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Bioaccumulation and Sub-lethal Effects in Marine Invertebrates

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Abstract

Contaminated surface (top 2 cm) sediments were collected from the nearshore waters of southern California and analysed for grain size, total organic carbon and nitrogen, dissolved sulfide in the interstitial water, 11 metals, PCBs, pesticides-(including ΣDDT), and 26 individual polynuclear aromatic hydrocarbons. In addition, biological effects (acute and chronic) of sediment exposures were measured on bacteria (Microtox), amphipods, shrimp, and urchins. Urchin gonads and whole shrimp were also analysed for bioaccumulation of contaminants. Neither urchins nor shrimps demonstrated bioaccumulation of trace metals from highly contaminated sediments. The short-term Microtox tests with interstitial water seemed to be responding to the more soluble components in the sediments, so the findings did not correlate well with the longer-term tests. Urchin growth was reduced when exposed to the most contaminated sediments, and gonad PCB concentrations showed a good correlation with sediment PCB content, when the latter were normalised to μg PCB g^{-1} organic carbon. DDE was also bioaccumulated by urchin gonads. Amphipod survival in 10 day exposures showed effects from the most contaminated stations, but amphipod growth over 28 days was a more sensitive measure of sediment effects. Both amphipods and urchins were recommended for toxicity testing with sediments, and urchin gonads were found suitable for measuring bioaccumulation of chlorinated organics.

Introduction

Except for petroleum hydrocarbons from oil spills, most pollutants, including atmospheric inputs, enter the near-shore marine environment bound to particulates. Just a few examples are the discharge of domestic wastes, dredged material disposal, and stormwater runoff. Long-lasting impacts that have been measured in the coastal waters are generally the result of toxic chemicals in sediments, or the presence of organic-rich particles. Hydrogen sulfide produced within these organic sediments may also be responsible for reducing the numbers of species and individuals inhabiting the area. Even the long-term effects of oil spills are associated with sediment contamination, as levels in the water seldom reach toxic concentrations and they are rapidly reduced over time. Several recent books and reports have considered the needs associated with understanding the potential for pollutant transport from sediment to biota, and the subsequent bioaccumulation or effects (NRC 1989; Long and Morgan 1990). Efforts are moving forward to produce regulations, either called sediment criteria or sediment objectives, for each individual toxicant in sediments. If we possessed such reliable values, they would provide a mechanism for managing the discharge of substances to the ocean (Anderson 1988).

Over the last 5 years, while Director of the Southern California Coastal Water Research Project (SCCWRP), my staff and I have designed and conducted studies to determine the potential for sediments from contaminated sites to produce toxicity and bioaccumulation in selected marine invertebrates. Organisms were chosen on a basis of: (1) their prominence at the depths of the municipal waste ocean outfalls (60 m); (2) their sensitivity to toxicants; (3) their potential for measuring both toxicity and bioaccumulation; and (4) the ability to maintain control animals in a healthy condition in the laboratory. The organisms considered in this paper are the shrimp, *Sicyonia ingentis*, the amphipod, *Grandidierella japonica*, and the sea urchin, *Lytechinus pictus*.

The objectives of the studies were to develop tests with local species to provide information on the acute and chronic toxicity of contaminated sediment, and when possible, also measure the bioaccumulation associated with the effects. We also wished to determine which sediments in the region produced impacts on these species, and if possible identify which of the multiple pollutants present were responsible for the adverse responses.

Methods

Site Locations and Sampling

The sites selected for this study were previously sampled during the Southern California Coastal Water Research Project (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 wt) were selected for this study (Fig. 1). Since the eight sites are situated in both open coastal and protected bay areas, two reference sites were selected, one off San Mateo Point and one at the Dana Point Marina.

Because of logistical limitations on both ship time 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 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.

