MET1	Ag, As, Cd, Cu, Cr,	
	Ni, and Sn	152-899
MET2	Pb, and Zn	275-809
	Pb	64-133
	Zn	211-675

Table 2. Summary of sediment contaminant response concentrations estimated from macrofaunal and toxicity test analyses. Values represent the summed concentration (in ppm) of the contaminants in each group.

More extensive field studies of sediment toxicity and macrofaunal effects should be conducted. The small size of the present investigation was insufficient to determine the spatial extent of sediment toxicity within the study area. Field studies should also focus upon areas exhibiting gradients of DDT and PAH contamination. Such studies would improve upon the statistical relationships between contaminant type and biological effects identified in this study.

Laboratory toxicity studies using spiked sediments are also needed to supplement and expand upon the information gained from the field surveys. These experiments provide the most straightforward method for determining the effects of specific contaminants.

Additional studies are also needed to better understand the relationship between changes in sediment characteristics and contaminant bioavailability. These studies should include measurements of interstitial water since strong correlations between contaminant concentrations in this phase and toxicity have been found by other investigators (3). Chemical analysis of both interstitial water and bulk natural sediments should be conducted to better understand the partitioning of contaminants between these two phases. Laboratory toxicity and bioaccumulation tests using interstitial water or water containing similar concentrations are also needed to provide a better understanding of effects resulting from exposure to contaminated environments.

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1. Anderson, J.W., S.M. Bay, and B.E. Thompson. Characteristics and effects of contaminated sediments from southern California. Final report to the California State Water Resources Control Board. Contribution No. C-297. Southern California Coastal Water Research Project, Long Beach, CA. 1988.

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## FINAL REPORT

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# CHARACTERISTICS AND EFFECTS OF CONTAMINATED SEDIMENTS FROM SOUTHERN CALIFORNIA

to

California State Water Resources Control Board Post Office Box 100 Sacramento, California 95801 Contract No. 6-214-250-0

from

J. W. Anderson, S. M. Bay, and B. E. Thompson Southern California Coastal Water Research Project 646 West Pacific Coast Highway Long Beach, California 90806

October 17, 1988

SCCWRP Contribution No. C-297

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Based on the relative concentrations of contaminant classes, macrofaunal communities, and toxicity for amphipods and urchins, the open sea and protected station groups can be ranked from most to least affected.

		Sediment	S		Toxicity		
	Metals	PAH	CHC	Macrofauna	Amphipod	Urchin	
Open sea							
Most affected	SMS PV	SMS PV	PV SMS	SMS PV	SMS PV	SMS PV	
4	OC	OC	OC	OC	OC	SMP	
Least affected	SMP	SMP	SMP	SMP	SMP	OC	
Protected							
Most affected	SD7	SD7	SD7	LAR	SDN	SD7	
	SDN	LAR	LAR	LAH	SD7	LAH	
	LAR	SDC	· LAH	SD7	LAH	SDC	
	SDC	LAH	SDN	SDC	DPM	SDN	
*	LAH	SDN	SDC	DPM	SDC	DPM	
Least affected	DPM	DPM	DPM	SDN	LAR	LAR	

While it is not surprising that the sludge outfall in Santa Monica Bay and the outfall at Palos Verdes are more impacted than the Orange County outfall and the reference station, it is gratifying that all analyses agree. Therefore, at the open sea sites, the two most contaminated outfall sites (SMS and PV) clearly separate themselves from the cleaner outfall at OC and the reference station. There is an obvious need

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## I. INTRODUCTION

In the past 15 years, enhanced environmental awareness has intensified the need for accurate and reliable methods to predict environmental impacts of marine discharges. Many scientists in academia, industry, and government have been concerned with the prediction of the environmental fate and effects of toxic chemicals with the potential to reach surface waters. Control strategies have led to the development of hazard assessment programs for new chemicals, effluent and discharge permitting, refinement of water quality criteria and standards, and other control programs. A general assumption made in the application of environmental safety assessments for water quality has been that pollutants were water-soluble. This led to an emphasis on pelagic species in toxicity testing and the application of water quality criteria. The ultimate fate and distribution of the pollutants were not considered and sediment particles were considered a safe repository for sorbed contaminants.

The overly simplistic view of potential pollutant effects from water only was discredited when it was found that chemicals on the U.S. Environmental Protection Agency (EPA) Priority Pollutant List were very water-insoluble and were observed at high concentrations in sediments. Environmental chemists expressed concern about the previous assumption of chemicals irreversibly sorbed to sediments. Other observations which emphasized the need to examine sediment effects were those of field studies showing organism impacts when water quality standards had not been exceeded. Investigations of spills, ocean outfalls, and non-point source contributions identified high levels of contamination of various types (trace metals, chlorinated organics, and aromatic hydrocarbons) and associated degradation to one or more species living in or on the sediments.

On the Pacific coast, perhaps the two best studied sites have been Puget Sound and the shelf off of the Palos Verdes Peninsula. A series of studies by Malins and his co-workers at the Northwest and Alaska Fisheries Center in Seattle have described pathological conditions in English sole collected from various sites in Puget Sound. The sediment contaminants which best correlated with the effects observed were the polynuclear aromatic hydrocarbons (PAH), but it was not possible to eliminate the possible contribution of co-occurring contaminants such as trace metals or polychlorobiphenyls. Other studies in the region were conducted by the State of Washington on the near-shore Commencement Bay Super Fund sites. These investigations included infaunal analyses, sediment chemistry and several toxicity bioassays. This approach, often referred to as the

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"Triad", was also used by the National Oceanic and Atmospheric Administration (NOAA) in other parts of Puget Sound. These data are presently used in one way by NOAA and in another way, called the Apparent Effects Thresholds (AETs), by the EPA (Region X). Another large data base on sediment contamination and biological effects was generated by Battelle Northwest for three bays considered to be clean and three others suspected of being contaminated in Puget Sound. We have not yet seen this final report which should be available from EPA Region X.

Sediment data for the shelf off Palos Verdes has been generated for several years by SCCWRP, the Los Angeles County Sanitation Districts (LACSD) and a group of EPA scientists from the Newport Oregon Laboratory. High DDT concentrations were reported in sediments during the 70s by SCCWRP scientists and concentrations of chlorinated organics and other contaminants have been described in surface and buried sediments in several reports and publications. Swartz and co-workers from EPA have defined (from collections in 1980 and 1983) the level of surface sediment contamination and the effects on the biota along a gradient to the northwest from the LACSD outfall on the Palos Verdes shelf (Swartz et al., 1985 and 1986). In 1980, there were significant reductions in amphipod survival produced by sediments from the three sites nearest the outfall, but the same stations tested in 1983 produced no significant effects. Chemical analyses conducted on sediments from both years showed the highest number of correlations between biological and chemical parameters measured were for total oil and grease, hydrocarbon oil and grease, and lead. The greatest changes in the chemistry of the most contaminated stations between 1980 and 1983 were a 71% reduction in BOD and a 67% decrease in oil and grease.

Recognizing the need to understand which sediment contaminants and what concentrations of single and multiple pollutants on sediments were producing the effects on organisms in the field and laboratory, a series of scientific workshops were held starting about 1983. National agencies organizing or sponsoring these meetings of experienced and interested scientists were the U.S. Army Corps of Engineers, EPA, and NOAA. The Office of Criteria and Standards of EPA set up a small program to establish "Sediment Quality Criteria". One of the best attended and significant meetings on the fate and effects of sediment bound chemicals was sponsored by EPA and the American Petroleum Institute, with the proceedings published by the Society of Environmental Toxicology and Chemistry (Dickson et al., 1987). A very recent meeting in Florida, involving most of the same

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participants, was sponsored by the Marine Board of the National Research Council, which plans to produce a proceedings volume.

The objective of state and federal programs on sediments is to establish quality criteria for sediments from sites ranging from Super Fund sites, to dredged material to deposits from outfalls. The scientific community, however, warns that only a small portion of the critical information needed is available to establish such criteria. The most straightforward findings available are from recent laboratory tests using only one to three contaminants added to sediments and extensive chemical analyses of sediments, pore water, and if possible organisms. These studies show that toxicity of a limited number of contaminants can be explained by the partitioning of pollutants (hydrophobic organics) from sediments to pore water and the subsequent exposure to the animals. In these studies, interstitial water concentrations of toxicants control the effects on the animals and they compare closely with water concentrations (without sediment) known to produce toxicity. Interstitial water concentrations of toxics are in turn controlled by the characteristics (organic carbon, etc.) of the sediments and the organics and redox potential of the pore water itself. While a few laboratory studies have demonstrated these relationships, we do not feel that criteria should be derived by simply applying predicted values from equilibrium partitioning to water quality criteria. There are no water quality criteria for many of the toxic compounds found in sediment and the complex interactions between bound toxicants and interstitial water can not easily be predicted. For some marine species, ingestion of sediment may be an important route of toxicant transport.

Field studies on polluted sediment are nearly always confounded by a mixture of a vast number of toxicants making an estimate of toxic concentrations of single compounds or compound classes (e.g., PAH) merely an approximation. Previous efforts in this field have recognized the weaknesses and have referred to their estimates as thresholds (including AETs) or screening level concentrations. Anderson (1988) and Chapman et al. (1987) have pointed out that for some pollutants such as PAH and PCB the various predictions fall within a reasonably small range. For PAH the estimates are between about 2 and 12 ppm and those for PCB are 0.06 to 0.13 ppm. For other compounds and trace metals, as well as other means of estimating toxic concentrations, there is little agreement.

With all the limitations in mind, this project for the California State Water Resources Control Board was conducted in Southern California to attempt to produce sediment toxicity values that could be compared to previous data and aid the state in their development of sediment quality criteria. As in previous projects, the three basic types of data collection were sediment chemistry, infaunal species analyses, and laboratory toxicity testing with collected sediment. While the infaunal and chemical analyses were approximately similar to other studies, toxicity testing involved two important differences. The first was the use of sea urchins (*Lytechinus pictus*) in chronic exposures to sediment for 35 days and the measurement of growth and bioaccumulation. The second difference was the use of a species of amphipod (*Grandidierella japonica*) which has not been used in previous studies, but is prominent in fine sediments of Southern California.

The presentation of our findings will proceed from descriptions of the methods used to results and then to relationships and finally conclusions. The results section will first treat the complexity of the sediment characteristics, then the benthic macrofauna, and finally the various toxicity tests. Within each of these subsections of Results there will be descriptions of the statistical analyses conducted on that particular set of data to demonstrate which factors are associated with the majority of the variations observed. We will also use statistical methods to identify relationships between the three types of measurements generated in the study. Finally, we will attempt to provide the state with a variety of mechanisms for utilizing the data to estimate the compounds and concentrations associated with sediment that are either predicted to be "safe" or predicted to be "harmful" to benthic marine species.

#### **II. METHODS**

## A. Site Locations and Sampling

The sites selected for this study were previously sampled during the SCCWRP/State Board PAH survey (Anderson and Gossett 1987). From that study, the eight sites that contained PAH concentrations above or near 5 ppm (dry) were selected for this study. Since the eight sites were situated in open coastal and protected bay areas, two reference sites were selected, one off San Mateo Pt. and one at the Dana Pt. marina. The sampling locations, dates, and depths are shown in Fig. 1 and Table 1. Detailed descriptions of these site localities are given by Anderson and Gossett (1987).

Because of logistical limitations on both shiptime and the size of the sediment toxicity tests that could be run simultaneously, the sampling was carried out in two phases. The reference sites were sampled during each phase.

Sediment and macrofaunal samples were collected with a  $0.1 \text{ m}^2$  chain-rigged Van Veen grab. A composite sediment sample for chemical analyses and toxicity tests was collected from the top 2 cm of 5 to 7 grab samples (total volume about 12 l) and stored in a bucket packed in ice. The sediment was maintained at about 5°C and transported to the laboratory.

Macrobenthic infauna were collected from four additional replicate grab samples at each site. The entire contents of each grab were sieved through a 1.0-mm screen. The animals and debris were preserved in 10% borax buffered formalin in seawater and transported to the laboratory.

#### **B.** Sediment Analyses

In the laboratory, the sediment samples were split into three fractions for separate analyses: (1) general constituents such as sediment grain size and organic material, (2) trace metals, and (3) trace organic contaminants.





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Table 1. Sediment collection sites, dates and locations. Station number refers to station examined in previous study (Anderson and Gosset 1987).

Collection Date	Station	Station Code	Station #	Depth(m)	Latitude/Longitude
Sept. Experin	nent				
9/19/87	San Mateo Pt.	SMP	19 18	60	33 <sup>0</sup> 23.91/117 <sup>0</sup> 39.49
9/19/87	Orange County Outfall	OC	12	60	33°34,49/118°00,51
9/21/87	San Diego Bay: NASSCO	SDN	22	9	32°41.42/117°08.66
9/21/87	San Diego Bay: Chollas Creek	SDC	23	12	32 <sup>0</sup> 41.12/117 <sup>0</sup> 08.01
9/21/87	San Diego Bay: 7th Street	SD7	24	8	32040.61/117006.99
	Newport Bay	NB		0	33°37.23/117°53.61
Nov. Experim	ent				
10/31/87	San Mateo Pt.	SMP	19	60	33023.91/117039.49
10/31/87	Dana Pt. Marina	DPM	18	5	33°27.60/117°41.20
10/27/87	L.A. Harbor East Turning Basin	LAH	6	15	33 <sup>0</sup> 45.94/118 <sup>0</sup> 15.18
10/27/87	L.B. Harbor Queensway Bay	LAR	7	5	33045.51/118011.68
10/27/87	Palos Verdes Outfall	PV	4	62	33042.30/118020.85
11/03/87	Santa Monica Bay Sludgeline	SMS	3	150	33°55.50/118°33.25

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#### 1. General constituents

Percent sand, silt, and clay (dry weight) were measured using wet and dry sieving with a 63-um screen for the sand fraction, and pipette analysis for the silt and clay fraction (for details, see Thompson et al. 1987). Total organic carbon in the samples was measured at Global Geochemistry, Canoga Park, using a LECO model WR12 Carbon Analyzer. Organic nitrogen was determined by Kjeldahl digestion at Galbraith laboratory, Chicago, IL. Total dissolved sulfides were measured by squeezing pore water (Kalil and Goldhaber 1973) and measuring dissolved sulfides using a modified methylene blue spectrophotometric method (APHA 1985).

#### 2. Trace metals

Samples for trace metal determinations were digested at SCCWRP (see below). The digestates were analyzed for the target trace metals (silver, arsenic, cadmium, chromium, copper, mercury, nickel, lead, tin, and zinc) by inductively coupled plasma-mass spectrometry (ICP-MS; VG Instruments Inc.) at the Institute of Molecular Ecology, California State University, Long Beach. This method of analysis was selected principally because of the need for simultaneous, multi-element capability due to the limited sample size of the tissues.

Sample preparation was as follows:

For tissues, 10 ml of 1:1 RDN:DDW (redistilled nitric acid:deionized, distilled water) were added to about one gram of freshly thawed, wet tissue in a watch glass (speed-evap) covered 150 ml beaker. The mixture was heated to incipient boiling and taken to near dryness, with particular care not to dry out (burn) the sample solution. If at this point the digestion was total, with no remaining lipid or undigested residue, the digestate was transferred to a Kimax glass, Teflon lined screw-top test tube, and brought to 15 ml with DDW. If the digestion was not complete, the addition of 10 ml of the 1:1 RDN:DDW was repeated. If there was still residue, the digestate was diluted to about 10 ml and filtered through a Whatman #40 filter and made up to 15 ml in the Kimax test tube as above. Process blanks were treated in an identical manner.

For sediments, with similar glassware as above, 2 to 5 grams of freshly thawed, thoroughly homogenized wet sediments were weighed into the beaker and 20 ml of 1:1 RDN:DDW

was added and heated at incipient boiling to near dryness. This step was repeated, and the mixture was cooled and filtered (Whatman #40) and brought up to 50 ml in a Nalgene screw-top polyethylene vial. The amount of initial wet sediment used was based on a dry/wet ratio determination made on a separate aliquot, so that the amount of wet sediment would yield approximately 1 gram of equivalent dry sediment. Freshly thawed, wet sediments were used to reduce the loss of volatile metals that could occur in a drying step.

The ICP-MS method ionizes the elements in the digestate by aspiration into an argon plasma. The heat source for the plasma is radio frequency inductance around a glass nebulizer torch. The ions generated in the plasma stream are passed into the mass spectrometer. Here a quadrupole mass filter measures the abundance of certain stable isotopes of the target elements. Stable isotopes readily detected and free of interference were selected. The data system computes the elemental concentrations in the digestate based on mass counts compared with mass counts of standards. Internal standards were used to calibrate the mass spectrometer with each run. The concentrations in the digestates were then used to compute the metal concentrations in the sample, based on sample weight and dilution volumes of the digestates. Five process blanks per suite of samples were run; three aliquots of reagent blanks were run.

Measurements of sediment organotin concentration were made by Moss Landing Marine Laboratories, California Department of Fish and Game, Moss Landing. Sediment samples were extracted with methylene chloride HCl, washed with sodium hydroxide, and analyzed by graphite furnace atomic absorption spectrometry. This procedure quantified tetra- and trialkyltin compounds in the sample, which are almost exclusively represented by tributyltin in harbor environments.

#### 3. Trace organic contaminants

Sediment samples were extracted following the protocol reported to the State Water Resources Control Board by Anderson and Gossett (1987). Also included in this report is the technique used to clean up and analyze the sediment extracts for PAH. A brief description will follow on how the tissue samples were extracted as well as how sediment and tissue extracts were cleaned up and analyzed for chlorinated hydrocarbons (CHCs). Tissue was extracted following the method of Bligh and Dyer (1959) which involved homogenization of the tissue with chloroform:methanol:water, removing the chloroform layer then re-extracting two more times with additional chloroform. The chloroform extracts were combined and roto-evaporated to dryness and placed in a dessicator for 24 hours. The residue weight was then used to determine percent lipid.

Sediment and tissue extracts for CHC analyses were cleaned up using activated Florisil eluted with 45 ml of 15% ether in hexane. These cleaned up extracts were then analyzed using a Varian Vista 44 GC equipped with an electron capture detector, helium carrier gas at 30 cm/sec flow velocity, and a 30 m X 0.025 mm ID DB5 fused silica capillary column which was temperature programmed from 150°C to 274°C at 4°C/min. Quantification was performed using the internal standardization method, with Mirex as the internal standard. The electron capture detector was calibrated weekly.

Measurement of phenols in sediments was performed by Global Geochemistry, Canoga Park, according to EPA method 8040. A gas chromatograph, equipped with a flame ionization detector, was used for these analyses.

C. Benthic Macrofauna

In the laboratory, the formalin in the macrofaunal invertebrate samples was replaced with 70% ethanol. Two of the replicates were sorted from the screened fraction and identified to the lowest taxon practicable, and two were archived. The sorters and taxonomists are all active members of the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) and are listed in the Acknowledgments.

Patterns in species composition and abundances were analyzed by ordination and classification methods at EcoAnalysis, Inc., as described by Smith et al. (1988) and as utilized by Thompson et al. (1987).

Species diversity (H') was calculated using the Shannon-Wiener index (Shannon and Weaver 1949) and evenness (J) was calculated using Pielou's (1966) index.

## D. Toxicity Tests

The toxicity test schedule followed the timing of the sediment collections. Two sets of experiments were conducted (beginning in September and November 1987). Sediment samples were stored at 5°C before use in bioassays. All sediment samples were used in toxicity tests within 9 days of sediment collection.

Toxicity tests with three species of marine organisms were conducted on each of the test samples. These tests were a bacterial luminescence test of the interstitial water (Microtox [Microbics Inc., Carlsbad, CA]), an amphipod survival test and a chronic sea urchin growth test, both using whole sediment.

An additional experiment was conducted in April 1988 to examine three of the most toxic stations in greater detail. Dilutions of sediment and interstitial water were prepared and tested with the amphipod and Microtox tests, respectively. The objective of these tests was to determine a toxicity threshold level for each station.

Microtox. Interstitial water samples for Microtox examination were prepared by centrifugation and tested within two days of preparation. Undiluted interstitial water from each station was assayed at 15°C using a 30-min exposure and standardized methods (Bulich et al. 1982). The luminescence of *Photobacteria* sp. following exposure was measured with a photometer. Toxic effects were identified as a reduction in light emission compared with bacteria incubated in control seawater. Interstitial water was prepared on the same day that sediment was mixed for the amphipod and urchin bioassays.

