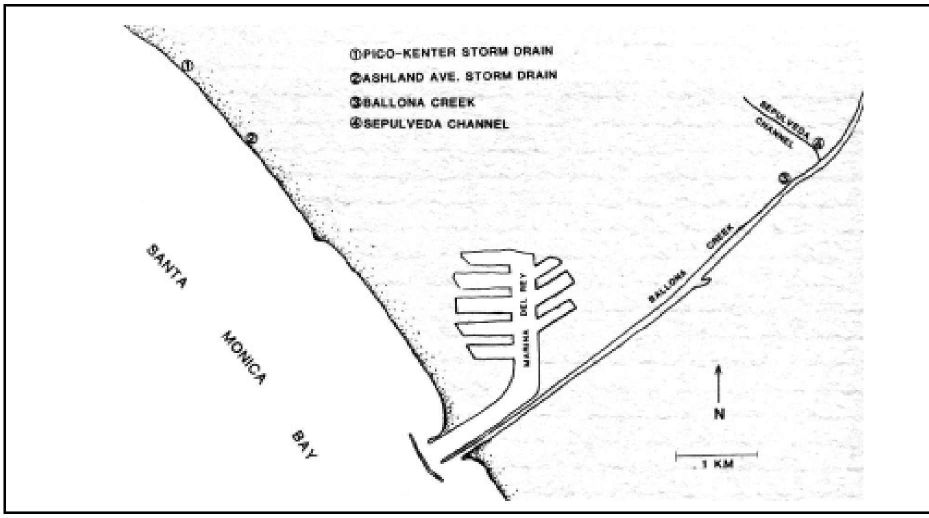
Preliminary Toxicity Identification Evaluation (TIE) of Dry-Weather Urban Discharge

Urban surface runoff accounts for a significant portion of the total mass of contaminants that enter the coastal ocean off Southern California (SCCWRP 1992). Approximately one-fourth of the total pollutant load delivered to Santa Monica Bay is the result of stormwater and urban runoff (SMBRP 1993). The largest contributor is Ballona Creek, which drains 232 km^2 of urbanized Los Angeles County (SCCWRP 1992). In toxicity tests with luminescent bacteria (Microtox™), the highest toxicity for Ballona Creek occurred during dry-weather and at the beginning of storms. Dry-weather flow occurred 95% of the days in 1987 accounting for 46% of the annual discharge; it occurred 89% of the days in 1988 accounting for 18% of the annual discharge (SCCWRP 1989). Dry-weather flow consists of groundwater from pumping and decontamination, swimming pool drainage, dehumidifier condensates, and excess runoff from landscape irrigation. These flows can mobilize household, industrial, and construction site wastes, used crankcase oil, and pesticides, and carry them untreated to the ocean through the storm drain system.

Figure 1.
Locations of selected stormdrains



Because nonpoint sources of contaminants are diffuse, they are difficult to trace, and end-of-the-pipe control is expensive. The best management strategy may be to rank the storm drains and pollutants by their potential for impact, and focus attention on those that pose the highest risk to natural resources and human health. The objective of this study was to measure spatial and temporal variations in toxicity in several Los Angeles storm drains during dry-weather. Samples from one storm drain were subjected to a toxicity identification evaluation (TIE) to characterize toxicity. This study was a collaborative effort between SCCWRP and the Department of Civil and Environmental Engineering at the University of California, Los Angeles.

Materials and Methods

Sample Locations, Times, and Sample Handling

Four storm drains were selected for this study: Ashland, Ballona Creek, Pico-Kenter, and Sepulveda Channel (Figure 1). These drains have consistent flows, are physically accessible, and their potential for environmental impact is high. Physical and chemical water quality parameters, and the spatial and temporal variability in toxicity were measured in dry-weather flows.