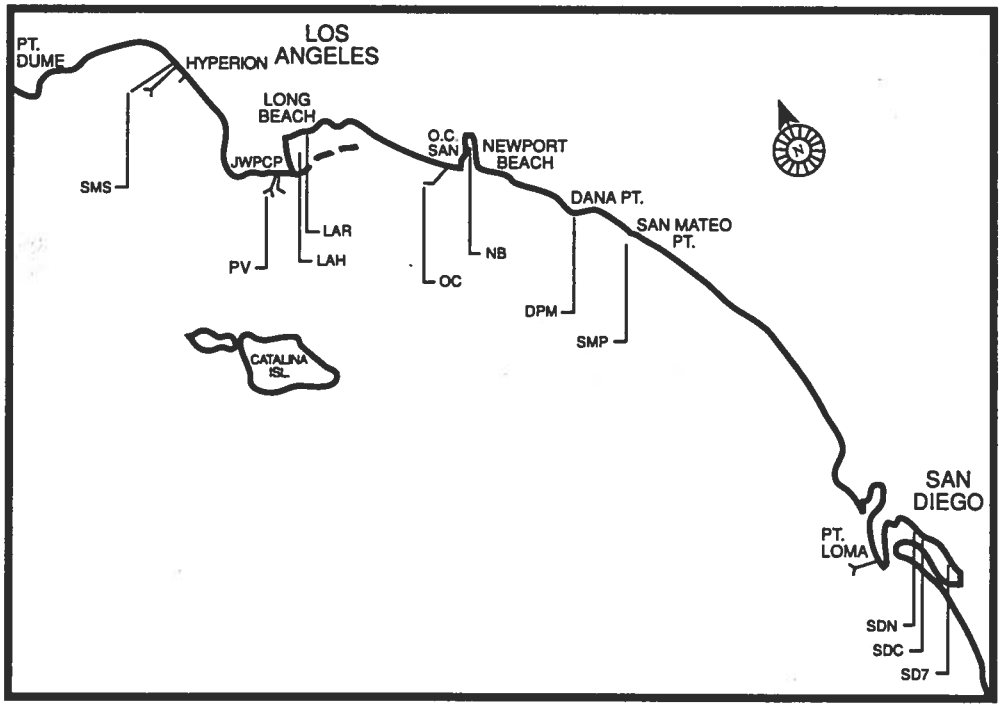

Figure 1. Location of test stations. See Table 1 for explanation of abbreviations.

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.

General constituents

Percent sand, silt, and clay (dry weight) were measured using wet and dry sieving with a 63-µm 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 Analyser. Organic nitrogen was by Kjeldahl digestion at Galbraith Laboratory, Chicago, Illinois. Total dissolved sulfides were measured by squeezing pore water (Kalil and Goldhaber 1973) and measuring dissolved sulfides using a modified methylene blue

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Trace metals

Samples for trace metal determinations were digested at SCCWRP. The digestates were analysed 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, California. 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.

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 analyse 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 analysed for chlorinated hydrocarbons (CHCs).

Tissue was extracted following the method of Bligh and Dyer (1959) which involved homogenisation 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 desiccator 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 analysed using a Varian Vista 44 GC equipped with an electron capture detector, helium carrier gas at 30 cm sec^{-1} flow velocity, and a 30 m x 0.025 mm i.d. DB5 fused silica capillary column which was temperature programmed from 150°C to 274°C at 4°C min^{-1}. Quantification was performed using the internal standardisation method, with Mirex as the internal standard. The electron capture detector was calibrated weekly,

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.

To examine the possible use of the shrimp, *Sicyonia ingentis*, these animals were exposed in aquaria to sediment collected from the Santa Monica Bay sludge outfall. A slow flow of seawater was passed through the aquaria and in addition to observing mortality tissue samples were taken at 0, 10, 20 and 30 days of exposure. 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, California), an amphipod survival test and a chronic sea urchin growth test, both using whole sediment.

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 minute exposure and standardised method (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.

Amphipod test

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°C under flow-through conditions.

Test procedures were adapted from those of Swartz *et al.* (1985).

Grandidierella japonica specimens were collected from Newport Bay (NB) at low tide. Amphipods were removed from the sediment by screening the material through a 1 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 homogenised prior to addition to the bioassay containers. A 2 cm layer of test sediment was added to three replicate 1 L 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^{-1} was established for each beaker, along with gentle aeration. A photoperiod of 12 hour light/12 hour 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 hour period was determined. This test was intended to evaluate the survivors' condition by observing their ability to respond normally to a favourable environment.