<u>Amphipod test</u>. Samples of whole sediment were used in toxicity tests with the amphipod (*Grandidierella japonica*). This test consisted of a 10-day exposure conducted at  $15^{\circ}$ C under flow-through conditions. Test procedures were adapted from those of Swartz et al. (1985b).

G. japonica specimens were collected from Newport Bay (NB) at low tide. Amphipods were removed from the sediment by screening the material through a 1.0-mm screen. Approximately 2-week old amphipods were used in the bioassays. These individuals were reared in the laboratory from ovigerous G. japonica collected from the field.

Each sediment was thoroughly homogenized prior to addition to the bioassay containers. A 2-cm layer of test sediment was added to three replicate 1-liter polypropylene beakers per station. A sample of Newport Bay sediment from the amphipod collection site was also used in these tests as a control for effects not related to contamination (the Newport Bay site was near to Station 16 of the previous PAH study by Anderson and Gossett 1987). There was approximately 0.7 l of water overlying the sediment in each beaker. Amphipods were added to the test beakers on the day following sediment addition. Twenty animals were randomly distributed to each of the replicate beakers. A seawater flow of approximately 0.12 l/h was established for each beaker, along with gentle aeration. A photoperiod of 12 h of light/12 h of dark was used during the 10-day exposure. No food was added to the beakers during the test.

Bioassays were terminated after 10 days by screening the test sediments and counting the surviving amphipods. Surviving G. japonica were tested for their reburial ability by adding the specimens to dishes containing control sediment and seawater. The number of amphipods able to rebury within a 1-h period was determined. This test was intended to evaluate the survivors' condition by observing their ability to respond normally to a favorable environment.

<u>Sea Urchin test</u>. The sea urchin toxicity test was a chronic (35-day) exposure, also at 15<sup>o</sup>C under flow-through conditions. White sea urchins (*Lytechinus pictus*) were collected by trawl from northern Santa Monica Bay. Urchins were allowed to acclimate for at least 2 weeks in the laboratory before being used in tests. Tests with urchins were conducted simultaneously with the amphipod bioassays.

Sea urchin tests were conducted in polyethylene tubs (29cm x 26cm x 14cm) containing a 2cm layer of test sediment. Approximately 2.3 l of water was above the sediment. A flow rate of approximately 1 l/hr was used for all sediments except those from the Santa Monica Bay sludge outfall; a higher flow (1.5 l/hr) was used in these containers to keep dissolved ammonia at levels similar to those in the other sediment types. Fifteen urchins ranging in size from 13 to 18 mm in diameter were randomly added to each test container. Three replicate containers were used for each sediment type. Urchins were fed every other day during the exposure. The feeding ration consisted of adding a seawater suspension containing 0.36 g of powdered fish food (Tetramin) to each container. This material settled rapidly, forming a dispersed layer of food on top of the sediment. Daily observations of sea urchin mortality and sediment avoidance were made during the test. Avoidance observations consisted of noting the number of urchins present on the sediment surface before feeding; animals could avoid the sediment by climbing up the sides of the test chamber. At the end of the test, each urchin was measured for total wet weight and test diameter, and then dissected in order to remove the gonad tissue for chemical analysis. Gonad tissue from each individual was removed, weighed, and divided in half to provide separate subsamples for metals and organics analyses. The gonad tissue from all animals within a replicate was composited into a single sample. The concentration of trace metals and chlorinated hydrocarbons in these samples was measured. Technical difficulties with the extraction procedure and the small size of the samples prevented measurement of PAH concentrations.

Data analysis. Analysis of variance followed by a multiple comparison test (Student-Neuman-Keuls or Dunnett's) was used to determine the statistical significance of differences in Microtox luminescence, amphipod survival, and sea urchin sediment avoidance. Rates of change for test diameter and gonad weight were calculated by subtracting the initial value for each parameter from the measurements after 35 days. Initial values for diameter were measured on each test animal. Initial gonad weight was determined from dissections of a representative subsample of the population at the start of the test. Nested analysis of variance followed by multiple pairwise comparisons with the San Mateo Pt. stations was used to evaluate the urchin chronic test results for diameter and gonad weight changes.

Bioassay data from the second series of experiments (November 1987) were adjusted to compensate for changes in response between experiments. Corrections were made by first expressing the data as a decimal fraction of the reference site response (San Mateo Pt. for urchins and Newport Bay for amphipods). These fractions were then multiplied by the reference value for the first experiment to yield the corrected data. Most analyses were conducted on a minicomputer using the SYSTAT package of statistical routines (Wilkinson, 1986).

E. Multivariate Analyses

Patterns in the occurrence of contaminants among the test sites were identified using principal components analysis (PCA). This procedure was used to reduce the data set from a large number of individual measurements which were often correlated with each other to

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a small number of uncorrelated factors, each of which represented a group of contaminants having a similar distribution pattern.

The PCA technique manipulates a matrix consisting of the standardized concentrations of several contaminants at each of the sites to produce a reduced number of variables, each of which represents a linear composite of the original concentrations (Green, 1978). Each composite (factor) was interpreted as representing the distribution pattern of the contaminants which were most highly correlated with it. Each data set was reduced to a set of two to four factors following PCA. PCA was used in a similar fashion to produce a set of three factors representing the principal patterns in the toxicity test endpoints.

Scores, which represented the standardized relative concentrations for each group of contaminants (or test results for toxicity tests), were calculated for each of the PCA factors generated. These values were used like conventional concentration values in scatterplots, correlation calculations, or subsequent PCA steps to identify potential relationships between biological responses and contaminant groups. The ordination and PCA scores from the initial analysis of each component, sediment, macrofauna, toxicity, were used to produce "second order" PCA axes. Scores from two of the components (sediment and macrofaunal, sediment and toxicity test, macrofaunal and toxicity test) were compared first; then all three sets of scores were used. These analyses were conducted at EcoAnalysis, Inc., using SAS procedures. This type of analysis allowed us to progressively build an understanding of patterns in each component separately, then 2-by-2, and finally in all 3 components.

In addition to ordination techniques, multiple regression and variable selection methods were used to evaluate relationships between various parameters and axis scores. These methods act as an independent check or verification of the ordination results. SAS procedure RSQUARE was used for this analysis as demonstrated by Smith et al. (1988).

Response concentrations (lowest concentrations of a group of contaminants at which a biological effect was always found) were calculated for several contaminant groups using both the toxicity test and macrofaunal data. Contaminant groups were identified based upon the PCA results for the sediment chemistry data. The sum of the concentrations of all compounds in a group was then calculated by station. To derive the response concentrations for a given contaminant group, the stations were ranked (highest to lowest) on the basis of the concentration sums. Each ranked site was then scored for the

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presence/absence of statistically significant biological effects. A separate response concentration was calculated for each biological effects parameter. This value was defined as the lowest summed concentration found at a station producing a significant effect, provided all higher concentrations also produced effects.

#### **III. RESULTS**

#### A. Sediment Characterization

#### 1. General constituents and contaminants

A summary of the general constituents of the sediment types examined in this study is shown in Table 2. The sediments encompassed a wide range of textural and organic characteristics. The stations were generally composed of silty sand, although some of the sites (NB, SMS, OC) had relatively high sand contents. The depth gradient present between these sites is reflected by the grain size differences; the harbor sites tended to have the highest clay contents, reflecting the depositional nature of these shallow, protected environments. Wide variations in the total organic carbon (TOC) and total organic nitrogen (TON) contents of the sediments were also observed. TOC levels were strongly elevated (above 4%) at three sites, SMS, PV, and LAR. TON levels generally paralleled the TOC values and were about an order of magnitude lower. The increased organic content at the SMS and PV sites is due to the deposition of sewage particulates from nearby outfalls. The increased organics at the LAR site appeared to result from the deposition of terrestrial organic material from the nearby mouth of the Los Angeles River. Twigs and leaves were often encountered in this sediment while preparing it for bioassay testing and chemical analysis.

The ratio of TOC to TON was calculated for these data (Table 2). This index serves to indicate the source and relative nutritive value of the organic material in the sediment. Low C/N ratios indicate that the sediment contains a larger fraction of labile (metabolizable) protein material. The outfall sediment from the SMS and OC sites had low C/N ratios as expected; sediment from several harbor and offshore locations also had similar values.

The organic enrichment at some of these sites was strongly associated with the presence of dissolved sulfide in the interstitial water. Correlations of sulfide with TOC were very high (r = 0.99; Table 3). Sulfide concentration was highest at SMS, which had a concentration of 56 mg/l (ppm). Dissolved sulfide levels were also elevated (above 15 ppm) at PV and LAR. Sulfide levels increased with time during the sea urchin bioassay with LAR and SMS sediment, suggesting that anaerobic metabolism was continuing during the experiment.

Table 2. Concentration of general constituents in sediments used for testing. Dissolved sulfide measurements were made on interstitial water at
times times during the sea treatm toxicity test. See Table 1 for explanation of station abbreviations.

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							SULFI	DE (mg	/1)
STATION	%SAND	%SILT	%CLAY	%TOC	%TON	C/N <sup>a</sup>	INITIAL	14 D	FINAL.
Sept. 1987 experiment									
SMP (112)	4.2	84.8	11.0	0.96	0.089	10.8	2.7	0.1	0.1
DPM Cite 1	27.7	55.1	17.2	0.73	0.089	8.7	0.2	NDD	ND
OC (12)	77.4	19.7	2.9	0.56	0.062	9.0	NAC	0.1	ND
SDC (3)	39.6	35.3	25.1	1.49	0.11	13.5	0.3	ND	ND
SDN (22)	16.1	39.2	44.7	1.71	0.17	10.1	0.9	ND	ND
SD7 (x-1)	37.6	31.8	30.6	1.74	0.11	15.8	0.1	ND	ND
Nov. 1987 experiment									
SMP (19)	4.5	85.3	10.2	1.11	NA		ND	ND	0.2
LAH (C)	42.5	38.2	19.3	1.12	0.086	13.0	0.3	7.8	ND
DPM (18)	4.6	69.2	26.2	0.91	NA		1.0	ND	0.2
LAR (3)	40.7	47.4	11.9	4.28	0.35	12.1	19.5	30.3	50.0
PV (c)	28 5	60.1	11.4	4.16	0.29	14.3	15.9	3.3	2.4
SMS ( ()	53.4	38.9	7.7	10.54	1.05	10.0	56.1	4.8	102.9
NB	96.5	2.0	15	0.11	NA		NA	NA	NA

aPercent TOC/Percent TON bSample below detection limit for analysis. <sup>c</sup>Sample not analyzed.

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Table 3. Pearson correlation matrix of selected sediment constituents.

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	%TOC	H <sub>2</sub> S	%SAND	PAH	DDT	PCB	Cu	Pb
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H <sub>2</sub> S	0.992							
%SAND	0.241	0.272						
PAH	0.809	0.762	0.319			4		
DDT	0.192	0.152	-0.109	-0.142				
PCB	0.513	0.457	0.034	0.249	0.921			
Cu	0.900	0.858	0.185	0.792	0.193	0.516		
Pb	0.732	0.662	0.230	0.814	0.326	0.634	0.668	
Zn	0.741	0.660	0.135	0.764	0.475	0.764	0.768	0.909

Elevated levels of chlorinated hydrocarbons were found at many of the sites (Appendix, Table A1), especially at two of the outfall sites (PV and SMS). The highest level of total DDT compounds (primarily p,p'-DDE) was present in PV sediment, which had a concentration of 5,966 ng/g dry wt (ppb). Levels at most of the other contaminated stations were relatively low (10-196 ppb). These data illustrate the magnitude of the historical input of DDT that occurred via the sewage outfall near the PV site.

Aroclor 1254 was the dominant PCB mixture present in the samples (Appendix, Table A1). These compounds were present in the highest concentrations at PV (1,548 ppb total PCB) and SMS (654 ppb).

PAH were present in high concentrations in many of the test samples. Total PAH concentrations greater than 1,000 ppb were found at all of the industrialized harbor sites and at two of the three outfall sites (Appendix, Table A2). The general pattern of PAH distribution differed from that of the chlorinated compounds, with the highest value (20,000 ppb) occurring at the SMS station. Total PAH levels in the harbors (4,711-12,109 ppb) surpassed that found at the PV site (3,209 ppb). These values are similar to those found at the same stations less than two years ago, during a study of PAH contamination along the coast (Anderson and Gossett 1987).

Analysis of the sediment samples for phenols resulted in detectable concentrations only for the SMS sample. Concentrations of phenol, 2-methyl phenol, 4-methyl phenol, and pentachlorophenol in this sample were 0.18, 0.21, 4.87, and 1.74 mg/g dry wt. (ppm), respectively. This analysis had a detection limit of 0.1 ppm for all compounds except pentachlorophenol (1.0 ppm).

Trace metal concentrations (Appendix, Table A3) were also highest at the PV and SMS outfall sites. These two sites had metal levels that were generally 10-100 times greater than those present at the SMP reference site. Some of the Los Angeles and San Diego harbor sites had relatively high levels of copper, lead, and zinc that rivaled those at the outfalls.

Measurements of organotin (tributyltin or similar compounds) yielded surprisingly high levels in the SMS and PV samples (Appendix, Table A3). Organotin concentrations in these outfall sediments ranged from 127 to 329 ppb, which were similar to levels found in the harbor sediment samples (28-423 ppb).
Although organotin concentrations were similar between outfalls and harbor sites, it is likely that significant differences is compound speciation existed between these two areas. Preliminary gas chromatography/mass spectrometry analysis of some of these samples indicated that tributyltin was the predominant compound measured in harbor sediments with the extraction technique used in this study (M.D. Stephensen, pers. comm.). Similar analyses of outfall sediments indicated that tributyltin was a minor component of the tetraand trialkyltin compounds present in these samples.

Detectable levels of mercury were not found in this study. This result was unexpected, as previous studies have found levels of mercury greater than 1 ppm at the PV (Eganhouse 1978) and LAH (Chen and Lu 1974) locations. Mercury concentrations in nearshore locations may have declined in recent years as a result of regulatory controls on discharges. Conversely, several technical factors in this study may have contributed to an unusually low detection sensitivity for mercury. First, sediment samples for analysis were taken from the flow-through exposure aquaria, which may have resulted in the loss of some mercury. Precautions were also not taken during the sample digestion procedure to minimize the loss of mercury to the atmosphere. In addition, a large background signal for mercury was present during the ICP/MS analysis, resulting in a high detection limit (0.5-0.8 ppm) for the analysis.

Sediment chemistry measurements were also made at the termination of the sea urchin toxicity tests in order to document changes in sediment composition during each 5-week exposure to flowing seawater. Declines in sediment contaminant levels were minor in most cases (Appendix, Tables A1-A3). Apparent increases in metal and hydrocarbon concentrations occurred nearly as frequently as losses, illustrating the variability inherent in the sampling and analytical methods.

Among the trace organics, losses during the exposure (up to 50% total PAH) were observed most consistently for the PAH compounds. This pattern was to be expected since many PAH compounds are more susceptible to microbial degradation and leaching than DDT or PCB compounds.

## 2. Contaminant distribution patterns

The data were examined numerically to determine the nature of the relationships between contaminants. Pearson's correlations were calculated between each of the chemical and

physical constituents of the sediments. A large number of high correlations was obtained, indicating the general similarity of contaminant distribution patterns (Table 3). Numerous pairwise comparisons of this type often produce many false or coincidental correlations; therefore, principal components analysis (PCA) was used to identify patterns of distribution among several groups of chemical constituents (metals, chlorinated hydrocarbons, PAH, and sulfide). Phenol measurements were not included in this analysis since this group of compounds was detectable at only one location (SMS). PCA was first conducted on groups of similar contaminants (e.g., PAH) using concentrations expressed on a dry weight basis. Similar analyses were also conducted on TOC-normalized PAH and chlorinated hydrocarbon values. The factors obtained from these analyses were analyzed by PCA once more, together with the dissolved sulfide and organotin data, in order to summarize overall contamination patterns. The results from all of these analyses are summarized in Table 4.

When the sediment inorganic metals data were factored by PCA, 92% of the variation in the standardized values was accounted for by two factors. The abundance patterns for most of the metals were strongly correlated with a general metals factor (MET1) as shown in Table 4. Lead and zinc grouped together on the second, independent, factor (MET2). The correlations of the metal concentrations with these factors are shown in the Appendix, Table A4.

The metals PCA results indicate that the pattern of distribution of lead and zinc among the stations differed from that of the other metals. These patterns can be seen by examining plots of the standardized scores (zero mean and unit variance) for each of these factors (Fig. 2). These scores are a composite of the relative abundances of the metals constituting each factor. Four groups of stations resulted from this analysis. The general metals factor scores were greatest at the group composed of the PV and SMS stations, reflecting the input of metals from wastewater. While the Pb-Zn factor scores also indicated high levels at these two sites, even greater scores were present at two harbor sites (LAR and SD7). This MET2 factor appeared to indicate that a substantial portion of the lead and zinc found at these two stations had a source (possibly stormwater) which was distinct from the sources responsible for the input of other trace metals to the sites. The third group represented contaminated harbor stations having metal distributions which were nearly equally influenced by each PCA factor. The final group was composed of relatively clean stations which had low concentrations of most metals.

Table 4. Summary of sediment contaminant groups (factors) identified using principal components analysis. Hydrocarbon data were analyzed on both a dry weight (ng/g) and TOC (ug/g TOC) basis. The sign of the correlation coefficients has been omitted for clarity.

Data Set	PCA Factor Name	Variables with highest correlations (r)
Inorganic Metals dry weight basis	MET1	Sn(0.94), Cd(0.90), As(0.88), Cr(0.88), Ni(0.85), Ag(0.80), Cu(0.78)
	MET2	Pb(0.94), Zn(0.82)
Chlorinated Hydrocarbons	DDTF	DDT(0.86), DDE(0.85), DDD(0.84)
dry weight basis	PCBF	Aroclor 1242(0.87), Aroclor 1254(0.78)
РАН	PAH1	Naphthalene(0.99), C1-C3 Methylnaphthalenes(0.98)
dry weight basis		Biphenyl(0.99), C1-C3 Phenanthrenes/Anthracenes(0.90)
	PAH2	Benzopyrenes(0.99), Anthracene(0.97), 2,3-Benzofluorene(0.97), Benzo(g,h,i)perylene(0.94) Benzoflouranthenes(0.93)
	PAH3	Acenaphthene(0.98), Fluorene(0.82), Phenanthrene(0.77)
	PAH4	Acenaphthylene(0.98), 9,10-Diphenylanthracene(0.97)
All contaminants	SED1	H2S(0.98), PAH1(0.94), MET1(0.86), PCBF(0.86)
dry weight basis	SED2	PAH4(0.94), DDTF(0.91)
	SED3 SED4	PAH2(0.94), Organotin(0.71), ME12(0.68) PAH3(0.96), MET2(0.65)
	0201	
Chlorinated Hydrocarbons	DDTTOC	DDE(0.92), DDT(0.92), DDD(0.85), Aroclor 1242(0.80)
TOC basis	PCRIOC	Aroclor 1254(0.92)
РАН	PAHTOC1	C1-C3 Methylnaphthalenes(0.95), Naphthalene(0.91),
TOC basis		Biphenyl(0.85)
	PAHTOC2	Pyrene(0.98), Benz(a)Anthracene(0.98), 2.3-Benzofluorene(0.98), Benzopyrenes(0.97), Eluoranthene(0.95), Anthracene(0.94)
	РАНТОСЗ	9.10-Diphenylanthracene(0.89), Acenaphthylene(0.85)
	PAHTOC4	Acenaphthene(0.88)

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# Table 4 continued.

Data Set	PCA Factor Name	Variables with highest correlations (r)
All contaminants dry or TOC basis	SEDTOC1 SEDTOC2 SEDTOC3 SEDTOC4	PAHTOC1(0.99), H <sub>2</sub> S(0.91) PCBTOC(0.88), PAHTOC2(0.84), MET2(0.75) DDTTOC(0.94), PAHTOC3(0.83), MET1(0.64) Organotin(0.91), MET1(0.55)

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Figure 2. Plot of PCA factor scores for metals data.

The patterns of DDT and PCB distribution were also studied using PCA (Table 4). Two independent patterns (factors) were found when the dry weight-normalized concentrations of these compounds were used. One factor (DDTF) was most strongly associated with the DDT compounds, while the second factor (PCBF) was most strongly influenced by the pattern of PCB distribution. The correlations of the individual compounds with these factors are shown in the Appendix (Table A5).

