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APPLICATION OF TIEs IN STUDIES OF URBAN STORMWATER IMPACTS ON MARINE ORGANISMS

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ABSTRACT: Urban stormwater runoff is a significant, yet poorly understood, source of contaminants to the marine environment. One of the largest sources of stormwater inputs to Santa Monica Bay (California) is the Ballona Creek watershed. Receiving water and runoff water samples were collected during several storms in both the 1995-96 and 1996-97 wet seasons. Sea urchin fertilization tests indicated toxicity in most Ballona Creek stormwater samples; EC₅₀ values were about 12-20%. Receiving water samples were also toxic, with the magnitude of effects generally corresponding to the concentration of runoff present. Selected phase I TIE (toxicity identification evaluation) manipulations were applied to samples showing toxicity. Ballona Creek samples had a consistent response pattern; EDTA addition removed virtually all toxicity, implicating divalent trace metals as the probable toxic constituents. Santa Monica Bay surface water samples showed a similar response pattern but other manipulations also removed some toxicity. Toxicity of receiving water samples tended to degrade with storage, while runoff sample toxicity was more stable. Chemical analysis of runoff and comparison to spiking studies showed that concentrations of zinc and occasionally copper were sufficient to produce toxicity. Evaluation of the relative effectiveness of EDTA versus sodium thiosulfate in toxicity removal also suggested zinc as a likely cause of toxicity.

KEYWORDS: stormwater, marine, TIE (toxicity identification evaluation), sea urchin, metals, Santa Monica Bay

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Urban stormwater runoff is a significant source of contaminants to the marine environment. The relative contribution of urban stormwater to the total contaminant load has increased in areas such as the Southern California Bight; this is partly due to improved treatment and control of point sources (e.g., municipal wastewater) and partly due to increased urbanization. Recent studies have detected toxicity in most samples of urban stormwater destined for marine receiving waters (SCCWRP 1990, Schiff and Stevenson 1996). However, virtually no information exists on contaminant fates and biological effects once wet weather discharges enter the marine environment. Most stormwater toxicity studies have employed freshwater test organisms. However, tests using marine organisms are essential for determining potential impacts from watersheds that discharge into marine waters.

Toxicity identification evaluations (TIEs) involve a suite of tools to enable researchers to go beyond the limitations of conventional toxicity testing and investigate the cause of toxicity. The initial phase of most TIEs includes various physical/chemical manipulations to separate a toxic sample into well characterized fractions. Once characterized, subsequent TIE procedures are applied to identify specific chemicals contributing to the toxicity. TIE techniques have been developed for, and applied mostly to, studies of freshwater systems. Applications have included municipal and industrial effluents, receiving water, and sediment interstitial water. A few stormwater samples have been evaluated with TIEs (Cooke and Lee 1993).

The use of TIEs in studies of marine systems, although needed, have been far less common. Some TIE procedures are compatible with marine toxicity test species (Burgess *et al.* 1995, EPA 1996) and have been used in preliminary investigations of dry weather urban runoff (Bay *et al.* 1996). Application of TIEs to stormwater has the potential to greatly benefit agencies responsible for stormwater management, as treatment or source control actions can be focused to reduce those chemicals contributing to the toxicity.

This report describes measurements of stormwater toxicity from the Santa Monica Bay (California) watershed to marine organisms. The proximity of Santa Monica Bay to a major metropolitan area (Los Angeles, California) and high public interest in maintaining the quality of this resource make it an important location to study the impacts of urban stormwater runoff. Our objectives were: 1) to compare the magnitudes of toxicity observed in runoff samples with those observed in the corresponding receiving water and 2) to characterize the toxicants present.

METHODS

Scope of Study

This study represents a portion of stormwater research conducted during the 1995-96 and 1996-97 wet seasons. The methods and results relevant to the toxicity characterization aspect of the projects are presented in this manuscript.

Site Selection

The Ballona Creek and Malibu Creek watersheds were selected as study sites because they are the two largest drainages in the Santa Monica Bay watershed; together they represent 58% of the total watershed area (Figure 1). Our toxicity characterization studies focused on Ballona Creek and the associated offshore receiving water. Ballona Creek is a concrete-lined flood control channel carrying runoff from highly urbanized areas (less than 20% open land). Previous studies have identified toxicity in dry and wet weather discharges (SCCWRP 1990, Bay *et al.* 1996).