Sea urchin test

The sea urchin toxicity test was a chronic (35-day) exposure, also at 15°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 (29 cm x 26 cm x 14 cm) containing a 2 cm layer of test sediment. Approximately 2.3 L of water was above the sediment. A flow rate of approximately 1 L h^{-1} was used for all sediments except those from the Santa Monica Bay sludge outfall; a higher flow (1.5 L h^{-1}) 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, weighted, and divided in half to provide separate sub-samples 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 the 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

Table 1
Concentration of general constituents in sediments used for testing.

Station	Station code	Percent sand	Percent silt	Percent clay	Percent TOC	Percent TON	C/N/a/	Sulphide (mg L^{-1}) /d/		
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								Initial	14 D	Final
September 1987										
San Mateo Point	SMP	4.2	84.8	11.0	0.96	0.089	10.8	2.7	0.1	0.1
Dana Point Marina	DPM	27.7	55.1	17.2	0.73	0.089	8.7	0.2	ND/b/	ND
Orange County outfall	OC	77.4	19.7	2.9	0.56	0.062	9.0	NA/c/	0.1	ND
San Diego Bay: Chollas Creek	SDC	39.6	35.3	25.1	1.49	0.11	13.5	0.3	ND	ND
San Diego Bay: NASSCO	SDN	16.1	39.2	44.7	1.71	0.17	10.1	0.9	ND	ND
San Diego Bay: 7th Street	SD7	37.6	31.8	30.6	1.74	0.11	15.8	0.1	ND	ND
November 1987										
San Mateo Point	SMP	4.5	85.3	10.2	1.11	NA		ND	ND	0.2
Los Angeles Harbor turning basin	LAH	42.5	38.2	19.3	1.12	0.086	13.0	0.3	7.8	ND
Dana Point Marina	DPM	4.6	69.2	26.2	0.91	NA		1.0	ND	0.2
Long Beach Harbor Queensway Bay	LAR	40.7	47.4	11.9	4.28	0.35	12.1	19.5	30.3	50.0
Palos Verdes outfall	PV	28.5	60.1	11.4	4.16	0.29	14.3	15.9	3.3	2.4
Santa Monica Bay sludgeline	SMS	53.4	38.9	7.7	10.54	1.05	10.0	56.1	4.8	102.9
Newport Bay	NB	96.5	2.0	1.5	0.11	NA		NA	NA	NA

/a/ Percent TOC/percent TON
/b/ Sample below detection limit for analysis
/c/ Sample not analysed
/d/ Dissolved sulfide measurements were made on interstital water at three times during the sea urchin toxicity test.

determined from dissections of a representative sub-sample of the population at the start of the test. Nested analysis of variance followed by multiple pairwise comparisons with the San Mateo Point 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 Point 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).

Results

Sediment Characterisation

A summary of the general constituents of the sediment types examined in this study is shown in Table 1. The sediment 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 harbour sites tended to have the highest clay contents, reflecting the depositional nature of the shallow, protected environments. Wide variations in the total organic carbon (TOC) and total organic nitrogen (TON) contents of the sediments were also observed. 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 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 ($r^2 = 0.99$). Sulfide concentration was highest at SMS, which had a concentration of 56 mg L^{-1} (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.

A detailed listing of all the metals, chlorinated organics and polyaromatic hydrocarbons in the test sediments may be found in Appendix Tables A1 to A3. Elevated levels of chlorinated hydrocarbons were found at many of the sites, 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^{-1}, 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. These compounds were present in the highest concentrations at PV (1,548 ppb total PCB) and SMS (654 ppb). PAHs 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 industrialised harbour sites and at two of the three outfall sites. 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 harbours (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 in an earlier survey, during a study of PAH contamination along the coast (Anderson and Gossett 1987).

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 *et al.* 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

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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 minimise 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 in flowing seawater. Declines in sediment contaminant levels were minor in most cases. 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.

Toxicity and Bioaccumulation Tests

Sicyonia ingentis

This species was observed to be very tolerant of the high concentrations of all classes of contaminants present in the sludge from Santa Monica Bay. There were no significant differences between the survival in these sediments and the reference sediments (SMP). Even more surprising was the fact that those animals exposed for 30 days to this material contained lower amounts of silver, cadmium, copper and zinc in their tissues than they originally contained (Table 2).