The chlorinated and polynuclear aromatic hydrocarbon data were also analyzed by PCA after normalization to sediment organic carbon content (ug/g TOC). When TOC-normalized DDT and PCB concentrations were factored, two groups were obtained. These groups were similar to those obtained after analysis of the dry weight data, except that Aroclor 1242 was now strongly associated with the DDT group (DDTTOC). The majority of the PCB concentration data was still represented by a separate factor (PCBTOC), since this group was highly correlated with Aroclor 1254, the most abundant PCB mixture present. The lack of a clear distinction between the PCB and DDT distribution patterns in this study may make it difficult to distinguish between the biological effects produced by each of these groups of compounds.

Examination of the dry weight-normalized PAH data by PCA resulted in the grouping of these compounds into four independent factors (Table 4). The first two factors (PAH1 and PAH2) represented most of the compounds and accounted for 76% of the variation in the data. PAH1 was composed of naphthalene compounds and alkylated phenanthrene/anthracenes. The compounds in this group are characteristic of petroleum. PAH2 was a composite of higher molecular weight compounds, such as the fluoranthenes and benzopyrenes. This group is most likely related to products of incomplete fossil fuel combustion.

The remaining two factors (PAH3 and PAH4) represented minor components of the PAH distribution pattern and were composed of a small number of compounds having intermediate molecular weights (Table 4). The composition of these two groups did not appear to be related to readily identifiable sources or biogeochemical processes.

Most of the PAH compounds correlated strongly with only one of the four PCA factors (Appendix, Table A6), indicating that it may be possible to distinguish between the biological effects of each of these groups. Perylene was unusual in that it was correlated with three of the four PAH factors. This pattern may reflect the fact that this compound is

produced by ubiquitous microbial processes (like petroleum), yet is resistant to metabolism by virtue of its large size (like combustion products). Consequently this compound is likely to be associated with PAH contamination resulting from a variety of processes.

Plots of the scores for the PAH factors indicated that several of the stations had very distinctive PAH composition patterns (Fig. 3). The petroleum pattern of PAH1 was present primarily at SMS and LAR. This may be the result of high petroleum inputs at these areas. This type of pattern may have also resulted from a high rate of particle deposition or the presence of anaerobic sediments, both of which would tend to reduce the loss of lower molecular weight compounds from the sediment. The combustion products characteristic of PAH2 were most prevalent at the San Diego Bay stations. Likely sources for these compounds include ship activity and urban runoff. Plots of the factor scores for PAH3 and PAH4 (Fig. 3) show that these groups reflect distinctive PAH patterns at the LAR and PV sites, respectively.

Statistical analysis of the TOC-normalized PAH concentrations produced a similar set of four PCA factors (Table 4). Many of the low molecular weight PAH compounds were again grouped together on a factor (PAHTOC1) different from the one representing most of the higher molecular weight compounds (PAHTOC2). A major difference between these two groups of PCA factors was a shift of the C1-C3 phenanthrene/anthracene compounds from the low molecular weight group to the high molecular weight group. Plots of the relative station scores for these two factors (not shown) were similar to those shown for PAH1 and PAH2.

The factor PAHTOC4 was distinguished only by a high correlation with acenaphthene. This compound was not detected at any of the sites, however, indicating that variability in detection limits between samples produced a spurious correlation. The PAHTOC4 factor was dropped from subsequent analyses because of its ambiguity and relatively minor contribution to the total PAH data set.

The bivariate correlation analyses (Table 3) showed that strong relationships often existed between the occurrence of different types of contaminants (e.g., metals and PAH), in addition to the within-group relationships described above. The patterns of association of different groups with each other were also identified using PCA. The PCA factor scores from the previous analyses were combined with values for dissolved sulfide and organotin and examined for groupings. When data expressed on a dry weight basis were analyzed,



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four independent factors were identified that together accounted for 94% of the variation in the data (Table 4).

The first PCA factor (SED1) represented a complex group of contaminants and was highly correlated with PCBF, MET1 (most metals), PAH1 (petroleum), and sulfide (Appendix, Table A7). DDT concentrations were closely associated with the PAH4 factor and made up the second sediment contamination factor (SED2). Plots of the station scores for these two factors (Fig. 4) show that these groupings reflect distinctive and very high levels of contamination at the SMS and PV outfall sites. The compounds in these groups showed relatively little variation between the other sites.

High molecular weight PAH compounds (PAH2) grouped with organotin and, to a lesser degree, with MET2 (lead and zinc) to form the third factor (SED3). The association of lead with this group of PAH may be due to the presence of lead-containing additives to some fossil fuels and potential atmospheric contributions of these compounds through runoff. This group showed a wide range of variation between the sites, with the San Diego Bay stations having the highest scores (Fig. 4). The fourth general sediment factor was strongly correlated with only the PAH3 factor, which was most elevated at the LAR site.

The PCA of all sediment contaminants can be used to identify the existence of different contaminant sources. A strong association of individual contaminants with different PCA factors implies that they have dissimilar patterns of distribution between stations. A likely cause for such differences would be variation in the nature of input (source) or rate of degradation for each contaminant between stations. Since contaminant sources are often complex mixtures of compounds, a group of chemicals that associates strongly with each other after PCA may have been input by similar sources. Four distinct patterns (factors) of contamination were indicated by the PCA of the dry weight-normalized data. Examination of the station plots (Fig. 4) indicates that factors SED1, SED2, and SED4 were each dominated by a single site. Each of these sites were located near large, chemically distinct, contamination sources (L.A. County sewage, L.A. City sludge, urban runoff). The remaining factor (SED3) appeared to represent a contamination pattern characteristic of other source(s) common within industrialized harbors.

A set of four factors was also obtained when the TOC-normalized trace organics data were used in a PCA of the combined sediment contamination groups (Table 4). Some of the resulting compound associations were similar to those obtained using the dry weight data,



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such as the H<sub>2</sub>S and low molecular weight PAH factor (SEDTOC1). High molecular weight PAH and MET2 were still grouped together (SEDTOC2), but this group now also contained most of the PCB data. The MET1 factor was now strongly associated with the DDT data (SEDTOC3). Organotin was now the principal component of the last factor, SEDTOC4.

These four general sediment contamination factors represent the maximum level of separation possible with the small sample size of 10 stations used in this study. Statistically, one cannot distinguish between the distribution patterns of the separate contaminants making up a single factor. Consequently, the biological effects of any one compound cannot be determined without consideration of the presence of many other contaminants. This is a common problem encountered when trying to determine the cause of toxicity in sediment samples collected from the field.

The contaminant groupings identified by the previous PCA analyses will be used in subsequent sections of this report which deal with the relationships between contaminants and biological effects. Biologically significant levels of metals and organics will be expressed as the total concentration of the contaminants characteristic of each PCA group. These concentrations are shown in Table 5.

B. Benthic Macrofauna

## 1. Species composition and abundances

a. Ordination and classification analyses

Ordination (principal coordinates analysis) of benthic macrofaunal data from each site produced four independent axes. Ordination scores of the sites plotted in the ordination spaces formed by these axes show the relationships of species composition and abundances among the sites (Fig. 5). These four axes accounted for a total of 70.8% of the variance among the sites.

The sites are arranged along the first axis with the 5 protected bay sites on the left and SMS on the right. Four groupings of sites with similar species and abundances are encircled. This pattern on the first 2 axes is quite strong and is generally unchanged by subsequent axes. Axis 3 best separates the protected sites and LAR and PV. For future reference,

						STATION				
Group	SMS	PV	LAH	LAR	oc	DPM	SMP	SDN	SDC	SD7
Dry weigh	nt-normalize	ed values								
DDT <sup>a</sup> PCB <sup>b</sup> PAH1 <sup>c</sup> PAH2 <sup>d</sup> PAH2 <sup>d</sup> PAH3 <sup>e</sup> PAH4 <sup>f</sup> MET1 <sup>g</sup> MET2 <sup>h</sup>	203 655 15800 3794 426 383 899 809	5956 1547 1288 1333 77 525 634 742	88 232 687 4183 181 278 164 275	87 310 4984 4109 683 152 152 520	12 58 32 66 21 3 53 74	8 10 28 59 9 25 58 79	22 8 15 33 6 6 50 64	18 212 153 4283 198 105 311 381	31 193 745 6460 289 160 190 305	80 353 1941 9597 326 267 206 685
TOC norn	nalized valu	es								
DDT PCB PAH1 PAH2 PAH3 PAH4	2 6 150 36 4 4	143 37 31 32 2 13	8 21 61 374 16 25	2 7 116 96 16 4	2 10 6 12 4 1	1 1 3 7 1 3	2 1 3 1 1	1 12 9 250 12 6	2 13 50 434 19 11	5 20 112 552 19 15

Table 5. Summed concentrations of sediment contaminants characteristic of groups having similar distribution patterns. Groups were selected based on principal components analysis results of data expressed on a dry weight basis. Values are ng/g dry weight or ug/g TOC except where noted.

Sum of 0,p and p,p' isomers of DDT, DDD and DDE. Sum of Aroclor 1242 and Aroclor 1254. а

c Low molecular weight PAH (sum of PAH #'s 1,2a,2b,3a,3b,4a,4b,5,10,11,12 from Table A2).
d High molecular weight PAH (sum of PAH #'s 13,14,15,16,17,18,19,20,21,22,25,26 from Table A2)
e Sum of PAH #'s 7,8,9 from Table A2.
f Sum of PAH #'s 6,23,24 from Table A2

Sum of Ag, As, Cd, Cu, Cr, Ni, and Sn, data are expressed in ug/g dry wt. Sum of Pb and Zn, data are expressed in ug/g dry wt.





vectors of increasing values for measured abiotic parameters are indicated on each plot, for example, site depth increases obliquely to the lower right in the plot of the first 2 ordination axes.

Classification analyses of ecological distances from the ordination space produced a dendrogram with 4 major site groups (Fig. 6). This analysis shows the same site groupings (based on species composition and abundances) as Fig. 5. The SDC and SDN sites were most similar to each other, and most similar to the DPM site (protected reference). The SD7 and LAH sites formed a subgroup of these sites. The only other protected site, LAR, was more similar to the PV outfall site than the other protected sites. In this analysis, the OC site was most similar to the open coastal reference site, SMP, however OC is not considered a reference site as it is known to be inhabited by infaunal species more typical of impacted areas (CSDOC, 1987). The SMS site was most similar to LAR and PV, but the macrofaunal assemblage there was very different from all other sites.

Examination of a two-way table of abundances and sites shows the differences in macrofaunal species at the site groups just described (Table 6). The protected sites (except LAR) all were inhabited by the polychaetes Theora lubrica and Tharyx spp. The polychaete Lumbrineris lagunae was most the most abundant species at SDC, SDN, and DPM, but the clam Lyonsia californica was most abundant at SD7, and Tharyx spp. was most abundant at LAH. These differences in dominant species produced the subgrouping of SD7 and LAH from the other 3 San Diego sites. The San Mateo Point and Orange County sites had numerous species in common, but the dominant species at each site were different. The ophiuroid Amphiodia urtica was most abundant species at the reference site (SMP), but the ostracod Euphilomedes carcharodonta was most abundant at the OC site. Both the polychaete Capitella capitata and the clam Parvilucina tenuisculpta were among the most abundant species collected at the OC site, and were also abundant at the PV site. Capitella was also collected at the LAR site, but not at the other protected sites. The PV and LAR sites formed an interesting combination of similar sites. Although one site is open sea and the other protected, and they are dominated by different species, they had species in common: the polychaetes Mediomastus sp. and Capitella, and the clam Macoma nasuta.

The macrofaunal assemblage at the Santa Monica sludge site was completely different from any of the other sites. Only the presence of *Capitella* linked this site with the other open sea sites. Only 5 species were collected at SMS and most of the animals belonged to one of three undescribed species of the polychaete genus *Ophryotrocha*. These worms are



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Table 6. Two-way table of species composition and mean abundances (n = 2 reps) at each site. This table includes at least the 5 most abundant species at each site (superscript = rank) and the 15 most commonly collected species among all sites. Station and species orders were determined from normal (sites) and inverse (species) classification analyses. PO = polychaete, PE = pelecypod, D = decapod crustacean, A = amphipod, OPH = ophuiroid, OST = ostracod, G = gastropod, CO = copepod.

				MEAN	ABUN	DANCE	PERM	2			-
Species	Taxon	SDC	SDN	DPM	SD7	LAH	SMP	OC	PV	LAR	SMS
Streblosnio benedicti	PO	405		į							1
Musculista senhousia	PE	1454	5101		5						
Euchone limnicola	PO	25	1854	10	153				1		1
Theora lubrica	PE	460 <sup>2</sup>	1405	35	202	50 <sup>3</sup>					
Cossura candida	PO	2503	1853	15			10		1		1
Lumbrineris lagunae	PO	4651	305 <sup>2</sup>	260 <sup>1</sup>		5					
Lumbrineris erecta	PO		50		154						
Lyonsia californica	PE	20	65	1.1	20 <sup>1</sup>	5	5				1
Prionospio heterobranchia	PO	15	95	40 <sup>5</sup>	15 <sup>5</sup>					5	1
Hemigrapsus oregonensis	D			105 <sup>2</sup>		1.00					
Leitoscoloplos pugettensis	PO		40	80 <sup>3</sup>		10		15			
Chaetozone corona	PO		5			35					
Tharyx spp.	PO	25	30	5	10	635 <sup>1</sup>	5	35	1		
Lumbrineris sp.	PO		5		15'	5	5	120			
Spiophanes missionensis	PO			10		5	595	90			1
Pectinaria californiensis	PO					15	155	200	1.1		1
Glycera americana	PO	10	5	10				65	5	1.1.2	
Mediomastus sp.	PO		105	30		30 <sup>5</sup>	15	430 <sup>5</sup>	5	40 <sup>3</sup>	1
Prionospio (Minuspio) lighti	PO		1100	604			35	35		5	
Byblis veleronis	A						60 <sup>S</sup>				
Heterophoxus oculatus	A						2103				
Amphiodia urtica	OPH						15651	5			
Prionospio sp. A	PO					5	20	735 <sup>2</sup>	1		1
Acmira catherinae	PO							4454	1.1.1.1		
Euphilomedes carcharodonta	OST			5		195 <sup>2</sup>	25	14351	2152		1
Capitella capitata	PO			2				503 <sup>3</sup>	5	15	54
Tellina carpenteri	PE	5					30	215	703		
Neverita recluziana	G						5	5	15		
Parvilucina tenuisculpta	PE						5	320	230		
Spiochaetopterus costarum	PO							20	304		
Pseudopolydora paucibranchiata	PO					10			1.12.2	20	
Microdeutopus schmitti	Α									185	
Oligochaetes	OLI		10							45 <sup>2</sup>	
Diopatra spiendissima	PO		10	12						304	
Ophryotrocha sp. B	PO		-	1	1						152
Ophryotrocha sp. A	PO										10 <sup>3</sup>
Ophryotrocha sp. C	PO										90 <sup>1</sup>
0 1 1 1 1 1 1 I	00										-5

only found in the most contaminated areas off southern California (D. Montaigne, L.A. County Sanitation District, personal communication).

It is not possible to determine from ordination and classification analysis alone which abiotic factors (including contaminants) may influence the macrofauna to produce the observed site groupings. Using principal components analysis we will show correlations between patterns of sediment "factors" and macrofaunal assemblages in a subsequent section (page 39).

### b. Indicator species

Percentages of total macrofaunal abundances of polychaetes (except capitellids), capitellids, amphipods, and ophiuroids at each site are shown in Fig. 7. Except at SMP and LAR, polychaetes contributed more to total abundances than the other taxonomic groups. Ophiuroids (*A. urtica*) were most abundant at SMP, were significantly lower at OC, and none were collected at any other site. Amphipods were dominant (*Microdeutopus schmittii*) at the LAR site, but were not collected from SMS, LAH, SDN, or SDC. The most abundant amphipod collected at the open sea reference site (SMP) was *Heterophoxus oculatus* ( $210/m^2$ ) and at the protected reference site (DPM) was *Listriella goleta* ( $15/m^2$ ). *Rhepoxinius bicuspidatus* was collected at SMP and OC. OC had the highest density of amphipods,  $545/m^2$ . Capitellids were collected at both the open sea and protected sites, but were most abundant at the LAR and OC sites.

Statistical comparisons of these parameters with the appropriate (open sea or protected) reference site values (Table 7) shows that for the open sea sites, SMS had significantly higher percentages of polychaetes that the reference site (SMP). At PV, percentages of amphipods and polychaetes were significantly lower than SMP. At OC, ophiuroid percentage abundance was significantly lower than at the reference site. Percentages of capitellids were not significantly different among the open sea sites.

At the protected sites, there were no significant differences in percentages of any of the indicator taxa between the protected reference (DPM) and any of the sites. Although no amphipods were collected at LAR, SDN, SDC, and no capitellids were collected from SDC, SD7, the proportions were not significant due to the variation in the reference site.



Figure 7. Plots of mean  $(\pm SD)$  percentages of total macrofaunal abundances for polychaetes (except capitellids), capitellids, amphipods, and ophiuroids at each site (N = 2 replicates). Reference sites for open sea (SMP) and protected sites (DPM) are cross-hatched. NC, None collected.

Table 7. Results of one-way ANOVA comparing percent of indicator taxa among four open-sea and six protected sites. N=2 replicates/site. Percentages were arcsine transformed. Significance level of pairwise comparisons with the appropriate reference site was corrected using Bonferroni's procedure (Neter and Wasserman, 1974) to  $\alpha = 0.017$ for open sea and  $\alpha = 0.010$  for protected sites.

	· · · · · · · · · · · · · · · · · · ·	OPEN S	SEA	PROTECTED			
Percent		Sig. Difference		Sig. erence		Sig. Difference	
Abundance	F	P	from Reference	F	P	from Reference	
a) Polychaetes	153.32	.001 <sup>a</sup>	SMS,PV	0.57	.722		
b) Amphipods c) Capitellid	19.23 2.46	.008 <sup>a</sup> .203	SMS,PV	0.62 5.04	.620 .037 <sup>a</sup>	[b	
d) Ophiuroids	79.61	.001 <sup>a</sup>	SMS, PV, OC	NONE	COLL	•	

<sup>a</sup>Significant value P < 0.05.</li>
 <sup>b</sup>Although overall one-way ANOVA shows significance among the sites, none of the paired comparisons to the reference site were significant ( a = 0.01). It was the differences between some of the non-reference sites that caused the significance of the overall ANOVA.

#### 2. Assemblage structure and diversity

Because the 10 sites sampled included sites from a range of depths and substrate types, it is difficult to observe trends in species composition and abundances that could be attributed to contamination alone. The use of measures of species diversity and biomass provides another way to evaluate effects of contamination on benthic macrofaunal assemblages that is independent of species composition (Smith, 1984).

The mean number of species, individuals, and biomass at each site is plotted in Fig. 8. At the open sea sites, SMS was significantly lower than the reference site (SMP) in all three parameters (Table 8). PV had significantly lower numbers of species and individuals, but not biomass. OC had higher average values for all 3 parameters but they were not significantly different from the reference site values.

At the protected sites, there were no significant differences between any of the sites and the protected reference (DPM) in any of the three parameters (Table 8). Average biomass was elevated (but not significant) at SDN due to large numbers of the mussel *Musculista stenhousia*.

Shannon-Wiener species diversity index (H') and Pielou's evenness (J) at each site are plotted in Fig. 9. At the open sea sites, H' was significantly lower at SMS than at the reference site, but H' was not significantly different at the other sites (Table 8). Evenness (J) was not significantly different among any of the open sea sites.

At the protected sites, only LAH had a significantly lower H' value than the protected reference site. Evenness (J) was not significantly different from the reference site at any of the protected sites.

3. Relationships between macrofauna and sediments

Second order PCAs were conducted using ordination scores of the four main axes identified in the macrofaunal principal coordinates analysis and (a) the 4 main sediment PCA axes (SED1-SED4) on a dry weight basis, and (b) the 4 main sediment PCA axes (SEDTOC1-SEDTOC4) on a TOC weight basis.



Figure 8. Plot of mean ( $\pm$  SD) number of species, individuals, and biomass per grab at each site (N = 2 replicates). Reference sites are cross-hatched

Table 8. Results of one-way ANOVA comparing assemblage structure parameters among four open-sea and six protected sites. N=2 reps/site. Values of J were arcsine transformed and the other parameters were  $\log_{10}$  transformed. Pairwise testing to reference sites as described for Table 7.