Malibu Creek was sampled during concurrent studies. In contrast to Ballona Creek, this watershed drains predominately undeveloped land in the Santa Monica Mountains (almost 90% open land); it served as a reference site.

Sample Collection

Ballona and Malibu Creeks--Samples were collected from Ballona Creek during four storm events occurring in January-February 1996 and one storm event in January, 1997. During the 1995-96 wet season, grab samples of Ballona Creek stormwater were obtained from the bridge at Sawtelle Blvd., located approximately 6 km upstream from the mouth. During the 1996-97 wet season, composite samples at the same Ballona Creek site were obtained from an autosampler maintained by the Los Angeles County Department of Public Works. Malibu Creek was sampled in the 1995-96 period during many of the same storm events as Ballona Creek. Samples from Malibu Creek were obtained from a bridge located just downstream of the Tapia Water Reclamation Facility, approximately 6 km upstream of the creek's discharge into Malibu Lagoon.

Water samples were collected from the surface of each creek using a stainless steel bucket and transferred to 3.8 L glass bottles. A polyethylene bucket was used to collect duplicate samples for metals analysis; these samples were stored in 250 ml polypropylene bottles. The samples were chilled with ice until delivered to the laboratory, where they were stored in the dark at 5°C until used for toxicity tests. Toxicity tests were initiated within 48 hours of the end of each storm sampling event.

Santa Monica Bay--Toxicity test samples were collected during three storm events occurring between January and March 1996 and two storm events in December 1996 and January 1997. Sampling coincided with stormwater collection at Ballona Creek for the January 31-February 1, 1996 and February 19-21, 1996 storm events. Surface water samples (approximately 10-30 cm depth) were collected up to 48 hours after the storm. Five to ten water samples were obtained offshore near the mouth of Ballona Creek area during each event.

The location of the water sampling stations was determined during each cruise and varied between events. Locations were selected to represent a gradient of stormwater concentration, based on salinity measurements. Stations were usually located along a transect extending offshore from the creek mouth. Deviations from the general sampling plan occurred in response to available ship time, weather conditions, and extent of the runoff plume. For example, one sample, collected February 21, 1996, was obtained from

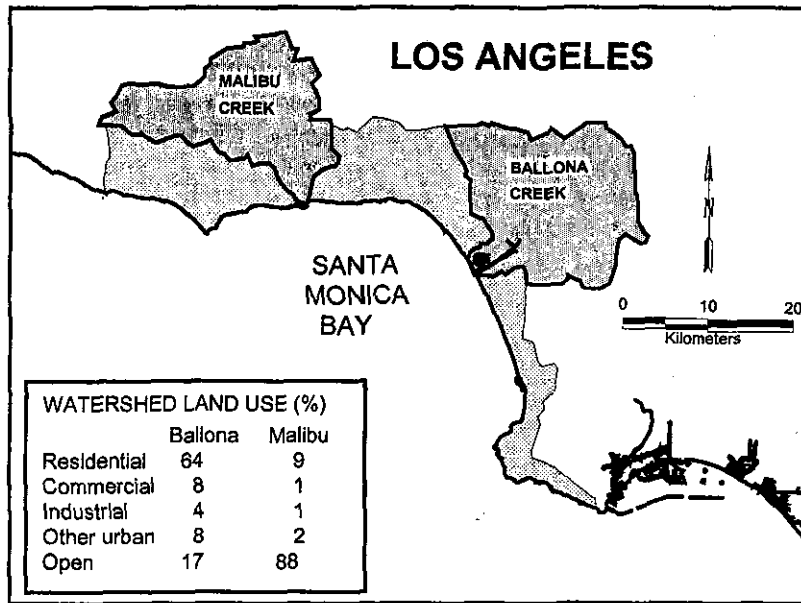


FIG. 1--Location of Ballona Creek and Malibu Creek sub-watersheds. Shaded areas represent the Santa Monica Bay watershed. Land use data are from Stenstrom and Stecker (1993).

the main channel of Marina del Rey in order to obtain a sample with intermediate salinity.

Water sampling methods varied because of the necessity to use different boats for some sampling. Surface water samples collected January 31 and February 1, 1996 were obtained by dipping a glass jar attached to the end of an aluminum pole. All other water samples were obtained with a submersible pump. Water samples were stored in glass bottles on ice immediately after collection. Samples were stored in the dark at 5°C and initial toxicity testing began within 48 hours.