Ashland, Ballona Creek, and Pico-Kenter were sampled three times during dry-weather with a stainless steel bucket; Sepulveda Channel was sampled once during dry-weather. The first samples were collected on 8/24/92 and 9/8/92; the second sample was collected on 9/29/92; and the third sample was collected on 10/12/92. Samples were collected after at least two weeks without precipitation. Morning and afternoon samples were composited and kept refrigerated in sealed 4-L glass bottles until they were tested. Samples were thoroughly mixed and a 2.5 L subsample was removed and filtered through a 1mm glass fiber filter (Whatman GF/B). Ashland and Pico-Kenter storm drain samples were centrifuged (3,200 x *g* for 10 min) to remove large particles before filtering. There was no significant difference in toxicity between filtered and unfiltered samples.

Seawater dilutions of dry-weather samples were prepared by adding seawater and brine to produce the desired concentrations and maintain salinity at 32-35 g/kg. Dilutions contained 5.6, 10, 18, 32, and 56% (v/v) storm drain samples for the first test; and 5.6, 12, 25, and 56% (v/v) stormdrain samples for the second and third tests. Salinity, dissolved oxygen, pH, and total ammonia were measured on each dilution at the start of the tests. The initial pH was high in samples from Ballona Creek and Sepulveda Channel; a small amount of HCl was added to reduce pH and minimize toxicity artifacts. Seawater, brine, and filter controls were included in the experiments; no controls were performed for pH adjustment. A concurrent reference toxicant was tested for each bioassay.

Toxicity Tests

Three marine toxicity tests described in the California Ocean Plan (SWRCB 1990) were used: the sea urchin (*Strongylocentrotus purpuratus*) fertilization test (Dinnel *et al*. 1987), red abalone (*Haliotus rufescens*) embryo development test (Anderson *et al*. 1991), and giant kelp (*Macrocystis pyrifera*) germination/germ tube growth test (Anderson *et al*. 1991). Toxic effects were: reduced fertilization of sea urchin eggs; increased incidence of abnormal shell development in abalone larvae; and reduced kelp germination and gametophyte length. Effects were assessed relative to a control. Three replicates were tested for each dry-weather sample concentration rather than five replicates recommended in the California Ocean Plan. Replication was reduced to increase the number of concentrations tested.

Storm channel.

Toxicity Identification Evaluation (TIE)

Preliminary TIE analyses were done on samples collected from Ballona Creek on 10/14/92 (3 days after 0.06 in of rain) and 1/19/93 (1 day after 1.37 in of rain). Short-term chronic tests with sea urchins were conducted on dry-weather samples collected just before the samples for TIE were collected. Morning and afternoon samples were tested for toxicity separately and samples with the highest toxicity were selected for the TIE.

The TIE manipulations and toxicity testing occurred within two days of sample collection for both periods. Runoff samples were tested again during TIE analyses to detect anomalies introduced by storage and differences among test animals. Time constraints imposed by the large number of samples forced us to reduce the number of replicates to two and to omit the reference toxicant series.

To characterize the constituents responsible for the toxicity, dry-weather runoff components were isolated by physical and chemical characteristics. A C18 solid phase extraction column was used to remove hydrophobic compounds (i.e., nonpolar organics). EDTA was added to the sample to chelate divalent metals. Oxidants were neutralized by adding sodium thiosulfate.

Solid Phase Extraction

A C18 column was used to evaluate dry-weather runoff for constituents resembling nonpolar organics (Mount and Anderson-Carnahan 1989). After filtration, a 1 L sample was pumped through a prepared C18 column and three solutions (50% MeOH, 100% MeOH, 50% MeOH-50% $MeCl_2$) were added sequentially to elute nonpolar constituents. Contaminants were removed based on their hydrophobicity (least to most). The solid phase elutriate solutions were added to the column in 2 mL aliquots. Post-column elutriates were diluted to 0.1% and 0.2% v/v with seawater to adjust nonpolar organic concentrations to 50% and 100% runoff. Elutriate blanks (solvent passed through a clean C18 column) were tested to control for solvent toxicity. Filtered pre-C18 samples, post-C18 samples, and column blanks were included in the testing at concentrations of 12, 25, and 56%.