Table 2

Lack of metals uptake by the shrimp, *Sicyonia ingentis*, after 30 days of exposure to highly contaminated sediments from the Santa Monica Bay sludge outfall. Values are mean ($\mu g\ kg^{-1}$) - concentrations in the whole body of shrimp.

Time period	Silver	Cadmium	Copper	Zinc
Initial	0.8	0.5	30	25
10 Days	0.8	0.5	25	20
20 Days	0.7	0.4	22	22
30 Days	0.6	0.35	20	19

Microtox Tests

Results of the Microtox tests on interstitial water are shown in Table 3. Significant reductions in light output compared with the San Mateo Point (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_2S) present in the interstitial water from this station (Table 1).

Amphipod Test

Exposure to whole sediment produced significant reductions in *G. japonica* survival at one outfall station (SMS) and most harbour stations (Table 4). 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 4). Only one or two amphipods failed to rebury within one hour in any of the replicates.

Table 3

Microtox test of interstitial water toxicity.

Values are mean and SE. Corrected values are data from the November experiment which have been adjusted to compensate for differences in San Mateo Point response between experiments.

Station	Percent control	Luminescence
	Actual	Corrected
September 1987 experiment		
SMP	93.1 ± 1.4	
DPM	87.0 ± 2.5	
OC	33.7 ± 4.1 /a/	
SDC	72.6 ± 0.9 /a/	
SDN	71.6 ± 1.5 /a/	
SD7	79.0 ± 5.7 /a/	
November 1987 experiment		
SMP	96.0 ± 1.3	93.1 ± 1.4
DPM	91.1 ± 1.9	88.4 ± 1.9
LAH	82.9 ± 4.1 /a/	80.4 ± 3.9 /a/
LAR	61.9 ± 0.8 /a/	60.0 ± 0.8 /a/
PV	72.4 ± 0.4 /a/	70.2 ± 0.3 /a/
SMS	0.3 ± 0.2 /a/	0.3 ± 0.1 /a/

Table 4

Amphipod survival and reburial following 10-day sediment exposure (mean ± 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.

Station	Percent survival		
	Actual	Corrected	Percent reburial
September 1987			
NB	88.3 ± 4.4		100 ± 0
SMP	85.0 ± 7.6		97 ± 3
DPM	63.3 ± 4.4 /a/		89 ± 2
OC	76.7 ± 3.3 /a/		98 ± 2
SDC	68.3 ± 1.7 /a/		100 ± 0
SDN	35.0 ± 5.0 /a/		89 ± 6
SD7	41.7 ± 6.0 /a/		97 ± 3
November 1987			
NB	91.7 ± 4.4	88.3 ± 4.2	96 ± 2
SMP	86.7 ± 1.7	83.5 ± 1.6	96 ± 4
DPM	60.0 ± 5.0	57.8 ± 4.8	100 ± 0
LAH	50.0 ± 7.6 /a/	48.1 ± 7.4 /a/	91 ± 3
LAR	91.7 ± 4.4	88.3 ± 4.2	100 ± 0
PV	70.0 ± 5.0	67.4 ± 4.8	93 ± 3
SMS	35.0 ± 20.2 /a/	33.7 ± 19.5 /a/	100 ± 0

/a/ Value is significantly less than survival in Newport Bay sediment ($p \leq 0.05$).

Survival of *G. japonica* was not affected by variations in sediment grain size present between the test sites. High survival (86-92%) 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* (Nipper *et al.* 1989). 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. 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.

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 5). Survival during the 35-day exposure period was high, except at SMS, where a 51% mortality occurred.

A significant behavioural 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 5).

Table 5

Sea urchin responses following 35-day exposure to sediment (mean ± 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.