Initial Toxicity Measurement

All samples were tested using the purple sea urchin (*Strongylocentrotus purpuratus*) fertilization test (Chapman *et al.* 1995). The test consists of a 20 minute exposure of sperm to 10 ml samples at 15°C. Eggs are then added and given 20 minutes for fertilization to occur. The eggs are preserved and examined later with a microscope to assess the percent fertilized. Effects are expressed as a reduction in fertilization percentage. Samples are considered "toxic" that have a statistically significant reduction relative to the control.

Creek stormwater samples were centrifuged (3 000 x g for 30 min.) in order to remove most particulates before testing. The salinity of the sample was then adjusted to

33-34 g/kg (typical seawater value) by the addition of brine produced by freezing seawater. Laboratory seawater was added to stormwater samples to produce concentrations of 50, 25, 12, 6, and 3%. The offshore samples were not centrifuged. The salinity was adjusted as required, resulting in sample concentrations ranging from 65% to 100%. No dilution series was prepared for the offshore samples.

Salinity, dissolved oxygen, pH, and total ammonia content of each test sample was measured at the start of the toxicity tests. Measurements were made using electrodes that were calibrated daily. Temperature was recorded at test initiation.

TIE Procedures

Selected samples from Ballona Creek and surface water locations were treated using modifications of EPA phase I TIE methods (Norberg-King *et al.* 1992, EPA 1996) in order to characterize the toxicants present. Usually, one stormwater and one surface water sample (representing the greatest toxicity) were selected for evaluation. TIE manipulations on samples collected during the 1995-96 wet season were conducted at UCLA and the samples returned to SCCWRP for toxicity testing within 5 to 10 days of collection. For the 1996-97 period, all TIE work was carried out at SCCWRP within 2 to 7 days of collection.

The phase I manipulations consisted of filtration through a 1.0 μm glass fiber filter (Whatman GF/B) followed by three treatments (Figure 2): solid phase extraction using a C-18 column (Analytichem, Harbor City, CA), addition of EDTA (1995-96: 3-30 mg/L, 1996-97: 15-30 mg/L) or addition of sodium thiosulfate (1995-96: 10-25 mg/L, 1996-97: 25 mg/L). In the first year of the study, TIE manipulations were conducted on stormwater samples prior to salinity adjustment and samples earmarked for the C-18 column were acidified to $\text{pH} < 2$ and then readjusted prior to toxicity testing. In the 1996-97 period, however, samples were salinity adjusted prior to TIE treatments, there was no acidification of C-18 samples, and the EDTA and thiosulfate samples were not filtered.

Sea urchin fertilization tests were used to evaluate the effectiveness of the TIE treatments. Each Ballona Creek treatment was diluted to produce concentrations of 50-12%. Offshore samples were tested at the highest concentration feasible. Toxicity removal was assessed by qualitative comparison of the fertilization percentages of baseline samples with the manipulated samples.

Chemical Analysis

Each Ballona Creek stormwater sample collected during the 1995-96 study period was analyzed to determine the concentration of dissolved trace metals using procedures described in EPA method 200.7. Water samples were filtered (1 μm glass fiber filter) within 24 hours of collection, acidified with nitric acid and stored at 4°C until digested. Samples were generally held two to three months before analysis. Hydrogen peroxide was added to the filtrates in order to digest particles that may have passed through the filter. Samples were analyzed using an inductively coupled plasma atomic emission spectrometer (ICP-AES).