EDTA Additions

To evaluate dry-weather runoff for constituents resembling divalent metals, EDTA was added to dry-weather samples at 3 and 8 mg/L (Norberg-King *et al*. 1992). Dry-weather samples were tested at concentrations of 12%, 25%, and 56%; EDTA controls were included.

Thiosulfate Additions

To evaluate dry-weather runoff for constituents resembling oxidants, 10 and 25 mg/L of sodium thiosulfate was added to the samples (Norberg-King *et* al. 1992). Samples were tested at concentrations of 12%, 25%, and 56%; thiosulfate controls were included.

Data Analyses

The no observed effect concentration (NOEC) was determined for each treatment by analysis of variance and Dunnett's multiple comparison test (Zar 1984). Taw data were transformed by arcsine for analysis for the sea urchin and abalone tests, but not for the kelp test. Probit analysis was used to estimate the sample concentration that produced the median toxic response (EC50).

Results

Spatial and Temporal Toxicity

Dry-weather samples from Ashland storm drain were usually the most toxic samples tested during each period (Table 1). Dry-weather samples from Pico-Kenter storm drain produced a consistent toxic response in abalone. Only one Pico-Kenter sample was toxic to kelp and urchins. Dry-weather samples from Ballona Creek were toxic only to sea urchins. Temporal variability was high and a different concentration of dry-weather flow produced toxicity in each of the three sea urchin tests (Table 1), which is evident in

Table 1.

The no observed effect concentration (NOEC) for samples of dry-weather flow from storm drains that discharge into Santa Monica Bay. Three marine toxicity test methods described in the California Ocean Plan were used: red abalone embryo development test, giant kelp germination and germ tube growth test, and sea urchin fertilization test. nd=no data; unacceptable results were obtained for the seawater control.

		NOEC (PERCENT EFFLUENT)			
LOCATION	SAMPLING DATE	Abalone	Kelp germination	Kelp length	Sea urchin
Ashland	8/24‘/92	<5.6	18	18	10
	9/29/92	nd	nd	nd	5.6
	10/12/92	5.6	5.6	5.6	<5.6
Ballona Creek	9/8/92	≥56	≥56	≥56	<5.6
	9/29/92	nd	nd	nd	12
	10/12/92	≥56	≥56	≥56	≥56
Pico-Kenter	8/24/92	18	≥56	≥56	≥56
	9/29/92	nd	nd	nd	≥56
	10/12/92	12	≥56	25	25
Sepulveda Channel	9/8/92	≥56	≥56	≥56	10

Table 2.

The median effect concentration (EC50) for dry-weather flow from storm drains that discharge into Santa Monica Bay. nd=no data; unacceptable results were obtained from the seawater control for these species. nc=not calculated; unusual dose-response prevented calculation.

		EC50 (PERCENT DRY-WEATHER FLOW)			
LOCATION	SAMPLING DATE	Abalone	Kelp germination	Kelp length	Sea urchin
Ashland	8/24/92	6.8	32	>56	17
	9/29/92	nd	nd	nd	14
	10/12/92	10	22	50	<5.6
Ballona Creek	9/8/92	>56	>56	>56	14
	9/29/92	nd	nd	nd	>56
	10/12/92	>56	>56	>56	>56
Pico-Kenter	8/24/92	42	>56	>56	>56
	9/29/92	nd	nd	nd	>56
	10/12/92	21	>56	>56	41
Sepulveda Channel	9/8/92	>56	>56	>56	nc

the EC50 data (Table 2). The dry-weather sample from Sepulveda Channel was only toxic in the sea urchin fertilization test (Table 1). Water quality data were measured in the storm drains (Table 3).