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		OPEN SE	4		PROTEC	TED
Parameter (per grab)	F	Р	Sig. Diff. From Ref.	F	Р	Sig.Diff. From Ref.
Mean No. Of Species	52.54	0.001 <sup>a</sup>	SMS,PV	3.36	0.086	
Mean No. Of	101.00	0.0010			0.0400	h
Individuals	126.28	< 0.001"	SMS,PV	7.12	0.0174	_0
Mean Biomass	12.41	0.017 <sup>a</sup>	SMS	3.76	0.069	· · · · · · · · · · · · · · · · · · ·
H'	10.11	0.024 <sup>a</sup>	SMS	8.12	0.012 <sup>a</sup>	LAH
1	0.28	0.837		7.00	0.017 <sup>a</sup>	b

<sup>a</sup>Significant  $\alpha < 0.05$ . <sup>b</sup>See Table 7.

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Figure 9. Plots of mean ( $\pm$  SD) species diversity (H') and evenness (J) at each site (N = 2 replicates). Reference sites are cross-hatched.

Using dry weight data, the analysis produced 5 new, second-order axes shown in Table 9A. The first three new axes had the highest loadings from one of the macrofaunal ordination axes and one of the sediment PCA axes. This analysis shows that the macrofaunal pattern from the first ordination axis (ORD1) is most highly correlated with the factors that compose SED1: sulfides, MET1, PAH1, and PCBF. As shown in section III.A, these factors occurred in highest concentrations at SMS and PV (see Fig. 4). Sulfide concentrations were the single most highly correlated sediment factor to ORD1 ( $R^2 = 0.81$ ). With 2 variables, sulfide and clay were most highly correlated to ORD1 ( $R^2 = 0.95$ ).

Similarly, ORD4 and SED3 provided the highest loadings on the second axis. SED3 was composed of PAH2 and MET2 factors which were highest at the protected bay sites; therefore, this analysis suggests that these factors may best separate the protected sites. ORD3 and SED4 provided the highest loadings on the third axis, but were not significantly correlated (r = 0.59). SED4 represented patterns in some of the intermediate weight PAH (PAH3) that were highest at the LAR site.

Interestingly, ORD2 loaded onto the fourth new axis and was not strongly associated with any of the sediment PCA axes. This indicates that the second ordination axis was influenced by some indistinct combination of sediment factors or by some unmeasured or non-sediment factor. No single sediment factor was significantly correlated to ORD2 scores. Organic material and depth were the 2 most highly correlated parameters ( $R^2 =$ 0.82). These two parameters were not used in the PCAs, and ORD2 was not associated with any of the sediment PCA factors; thus ORD2 may represent site differences due to depth and grain size (Fig. 5).

Using TOC standardized organic contaminant factors, second-order PCA axis loadings similar to the dry wt. factors were obtained (Table 9B). ORD1 was most highly correlated with SEDTOC1 which represents concentrations of sulfides and PAHTOC1. It is noteworthy that low molecular weight PAH and sulfides were highly correlated with ORD1, regardless of whether TOC normalization of the data was performed. The use of TOC normalized hydrocarbon data did eliminate the relationship found between ORD1 and MET1, however. The other new second-order axes associated ordination axes and TOC standardized sediment factors the same as they did on a dry weight basis, and ORD2 and SEDTOC4 were not associated with any other factors. Table 9. Associations between macrofaunal ordination axes and sediment PCA axes as determined by second order PCA. Components shown provided the highest loadings on their respective second order axes. Complete listing of correlations is shown on Appendix Table A11.

	MACROF	AUNA - CONTA	MINATION A	CIS	
A. Dry Wt. Basis	1	2	3	4	5
	ORD1 SED1	ORD4 SED3	ORD3 SED4	ORD2 -	SED2
% Variance Explained	24	22	21	17	15
B. TOC Wt. Basis	1	2	3	4	5
	ORD1 SEDTOC1	ORD3 SEDTOC3	ORD4 SEDTOC2	ORD2 -	SEDTOC4
% Variance Explained	25	19	19	16	16

The relationship between macrofauna and sediments was generally the same using dry weight or TOC standardized sediment concentrations. We will therefore only consider the dry weight data in the remainder of the report as they represent the least permuted values and we are not sure how to interpret TOC standardized sediment values.

Estimated response concentrations

Sediment contaminant concentrations that correspond to significant responses by macrobenthic assemblages were calculated using information on significant deviations (or lack of deviations) from reference values of indicator species and diversity parameters (Tables 7 and 8), and differences in species composition and abundances shown in classification analysis (Fig. 6). Estimated response concentrations of each sediment factor were calculated for several macrobenthic assemblage parameters. Concentrations of factor components (i.e., MET2 included Pb and Zn) were summed at each site and are listed in Table 5. Individual contaminants were not used in this analysis because our analysis showed that the factors used provide the best resolution possible. Even estimated response values calculated using separate factors (i.e., PAH1, MET1) should be evaluated with caution; SED1 through SED4 is the limit of resolution of sediment factors in this study. Since PCAs showed that benthic responses patterns were similar to dry weight and TOC standardized sediment factors, we did not calculate estimated response thresholds for TOC standardized sediment factor concentrations.

Estimated response concentrations for each sediment factor are shown in Table 10. Most of the significant deviations from reference conditions occurred at the open sea sites; thus the estimated values generally represent PV or SMS concentrations. Concentrations for significant changes in percent amphipods are the same as for percent polychaetes, total species, and individuals per grab. Since no ophiuroids were collected at the protected sites, the estimated concentrations are over estimates. Significant ophiuroid reductions were shown at all three open water outfall sites, thus open water estimated response concentrations would usually be those at the OC site. No estimates were made for phenols as they were only detected at SMS.

Table 10. Estimated response concentrations for several contaminant groups based on macrobenthic assemblage parameters. The response concentration indicates the lowest concentration at which a significant difference from the appropriate reference site was consistently found (i.e., higher concentrations always produced a significant effect). The hydrocarbon and inorganic metal group values represent the summed concentrations of their components (listed in Table 5).

	RESP	ONSE CONCE	VTRATION (ppm)	FOR:	
group	Species Comp.	Amphipods	Ophiuroids	H'	
H_Sª	15.9	56.1	56.1	56.1	
DDT	0.20	0.20	0.20	NC	
PCB	0.66	0.66	0.66	NC	
PAH1	5.0	15.8	15.8	15.8	
PAH2	NC	NC	NC	NC	
PAH3	0.43	NC	NC	NC	
PAH4	0.38	0.38	0.38	NC	
MET1	634	634	634	899	
MET2	742	742	742	809	
ORGANOTIN	NC	NC	NC	NC	

<sup>a</sup>Concentrations for H<sub>2</sub>S were measured in milligrams per liter.

<sup>b</sup>NC, No correspondence between significant responses and concentrations.

#### Summary

Previous studies of animal-sediment relationships in the region have repeatedly identified depth, sediment grain-size, and organic material as the most important factors associated with differences in macrofaunal assemblages (Smith and Greene 1976, Thompson et al. 1987, Cimberg and Smith [in press]). Our analyses also identified these parameters as important; however, due to the high intercorrelations of percent clay, organic material, and sulfide, we cannot conclude which had the most influence on the macrofaunal patterns observed. Additionally, the PCAs showed that contaminant concentrations are important, but again due to the high degree of intercorrelations and low numbers of sites, we cannot conclude which individual contaminants have the most influence on species composition. We can conclude that the distribution of compounds that compose PAH1, MET1, and PCBF correlate best with the distribution of macrofaunal assemblages that we sampled.

In open sea reference areas off southern California, the dominant species is Amphiodia urtica. Closer to sewage outfalls, in transition areas, A. urtica decreases in abundance and Parvilucina tenuisculpta becomes most abundant. In areas nearest to outfalls, Capitella capitata is most abundant (Word et al. 1977, Thompson 1982). This pattern was observed in the present study and facilitates evaluation of macrobenthic effects. Polychaetes, ophiuroids, and amphipods were also good indicators of macrobenthic effects at open sea sites (Fig. 7, Table 7).

Similar patterns of indicator species were not observed at the protected sites. Changes in species composition in bays and harbors related to contamination have not been studied as much as in the open sea off southern California; however, species such as *Capitella* and *Tharyx* may occur in both areas. A few of the indicator species listed by Pearson and Rosenberg (1978) were collected at some of the protected sites: *Streblospio benedicti, Mediomastus* sp., and *Prionospio heterobranchia* (see Table 6).

Assemblage structure parameters (i.e., species diversity) have also been shown to change over contamination gradients (Pearson and Rosenberg 1978, Swartz et al. 1986). In general, diversity increases in transition areas, approaching outfalls, then decreases to below reference levels nearest the contamination source. This elevation in diversity in the transition zone is a demonstration of the intermediate disturbance principal (e.g., Hughes 1984) and was observed at OC. Some sites (SMS, LAH) had species diversity (H') values significantly lower than the reference sites; therefore, these assemblages are considered to be affected by contamination. Evenness (J) reflects the fact that as contamination affects the assemblage the distribution of individuals among species increases, or some species become more dominant (usually the contamination tolerant ones). However, none of the stations sampled showed significant shifts in J compared with reference sites. Evenness was not shown to be a sensitive indicator of macrobenthic assemblage effects at the sites we sampled.

The open sea assemblages showed much stronger responses to sediment characteristics than those of the protected sites. Depth, grain size, sulfides, and the low molecular weight PAH appear to be responsible for the differences in macrofaunal assemblages among the ten sites sampled.

Significant effects at the protected sites were harder to find. LAR was dissimilar in species composition to the other protected sites, but usually had similar or lower sediment contaminant concentrations than other protected sites in San Diego Bay and Los Angeles Harbor. LAR also had the highest TOC of any protected site and perhaps the macrofaunal dissimilarity is due more to organic enrichment than to contamination. Species diversity at LAH was significantly reduced and that site was highest of the protected sites in PAH4 and DDT. Patterns of species composition and abundance at the protected sites were most highly associated with the higher molecular weight PAH, Pb, and Zn - compounds which are likely related to storm runoff.

C. Toxicity Tests

1. Toxicity tests of stations

#### a. Microtox tests

Results of the Microtox tests on interstitial water are shown in Table 11. Significant reductions in light output compared with the San Mateo Pt. (SMP) sample were found for each sample except DPM. By far the greatest effect on the Microtox bacteria was seen with the SMS (sludge outfall) sample. Light output in this sample was virtually eliminated, possibly the result of the very high level of dissolved sulfide (mainly H<sub>2</sub>S) present in the interstitial water from this station (Table 2).

Table 11. Microtox test of interstitial water toxicity. Values are mean  $\pm$  SE.  $4^{\pm3}$ Corrected values are data from the November experiment which have been adjusted to compensate for differences in San Mateo Pt. response between experiments.

- Time 5 2.

- - 14.

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100 C C C C C C C C C C C C C C C C C C	%CONTROL L	UMINESCENCE	
STATION	ACTUAL	CORRECTED	
Sept. 1987 experiment			
SMP	93.1 + 1.4		
DPM	87.0 + 2.5		
OC	$33.7 \pm 4.1^{a}$		
SDC	$72.6 \pm 0.9^{a}$		
SDN	$71.6 \pm 1.5^{a}$		
SD7	$79.0 \pm 5.7^{a}$		
Nov. 1987 experiment			
SMP	$96.0 \pm 1.3$	93.1 + 1.4	
DPM	91.1 <u>+</u> 1.9	88.4 + 1.9	
LAH	$82.9 \pm 4.1^{a}$	$80.4 \pm 3.9^{a}$	
LAR	$61.9 \pm 0.8^{a}$	$60.0 \pm 0.8^{a}$	
PV	$72.4 \pm 0.4^{a}$	$70.2 \pm 0.3^{a}$	
SMS	$0.3 \pm 0.2^{a}$	$0.3 \pm 0.1^{a}$	

<sup>a</sup>Value is significantly different from San Mateo Pt. ( $p \le 0.05$ ).

## b. Amphipod tests

Exposure to whole sediment produced significant reductions in G. japonica survival at one outfall station (SMS) and most harbor stations (Table 12). The greatest reductions in survival were found for SDN and SMS. Data from the SMS station were highly variable; survival in each of the replicates from this station ranged from 0 to 70%. An unexpected result was the detection of toxicity at DPM. Reduced survival at this station was found in each experiment even though hydrocarbon and metal concentrations were very low at this site. No differences or trends were seen in the amphipod reburial data (Table 12). Only one or two amphipods failed to rebury within one hour in any of the replicates.

Survival of G. japonica was not affected by variations in sediment grain size present between the test sites. High survival (85-92%) of amphipods was obtained for sediment types where sand content ranged from 4 to 96%.

Several of the sediment samples were also tested under chronic exposure conditions with G. japonica. These experiments were conducted at 20°C, using newly hatched amphipods which were fed during the experiment. Measurements of survival and growth were made after 28 days of exposure (Appendix, Table A9). A different pattern of response was obtained, compared to the 10-day survival results described previously. Strong effects on both survival and growth were found for animals exposed to sediment from PV, whereas the 10-day results indicated minor effects at this site.

The differences between the short-term and chronic test results for G. *japonica* indicate that the nature of the toxic material at PV (presumably DDE) is different from that at the other contaminated stations tested.

#### c. Sea urchin tests

A variety of both short- and long-term responses to the test sediments was observed in the tests with *L. pictus* (Table 13). Survival during the 35-day exposure period was high, except at SMS, where a 51% mortality occurred.

A significant behavioral response of the urchins was noted during the tests. Measurements of sediment preference (percentage of urchins on sediment) during the first week of the tests indicated a rapid recognition of some sediment types as unattractive (Table 13).

States and		%SURVIVAL		
STATION	ACTUAL	CORRECTED	%REBURIAL	_
Sept. experiment				
NB	88.3 <u>+</u> 4.4		$100 \pm 0$	
SMP	$83.0 \pm 1.0$		$97 \pm 3$	
DPM	03.3 + 4.4 767 + 33		$09 \pm 2$ 08 ± 2	
SDC	$683 \pm 17^{\circ}$		$100 \pm 0$	
SDN	$35.0 + 5.0^{\circ}$		$\frac{100}{89+6}$	
SD7	$41.7 \pm 6.0^{a}$		$97 \pm 3$	
Nov. experiment				
NB	$91.7 \pm 4.4$	88.3 <u>+</u> 4.2	$96 \pm 2$	
SMP	$\frac{80.7 + 1.7}{50}$	$83.2 \pm 1.0$	96 + 4	
DPM	$50.0 \pm 3.0$	$57.8 \pm 4.8$	$100 \pm 0$	
LAR	$50.0 \pm 1.0^{-1}$	40.1 + 1.4	$91 \pm 3$	
DV	$70.0 \pm 5.0$	$67.4 \pm 4.2$	$100 \pm 0$ 03 ± 3	
SMS	$35.0 \pm 20.2^{\circ}$	$33.7 \pm 19.5^{a}$	$100 \pm 0$	

Table 12. Amphipod survival and reburial following 10 day sediment exposure (mean  $\pm$  SE; N = 3). Corrected values are data from the November experiment which have been adjusted to compensate for differences in control (Newport Bay) survival between experiments.

<sup>a</sup>Value is significantly less than survival in Newport Bay sediment  $(p \le 0.05)$ .

	and the first	SEDIMENT	Constanting of	GROWTH		CORRECTED
STATION	SURVIVAL. (%)	PREFERENCE (%)	WEIGHT (g)	GONAD (g)	TEST (inm)	TEST GROWTH (mm)
September Experiment		ŧ				
SMP DPM OC SDC SDN SD7	97.8 ± 2.2 100 ± 0 100 ± 0 97.8 ± 2.2 97.8 ± 2.2 100 ± 0	$75.3 \pm 2.880.7 \pm 2.988.0 \pm 1.755.0 \pm 3.5452.0 \pm 4.34448.0 \pm 3.244}$	$\begin{array}{c} 0.39 \pm 0.04 \\ 0.43 \pm 0.02 \\ 0.42 \pm 0.01 \\ 0.29 \pm 0.03 \\ 0.30 \pm 0.02 \\ 0.21 \pm 0.02 \end{array}$	$\begin{array}{c} 0.114 \pm 0.016 \\ 0.136 \pm 0.004 \\ 0.163 \pm 0.006 \\ 0.140 \pm 0.012 \\ 0.126 \pm 0.001 \\ 0.119 \pm 0.004 \end{array}$	$\begin{array}{c} 0.63 \pm 0.11 \\ 0.75 \pm 0.19 \\ 0.66 \pm 0.14 \\ 0.48 \pm 0.10 \\ 0.51 \pm 0.08 \\ 0.33 \pm 0.06^{\prime\prime} \end{array}$	
November Experiment						
SMP DPM LAH LAR PV SMS	$100 \pm 0 100 \pm 0 100 \pm 0 100 \pm 0 100 \pm 0 48.9 \pm 5.9$	$\begin{array}{c} 65.0 \pm 4.0 \\ 67.3 \pm 5.3 \\ 70.7 \pm 1.2 \\ 85.7 \pm 2.9 \\ 77.7 \pm 1.2 \\ 30.3 \pm 2.6^{\prime\prime\prime} \end{array}$	$\begin{array}{c} 0.26 \pm 0.02 \\ 0.29 \pm 0.03 \\ 0.25 \pm 0.02 \\ 0.36 \pm 0.06 \\ 0.18 \pm 0.02 \\ 0.04 \pm 0.07 \end{array}$	$\begin{array}{c} 0.124 \pm 0.009 \\ 0.113 \pm 0.010 \\ 0.118 \pm 0.012 \\ 0.151 \pm 0.030 \\ 0.138 \pm 0.004 \\ 0.048 \pm 0.026^{a} \end{array}$	$\begin{array}{c} 0.55 \pm 0.18 \\ 0.56 \pm 0.17 \\ 0.41 \pm 0.13 \\ 0.92 \pm 0.18 \\ 0.23 \pm 0.12^{41} \\ -0.02 \pm 0.22^{41} \end{array}$	$\begin{array}{c} 0.63 \pm 0.21 \\ 0.65 \pm 0.19 \\ 0.47 \pm 0.15 \\ 1.06 \pm 0.21 \\ 0.27 \pm 0.14^{44} \\ -0.02 \pm 0.25^{44} \end{array}$

Table 13. Sea urchin responses following 35-day exposure to sediment (mean  $\pm$  SE; N=3). Sediment preference is percentage of sea urchins on the sediment during the first week of exposure. The survival and wet weight data were not tested for statistically significant differences.

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<sup>a</sup>Value is significantly less than growth on San Matco Pt. sediment ( $p \le 0.05$ ).

Fewer urchins exposed to sediment from the San Diego Bay and sludge outfall stations were observed in contact with the sediment, compared to the reference (SMP). Sediment preference was also observed to change during successive weeks of each test, as shown in Fig. 10. By three weeks, animals exposed to the San Diego Bay sediments showed a similar preference for these sediments as did control animals. Urchins exposed to sediment from LAR increased their avoidance of the sediment after four weeks. This response may have been related to changes in microbial populations on the sediment, as LAR interstitial water sulfide levels (produced by bacteria) increased by the end of the experiment.

Measurements of sea urchin growth (changes in wet weight and test diameter) after the 35day sediment exposure (Table 13) revealed similar trends to those found with the amphipods. The greatest inhibition of growth occurred at SMS, where a negative rate of growth (reduction in size) was found. Urchins exposed to sediment from the LAR site had a growth rate much greater than the controls. This result was unexpected, as this station had high concentrations of sulfide, trace metals, PAH, and PCB. This stimulation in growth may have resulted from the high level of organic material present in the sediment (4.3% TOC), reducing contaminant bioavailability and providing an enhanced food source.

A reduction in gonad production was observed only for urchins exposed to SMS sediment (Table 13). An unexpected result was the lack of an effect on gonad growth from the PV sediment. A previous study with sediment from this location found a strong inhibition of gonad production (Thompson et al. [submitted]). This discrepancy in results may have been due to differences in experimental methods (duration, time of year) between the two studies.

Exposure of *L. pictus* to contaminated sediments resulted in the bioaccumulation of chlorinated hydrocarbons by the gonad (Table 14), indicating that DDT and PCB compounds in the sediment were bioavailable to the urchins and thus had the potential to cause toxicity. In general, accumulation of DDT and PCB appeared to be proportional to sediment concentration of these compounds; the greatest gonad concentrations of DDT and PCB were found in urchins exposed to sediment from PV, which had the highest levels of these compounds. It is not known if these gonad contaminant concentrations represent equilibrium values, since measurements were made for only one exposure time.