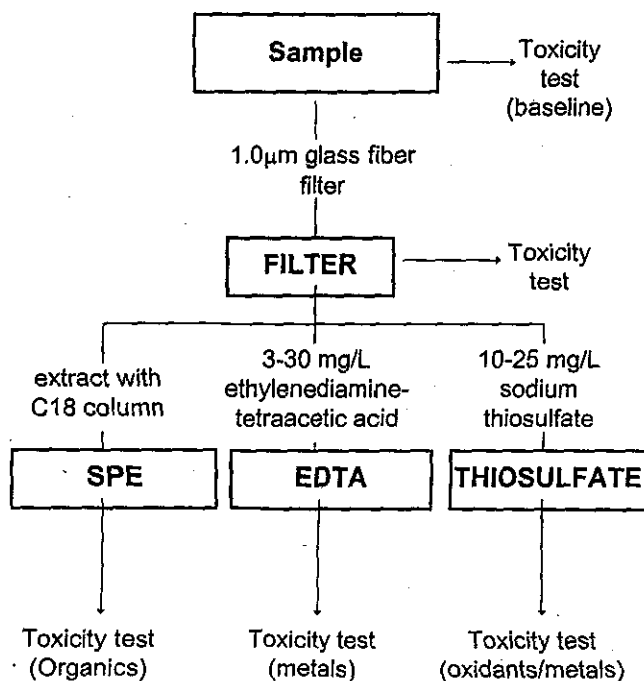


FIG. 2--Flow chart of TIE manipulations applied to most samples.

Quality Assurance

Concurrent brine control treatments were included in all toxicity tests. Blanks were included for each TIE manipulation to assess compatibility of the treatment with the test organism.

For dissolved metals analysis, blanks, consisting of deionized water spiked with nitric acid and hydrogen peroxide, were digested and analyzed under the same conditions as the sample. Final data were blank corrected by subtracting the blank concentration for each metal from the sample value. Percentage recovery was measured for six metals (Zn, Mn, Pb, Se, Cd, and Be) by spiking standards into duplicate stormwater samples. Recovery was 88-100% for all metals except Se, which was 23%. Detection limits for the analyses were $\leq 0.5 \mu\text{g/L}$ for Mn and Zn, $\leq 1.5 \mu\text{g/L}$ for Cd, Cu and Ni, and $4.9 \mu\text{g/L}$ for Pb.

Data Analysis

The concentration of runoff in surface water samples was calculated from initial salinity measurements and included dilution from salinity adjustment. This calculation assumed that the percent runoff present in the original sample was inversely proportional

to the relative salinity, expressed as a percentage of the background value (outside of plume). Median response values (EC_{50} , effective concentration producing a 50% toxic response) were calculated by probit analysis in most cases. Some data could not be analyzed by probit analysis; EC_{50} s for these samples were estimated by nonlinear interpolation. Pearson product moment correlation coefficients were calculated to identify potential relationships between toxicity and trace metal concentrations.

RESULTS

Magnitude of Toxicity

Ballona Creek stormwater runoff was consistently toxic, with fertilization test EC_{50} values usually between 12 and 20% stormwater. Toxicity was detected in Malibu Creek stormwater on a less frequent basis and was usually of lower magnitude. The permeability of the Malibu watershed resulted in storm flow conditions being present during just two of the four storms sampled. Four of the five Malibu Creek samples collected during these two storms had notably less toxicity than the Ballona Creek samples.

Many offshore samples were also toxic, with the greatest toxicity usually occurring nearest the mouth of Ballona Creek. For surface water, the magnitude of toxicity (as indicated by fertilization percentage) was proportional to the amount of runoff present in the sample (as indicated by plume concentration) (Figure 3). This relationship was also similar to the dose response observed for Ballona Creek stormwater (Figure 3).

Toxicant Characterization

One Ballona Creek sample was selected from each of the four 1995-96 storm events for TIE analysis. A range of flow conditions (400-4 200 cfs) was represented by these samples. Each of the Ballona Creek samples showed a remarkably similar pattern of response to the TIE treatments (Figure 4). Addition of EDTA removed virtually all the toxicity in each case, while filtration and sodium thiosulfate addition were not effective on any of the samples. Solid phase extraction (SPE) with a C-18 column partially removed toxicity on only one sample. Baseline toxicity (prior to filtration) was usually similar to initial toxicity measured 6-10 days earlier, indicating that the toxicants were relatively stable in solution.

A composite sample was tested from a January 13, 1997 storm using EDTA and sodium thiosulfate addition only. It was only slightly toxic (77% fertilization in the baseline sample versus 99% fertilization in the control). Once again EDTA removed all of the toxicity (100% fertilization); in this case sodium thiosulfate also removed much of the toxicity (91% fertilization).