Station	Percent survival	Sediment preference (percent)	Growth			Corrected test growth (mm)
			Weight (g)	Gonad (g)	Test (mm)	
September 1987						
SMP	97.8 ± 2.2	75.3 ± 2.8	0.39 ± 0.04	0.114 ± 0.016	0.63 ± 0.11	
DPM	100 ± 0	80.7 ± 2.9	0.43 ± 0.02	0.136 ± 0.004	0.75 ± 0.19	
OC	100 ± 0	88.0 ± 1.7	0.42 ± 0.01	0.163 ± 0.006	0.66 ± 0.14	
SDC	97.8 ± 2.2	55.0 ± 3.5 /a/	0.29 ± 0.03	0.140 ± 0.012	0.48 ± 0.10	
SDN	97.8 ± 2.2	52.0 ± 4.3 /a/	0.30 ± 0.02	0.126 ± 0.001	0.51 ± 0.08	
SD7	100 ± 0	48.0 ± 3.2 /a/	48.0 ± 0.02	0.119 ± 0.004	0.33 ± 0.06 /a/	
November 1987						
SMP	100 ± 0	65.0 ± 4.0	0.26 ± 0.02	0.124 ± 0.009	0.55 ± 0.18	0.63 ± 0.21
DPM	100 ± 0	67.3 ± 5.3	0.29 ± 0.03	0.113 ± 0.010	0.45 ± 0.17	0.65 ± 0.19
LAH	100 ± 0	70.7 ± 1.2	0.25 ± 0.02	0.118 ± 0.012	0.41 ± 0.13	0.47 ± 0.15
LAR	100 ± 0	85.7 ± 2.9	0.36 ± 0.06	0.151 ± 0.030	0.92 ± 0.18	1.06 ± 0.21
PV	100 ± 0	77.7 ± 1.2	0.18 ± 0.02	0.138 ± 0.004	0.23 ± 0.12 /a/	0.27 ± 0.14 /a/
SMS	48.9 ± 5.9	30.3 ± 2.6 /a/	0.04 ± 0.07	0.048 ± 0.026 /a/	-0.02 ± 0.22 /a/	-0.02 ± 0.25 /a/

/a/ Value is significantly less than growth on San Mateo Point sediment ($P<0.05$).

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. By three weeks, animals no longer avoided the San Diego Bay sediments. 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 or the increased interstitial water sulfide levels.

Measurements of sea urchin growth (changes in wet weight and test diameter) after the 35-day sediment exposure (Table 5) revealed similar trends to those found with the amphipods. The greater 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 5). 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.* 1989). 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 6), 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 greater 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 (35 days).

Table 6

Chlorinated hydrocarbons in *L. pictus* gonad tissues after 35 days of exposure to test sediment.

Values are expressed on a dry weight basis (mean ± SE; N = 3).
Bioconcentration factor (BCF) is the quotient: tissue concentration/sediment concentration.

Station	Tissue concentration (ng g^{-1})		BCF	
	DDT	PCB	DDT	PCB
September 1987				
SMP	406 ± 16	ND /a/	31	ND
DPM	221 ± 7	481 /b/	44	48
OC	579 ± 11	1.560 ± 87	83	28
SDC	284 ± 2	2.076 ± 92	9	11
SDN	201 ± 8	2.005 ± 21	20	10
SD7	457 ± 14	2,904 ± 47	6	8
November 1987				
SMP	693 ± 16	603 ± 11	25	ND /a/
DPAM	532 ± 22	567 /b/	133	ND /a/
LAH	2,092 ± 51	4,239 ± 438	24	20
LAR	489 ± 24	1.040 ± 73	5	3
PV	47,870 ± 447	8,097 ± 213	8	5
SMS	1,474 ± 130	2,628 /b/	8	4

/a/ 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.

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 greatest tissue concentrations. The LAH sediment consistently produced outlying values in these plots. This situation could result from variations in sediment composition, such as high organic carbon content, which changed contaminant bioavailability. The sediment chemistry data were normalised to TOC and re-plotted to see if these values had a better relationship to tissue levels. 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 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. 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. However, trends towards increased metals levels were consistently found in urchins from the PV and SMS stations. 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, yet gonad tissue from this station had a relatively low concentration of this metal.

Relationships Between Tests and Stations

Since the results of exposure of the shrimp, *Sicyonia ingentis*, to highly contaminated sediment produced neither significant mortality nor bioaccumulation it was eliminated from further consideration in testing the impacts of sediment. The most responsive endpoints in each of the three tests (luminescence, amphipod survival, and urchin test growth) have been compared. 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 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.

Discussion

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.