Plots of sediment DDT or PCB level (dry weight basis) versus tissue concentration indicated that some of the most contaminated sediments did not always produce the





Table 14. Chlorinated hydrocarbons in Lytechinus gonad tissues after 35 days of exposure to test sediment. Values are expressed on a dry weight basis (mean  $\pm$  SE; N=3). Bioconcentration factor (BCF) is the quotient: tissue concentration/sediment concentration.

C2.3.77	TISSUE CONCE	NTRATION (ng/g)	B	CF	
STATION	DDT	PCB	DDT	PCB	
Sept. experiment SMP DPM OC SDC SDN SD7	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$ND^{a}$ $481^{b}$ $1,560 \pm 87$ $2,076 \pm 92$ $2,005 \pm 21$ $2,904 \pm 47$	31 44 83 9 20 6	ND <sup>4</sup> 48 28 11 10 8	
Nov. experiment SMP DPM LAH LAR PV SMS	$\begin{array}{r} 693 \pm 16 \\ 532 \pm 22 \\ 2,092 \pm 51 \\ 489 \pm 24 \\ 47,870 \pm 447 \\ 1,474 \pm 130 \end{array}$	$\begin{array}{r} 603 \pm 11 \\ 567^{9} \\ 4,239 \pm 438 \\ 1,040 \pm 73 \\ 8,097 \pm 213 \\ 2,628^{9} \end{array}$	25 133 24 5 8 8	ND <sup>4</sup> ND <sup>4</sup> 20 3 5 4	

"Value is less than detection limit for either tissue or sediment.

 $^{b}N = 1$ ; replicates with values below the detection limit are not included in mean due to high detection limits resulting from the small amount of tissue available for analysis.
greatest tissue concentrations (Fig. 11). The LAH sediment consistently produced outlying values in these plots. This situation could result from variations in sediment composition, such as organic carbon content, which changed contaminant bioavailability. The sediment chemistry data were normalized to TOC and replotted to see if these values had a better relationship to tissue levels (Fig. 11). An improved relationship was obtained for PCB, but not for the DDT values. Data for PV was eliminated from the DDT plots because the extremely high tissue and sediment concentrations found at this site would have obscured any relationships present for the other sites. It appears that the strength of the relationship between contaminant bioavailability and sediment organic content is variable, dependent upon compound or sediment type.

Trace metal concentrations in gonad tissue were also measured. These data were quite variable and had high detection limits (especially for SMS), due to the small amount of tissue available for analysis. Bioaccumulation of metals in the strict sense was not observed because gonad metal concentrations at the end of the experiments were usually lower than the initial value (Table 15). This response was observed for urchins exposed to both reference and contaminated sediment. Reductions in metal levels may have been related to the substantial increase in gonad size that occurred during each experiment. This increase in tissue mass may have diluted the metal present initially, resulting in an apparent loss of metals. Alternatively, the gonad tissue produced during the test may have been of a different cell type, and had a very different characteristic level of metals within it.

Assuming that the initial tissue metal measurement is not an appropriate reference value, the extent of metal bioaccumulation in the tissues can be determined by comparing tissue levels to those of the SMP samples. Analysis of variance tests indicated that there was no statistically significant metal bioaccumulation compared to SMP. Trends towards increased metal levels were consistently found in urchins from the PV and SMS stations, however. The highest tissue concentrations of a particular metal were usually found in samples from sediments having high concentrations of that metal. A major deviation from this pattern was found for lead. Sediment from LAR had one of the highest lead levels measured (Appendix, Table A3), yet gonad tissue from this station had a relatively low concentration of this metal.



Figure 11. Plots of sea urchin gonad PCB and DDT concentration versus sediment concentration. Sediment concentration is expressed on a dry weight (A and C) and a TOC basis (B and D). Data for the PV station are not included in the DDT plots.

ST

Station	Ag	As	Cd	Cr	Cu	Ity	Ni	Pb	Sa	Za
Initial	2()	66	5.35	5.1	11.5 -	3.2	<1.22	< 2.2	0.256	1420
Sept. experiment										
SMP	1.9 ± 0.5	12 ± 0.4	0.75 ± 0.28	1.8 ± 0.2	2.6 ± 0.7	<1.22	<2.2	< 0.96	0.11 ± 0.06	345 ± 123
DPM	1.4 ± 0.2	13 ± 1.8	0.70 ± 0.14	2.1 ± 0.5	4.2 ± 0.5	<0.96	1.2 ± 0.4	0.61 ± 0.14	0.31 ± 0.01	165 ± 19
OC	1.4 ± 0.4	7.9 ± 0.2	0.47 ± 0.06	1.9 ± 0.3	3.8 ± 0.6	<0.72	0.86 ± 0.3	0.59 ± 0.18	0.14 ± 0.04	245 ± 59
SDC	1.3 ± 0.02	13 ± 0.5	0.62 ± 0.03	1.8 ± 0.2	5.8 ± 1.0	< 0.92	2.5 + 0.8	1.3 ± 0.6	0.43 ± 0.11	272 ± 14
SDN	2.6 + 0.5	13 ± 1.0	0 67 ± 0.10	3.1 1 0.1	8.6 ± 0.6	<1.04	1.4 ± 0.4	2.6 ± 0.7	0.85 + 0.08	388 ± 66
SD7	$2.0 \pm 0.5$	11 + 1.2	0.66 ± 0.09	2.2 ± 1.5	5.3 ± 4.4	<1.62	1.9 ± 0.6	1.8 ± 1.4	0.19 ± 0.32	301 ± 177
Nov. experiment										
SMP	2.0 + 0.8	14 1 0.5	0.70 ± 0.06	1.9 1 0.6	3.8 + 0.3	<1.30	<2.2	0.67 ± 0.21	0.82 + 0.69	317 + 45
DPM	3.5 + 0.9	16 ± 1.4	0.96 ± 0.25	2.7 + 0.2	6.1 ± 1.0	<1.92	<3.0	<1.42	0.37 ± 0.12	523 + 145
IAH	2.6 + 1.0	15 + 0.7	0.76 ± 0.18	2.3 + 0.7	5.5 ± 1.4	<1.54	<2.6	2.2 + 0.9	0.88 1 0 46	393 + 37
LAR	14 + 0.2	10 + 1.2	065 + 003	1.2 + 0.2	6.2 + 2.8	<0.82	<1.36	0.79 ± 0.35	0.48 + 0.27	289 + 78
PV	18 + 01	14 + 1.0	094 + 0.06	34103	46 + 0.2	<0.94	<1.32	16 103	22 1 22	377 + 45
SMS	53+22	10 + 5.5	18 + 11 92	68138	13 1 7.0	6.0 1 29	<15.8	<7.0	-2.4	1030 1 481

Table 15. Lytechinus gonad metals data. Values are mg/kg dry weight (mean  $\pm$  SE; N = 3). Data preceded by a "<" are the average detection limit for that station, as all three replicates had nondetectable concentrations.

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## 2. Dilution experiments

Seawater dilutions of the interstitial water samples from the November 1987 Microtox bioassay were tested to determine the no observable effect concentration (NOEC). Statistically significant reductions in luminescence were still present, even though the samples were diluted to concentrations of 6% (DPM, LAH, and PV) or 1% (SMS; Table 16).

Additional dilution experiments were conducted in April 1988. Fresh sediment samples from three toxic stations (SD7, PV, and SMS) were collected for tests with Microtox and amphipods. The Microtox results showed much less toxicity at these stations than had been measured previously (Table 16). For example, the 12% SMS sample had 86% of the control luminescence in April, compared to 37% in November. This change in toxicity was probably due to changes in interstitial water composition during its preparation. A larger quantity of interstitial water was prepared in April so that additional chemical and bioassay measurements could be made on the samples. This change in procedure increased the length of time that the samples were stored at room temperature and exposed to air. It is probable that this situation led to an alteration of sample composition through oxidation or volatilization. Measurements of the bioassay sample for dissolved sulfide concentration in April provided support for this hypothesis. Sulfide levels in PV or SMS interstitial water in April were less than 0.5 mg/l, less than 1% of the concentration found in samples from the same stations tested in November.

An amphipod bioassay was also run on dilutions of the sediment samples collected in April. Sediment samples were diluted with Newport Bay sediment to result in concentrations of 80, 50, and 20% on a dry weight basis. Each dilution was subsampled and sent to the EPA Environmental Research Laboratory in Newport, Oregon. The EPA conducted an amphipod test using *Rhepoxynius abronius* on the samples. Bioassays with *Grandidierella* and *Rhepoxynius* were conducted at the same time, so that a comparison of relative sensitivity between these amphipod species could be made.

Analysis of the sediment samples for the concentrations of selected metals indicated a close similarity in chemical composition to earlier collections of sediment from the same sites (Table 17). In most cases, concentrations did not vary by more than 50% between each set of samples.

STATION	%	SEAWATER LUM	<b>INESCENCE IN D</b>	ILUTED SAMPLE	
	50%	25%	12%	6%	1%
Nov. experiment					
DPM .	$92.1 \pm 0.5^{a}$	$92.9 \pm 0.6^{a}$	$96.3 \pm 0.8^{a}$	96.9 ± 0.7 <sup>a</sup>	NA <sup>b</sup>
LAH	$89.8 \pm 0.6^{a}$	89.8 ± 0.7 <sup>a</sup>	$93.6 \pm 0.6^{a}$	$95.2 \pm 0.7^{\circ}$	NA
LAR	$75.4 \pm 0.4^{a}$	$85.6 \pm 0.5^{a}$	$92.3 \pm 0.8^{a}$	$96.1 \pm 0.3^{a}$	NA
PV	$77.5 \pm 0.5^{a}$	85.0 + 0.1 <sup>a</sup>	$90.3 \pm 0.3^{a}$	$92.2 \pm 0.4^{a}$	NA
SMS	NĀ	$21.4 \pm 0.1^{a}$	$37.3 \pm 0.1^{a}$	58.4 <u>+</u> 2.0 <sup>a</sup>	87.1 <u>+</u> 1.0 <sup>4</sup>
Apr. experiment					
SD7	$91.5 + 0.4^{\circ}$	NA	$93.8 \pm 0.6^{a}$	NA	97.3 + 1.4
PV	$91.9 \pm 0.9^{a}$	NA	$92.0 \pm 1.4^{a}$	NA	$96.0 \pm 0.9$
SMS	NĀ	NA	85.6 ± 0.8 <sup>a</sup>	90.2 <u>+</u> 0.7 <sup>a</sup>	91.7 <u>+</u> 0.7 <sup>a</sup>

Table 16. Results of Microtox tests of interstitial water diluted with seawater. Values are mean  $\pm$  SE (N = 3).

<sup>a</sup>Values significantly different from seawater control ( $p \le 0.05$ ). <sup>b</sup>Sample not analyzed.

Station	Ag	Ratio <sup>a</sup>	Cu	Ratio	Zn	Ratio	Cd	Ratio	Pb	Ratio
SMS	17.7	4.7	540	1.1	. 672	1.0	29	1.0	153	1.2
PV	3.4	0.6	188	0.9	; 561	0.9	15	1.0	126	1.1
SD7	1.6	1.5	193	1.6	542	0.9	2	1.4	205	2.0
NB	< 0.3		2	1.0	17	1.3	< 0.2		2	0.6

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Table 17. Metals concentrations (ug/g dry wt.) of samples tested in sediment dilution experiment, with comparison to earlier sampling.

<sup>a</sup> Concentration/initial concentration during sea urchin experiment

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Results from the G. japonica tests are shown in Table 18. The amphipod's response to undiluted sediment was nearly identical to that observed in the earlier tests (Table 12), again indicating the similarity in composition of the samples from each collection. The same NOEC level of 20% was obtained for the SD7 and SMS samples, even though SMS was much more toxic in its undiluted form. A statistically significant effect was not found for the PV sediment, resulting in a NOEC of 100%.

Good agreement was obtained between the patterns of survival for *Rhepoxynius* and *Grandidierella* (Fig. 12). In most cases, survival was usually not significantly different between the two species. However, there was a consistent trend for *Grandidierella* to be more affected by PV sediment than *Rhepoxynius*. These results indicate that *G. japonica* is a satisfactory alternative species to *R. abronius* for conducting 10-day sediment bioassays.

Survival of *Rhepoxynius* in the sediment dilutions was usually less variable than was observed for *Grandidierella*. As a result, statistically significant reductions in *Rhepoxynius* survival were found at greater sediment dilutions than for *Grandidierella*. Significant toxicity to *Rhepoxynius* was found at all of the dilutions tested for the SMS and SD7 stations, resulting in a NOEC of <20% for these sites. A significant reduction in *Rhepoxynius* survival following exposure to 50% PV sediment was also found. Survival in 80% and 100% PV sediment was not significantly reduced, however, resulting in a NOEC of 100% for this station.

It is unlikely that the fine grain size of the test sediments had a significant influence on the *Rhepoxynius* test results. This species is sensitive to sediments of high silt and clay content (Swartz et al., 1985b), but previous tests by Swartz et al. (1985a) found good amphipod survival in sediment types similar to those used for the dilution study. Potential grain size effects would have been even more unlikely at greater sediment dilutions, since the addition of Newport Bay sediment to the samples increased their sand content to levels easily tolerated by *Rhepoxynius*.

## 3. Relationships between tests and stations

The most responsive endpoints in each of the three tests (luminescence, amphipod survival, and urchin test growth) have been plotted for comparison of the general pattern of results (Fig. 13). Each of the three test methods yielded consistent results in that statistically significant toxicity was found at the SMS and SD7 stations. Similar patterns were also

Table 18. Results of sediment dilution toxicity tests with *Grandidierella japonica*. Concentration values represent the percentage of dry sediment from the test station; actual concentrations were calculated from grain size measurements. Survival values are mean  $\pm$  SE (N = 4).

Same and a second second	%CONCENTRATION		
STATION	NOMINAL ACTUAL	%SURVIVAL	
NB	100 100	87.5 <u>+</u> 4.3	
SD7 SD7 SD7 SD7 SD7	100100806750502020	$\begin{array}{r} 43.8 \pm 6.9^{a} \\ 38.8 \pm 3.8^{a} \\ 46.2 \pm 2.4^{a} \\ 61.2 \pm 6.6 \end{array}$	
PV PV PV PV	100100807750502018	$\begin{array}{r} 68.8 \pm 8.3 \\ 61.2 \pm 6.6 \\ 71.2 \pm 5.5 \\ 81.2 \pm 2.4 \end{array}$	
SMS SMS SMS SMS	100 100 80 72 50 43 20 16	$7.5 \pm 7.5^{a}$ $55.0 \pm 18.9$ $47.5 \pm 18.3^{a}$ $77.5 \pm 14.8$	

<sup>a</sup>Value is significantly different from Newport Bay control ( $p \le 0.05$ ).





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often found between the responses of the sea urchin and amphipod tests. These two tests indicated a lack of toxicity at LAR and OC and usually found toxicity at the LAH, PV, SDN, and SDC stations.

The strengths of these interrelationships were examined by calculating bivariate correlations between each of the principal bioassay responses (Table 19). The three measures of sea urchin growth (change in test diameter, gonad weight, and wet weight) correlated most highly with each other. Reduced Microtox luminescence correlated most strongly with sea urchin mortality, both of which had strong responses at SMS. Amphipod survival correlated best with sea urchin growth, and most poorly with Microtox luminescence.

Principal components analysis (PCA) was used to determine the patterns present in the bioassay responses. Sea urchin survival and growth (wet weight, test diameter, and gonad), amphipod survival and Microtox luminescence data were used in this analysis. This analysis indicated that 94% of the variation in the results could be accounted for by three PCA axes or factors (Table 20). The first factor (TOX1) was indicative of chronic urchin growth effects, correlating highly with all three growth endpoints (Appendix, Table A10). The second factor, statistically uncorrelated with the first, reflected the Microtox results most strongly and served to indicate the pattern of interstitial water toxicity. The third PCA factor was most strongly correlated with amphipod survival.

The PCA results indicate that each test species had distinctive patterns of response to the same test sediments. This implies that different aspects of sediment composition (presumably contamination) were most effective in each test. These differences in test response reflect variations in the nature of the test sample (sediment or interstitial water), duration of exposure, and species-specific sensitivity to contaminants.

Differences in sample type certainly had a large effect on the Microtox results. Interstitial water will have very different levels of dissolved and colloidal contaminants than those measured in the bulk sediment. Levels of dissolved constituents such oxygen, pH, and ammonia may have varied greatly between samples. Variations in these parameters (and also  $H_2S$ ) are likely to have a pronounced effect on the Microtox bacteria, which were not exposed in the presence of clean, flowing seawater like the other species.

		L. pict	us responses		G. japonica	
	Diameter	Wet wt.	Gonad Wt.	Mortality	Survival	
Urchin wet wt.	0.931					
Urchin gonad wt.	0.748	0.827				
Urchin mortality	-0.671	-0.765	-0.876			
Amphipod survival	0.683	0.609	0.640	-0.463		
Microtox (luminescence)	0.419	0.458	0.446	-0.801	0.252	

Table 19. Pearson correlation matrix of toxicity test responses.

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Table 20. Summary of principal components analysis of bioassay results. Those test responses correlating strongest (r > 0.74) with each of the three factors are shown.

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		PCA FACTOR	
	TOX1	TOX2	TOX3
Urchir Urchir Urchir	i diameter i total wt. i gonad wt.	Microtox Urchin survival	Amphipod survival
% Variance explained	45	31	21

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Because different response patterns were evident for each bioassay, it is most appropriate to use the tests independently in ranking the toxicity of the test sites. The bioassay data presented in this report indicate the value of a multi-species approach to toxicity testing. If only one of the three tests described in this study had been used, a very different pattern of effects would have resulted.

#### 4. Relationships between tests and contaminants

The relationships between toxicity test responses and sediment contamination were examined using PCA in a manner similar to that used for the macrofaunal data. The factor scores resulting from the toxicity test PCA (TOX1-3) and from the dry weight basis contamination PCA (SED1-4) were analyzed together. Five PCA factors were identified that accounted for 98% of the variation in the data (Table 21). These results indicated that different groups of contaminants were correlated most strongly with each group of toxic responses.

The Microtox and urchin mortality group scores correlated strongly on the same factor with the scores for SED1 (PCBF, PAH1, MET1,  $H_2S$ ).

Amphipod survival scores correlated most strongly with the SED3 scores (MET2, PAH2). Amphipod survival was also weakly correlated with SED4 (PAH3, MET2) on a separate PCA axis.

The urchin growth scores (TOX1) loaded on an axis not strongly dominated by any one sediment contamination group. This axis had a moderate correlation with SED1, the same group associating with the Microtox results. The remaining contaminant correlations in this axis were evenly distributed among the other sediment groups.

The preceding analysis indicated that the Microtox and amphipod tests were responding to different groups of contaminants. The urchin growth tests did not associate as strongly with a single contaminant group, indicating that these responses may have been influenced by a greater variety of contaminants. These results may also indicate that the urchin test results were strongly affected by sediment factors other than contamination (e.g., TOC) at some sites, producing a more variable contaminant-response relationship.

Table 21. Association between sediment contamination (SED1-SED4) and toxicity (TOX1-TOX3) factors as determined by second order PCA. Those factors having the highest correlations (r > 0.41) with each of the second order axes are shown. A complete listing of the correlations obtained with this analysis is shown in Table A12.

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		Tox	city-Contamination	Axis	
A. Dry weight basis	-1	2	3	4	5
	Microtox Urchin mortality (TOX2)	Amphipod survival (TOX3)		Amphipod survival	Urchin growth (TOX1)
	PCBF, PAH1, MET1 H <sub>2</sub> S (SED1)	MET2, PAH2 Organotin(SED3)	DDTF, PAH4 (SED2)	PAH3, MET2 (SED4)	PCBF, PAH1, MET1 H <sub>2</sub> S
%Variance explained	23	23	15	17	20
B. TOC basis	_1	2	3	4	5
	Microtox Urchin mortality	Amphipod survival	Urchin growth		
	PAHTOC1, H <sub>2</sub> S (SEDTOC1)	Organotin, MET1 (SEDTOC4)	PAHTOC1, H <sub>2</sub> S	PCBTOC, PAHTOC2, MET2 (SEDTOC2)	DDTTOC, PAHTOC3, MET1 (SEDTOC3)
%Variance explained	24	24	17	16	15

The small sample size available in this study makes a more specific determination of the association of effects with contaminant types using PCA difficult. Pearson correlation coefficients were calculated to determine if selected bioassay responses were strongly related to more specific groups within each of the general sediment contamination factors. The results of this analysis confirmed the patterns identified by PCA. Among the groups constituting SED1, the Microtox results had significant correlations with  $H_2S$  and PAH1 (Table 22). This result indicates that  $H_2S$  and relatively soluble PAH compounds are likely candidates for interstitial water toxicity.