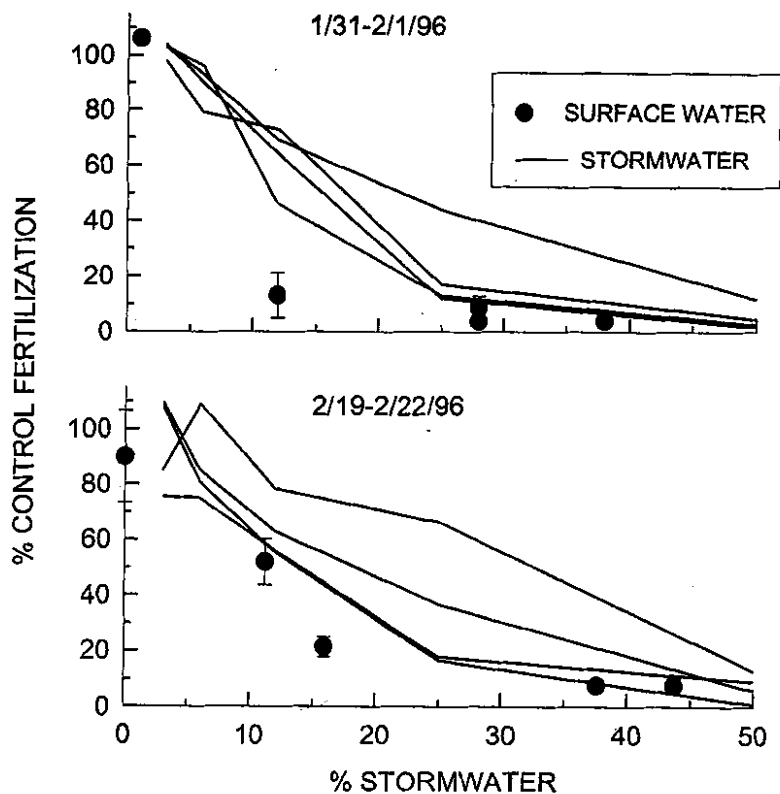


FIG. 3--Comparison of sea urchin fertilization test results for Ballona Creek stormwater and nearby surface water. Error bars are one standard deviation.

One or two offshore samples from each storm were selected for TIE analysis. There was a greater change in toxicity between the initial and baseline toxicity tests for the Santa Monica Bay samples than for the Ballona Creek stormwater samples (Figure 5). Three of the four samples that were stored between initial and baseline testing had at least 50% greater mean fertilization after storage. The elapsed time between collection and TIE testing for all of the samples collected during 1996 was 6-7 days. TIE tests and initial toxicity tests took place concurrently (within three days of collection) for samples collected January 25-26, 1997.

EDTA consistently removed virtually all toxicity in Santa Monica Bay samples (Figure 5). There were sporadic toxicity reductions for other treatments. Filtration reduced toxicity in three of the six samples. Solid phase extraction removed some toxicity (beyond that accounted for by filtration) in four samples. Sodium thiosulfate removed toxicity in two samples.

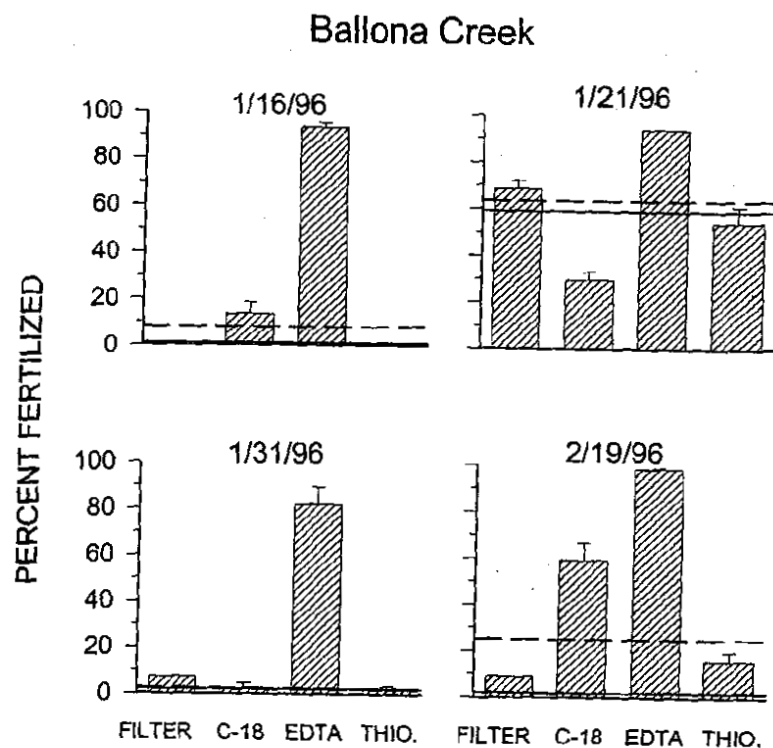


FIG. 4--TIE treatment results for Ballona Creek stormwater. The solid horizontal lines represent the initial test fertilization; the dotted horizontal lines represent the baseline fertilization. Error bars are one standard deviation.