The laboratory toxicity tests identified several stations as being harmful. There was generally good agreement among 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 favourably to the *Rhepoxynius* amphipod test (Swartz *et al.* 1985). Differences in sensitivity between the amphipod and sea urchin tests were evident at the harbour (amphipod most sensitive) and LA 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. 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 emphasises the necessity of using multiple species and different test strategies in order to accurately assess sediment toxicity. The sea urchin growth test has the advantage of producing data on the sub-lethal effects of contaminants and bioaccumulation data for at least chlorinated organic compounds.

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Table A1

Concentrations of chlorinated hydrocarbons 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 ng g^{-1}, dry wt.

Compound	STATION												
	SMS	PV	LAH	LAR	OC	DPM[a]	DPM[b]	SPM[a]	SPM[b]	SDN	SDC	SD7	NB

Table A1

Concentrations of chlorinated hydrocarbons 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 ng g^{-1}, dry wt.

Compound		STATION											
		SMS	PV	LAH	LAR	OC	DPM[a]	DPM[b]	SPM[a]	SPM[b]	SDN	SDC	SD7	NB
Hexachlor-obenzene	I	<7	2	<2	2	<1	<2	<2	<2	<2	<3	<2	<2	<1
	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	I	<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	I	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	I	<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	I	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	<3	<2	<2	10	<5	6	10	<1
	F	<6	388	3	42	<3	<3	<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 1242	I	197	368	35	84	<6	<5	<6	<5	<7	<8	<8	42	<3
	F	209	302	38	105	<6	10	<8	<7	<5	<18	<8	49	<3
Aroclor 1254	I	459	1178	197	227	55	10	<6	7	<7	208	188	311	<3
	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

[a]Data from experiment in September 1987.
[b]Data from experiment in November 1987.

Table A2

Concentrations of polynuclear aromatic hydrocarbons (PAH) in test sediments (ng g^{-1}, dry wt). Measurements were made at the beginning (I) and end (F) of each sea urchin bioassay. The compounds corresponding to each PAH number are listed at the end of the tables.

PAH#		STATION												
		SMS	PV	LAH	LAR	OC	DPM[a]	DPM[b]	SPM[a]	SPM[b]	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	508508	53	13	103	<4	<5	<2	<3	<2	<8	<6	<4	<1
2b	I	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	I	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	I	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	I	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
10	I	1101	322	200	721	<3	<2	12	<2	3	96	209	513	<1
	F	379	160	186	517	<3	<3	16	<3	31	<5	149	364	<1
11	I	1953	253	195	1025	<3	<2	6	<2	5	21	216	401	<1
	F	825	287	196	1074	<3	<3	8	<3	9	<5	135	335	<1
12	I	3113	572	255	1197	<3	<2	12	<2	3	10	300	1006	<1
	F	1768	721	333	931	<3	<3	2	<3	<2	<5	278	232	<1
13	I	183	96	240	110	<3	<2	6	<2	<2	260	260	403	<1
	F	85	79	212	96	12	<5	8	<5	9	68	235	343	<1

Table A2 continued

PAH#		STATION												
		SMS	PV	LAH	LAR	OC	DPM[a]	DPM[b]	SPM[a]	SPM[b]	SDN	SDC	SD7	NB
14	I	629	115	240	774	9	21	16	3	3	273	582	545	<1
	F	412	96	281	725	44	19	20	<2	7	151	512	315	<1

10	I	1101	322	200	721	<3	<2	12	<2	3	96	209	513	<1
	F	379	160	186	517	<3	<3	16	<3	31	<5	149	364	<1
11	I	1953	253	195	1025	<3	<2	6	<2	5	21	216	401	<1
	F	825	287	196	1074	<3	<3	8	<3	9	<5	135	335	<1
12	I	3113	572	255	1197	<3	<2	12	<2	3	10	300	1006	<1
	F	1768	721	333	931	<3	<3	2	<3	<2	<5	278	232	<1
13	I	183	96	240	110	<3	<2	6	<2	<2	260	260	403	<1
	F	85	79	212	96	12	<5	8	<5	9	68	235	343	<1