Amphipod survival was significantly correlated only with organotin, suggesting that this group of compounds was more strongly associated with toxicity than the high molecular weight PAH and trace metals (lead and zinc) found in the SED3 contamination factor.

Urchin growth (test diameter) had high correlations with MET1 and PCBF and had moderately high correlations with many of the other contaminant groups. This analysis confirmed that the urchin growth test was responding to a different, and perhaps broader group of contaminants than were the other tests.

A significant correlation between toxic effects and concentration was not identified for the DDTF, PAH4, and PAH3 factors. The lack of a relationship to DDT is surprising, given the extremely high concentrations of this group of compounds at PV. This situation illustrates a limitation of using the PCA technique to identify the "most important contaminants". PCA identifies patterns that account for the majority of the variation in the data. In the case of DDT, high concentrations were found only at PV, in the presence of many other contaminants that were more widely distributed among the other stations. The sea urchin gonad chemistry data indicated that DDT compounds were greatly accumulated from PV sediment and therefore DDT was a likely source of toxicity, but only at PV. The PCA strategy may have been unable to separate this pattern from the larger pattern of sea urchin toxicity present for the entire study. The PV sediment was also shown to be highly toxic to amphipods (*G. japonica*) under chronic exposure conditions (Table A9).

PCA factors and correlation coefficients were also calculated for the toxicity and contamination data using TOC-normalized hydrocarbon data. The PCA results (Table 21) again showed a strong association between contaminants characteristic of the SMS site (PAHTOC1, H<sub>2</sub>S) and Microtox and urchin growth effects. The association between amphipod survival and organotin was also present in this analysis. The sediment

Table 22. Pearson correlation matrix of selected bioassay responses and contamination factor scores, sulfide, and organotin. Correlations with r > 0.648 are significant at  $\alpha = 0.05$ .

	Microtox	Amphipod survival	Urchin diameter	
PAH1	-0.808	-0.403	-0.615	
PAH2	0.090	-0.539	-0.307	
PAH3	0.055	0.479	0.549	
PAH4	0.024	-0.004	-0.464	
MET1	-0.566	-0.326	-0.770	
MET2	-0.214	-0.233	-0.239	
PCBF	-0.595	-0.301	-0.694	
DDTF	0.460	0.339	0.021	
H <sub>2</sub> S	-0.782	-0.232	-0.547	
Organotin	-0.442	-0.717	-0.517	
PAHTOC1	0.753	0.200	0.365	
PAHTOC2	0.179	-0.446	-0.176	
PAHTOC3	0.161	0.074	-0.335	
PCBTOC	0.109	-0.374	-0.351	
DDTTOC	0.053	0.310	-0.230	

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contamination factor representing DDT contamination still did not show a strong relationship to any of the toxicity tests. Several groups of contaminants that showed an association with toxicity when factored on a dry weight basis, lost that relationship after TOC normalization; these groups represented lead and zinc, PCB, and high molecular weight PAH.

Examination of the correlation coefficients between the factor scores and the toxicity tests also indicated a strong relationship between Microtox and PAHTOC1 (Table 22). No other significant correlations were present between the TOC-normalized hydrocarbon values and biological effects. In most cases, higher correlations were found when the hydrocarbon data were expressed on a dry weight basis.

#### Estimated response concentrations

The sediment concentrations producing toxic effects (response concentrations) were estimated for each of the contaminant factor groups using methods similar to those used for the macrofauna data. The most responsive endpoints for each of the three test methods were used in this calculation (Microtox luminescence, amphipod survival, and sea urchin diameter change). Response concentrations were calculated for each of the contaminant groups identified by PCA.

A response concentration could not be calculated for a particular contaminant-toxicity test combination in some cases. This situation arose when there was no significant toxicity at the station containing the highest concentration of that contaminant. Even though response levels have been calculated for all possible contaminant-test combinations, the greatest emphasis should be placed upon values derived from contamination-toxicity test associations identified by PCA (Table 21).

Response levels based on amphipod or sea urchin test results were always greater than concentrations estimated from the Microtox data (Table 23). This difference was primarily due to the detection of toxicity at the less contaminated OC and LAR sites by Microtox, but not by the two other tests. The response concentrations estimated from the sea urchin or amphipod results are probably a more accurate indication of the effective levels in the sediment since these tests involved a direct sediment exposure. The Microtox test used an interstitial water exposure; consequently, the bacteria may have been reacting to variations in water quality not related to contaminant concentrations (e.g., DO, pH, NH<sub>3</sub>). The

Table 23. Estimated response concentrations for selected toxicity test responses. Significant test responses were always found at concentrations equal to or greater than the response concentration.

77.5	Respo	onse concentration (r	m) for:	
Factor or Compound	Microtox Luminescence	Amphipod Survival	Urchin Diameter	
Sulfide <sup>4</sup> DDT PCB PAH1 PAH2 PAH3 PAH4 MET1 MET2 Organotin	16 0.03 0.06 0.03 0.07 0.02 0.10 152 275 0.06	56 NC NC 15.8 4.2 NC NC 809 809 809 0.16	56 0.20 0.35 15.8 9.6 NC 0.38 634 685 0.19	

<sup>a</sup>Concentrations of sulfide were measured in milligrams per liter. <sup>b</sup>NC, No correspondence of concentration changes with toxic effects.

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extent of this interference is unknown, as interstitial water quality measurements were not made during this study.

#### D. Relationships between Macrofauna and Toxicity Tests

The collection of species composition and abundance data along with laboratory measurements of sediment toxicity for the same stations permits the evaluation of the correspondence between these two types of biological assessment methods. These comparisons can help determine the ecological relevance of the toxicity test results, by determining if responses of a test species correspond to observed abundances of related species in the field. In addition, a correspondence between trends in abundances and laboratory tests provides a verification that changes in macrofauna were related to contamination, instead of to other environmental or ecological changes (e.g. TOC or predation).

The amphipod (G. japonica) represented a taxonomic group having a common enough occurrence in the macrofaunal samples to permit a direct comparison of results. There was a significant correlation between G. japonica survival and the percentage of amphipods at each site (Fig. 14). Sites with the highest proportions of amphipods in the assemblage also permitted the highest survival in the laboratory. The strength of this relationship was determined to a large extent by the high amphipod survival and abundance at the LAR and SMP sites, as shown in Fig. 14. A similar relationship has been demonstrated previously for another amphipod, Rhepoxynius abronius (Swartz et al., 1982).

The presence of this relationship provides evidence that laboratory amphipod survival test results can serve as predictors of sediment quality for related species groups. Similar direct comparisons of the Microtox and sea urchin test results with macrofaunal abundances cannot be made because these species groups were not adequately represented in the macrofaunal samples.

The data were also examined for patterns of association between the toxicity test results and macrofauna species composition and abundance. These patterns were identified using second order PCA in a manner similar to that used to examine patterns between sediment contamination and biological effects. This analysis was performed on a combination of the scores from the original macrofaunal ordination axes (ORD1-4) and the scores from the





toxicity PCA factors (TOX1-3). The PCA resulted in five axes which accounted for 98% of the variation in the data (Table 24).

The first axis showed a strong association between ORD1 (dominant axis for macrofauna composition) and TOX2 (Microtox + urchin mortality). A similar pattern was indicated by correlation analysis of the ordination scores against the individual test responses. Significant correlations were found between ORD1 and Microtox or urchin mortality ( $\mathbb{R}^2 = 0.57$  and 0.55, respectively). Separate analyses of the toxicity and macrofaunal results showed these groups were responding most strongly to the SMS site.

An association between macrofauna (ORD4) and urchin growth changes (TOX1) was indicated by the second PCA axis. Correlation analysis also indicated a similar relationship; strong correlations were found between ORD4 and measurements of urchin diameter or wet weight ( $R^2 = 0.49$  and 0.41, respectively). This grouping may be related to similar types of responses to the San Diego and Los Angeles Harbor sites, which was the principal gradient associated with the macrofaunal data.

The remaining toxicity and ordination groups (amphipod survival, ORD2, ORD3) correlated strongly with separate PCA axes, indicating that they responded in a dissimilar fashion to the stations. The lack of a strong association between the amphipod test and any one of the ordination axes was surprising in light of the correlation between these measurements shown in Fig. 14. Amphipod abundances were relatively low at eight of the stations (less than 15% of total individuals). Consequently, the amount of macrofauna variation represented by this group may have been too small for the PCA to confirm the relationship indicated by the regression analysis. Instead amphipod survival was correlated weakly with several of the axes.

The associations indicated by this PCA suggest a correspondence between some aspects of the benthic invertebrate community and the toxicity test results. This helps to foster confidence that both methods of biological assessment are responding to similar characteristics of the sediment, presumably contamination.

E. Evaluation of Toxicity Tests

Each of the three toxicity tests used in this project was useful in that they were responsive to at least some of the different sediment samples tested. The following discussion of these Table 24. Summary of principal components analysis of ordination (ORD1-ORD4) and toxicity (TOX1-TOX3) factor scores. The components of these groups correlating strongest (r > 0.85) with each of the five resulting axes are shown. The correlations of the groups with these axes are listed in the Appendix (Table A13).

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	Macrofauna-Toxicity Axis						
	1	2	3	4	5		
	Microtox Urchin mortality	Urchin growth	Amphipod survival				
	ORD1	ORD4		ORD3	ORD2		
%Variance	25	23	18	16	16		

tests is intended to identify the principal advantages and limitations of the methods and thus aid in decisions regarding their future use.

<u>Microtox</u>. This test was the most responsive of the three methods in that it identified statistically significant effects at the greatest number of stations. This was the only test which identified toxicity at the OC and LAR stations.

The principal advantages of this method are its speed and good reproducibility between tests and within replicates. Low variability in the results is an advantage if a NOEC value is desired from the data, as it improves the test's ability to detect low levels of toxicity. Microtox is the only one of the three methods able to directly measure the toxicity of interstitial water. A small volume of aqueous sample is required for the test. The test apparatus is used throughout the nation and there is a large database of single compound toxicity data with which to compare results. Microtox has been used in other studies to assess sediment quality, but different methods of sample preparation are often used, complicating the comparison of results.

A major disadvantage of the Microtox method is that the endpoint (luminescence) may not relate directly to negative impacts on organism survival or reproduction. Consequently, it is difficult to determine the importance of the relatively small, yet statistically significant, effects often found in this study. A potential problem is the impact of changes in water quality parameters not representing contamination. Differences in interstitial water concentrations of constituents such as oxygen or ammonia may have significant effects on the test results. These should be measured and controlled for in future applications of this method in order to determine their contribution to any observed effects. Another difficulty encountered in this study was sample instability. Samples from PV and SMS were much less toxic when tested a second time; presumably from the unintended oxidation of dissolved sulfide during sample preparation. It appears that greater care is needed in handling interstitial water samples than is required for bulk sediment.

<u>Amphipod survival</u>. The 10-day amphipod survival test was the most responsive of the two bulk sediment toxicity test methods. This test detected toxicity at six of the stations and was the only test to find an effect at the DPM site. Greater responses to harbor sediments were usually found with the amphipod test, compared to the other two test methods. A major advantage of the amphipod test is that this general method is used throughout the nation to assess sediment quality. Consequently, results obtained locally will be comparable to data resulting from other studies. The species (G. japonica) used for these tests is easily collected from both southern and central California, which facilitates the statewide use of this organism. The endpoint used in the 10-day test (survival) is clearly of ecological significance and the animal's tube-dwelling habit assures contact with both particulate and dissolved sediment constituents.

The principal disadvantage of the amphipod survival test is its relative lack of sensitivity to the PV sediment sample. Data from this and other studies indicates that the nature of contamination at PV has little effect on amphipod survival yet produces strong chronic effects on the growth of urchins and amphipods. The 10-day test with *G. japonica* also does not currently have any useful sublethal endpoints of toxicity. Measurements of reburial activity were not sensitive to contamination and daily observations of the animals are not possible because they reside in the sediment during the test.

<u>Sea urchin growth</u>. The sea urchin growth test was the least responsive of the toxicity tests used in this study. Statistically significant effects were observed at only three sites, although strong trends of growth inhibition were also evident at an additional three stations.

A major advantage of the sea urchin growth test is that this method utilizes a chronic exposure. Consequently, this test should produce the most accurate assessment of toxicity since it incorporates both acute (daily survival) and chronic (growth, gonad production) responses. The species used in this test (*L. pictus*) is indigenous to southern California sewage outfall discharge zones, lives in close contact with the sediment, and is also an active surface deposit feeder. These ecological characteristics enhance the species' contact with contaminants and give strong environmental relevance to the results obtained. In addition, the relatively large size of the sea urchin permits chemical analysis of the gonad tissue so that the relationship between bioaccumulation and effects can be studied.

The sea urchin's large size and the length of exposure used for this test also create technical difficulties with the method. This test requires a relatively large amount of sediment per replicate (approximately 1.21) and needs more flowing seawater and lab space than does the amphipod test. The acute survival response of this species is not nearly as sensitive as that for the amphipod. Growth is a sensitive endpoint (especially to conditions

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at PV), but this parameter often has high variability in the response. As a result, a longer exposure time or greater replication is needed to produce satisfactory sensitivity to contamination in harbor sediment samples.

Each of these tests has unique strengths and weaknesses and are therefore best suited to different applications. The amphipod test is probably the best overall choice for the assessment of sediment samples collected from the field. This test is widely used, requires moderate amounts of time and laboratory facilities, and has demonstrated adequate sensitivity to contaminant concentrations present in the environment. The amphipod test, like any test, will not have acceptable sensitivity to all forms of contamination. This 10-day test appears to underestimate chronic toxicity. It is therefore advisable to combine this test with a sensitive measure of chronic toxicity, such as a sea urchin or amphipod growth test, in order to make the most accurate assessment of sediment toxicity.

The Microtox test has many potential advantages, but further research should be conducted to evaluate the dependability of the method. Of principal concern is whether all of the effects found in this study can be attributed to contaminant or sulfide effects. This method is best suited to studies that focus upon interstitial water.

The sea urchin growth test method enables the most comprehensive measurement of biological responses and is best suited for comprehensive studies of contaminant fate and effects. A wide variety of both acute and chronic responses can be measured over a variable exposure time. This method utilizes a large deposit feeding species and therefore provides the opportunity to assess effects resulting from sediment ingestion and to measure contaminant accumulation in the test organism.

# IV. CONCLUSIONS

Chemical analysis of the sediment samples found high concentrations of chlorinated hydrocarbons, polynuclear aromatic hydrocarbons and inorganic metals at many stations. The high concentrations found were within the range expected, as most of the stations had been identified in prior studies to represent areas of high contamination.

An unexpected result was the detection of organotin compounds in sewage outfall sediments at levels similar to those found in harbor sediments. Preliminary studies of the speciation of the organotin compounds from the outfall sites indicate a composition pattern different from that of harbor sediments. Tributyltin appears to represent a minor fraction of the organotin compounds in the outfall sediments. Therefore, the toxicological effects of similar organotin concentrations may be different between harbor and outfall sites.

Multivariate analysis (principal components analysis) was used to identify distribution patterns in the chemistry data. This method identified two principal groups (each having independent distribution patterns) within each of three major categories of contaminants. One of the two major PAH groups identified was composed primarily of low molecular weight compounds, while high molecular weight compounds predominated in the other; these groups represented hydrocarbons characteristic of petroleum and fossil fuel combustion, respectively. The remaining four contaminant groups identified were characteristic of PCB, DDT, lead and zinc, and other inorganic metals.

Further analysis of the contaminant data indicated that only four statistically independent patterns of contaminant distribution could be distinguished from the data. These patterns indicated associations between different groups of metal and hydrocarbon contaminants. One group represented the combined distribution pattern of PCB, petroleum PAH, most inorganic metals, and dissolved sulfide. This pattern was dominated by the contamination present at the sludge outfall (SMS) site. Fossil fuel PAH and lead and zinc were grouped together as having similar patterns, forming a group that characterized contamination patterns among most of the harbor stations. The remaining two groups represented contamination patterns that were characteristic of single stations. These stations were the Los Angeles County outfall, which had high DDT concentrations, and the Los Angeles River mouth, which had distinctive concentrations of several PAH. Analysis of the benthic macrofauna data produced four groups of stations having similar species composition and abundance values. The sludge outfall site formed a group by itself and was most dissimilar in species composition and abundance when compared to the reference site. The Orange County Outfall site was found to be most similar to the open water reference site. All but one of the harbor stations were grouped together with the protected reference site, even though these sites had high levels of some contaminants.

The Los Angeles River mouth site was unusual in that this station's benthos were different from the other protected sites and most similar to the L.A. County Outfall site. This result was unexpected because of large differences in depth, contamination, and laboratory toxicity between these two sites. These two stations did have similar organic carbon and dissolved sulfide contents, however. It appears that the river station represents a transition between an organically enriched site and a site having toxic levels of contamination in addition to enrichment.

Multivariate analysis of the macrofauna data identified differences between the open water sites that corresponded to a gradient of contamination and organic enrichment. Similar results were also found when indicator taxa abundance or measures of assemblage structure and diversity were compared statistically. With the exception of the river mouth site, significant differences between the protected stations (including the protected reference site) were not found. Greater power in detecting differences between indicator species abundance could have been obtained by analyzing additional replicates. Increased replication probably would not have substantially altered the results of the species composition and abundance analyses, however. Previous studies have shown that the two replicate samples examined in this study should have had sufficient power to detect meaningful differences in these multivariate statistics (Bernstein et al., 1984).

The relative lack of sensitivity of the macrofaunal analyses of the protected stations may be explained by ecological differences between the benthos present in these areas and the deeper open water sites. Environmental conditions such as temperature and salinity are likely to be more variable in shallow water due to the influence of predictable (seasonal) and unpredictable (storms) perturbation. Such a variable environment may result in a greater proportion of the macrofauna inhabiting shallow are to be adaptable to additional stresses resulting from contamination, causing less discernible changes in species composition and abundance compared to macrofauna adapted to the more constant conditions of deeper water. The laboratory toxicity tests usually identified more stations as being harmful than did macrofaunal analysis. There was generally good agreement among all three of the test methods in identifying the most toxic sites (SMS, PV, SD7).

Each toxicity test showed a somewhat different pattern of responses for the remaining stations. Some of these differences were related to test methodology. The Microtox test was a measure of interstitial water and was probably highly sensitive to contaminants with high water solubilities, such as sulfide and low molecular weight PAH. The results of this test may have been influenced by variations in interstitial water quality parameters which were not of concern in this study (e.g., oxygen, ammonia). Changes in these constituents may have been responsible for some of the effects observed with this test, such as the large reduction in luminescence produced by sediment from near the Orange County Outfall.

The sea urchin growth and amphipod survival tests used species which have not been widely used previously for sediment toxicity tests. These methods performed well, showing strong responses to some of the contaminated stations and comparing favorably to the *Rhepoxynius* amphipod test. Differences in sensitivity between the amphipod and sea urchin tests were evident at the harbor (amphipod most sensitive) and L.A. County Outfall (urchin most sensitive) sites. These differences probably reflect species specificity in contaminant tolerance in addition to differences in test duration and the organism's mode of exposure to the contaminants.

The sea urchin and amphipod test methods using moderate or long-term exposures to bulk sediment are appropriate for use in future sediment assessment studies in California. The results from this study indicate that each of the toxicity test methods used responded in a unique way to the sediment samples. This finding emphasizes the necessity of using multiple species and different test strategies in order to accurately assess sediment toxicity.

Three different approaches to sediment quality assessment (chemical analysis, macrofauna examination, toxicity testing) have been used in this project. Under ideal circumstances, each of these methodologies should correlate fully with each other, but this is seldom the case. It is important to examine the relationships which exist between each method, for these often provide useful information about the significance of contaminant concentrations or the relevance of biological measurements. Strong relationships between pairs of the various data sets were identified using principal components analysis (PCA)

and other correlational methods. The results from these analyses have been presented in the Results.

The three data sets were also combined and analyzed by a single PCA in order to identify additional associations between chemical and biological measurements. The results from this analysis are shown in Table 25. This analysis generally showed patterns which had been identified earlier through multiple PCA of data set pairs. A strong association between the dominant macrofauna ordination axis (ORD1), Microtox results (TOX2), and many contaminants (PCB, most metals, petroleum PAH, and sulfide) was found. Amphipod survival (TOX3) retained its strong association with organotin, combustion PAH, lead, and zinc (SED3). The macrofaunal pattern in the protected sites (ORD4) was best correlated with sea urchin growth.