Chemistry-Toxicity Relationships

Dissolved metal concentrations in stormwater were examined for cadmium, copper, manganese, lead, nickel, and zinc; trace elements which were most frequently detected in the samples and/or associated with toxicity to aquatic life. Cadmium, lead, and nickel were detected sporadically in the samples, with similar concentrations in samples from both creeks (Table 1). In contrast, dissolved copper, manganese, and zinc were detected in all Ballona Creek samples at concentrations generally much higher than those observed for Malibu Creek.

Fertilization test EC_{50} values for copper, cadmium, zinc, and manganese were determined from independent experiments conducted at SCCWRP. Based on these EC_{50} values, in some Ballona Creek samples, the concentrations of zinc, and to a lesser extent copper, were of a magnitude where toxic effects on sea urchin sperm would be expected even after accounting for dilution of the sample for testing.

Santa Monica Bay

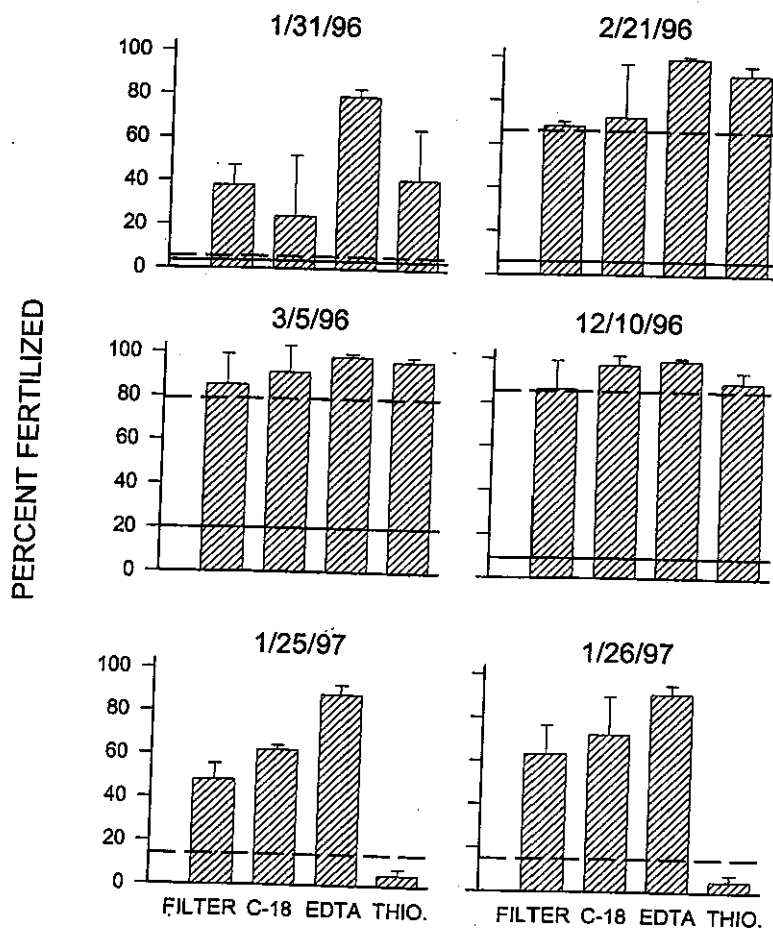


FIG. 5--TIE treatment results for Santa Monica Bay. The solid horizontal lines represent the initial test fertilization; the dotted horizontal lines represent the baseline fertilization. Error bars are one standard deviation.

Correlation coefficients were calculated between the nine Ballona Creek samples having calculable EC_{50} s and the corresponding concentration of each metal. Negative correlation coefficients (indicating greater toxicity as concentration increased) were obtained for cadmium, copper, manganese, nickel, and zinc (Table 1). Correlation coefficients were relatively low (-0.268 to -0.435) and not statistically significant ($r_{0.05,1,7}=0.582$).