Table A2 continued

		STATION												
PAH#		SMS	PV	LAH	LAR	OC	DPM[a]	DPM[b]	SPM[a]	SPM[b]	SDN	SDC	SD7	NB
14	I	629	115	240	774	9	21	16	3	3	273	582	545	<1
	F	412	96	281	725	44	19	20	<2	7	151	512	315	<1
15	I	583	263	405	798	12	<2	19	5	5	388	698	1119	<1
	F	384	36	452	748	41	32	34	5	15	249	767	841	<1
16	I	66	<2	318	193	<3	<3	<2	<2	<7	242	501	749	<1
	F	282	102	256	201	15	5	<2	<5	<2	164	265	585	<1
17	I	406	29	290	322	<1	3	4	<2	<2	231	423	507	<1
	F	345	94	311	331	37	15	4	<2	<2	166	496	382	<1
18	I	531	157	605	571	16	<1	8	2	5	468	781	753	<1
	F	384	173	624	586	43	22	6	3	10	322	933	598	<1
19	I	668	204	870	521	13	10	8	7	5	629	929	1874	<1
	F	441	183	950	603	103	69	22	10	9	803	2063	1988	<1
20	I	<7	<2	<2	<2	<1	<2	<2	<2	<2	299	489	622	<1
	F	<6	<3	<2	<2	<1	<2	<2	7	<2	<3	<2	<2	<1
21	I	301	152	387	248	4	<2	6	2	3	401	548	968	<1
	F	198	137	436	304	35	19	8	2	3	345	818	746	<1
22	I	387	145	423	222	<1	<2	6	3	2	518	641	1083	<1
	F	237	132	458	252	47	31	14	5	5	421	971	817	<1
23	I	341	297	247	141	<1	<2	47	3	5	101	138	221	<1
	F	243	231	289	179	7	19	12	<2	5	75	235	159	<1
24	I	<7	88	7	<2	<1	<2	<2	<2	<2	<3	<2	<2	<1
	F	45	<3	<2	<2	<1	<2	<2	<2	<2	<3	<2	14	<1
25	I	33	59	48	81	<1	<2	<2	<2	<2	94	118	102	<1
	F	96	46	194	115	7	<2	<2	<2	<2	91	192	83	<1
26	I	<7	110	355	267	6	<2	<2	3	3	481	489	870	<1
	F	175	107	366	368	40	17	65	<2	3	413	716	663	<1
Total PAH	I	20387	3209	5310	9914	90	38	153	28	59	4711	7626	12109	<3
	F	10051	2987	5794	9419	495	268	235	32	114	3369	9006	8715	<3

[a]Data from experiment in September 1987.
[b]Data from experiment in November 1987.

Table A2 continued

1	Naphthalene	13	Anthracene
2a	1-Methylnaphthalene	14	Fluoranthene
2b	2-Methylnaphthalene	15	Pyrene
3a	2,6-Dimethylnaphthalene	16	2,3-Benzofluorene
3b	Other C2-Naphthalenes	17	Benz(a)anthracene
4a	2,3,5-Trimethylnaphthalenes	18	Chrysene/Triphenylene
4b	Other C3-Naphthalenes	19	Benzo(b)fluoranthene
5	Biphenyl	20	Benzo(k)fluoranthene
6	Acenaphthylene	21	Benzo(e)pyrene
7	Acenaphthene	22	Benzo(a)pyrene
8	Fluorene	23	Perylene
9	Phenanthrene	24	9,10-Diphenylanthracene
10	C1-Phenanthrenes/Anthracenes	25	Dibenz(a,h)anthracene
11	C2-Phenanthrenes/Anthracenes	26	Benzo(g,h,i)perylene
12	C3-Phenanthrenes/Anthracenes		

Table A3

Concentrations 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 $\mu g\ g^{-1}$, dry wt except for organotin which is in $ng\ g^{-1}$.

Compound	STATION											
	SMS	PV	LAH	LAR	NB	OC	DPM[a]	DPM[b]	SPM[a]	SPM[b]	SDN	SDC	SD7
Ag													
Al													
As													
Cd													
Cr													
Cu													
Fe													
Hg													
Mn													
Ni													
Pb													
Sn													
Zn													
Organotin													

Table A3

Concentrations 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 $\mu g\ g^{-1}$, dry wt except for organotin which is in $ng\ g^{-1}$.

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

[a]Data from experiment in September 1987.
[b]Data from experiment in November 1987.