The multivariate statistical analyses used in this study have indicated the presence of relationships between biological affects and sediment contamination. The dominant pattern in macrobenthic assemblages was most clearly related to patterns in the concentration of organic material, sulfides, low molecular weight, PAH, and most metals (except lead and zinc). These macrofaunal and contaminant patterns were also closely related to the Microtox results, suggesting that changes in interstitial water quality have a strong impact on the benthic macrofauna. Amphipod survival was most strongly associated with a different group of contaminants, organotin, combustion PAH, lead, and zinc. Urchin growth was not as strongly associated with a different group of contaminants, organotin, compounds at each toxic station. Because several chemical groups were highly correlated with one another and each of the three toxicity tests showed a different pattern of response to the contaminants, it is not possible to identify specific contaminants as being most important to toxicity overall. Rather, these results indicate potential relationships between contaminants and test organisms that should be explored in future laboratory studies.

Our analyses have shown that the patterns in sediment contamination, macrofauna, and toxicity correlates well in some cases, but not all. Most notably, DDT concentration did not correlate with short-term toxicity or macrofaunal patterns. Both DDT and PCB concentrations were highest at the PV site (L.A. County Outfall) and significant biological effects (macrofaunal changes and toxicity) were found at this station. In spite of these responses, PCA did not show any strong associations between concentration and biological effects. We believe that the gradients of other contaminants present at the sludge outfall

Table 25. Second order PCA using factor scores from macrofaunal ordination, sediment, and toxicity PCAs. Those factors having the highest correlations (r > 0.60) with each of the axes are shown. See Appendix Table A14 for loadings.

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	Macr	ofauna-Toxicity-C	ontamination Axis	
1	2	3	4	5
PCPF,MET1, PAH1,Sulfide (SED1)	Organotin, MET2,PAH4 (SED3)	PAH3 (SED4)	SED3 <sup>a</sup>	DDTF,PAH4 (SED2)
ORD1	ORD2	ORD3	ORD4	
Microtox, Urchin survival (TOX2)	Amphipod survival (TOX3)	TOX3 <sup>a</sup>	Urchin Growth (TOX1)	
6 Variance xplained 26	18	18	16	15

<sup>a</sup>weak loading.

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site overshadowed most of the chlorinated hydrocarbon effects. In addition, there was no real gradient of DDT concentration over several stations that would lend itself to examination by PCA.

Conflicting information regarding the importance of sediment organic carbon (TOC) in modifying contaminant toxicity was found in this study. The toxicity tests indicated that the high TOC content of the L.A. River Mouth sediment may have reduced the toxicity of this relatively highly contaminated station. Bioaccumulation of chlorinated hydrocarbons and the relationship between contamination and macrofauna appeared to be influenced by sediment organic content in a few cases. Such relationships were inconsistent, however.

Most of the statistical analyses were conducted using hydrocarbon concentrations expressed on both a dry weight and TOC-normalized basis. In general, expression of the data on a TOC basis rarely changed the nature of the patterns observed by PCA. In some cases, fewer correlations between toxicity and sediment contamination factors were obtained when TOC-normalized data were used.

This report has emphasized data expressed on a dry weight basis because of the ambiguities described above. Organic content is certainly an important modifier of contaminant partitioning, but a better understanding of this process is needed before normalization of concentrations from field samples can be made with confidence. The inconsistent results obtained with organic carbon normalization in this study suggest that this method may not be as useful for organic material derived from sewage. It is also possible that the range in sediment TOC found in this study was too great for accurate normalization.

Determination of sediment metals bioavailability is an additional problem which must be resolved. It is likely that factors other than organic carbon will have major effects on the availability of sediment-bound metals to organisms. Unfortunately, the factors controlling metal partitioning in marine systems are less well understood than are those for some organics. Normalizing one category of contaminants without adjusting others may create artificial patterns in the data and complicate the determination of which chemical components are responsible for sediment toxicity.

We have presented empirically determined response concentration estimates (concentration at or above which significant biological effects were always found) for benthic macrofauna and toxicity test results (Section III). Estimated concentrations for many individual contaminants were not calculated because our analysis showed that we could not separate patterns in greater detail than the contaminant groups listed. The small number of sites examined in this study was a major factor in preventing the determination of more specific and precise concentration estimates. Consequently, these values should be considered preliminary estimates and used with caution.

The ranges of response concentrations derived from our data are shown in Table 26. Others, as summarized by Chapman et al. (1987), have calculated toxicity thresholds for PAH groups similar to those used in this study. These investigators calculated toxicity thresholds for total PAH ranging from 2.0 to 12.0 ppm. Response concentrations for the PAH groups examined in this study generally fall within this range. A large range of response concentrations (0.03-15.8 ppm) was calculated for the PAH groups. The low end of this range was determined by the Microtox data. As discussed previously, these data may not be as reliable as those derived from the bulk sediment toxicity tests.

Chlorinated hydrocarbon thresholds have also been reported in previous studies. The Apparent Effects Threshold for DDT in Puget Sound studies was 0.062 ppm, which falls within the range we show in Table 26 (0.03-0.20). Our values may be underestimates as the stations selected for this study did not provide an adequate DDT concentration gradient. Swartz et al. (1985a) studied benthic macrofauna and sediment toxicity in 1980 along a concentration gradient of DDT and other contaminants originating at the PV site. The authors found no correlation between amphipod toxicity and sediment DDT concentrations as high as 8 ppm. Degradation of the macrofaunal community was found at DDT concentrations of 4 ppm and greater, however.

The PCB response concentration range reported here (0.06-0.66 ppm) is slightly higher than previous estimates (0.06-0.13 ppm) as summarized by Chapman et al. (1987). As concentrations of PCB in Puget Sound and San Francisco Bay are generally lower than those of the stations examined in this study, it is not surprising that our PCB response concentration range encompasses greater values.

Comparison of the response concentrations determined for the MET1 factor with other data is not appropriate because this group contains numerous metals which cannot be validly separated from each other. The other metals group (MET2) consisted only of lead and zinc, however, and the estimated response levels for these two elements have been listed separately on Table 26. The response concentration range derived from this study

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Table 26. Summary of sediment contaminant response concentrations estimated from macrofaunal and toxicity test analyses. Values represent the summed concentration (in ppm) of the contaminants in each group.

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Contaminant group	Group members	Response concentration (range, ppm)
Sulfide Organotin DDT PCB PAH1 PAH2 MET1 MET2	dissolved sulfides tetra- and trialkyltin DDE, DDD, and DDT Arociors 1242 and 1254 petroleum PAH combustion PAH Ag, As, Cd, Cu, Cr, Ni, and Sn Pb, and Zn Pb Zn	$\begin{array}{r} 16\text{-}56\\ 0.06\text{-}0.19\\ 0.03\text{-}0.20\\ 0.06\text{-}0.66\\ 0.03\text{-}15.8\\ 0.07\text{-}9.60\\ 152\text{-}899\\ 275\text{-}809\\ 64\text{-}133\\ 211\text{-}675\end{array}$

(64-133 ppm) falls within the threshold range of 50 to 300 ppm reported by Chapman et al. (1987) for other areas of the nation.

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## V. RECOMMENDATIONS

This study provided an initial evaluation of contaminated sediment effects for several coastal areas in southern California; however, this study was limited in scope. Following are our recommendations on future studies that are needed to supplement the efforts of the State Water Resources Control Board to develop sediment quality criteria.

1. It would be useful to conduct additional statistical analyses of the data contained in this report. Alternative statistical methods for identifying harmful levels of specific contaminants (e.g., Apparent Effects Threshold, Screening Level Concentration) should be tried. Caution should be exercised, however, on the adoption of regulatory criteria based on such evaluations. These methods will generate levels of response for many contaminants, regardless of whether there is any real effect of that chemical.

The data base from this study should be modified and reanalyzed to yield additional information about the relationships between contaminant levels and biological effects. Statistical analyses should be conducted after deletion of the data from the sludge outfall station. This station dominated some of the contaminant groupings and may have obscured interesting relationships present among the other data. In addition, data from comparable studies in southern California could be combined with the results from this study. SCCWRP is currently compiling a regional database of recent chemistry and benthic macrofauna measurements from contaminated and reference areas in southern California. The statistical analysis of these combined data would result in greater power to discriminate relationships between contamination and macrofaunal changes.

2. More extensive field studies of sediment toxicity and macrofaunal effects should be conducted. A large and detailed data base of sediment chemistry and macrofauna information exists for southern California, but sediment toxicity studies have been much more limited in scope. The current study focused on highly contaminated "hot spots" and found many effects. Future studies should include several sites spanning a gradient of contamination (and toxicity) within these areas. More precise threshold concentrations of effects could then be determined from field sediments and compared to similar values derived from laboratory studies and calculations. Particular emphasis should be given to the L.A. County Outfall area, since the present study did not include a satisfactory gradient of DDT concentrations. A gradient dominated by PAH should also be studied.
3. Additional laboratory studies of toxicity using spiked sediments should also be conducted. The complex nature of environmental contamination in southern California makes it impossible to determine cause and effect relationships of individual contaminants, even with more extensive field samplings. Controlled laboratory experiments are the only way to confidently determine the effects of specific contaminants. Hydrogen sulfide, though not a contaminant in the strict sense, should be included in these studies as it was identified as an important factor in the present study. Emphasis should also be placed on studying compounds with varying chemical forms so that structure-activity relationships can be identified and applied to other, untested forms.

4. The organotin compounds found near the outfalls should be studied in greater detail. These studies should determine the composition, toxicity, and sources of the organotin compounds in outfall sediments. Sediment and sewage effluent samples should be analyzed using mass spectrometry methods to identify the specific compounds present. Laboratory studies with spiked sediments should then be conducted with these compounds to determine their toxicity, bioaccumulation, and persistence in the environment.

5. A better understanding of contaminant bioavailability in sediments is needed. The factors that control hydrocarbon and metal partitioning between sediments, interstitial water, and organisms should be studied using both natural and spiked sediments. These studies should examine the relative influence of organic material derived from wastewater and other sources on contaminant bioavailability and use species indigenous to the areas of concern. Large interstitial water samples should be collected during future field studies and analyzed for soluble toxicants. These data should then be compared to sediment contaminant concentration and biological effect measurements to better understand the relationships present.

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# APPENDIX

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## GLOSSARY OF ABBREVIATIONS

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BOD	Biological oxygen demand.
DDT	Sum of DDT (1,1-Bis[4-chlorophenyl]-2,2,2-tichhloroethane) isomers and its metabolites (DDE, DDD).
DDTF	PCA factor correlating with DDT concentrations.
DDTTOC	PCA factor correlating with TOC-normalized DDT concentrations.
DPM	Test station inside Dana Pt. Marina.
H'	Shannon-Wiener species diversity.
I	Evenness.
LAH	Test station in Los Angeles inner harbor.
LAR	Test station in Long Beach Harbor near mouth of Los Angeles River.
MET1	PCA factor correlating with concentrations of inorganic trace metals except lead and zinc.
MET2	PCA factor correlating with lead and zinc concentrations.
NB	Reference station in Newport Bay.
NOEC	No Observable Effect Concentration.
OC	Test station near Orange Count Sanitation District outfall.
ORD1-4	Ordination axes resulting from principal coordinates analysis benthic macrofaunal data.
РАН	Polynuclear aromatic hydrocarbon (sum of compounds having two to six aromatic rings).
PAH1	Major PCA factor correlating with low molecular weight PAH concentrations.
PAH2	Major PCA factor correlating with high molecular weight PAH concentrations.
PAH3&4	Minor PCA factors correlating with PAH concentrations.
PAHTOC1	PCA factor correlating with TOC-normalized concentrations of low molecular weight PAH.

PAHTOC2	PCA factor correlating with TOC-normalized concentrations of high molecular weight PAH.
PAHTOC3&4	Minor PCA factors correlating with TOC-normalized PAH concentrations.
PCA	Principal components analysis.
РСВ	Polychlorinated biphenyl (sum of Aroclor 1242 and 1254 concentrations).
PCBF	PCA factor correlating with PCB concentrations.
PCBTOC	PCA factor correlating with TOC-normalized PCB concentrations.
ppb	Parts per billion (ug/dry kg).
ppm	Parts per million (mg/dry kg or mg/l).
PV	Test station near Los Angeles County Sanitation District outfall.
SAS	Statistical Analysis System software.
SD7	Test station in San Diego Bay near Navy fuel dock.
SDC	Test station in San Diego Bay near Chollas Creek drainage.
SDN	Test station near National Steel and Shipbuilding Co. shipyard in San Diego Bay.
SED1	Second order PCA factor correlating with PCBF, MET1, PAH1, and sulfide.
SED2	Second order PCA factor correlating with DDTF and PAH4.
SED3	Second order PCA factor correlating with organotin, MET2, and PAH2.
SED4	Second order PCA factor correlating with PAH3.
SEDTOC1	Second order PCA factor correlating with PAHTOC1 and sulfide.
SEDTOC2	Second order PCA factor correlating with PCBTOC, PAHTOC2, and MET2.
SEDTOC3	Second order PCA factor correlating with DDTTOC, PAHTOC3, and MET1.
SEDTOC4	Second order PCA factor correlating with organotin, and MET1.

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SMP	Reference station near San Mateo Pt.
SMS	Test station near the end of Santa Monica Bay sludge outfall.
TOC	Total organic carbon.
TON	Total organic nitrogen.
TOX1	PCA factor correlating with measures of sea urchin growth.
TOX2	PCA factor correlating with Microtox luminescence and sea urchin survival.
TOX3	PCA factor correlating with amphipod survival.

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Compound		SMS	PV	LAH	LAR	OC	DPM <sup>a</sup>	DPM <sup>b</sup>	SMP <sup>a</sup>	SMP <sup>b</sup>	SDN	SDC	SD7	NB	
Hexachlor-	1	<7	2	<2	2	<1	<2	<2	<2	<2	<3	<2	<2	<1	
obenzene	F	6	<3	<2	17	<1	<2	<2	<2	<2	<3	<2	<2	<1	
Lindane	I	<7	<2	<2	2	<1	<2	<2	<2	<2	<3	<2	<2	<1	
	F	<6	<3	<2	<2	<1	<2	<2	<2	<2	<3	<2	<2	<1	
o,p'-DDE	T	<7	692	3	<2	<1	<2	<2	<2	<2	<3	<2	<2	3	
	F	<6	642	5	<2	<1	<2	<2	<2	<2	<3	<2	<2	3	
p,p'-DDE	1	170	4309	50	53	7	5	4	10	12	10	11	23	<1	
	F	153	4510	70	59	6	3	4	7	9	8.	12	24	<1	
o,p'-DDD	1	<7	<2	<2	<2	<4	<3	<2	<3	<2	<8	<6	<4	<1	
	F	<6	<3	<2	<2	<4	<3	<2	<3	<2	<10	<4	<4	<1	
p,p'-DDD	1	26	599	32	24	<4	<3	<2	3	5	<8	13	46	1	
	F	23	584	37	29	<4	<5	<2	<5	3	<13	8	45	1	
o,p'-DDT	I	<7	5	<2	<2	<3	<2	<2	<2	<2	<5	<4	<2	<1	
	F	<6	<3	<2	<2	<3	<3	<2	<3	<2	<8	<4	<4	<1	
p,p'-DDT	I	<7	356	3	10	<3	<2	<2	<2	10	<5	6	10	<1	
	F	<6	388	3	42	<3	<2	<2	<2	10	<8	<2	16	<1	
Total DDT	I	196	5966	88	91	7	5	4	13	28	10	30	79	4	
	F	175	6124	115	130	6	3	4	7	22	8	20	85	NA	
Aroclor	I	197	368	35	84	<6	<5	<6	<5	<7	<8	<8	42	<3	
1242	F	209	302	38	105	<6	10	<8	<7	<5	<18	<8	49	<3	
Aroclor	I	459	1178	197	227	55	10	<6	7	<7	208	188	311	<3	
1254	F	508	1124	242	245	18	10	14	<5	<5	158	204	323	<3	
Total PCB	I	654	1548	217	310	55	10	ND	7	ND	208	188	353	ND	
	F	718	1426	281	350	18	20	14	ND	ND	158	204	372	NA	

Table A1. Concentrations of chlorinated hydrocarbons in sediments from the test stations. Measurements were made at the begining (1) and end (F) of each sea urchin bioassay experiment. All values are in ng/g dry weight.

<sup>a</sup> Data from experiment in September 1987. <sup>b</sup> Data from experiment in November 1987.

	-						ST	ATION						
PAII#		SMS	PV	LAII	I.AR	OC	DPM <sup>a</sup>	DPM <sup>b</sup>	SMP <sup>a</sup>	SMPb	SDN	SDC	SD7	NB
1	I	446	<2	10	41	4	<3	<2	<2	<2	<5	<4	<4	<1
	F	91	20	10	44	<4	<5	<2	<3	<2	<8	<6	<4	<1
2a	I	1134	37	15	167	10	<3	<2	<2	<2	<5	<4	<4	<1
	F	508	53	13	103	<4	<5	<2	<3	<2	<8	<6	<4	1
2b	l	380	10	<3	84	<3	<3	<2	<2	<2	<5	<4	<4	<1
	F	158	15	<2	29	<4	<5	<2	<3	<2	<8	<6	<4	<1
3a	I	682	59	<3	275	<3	<3	<2	<2	<2	<5	<4	<4	<1
	F	277	48	12	284	<4	<5	<2	<3	<2	<8	<6	<4	<1
3b	l	1625	12	<3	437	<3	<3	<2	<2	<2	<5	<4	<4	<1
	F	452	30	<2	292	<4	<5	<2	<3	<2	<8	<6	<4	<1
4a	I	406	<5	<5	201	<7	<5	<4	<2	<3	<10	<6	<8	<3
	F	11	<5	<3	199	<7	<10	<4	<7	<3	<13	<10	<6	<3
4b	I	2313	7	<5	817	<7	<5	<4	<2	<3	<10	<6	<8	<3
	F	802	<5	<3	831	<7	<10	<4	<7	<3	<13	<10	<6	<3
5	I	2647	12	<3	21	<3	<3	<2	<2	<2	<5	<4	<4	<1
	F	1096	20	<2	27	<4	<5	<2	<3	<2	<8	<6	<4	<1
6	l	39	140	25	10	<3	<3	<2	<2	<2	<5	21	44	<1
	F	11	137	45	10	<4	<5	<2	<3	<2	<8	41	45	<1
7	I	<13	<5	<5	31	<7	<5	<4	<2	<3	<10	<6	<8	<3
	F	<28	<5	<3	12	<7	<10	<4	<7	<3	<13	<10	6	<3
8	I	26	<2	10	41	<3	<2	<2	<2	<2	8	13	10	<1
	F	17	<3	8	39	<3	<3	<2	<3	<2	<5	<4	<2	<1
9	l	393	74	168	611	16	3	8	2	5	185	273	311	<1
	F	220	79	161	522	59	25	16	<3	9	99	196	205	<1

Table A2. Concentrations of polynuclear aromatic hydrocarbons (PAH) in test sediments (ng/g dry weight). Measurements were made at the beginning (1) and end (F) of each sea urchin bioassay. The compounds corresponding to each PAH number are listed at the end of the table.