TABLE 1--Dissolved metals concentration in stormwater samples. Pearson correlation coefficients with corresponding Ballona Creek sea urchin fertilization EC_{50} data are also shown, along with comparative toxicity data for selected metals.

Sample Date	Storm flow (cfs)	Concentration (mg/L)					
		Cadmium	Copper	Zinc	Manganese	Lead	Nickel
Malibu Creek							
1/16/96	0	nt ^a	nt	nt	nt	nt	nt
1/21/96	0	nd ^b	nd	0.050	0.019	nd	nd
1/31/96	0.7	nd	nd	0.058	0.009	nd	nd
1/31/96	1.2	nd	nd	0.079	nd	0.004	nd
2/01/96	2.4	nd	nd	0.015	0.012	nd	nd
2/01/96	1.3	nd	nd	0.042	0.010	nd	nd
2/19/96	0	nd	nd	0.058	0.009	nd	nd
2/20/96	4.5	0.002	nd	0.084	0.019	0.014	nd
2/20/96	70	nd	nd	0.015	0.012	nd	nd
2/21/96	77	0.002	nd	0.006	0.016	0.013	0.002
2/22/96	0	0.001	nd	0.010	0.021	0.006	0.002
Ballona Creek							
1/16/96	na ^c	nd	0.021	0.171	0.084	nd	0.005
1/21/96	2,600	0.001	0.017	0.183	0.068	0.016	0.003
1/31/96	325	nd	0.003	0.045	0.015	nd	nd
1/31/96	4,200	0.001	0.006	0.036	0.012	0.003	0.002
1/31/96	7,875	nd	0.006	0.047	0.006	nd	nd
1/31/96	275	nd	0.022	0.077	0.013	nd	nd
2/19/96	400	0.001	0.028	0.107	0.114	0.001	0.003
2/19/96	3,250	nd	0.005	0.044	0.001	0.001	0.001
2/20/96	1,900	nd	0.009	0.076	0.005	nd	0.001
2/21/96	250	nd	0.005	0.045	0.015	nd	nd
Correlation with Ballona Creek toxicity		-0.268	-0.372	-0.379	-0.330	0.299	-0.435
EC50 (mg/L) ^d		12.4	0.031	0.022	>40

^a nt: sample not tested for this parameter.

^b nd: non-detectable.

^c na: data not available.

^d SCCWRP sea urchin fertilization test data.

DISCUSSION

Relative Toxicity

Toxicity associated with storm events was consistently detected in both Ballona Creek runoff and in the corresponding receiving water. The detection of toxicity in Ballona Creek stormwater is consistent with the limited prior data available (SCCWRP 1990). However, this study was the first to investigate stormwater related toxicity in Santa Monica Bay surface waters.

The dose response relationship was similar for both sample types (Ballona Creek stormwater and offshore surface water). For the surface water, the occurrence of greater toxicity at higher concentrations of runoff is consistent with the relationship expected if

Ballona Creek was the source of toxicity. The results indicate that surface water toxicity in the area offshore of the mouth of Ballona Creek can be expected whenever runoff concentrations exceed 10%.

Toxicant Characterization

General considerations--The TIE manipulations performed in the study were generally compatible with the sea urchin fertilization test. The test was sensitive enough to both detect significant toxicity in the samples and to reflect toxicity reduction after manipulations. The sensitivity, short exposure duration, and easily distinguished endpoint make the sea urchin fertilization test a good candidate for marine TIEs.

Some samples, principally those from Santa Monica Bay, lost a major portion of their initial toxicity with storage, which limits the ability to compare toxicity reductions for TIE manipulations. However, even for these samples, positive effects of certain treatments can be noted.

Ballona Creek--The consistency of the TIE results for Ballona Creek stormwater suggests that most toxicity was caused by the same general class of toxicants during each event. The observation that EDTA removed virtually all the toxicity in Ballona Creek stormwater samples while other treatments were generally not effective suggests trace metals as the causative component. EDTA is a ligand that chelates divalent cations and renders them nontoxic. It is not known to significantly affect the toxicity of constituents other than metals (EPA 1996).

Santa Monica Bay--The TIE toxicity removal patterns for Santa Monica Bay samples were more variable than for Ballona Creek samples. However, the response to EDTA addition was again consistent; virtually all toxicity was removed in every case--again implicating trace metals. The effectiveness of other treatments varied from storm to storm, with some notable toxicity reductions for filtration, solid phase extraction, and addition of sodium thiosulfate.

The TIE results were remarkably similar for the two offshore samples collected about a day apart during the same storm (January 25-26, 1997). This is one indication that the toxicity "fingerprint" may be stable temporally for a major portion of a storm's duration. More TIE testing of multiple samples from the same storm would be required in order to confirm this result.

Differences in partial toxicity reduction and sample storage stability patterns were found between the stormwater and offshore samples. Comparisons are especially useful for the two storms where TIE tests were applied to matching samples of Ballona Creek and offshore waters (January 31-February 1, 1996 and February 19-21, 1996). The partial responses did not correspond for these samples, especially for the filtration and thiosulfate treatments. Moreover, toxicity was less stable in the Santa Monica Bay samples, as indicated by the large differences in baseline and initial toxicity in three of four stored samples. These differences between stormwater and receiving water toxicity patterns may stem from: 1) alteration of runoff characteristics upon integration into the marine matrix, 2) the effectiveness of TIE treatments being matrix dependent, 3) potential interactions between sample constituents not assessed in this study, such as total and

dissolved organic carbon, or total suspended solids, or 4) toxicants from sources other than runoff affecting the receiving water.

Identification of Toxicants

The consistent effectiveness of EDTA in toxicity removal from both runoff and offshore surface water samples is strong indirect evidence that a metal or metals are the causative toxicant(s). However, findings from a phase I TIE are not conclusive and should be confirmed by supporting data (e.g., chemical analyses and dose response information).

Evaluation of stormwater dissolved metals data identified zinc and perhaps copper as often present in concentrations sufficient to cause toxicity. We excluded cadmium, lead, and nickel based on the assumption that since Ballona Creek runoff was usually significantly more toxic than Malibu Creek runoff, Ballona Creek samples would generally be expected to contain higher levels of the causative toxicants than Malibu Creek samples. Manganese is not a likely toxicant based on the fertilization test EC₅₀ value.

Toxicity removal by EDTA and usually not by sodium thiosulfate is consistent with the pattern expected if zinc were responsible for toxicity. The relative effectiveness of EDTA and sodium thiosulfate in removing the toxicity of specific metals has been assessed by Hockett and Mount (1996) for freshwater TIEs. Three metals, zinc, lead, and nickel, match the pattern we observed for all runoff samples and most receiving water samples—strong toxicity removal by EDTA and little or no removal by thiosulfate. On the other hand, both EDTA and sodium thiosulfate are reported to be effective at removing copper toxicity in freshwater.

Sea urchin test-specific data for toxicity removal by EDTA and thiosulfate add further evidence that zinc and possibly copper are probable causative toxicants. SCCWRP has generated limited data for the sea urchin fertilization test analogous to the work of Hockett and Mount. We spiked laboratory seawater samples with toxic concentrations of copper and zinc and treated these samples with concentrations of EDTA and thiosulfate equal to those in our TIE procedures. Our results indicate that both zinc and copper toxicity to sea urchin sperm is strongly reduced by EDTA, but largely unaffected by thiosulfate, which matches the pattern observed in most of the storm-related TIEs.

The fact that filtration and solid phase extraction often resulted in partial toxicity removal is not inconsistent with a conclusion that metals were responsible for the toxicity. Researchers have observed that these two manipulations can remove metals to varying extents (EPA 1996).

Interactions between toxicants may have contributed to the overall toxicity and resulted in variable partial toxicity removal patterns. Such interactions could also explain why we did not find any statistically significant correlations between toxicity and individual metal concentrations. The loss of toxicity with storage (not usually expected with metals toxicity) for most of the offshore samples and the effectiveness of sodium thiosulfate addition on two offshore samples may be other indications of complex interactions.

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Based on the evidence we have obtained thus far it appears that metals, most likely zinc and copper, are a probable cause of toxicity for both Ballona Creek stormwater runoff and receiving water in Santa Monica Bay. Further study is required in order to fully understand the cause(s) of toxicity and to assess the effects of different matrices (fresh and salt water) on the behavior of toxicants and the effectiveness of TIE treatments.

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