Table A2 continued

							ST	ATION							1
PAH#		SMS	PV	LAH	LAR	OC	DPM <sup>a</sup>	DPM <sup>b</sup>	SMP <sup>a</sup>	SMP <sup>b</sup>	SDN	SDC	SD7	NB	
10	I F	1101 379	322 160	200 186	721 517	<3 <3	<2 <3	12 16	<2 <3	3 31	96 <5	209 149	513 364	<1 <1	
11	l F	1953 825	253 287	195 196	1025 1074	<3 <3	<2 <3	6 8	<2 <3	5 9	21 <5	216 135	401 335	<1 <1	
12	I F	3113 1768	572 721	255 333	1197 931	<3 <3	<2 <3	12 2	<2 <3	3 <2	10 <5	300 278	1006 232	<1 <1	
13	l F	183 85	96 79	240 212	110 96	<3 15	<2 <5	6 8	<2 <5	<2 9	260 68	260 235	403 343	<1 <1	
14	I F	629 412	115 96	240 281	774 725	9 44	21 19	16 20	3 <2	3 7	273 151	582 512	545 315	<1 <1	
15	I F	583 384	263 36	405 452	798 748	12 41	<2 32	19 34	5 5	5 15	388 249	698 767	1119 841	<1 <1	
16	I F	66 282	<2 102	318 256	193 201	<3 15	<3 5	<2 <2	<2 <5	7 <2	242 164	501 265	749 585	<1 <1	
17	I F	406 345	29 94	290 311	322 331	<1 37	3 15	4	<2 <2	<2 <2	231 166	423 496	507 382	<1 <1	
18	l F	531 384	157 173	605 624	571 586	16 43	<1 22	8 6	2 3	5 10	468 322	781 933	753 598	<1 <1	
19	I F	668 441	204 183	870 950	521 603	13 103	10 69	8 22	7 10	5 9	629 803	929 2063	1874 1988	<1 <1	
20	l F	<7 <6	<2 <3	<2 <2	<2 <2	<1 <1	<2 <2	<2 <2	<2 7	<2 <2	299 <3	489 <2	622 <2	<1 <1	
21	l F	301 198	152 137	387 436	248 304	4 35	<2 19	6 8	2 2	3 3	401 345	548 818	968 746	<1 <1	
22	l F	387 237	145 132	423 458	222 252	<1 47	<2 31	6 14	3 5	2 5	518 421	641 971	1083 817	<1 <1	

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## Table A2 continued

	STATION														
PAH#		SMS	PV	LAH	LAR	ос	DPM <sup>a</sup>	DPM <sup>b</sup>	SMP <sup>a</sup>	SMP <sup>b</sup>	SDN	SDC	SD7	NB	
23	I F	341 243	297 231	247 289	141 179	<1 7	<2 19	47 12	3 <2	5 5	101 75	138 235	221 159	<1 <1	4
24	I F	<7 45	88 <3	7 <2	<2 <2	<1 <1	<2 <2	<2 <2	<2 <2	<2 <2	<3 <3	<2 <2	2 14	<1 <1	
25	I F	33 96	59 46	48 194	81 115	<1 7	<2 <2	<2 <2	<2 <2	<2 <2	94 91	118 192	102 83	<1 <1	
26	l F	<7 175	110 107	355 366	267 368	6 40	<2 17	<2 65	3 <2	3 3	481 413	489 716	870 663	<1 <1	
Total PAH	I F	20387 10051	3209 2987	5310 5794	9914 9419	90 495	38 268	153 235	28 32	59 114	4711 3369	7626 9006	12109 8715	<3 <3	

<sup>a</sup>Data from experiment in September 1987. <sup>b</sup>Data from experiment in November 1987.

- Naphthalene 1
- 2a 1-Methylnaphthalene
- 2b
- 3a
- 3b
- 2-Methylnaphthalene 2,6-Dimethylnaphthalene Other C2-Naphthalenes 2,3,5-Trimethylnaphthalenes Other C3-Naphthalenes 4a
- 4b
- 5 Biphenyl
- 6 Acenaphthylene
- 7 Acenaphthene
- Fluorene 8
- 9 Phenanthrene
- 10 C1-Phenanthrenes/Anthracenes
- C2-Phenanthrenes/Anthracenes C3-Phenanthrenes/Anthracenes 11
- 12

- 13 Anthracene
- 14 Fluoranthene
- 15 Pyrene
- 2,3-Benzofluorene 16
- 17
- Benz(a)anthracene Chrysene/Triphenylene Benzo(b)fluoranthene 18
- 19
- 20 Benzo(k)fluoranthene
- Benzo(e)pyrene Benzo(a)pyrene 21
- 22
- 23 Pervlene
- 9,10-Diphenylanthracene 24
- Dibenz(a,h)anthracene 25
- Benzo(g,h,i)perylene 26

Table A3. Concentration of trace metals in sediments from the test stations. Measurements were made at the beginning (I) and end (F) of each sea urchin bioassay experiment. All values are in ug/g dry weight except for organotin which is in ng/g.

		STATION												
Com	pound	SMS	PV	LAH	LAR	NB	OC	DPM <sup>a</sup>	DPM <sup>b</sup>	SMP <sup>a</sup>	SMP <sup>b</sup>	SDN	SDC	SD7
Ag	l F	3.80 20.36	4.94 7.96	0.046 0.052	1.34 1.27	< 0.003	0.46 0.69	0.01 0.01	0.01 0.01	0.01 0.02	0.02 0.02	1.88 1.77	0.90 0.83	1.04 1.10
As	I F	18.4 19.5	18.7 20.6	7.4 7.4	4.0 4.4	1.2	1.8 2.0	3.3 3.6	3.7 4.4	3.3 2.6	3.3 3.5	8.0 8.0	6.9 6.3	5.9 4.7
Cd	I F	28.64 30.27	15.15 14.00	0.47 0.51	3.15 2.93	< 0.05	0.80 0.86	0.14 0.14	0.30 0.27	<0.05 <0.05	<0.05 <0.05	0.61 0.80	0.41 0.46	1.66 1.15
Cr	l F	258.4 281.3	326.8 303.2	49.8 48.7	32.4 32.0	2.5	18.6 18.4	17.0 17.4	18.3 23.2	20.6 20.1	21.3 23.3	64.3 63.4	36.9 37.3	62.1 42.6
Cu	I F	510.9 558.4	213.1 197.7	82.1 79.3	83.4 74.1	1.9	23.8 18.8	26.7 28.9	26.2 30.9	13.1 14.1	14.2 14.9	214.1 214.1	131.7 142.5	122.1 130.1
Hg	l F	<0.5 <0.5	<0.7 <0.6	<0.7 <0.7	<0.7 <0.7	<0.6	<0.7 <0.7	<0.6 <0.7	<0.7 <0.7	<0.6 <0.7	<0.7 <0.7	<0.7 <0.6	<0.7 <0.7	<0.8 <0.6
Ni	l F	67.9 74.7	46.9 42.1	23.3 24.7	28.0 24.6	2.2	7.9 7.2	9.1 10.4	12.0 14.1	11.3 11.6	12.1 13.3	20.2 19.1	12.2 13.0	12.4 12.5
Pb	l F	133.3 153.5	112.4 107.4	64.1 60.6	130.3 122.9	4.1	12.0 6.5	8.2 7.5	8.1 9.0	5.4 5.5	5.7 6.1	60.1 64.7	69.5 63.6	103.6 99.6
Sn	I F	10.62 25.26	7.89 7.79	1.21 1.18	0.13 0.66	< 0.04	<0.05 0.36	0.08 0.13	0.05 0.11	0.05 0.10	<0.04 0.12	1.44 0.98	0.66 0.69	1.04 1.04
Zn	l F	675 742	630 587	211 223	389 358	13	62 52	66 69	75 92	57 60	61 67	321 286	235 279	581 333
Orgai	notin I	329	127	28	62		11	56		<16		423	162	189

<sup>a</sup>Data from experiment in September 1987. <sup>b</sup>Data from experiment in November 1987.

	Metal	Factor	
VARIABLE	MET1	MET2	
Cr	0.884	0.367	
Ni	0.849	0.448	
Cu	0.784	0.451	
As	0.885	0.400	
Ag	0.796	0.492	
Cď	0.896	0.349	
Sn	0.938	0.336	
Pb	0.317	0.936	
Zn	0.518	0.828	
%Variance			
explained	62	30	

Table A4. Correlations (loadings) of trace metals with principal component analysis factors. A VARIMAX rotation was used in the analysis.

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Table A5. Correlations (loadings) of chlorinated hydrocarbons with principal component analysis factors. A separate PCA was conducted on the TOC-normalized data. A VARIMAX rotation was used in each analysis.

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VARIABLE	FACTOR LC	DADING	
A. Dry wt. basis	DDTF	PCBF	
o,p'-DDE	0.858	0.513	
p.p'-DDE	0.841	0.540	
p.p'-DDD	0.835	0.548	
p.p'-DDT	0.857	0.515	
Aroclor 1242	0.486	0.867	
Aroclor 1254	0.602	0.784	
% Variance			
explained	58	41	
B. TOC basis	DDTTOC	PCBTOC	
o.p'-DDE	0.926	0.370	
p.p'-DDE	0.923	0.380	
p.p'-DDD	0.848	0.525	
p.p'-DDT	0.918	0.386	
Aroclor 1242	0.799	0.537	
Aroclor 1254	0.387	0.919	
%Variance explained	68	31	

VARIABLE		PAH	Factor		
A. Dry weight basis	PAH1	PAH2	РАНЗ	PAH4	
Naphthalene	0.997	-0.021	-0.037	-0.015	
2-Methylnaphthalene	0.998	-0.036	0.020	0.011	
1-Methylnaphthalene	0.994	-0.036	0.092	-0.002	
2.6-Dimethylnaphthalene	0.960	-0.065	0.263	0.045	
Other C2-Naphthalenes	0.988	-0.039	0.142	-0.020	
2.3.5-Trimethylnaphthalene	0.937	-0.050	0.341	-0.040	
Other C3-Naphthalenes	0.973	-0.045	0.221	-0.029	
Biphenvi	0.991	-0.017	-0.114	-0.005	
Acenaphthylene	0.090	0.077	-0.091	0.983	
Acenaphthene	0.135	-0.021	0.976	-0.086	
Fluorene	0.513	0.226	0.817	-0.107	
Phenanthrene	0.440	0.463	0.768	-0.037	
C1-Phenanthrenes/Anthracenes	0.830	0.290	0.406	0.201	
C2-Phenanthrenes/Anthracenes	0.926	0.110	0.348	0.074	
C3-Phenanthrenes/Anthracenes	0.939	0.192	0.200	0.144	
Anthracene	0.082	0.973	-0.025	0.075	
Fluoranthene	0.457	0.617	0.619	-0.037	
Pyrene	0.219	0.866	0.415	0.081	
2.3-Benzofluorene	-0.170	0.969	0.053	-0.091	
Benz(a)anthracene	0.375	0.877	0.257	-0.087	*
Chrysene/Triphenvlene	0.206	0.885	0.309	-0.011	
Benzo(b)fluoranthene	0.092	0.974	0.030	0.017	
Benzo(k)fluoranthene	-0.221	0.892	-0.125	-0.108	
Benzo(e)pyrene	0.023	0.993	0.014	0.044	
Benzo(a)pyrene	0.057	0.994	-0.043	0.012	
Perviene	0.573	0.425	0.045	0.646	
9.10-Diphenvlanthracene	-0.104	-0.196	-0.067	0.968	
Dibenz(a,h)anthracene	-0.136	0.831	0.351	0.195	
Benzo(g,h,i)perylene	-0.283	0.944	0.087	-0.008	
%Variance					
explained	40	36	13	9	

Table A6. Correlations (loadings) of polynuclear aromatic hydrocarbons with principal component analysis factors. A separate PCA was conducted on the TOC-normalized data. A VARIMAX rotation was used in each analysis.

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## Table A6 continued.

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в. 1	FOC basis	PAHTOC1	PAHTOC2	PAHTOC3	PAHTOC4
	Naphthalene	-0.906	-0.111	-0.110	0.281
	2-Methylnaphthalene	-0.954	-0.172	-0.051	0.159
	1-Methvinaphthalene	-0.982	-0.149	-0.085	0.029
	2.6-Dimethylnaphthalene	-0.930	-0.175	0.050	-0.266
	Other C2-Naphthalenes	-0.981	-0.133	-0.085	-0.073
	2.3.5-Trimethylnaphthalene	-0.892	-0.124	-0.104	-0.409
	Other C3-Naphthalenes	-0.958	-0.127	-0.084	-0.215
	Biphenyl	-0.854	-0.123	-0.116	0.479
	Acenaphthylene	0.178	0.418	0.853	0.186
	Acenaphthene	-0.009	-0.083	-0.293	-0.877
	Fluorene	-0.167	0.750	-0.052	-0.551
	Phenanthrene	-0.044	0.934	-0.014	-0.329
	C1-Phenanthrenes/Anthracenes	-0.282	0.856	0.323	-0.157
	C2-Phenanthrenes/Anthracenes	-0.629	0.661	0.261	-0.262
	C3-Phenanthrenes/Anthracenes	-0.958	-0.127	-0.084	-0.215
	Anthracene	0.212	0.945	0.069	0.093
	Fluoranthene	0.023	0.947	-0.057	-0.162
	Pyrene	0.096	0.982	0.076	-0.047
	2,3-Benzofluorene	0.187	0.975	0.038	0.051
	Benz(a)anthracene	0.107	0.984	0.028	0.002
	Chrysene/Triphenylene	0.157	0.939	0.052	-0.022
	Benzo(b)fluoranthene	0.153	0.960	0.119	0.064
	Benzo(k)fluoranthene	0.226	0.816	-0.208	0.139
	Benzo(e)pyrene	0.184	0.971	0.064	0.082
	Benzo(a)pyrene	0.194	0.968	0.026	0.107
	Pervlene	0.114	0.738	0.459	0.076
	9,10-Diphenylanthracene	0.199	-0.232	0.892	0.145
	Dibenz(a,h)anthracene	0.239	0.899	-0.061	-0.002
	Benzo(g,h,i)perylene	0.234	0.956	0.017	0.051
	%Variance				
	explained	28	51	8	7

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VARIABLE	C	ontamination	Factor	
A. Dry wt. basis _	SED1	SED2	SED3	SED4
PCBF DDTF MET1 MET2 PAH1 PAH2 PAH3 PAH4 Sulfide	0.855 -0.284 0.856 0.282 0.935 -0.173 0.008 0.283 0.976	-0.379 -0.910 -0.343 -0.189 0.304 0.081 0.068 -0.944 0.073	0.253 -0.230 -0.090 0.677 0.106 0.941 -0.044 0.103 0.030	0.224 -0.139 -0.365 0.646 0.014 0.095 0.961 0.089 0.194
Organotin % Variance explained	0.429 37	0.094 21	0.706 20	-0.232 17
B. TOC basis	SEDTOC1	SEDTOC2	SEDTOC3	SEDTOC4
PCBTOC DDTTOC MET1 MET2 PAHTOC1 PAHTOC2 PAHTOC3 Sulfide Organotin	-0.259 0.036 0.394 0.566 -0.986 -0.044 0.042 0.908 0.231	0.886 -0.158 -0.248 0.751 -0.018 0.839 0.469 -0.130 0.226	0.233 0.942 0.634 0.036 0.039 -0.462 0.834 0.239 -0.160	0.118 -0.011 0.555 0.048 -0.105 0.071 -0.247 0.302 0.906
%Variance explained	27	27	26	15

Table A7. Correlations (loadings) of contaminant factors or measurements with principal component analysis factors. A separate PCA was conducted on the TOC-normalized data. A VARIMAX rotation was used in the analysis.

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Macrofaunal ordination axis scores							
Site	ORD1	ORD2	ORD3	ORD4			
SMS	3.002	1.097	-0.163	0.155			
PV	1.106	-0.494	-1.246	0.055			
LAH	-0.693	-0.277	-0.752	0.027			
LAR	0.921	0.670	1.522	-0.251			
OC	0.372	-1.761	-0.207	-0.082			
DPM	-0.651	0.579	-0.570	-0.299			
SMP	-0.109	-1.849	0.363	0.033			
SDN	-1.277	0.804	-0.347	-0.075			
SDC	-1.455	0.832	-0.672	0.015			
SD7	-1.215	0.400	0.569	0.421			
%Vari	ance						
explain	ied 32	19	11	10			

Table A8. Macrofaunal principal coordinates analysis axis scores.

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Station	%Survival	Growth (mm)		
NB	62 + 4	4.0 + 0.6		
PV	17 + 4	0.6 + 0.4		
LAH	40 + 4	$2.0 \pm 0.8$		
OC	60 + 3	$2.7 \pm 0.6$		
SMP	49 + 7	$1.9 \pm 0.7$		
SDC	$45 \pm 2$	$1.8 \pm 0.3$		

Table A9. Amphipod survival and growth following chronic exposure to contaminated sediments. Values are mean  $\pm$  SE (N=3).

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Table A10. Correlations (loadings) of toxicity test responses with principal components analysis factors. A VARIMAX rotation was used in the analysis.

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	Toxicity Test Factor					
Variable	TOX1	TOX2	TOX3			
Urchin diameter Urchin wet wt. Urchin gonad weight Urchin mortality Amphipod survival Microtox	0.839 0.905 0.792 -0.627 0.379 0.177	0.188 0.255 0.359 -0.740 0.112 0.962	0.388 0.257 0.317 -0.149 0.917 0.090			
% Variance explained	45	31	21			

\*

Group	Macrofauna-Contamination Axis						
	1	2	3	4	5		
ORD1	0.961	-0.113	0.178	0.106	0.131		
ORD2	0.115	0.091	0.088	-0.977	-0.080		
ORD3	0.022	0.134	0.864	0.089	-0.451		
ORD4	0.132	0.970	-0.173	0.089	0.045		
SED1	0.960	0.117	-0.089	-0.228	-0.055		
SED2	0.059	0.065	-0.096	0.083	0.983		
SED3	-0.208	0.831	0.101	-0.496	0.019		
SED4	0.070	-0.288	0.927	-0.212	0.188		
%Variance	24	22	21	17	15		

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Table A11. Principal components analysis (PCA) axis loadings using macrofaunal ordination (ORD) and sediment contamination (SED) PCA scores.

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Table A12. Correlations (loadings) of toxicity test and sediment contamination factors with principal components analysis factors (axes). A separate PCA was conducted on the TOC-normalized data. A VARIMAX rotation was used in the analysis.

Group		Toxicity-0	Contaminat	Variables in Group		
A. Dry wt. basis	1	2	3	4	5	
SED1	-0.800	0.009	0.063	0.001	0.583	H-S, PAH1, MET1, PCBF
SED2	-0.177	-0.058	-0.971	-0.020	-0.144	PÁH4, DDTF
SED3	0.106	-0.947	0.001	0.129	0.192	PAH2, Organotin, MET2
SED4	-0.065	0.072	0.008	0.978	-0.115	PAH3, MET2
TOX1	0.099	0.089	-0.148	0.111	-0.972	Urchin growth
TOX2	0.946	-0.049	0.271	-0.066	0.043	Microtox/Urchin mortality
TOX3	0.074	0.839	0.098	0.464	0.129	Amphipod survival
%Variance						
explained	23	23	15	17	20	
B. TOC basis	1	2	3	4	5	
SEDTOC1	-0.866	0.171	0.416	-0.093	-0.056	PAHTOC1, H.S
SEDTOC2	0.102	-0.087	0.065	-0.979	0.002	PCBTOC, PAHTOC2, MET2
SEDTOC3	0.074	0.093	0.221	0.003	0.964	DDTTOC, PAHTOC3, MET1
SEDTOC4	-0.105	-0.933	0.196	0.114	0.071	Organotin, MET1
TOX1	0.107	0.136	-0.941	0.065	-0.255	Urchin growth
TOX2	0.934	0.172	0.125	-0.234	0.039	Microtox/Urchin mortality
TOX3	-0.093	0.855	0.078	0.331	0.250	Amphipod survival
%Variance						
explained	24	24	17	16	15	

		Macrofauna-Toxicity Axis					
Group	1	2	3	4	5		
TOX1-	-0.160	-0.858	-0.249	0.294	0.258		
TOX2	-0.957	0.106	0.142	-0.139	0.055		
TOX3	0.079	-0.101	0.904	0.192	0.289		
ORD1	0.872	0.230	0.380	-0.035	-0.034		
ORD2	0.064	0.038	-0.225	0.053	-0.962		
ORD3	0.095	-0.065	0.151	0.973	-0.050		
ORD4	-0.063	0.903	-0.346	0.118	0.139		
%Varia	unce						
explain	ed 25	23	18	16	16		

Table A13. Correlations (loadings) of toxicity PCA (TOX1-TOX3) and macrofauna ordination scores (ORD1-ORD4) with second order PCA axes. A VARIMAX rotation was used in the analysis.

	Macrofauna-Toxicity-Contamination Axis					
	1	2	3	4	5	
ORD1	0.953	-0.187	0.165	0.005	0.061	
ORD2	0.191	0.940	0.147	-0.058	0.002	
ORD3	-0.028	-0.062	0.826	0.099	-0.501	
ORD4	0.018	0.095	-0.095	0.972	0.026	
SED1	0.971	0.150	-0.052	0.145	0.045	
SED2	0.040	0.099	0.065	-0.083	-0.921	
SED3	-0.197	0.762	0.021	0.578	-0.035	
SED4	0.064	0.122	0.947	-0.184	0.071	
TOX1	-0.528	-0.088	0.143	-0.619	-0.396	
TOX2	-0.776	-0.050	-0.034	0.148	0.508	
TOX3	0.171	-0.670	0.542	-0.203	0.277	
%Variance explained	26	18	18	16	15	

Table A14. Axis loadings from second order PCA of macrofaunal ordination, contamination, and toxicity.