Toxicity Identification Evaluation

The afternoon sample collected from Ballona Creek in December 1992 was more toxic than the morning sample; the afternoon sample was used for the toxicity identification evaluation. The C18 column reduced toxicity and the thiosulfate treatments completely removed it (Table 4). The EDTA addition partially removed toxicity from the 56% runoff samples. Elution of the C18 column with 100% methanol successfully recovered a portion of the toxicity in the aqueous sample.

The morning sample collected from Ballona Creek in January 1993 was more toxic than the afternoon sample; the morning sample was used for the TIE. The C18 column reduced toxicity slightly, addition of 3 mg/L EDTA completely removed it, and addition of thiosulfate had no effect (Table 4).

Discussion

Toxicity in the dry-weather storm drain samples was spatially and temporally variable. Ashland samples were usually the most toxic and the

Table 3.

Physical and chemical water quality parameters for dry-weather flow samples collected in August, September, and October 1992 from storm drains that discharge into Santa Monica Bay. Data are means and standard deviations. COD = chemical oxygen demand, DO = dissolved oxygen, TDS = total dissolved solids, TSS = total suspended solids, VSS = volatile suspended solids, DOC = dissolved organic carbon. The pH of Ballona Creek and Sepulveda Channel samples was adjusted to 7.9-8.2 before testing. Data were collected by UCLA.

CONSTITUENTS	ASHLAND STORM DRAIN	BALLONA CREEK	PICO-KENTER STORM DRAIN	SEPULVEDA CHANNEL
pH	7.6 ± 0.2	8.8 ± 0.2	7.6 ± 0.1	8.7
COD (mg/L)	252 ± 64	51 ± 29	88 ± 38	73
DO (mg/L)	1.6 ± 0.3	>15	6.6 ± 0.8	>15
TDS (mg/L)	6,058 ± 4,045	1,625 ± 624	1,493 ± 841	4,071
TSS (mg/L)	299 ± 476	8 ± 5	103 ± 71	13
VSS (mg/L)	86 ± 117	5 + 4	42 + 33	7
DOC (ppm)	34 + 14	9 + 3	15 + 1	16
Salinity (ppt)	2.2 + 1.1	1.2 + 0.1	0.9 + 0.6	2.1
Ammonia (mg/L as NH_3-N)	0.76 + 0.46	0.05 + 0.05	0.11 + 0.10	0.06
Alkalinity (mg/L as $CaCO_3$)	357 + 13	212 + 6	260 + 16	145
Hardness (mg/L as $CaCO_3$)	1,080 + 725	722 + 315	353 + 160	1,434
Detergent (ppm as LAS)	2.50 ± 1.80	0.75 ± 0.66	0.75 ± 0.25	0.50

magnitude of toxicity was similar between sampling times. There was no clear distinction between the levels of toxicity in samples from Ballona Creek and Pico-Kenter storm drain. Samples from Pico-Kenter were toxic to abalone embryos in all tests, but only one sample was toxic to sea urchin sperm. The toxicity of Ballona Creek dry-weather to sea urchin sperm ranged from strong (NOEC <5.6%, EC50=14) in the first sample to nontoxic (NOEC >56%, EC50 >56) in the last sample. Abalone and kelp were unaffected by samples from Ballona Creek. Sepulveda dry-weather flow was toxic only in the sea urchin test. The responses of the test species indicate that one organism cannot predict the relative toxicity of dry-weather flows among storm drains.

Temporal variability in toxicity occurred over short time scales. In December 1992, dry-weather samples collected in the afternoon from Ballona Creek were more toxic than samples collected in the morning. In January 1993, dry-weather samples collected in the morning were only slightly more toxic than samples collected in the afternoon.

Spatial and temporal differences in storm drain sample toxicities indicated that the composition of dry-weather urban runoff was variable. This could reflect the transience of particular toxicants, or synergistic or antagonistic interactions between runoff constituents. Changes in chemical speciation may also influence the effect of toxic contaminants. For example, after two days of storage, toxicity in the December dry-weather sample collected for TIE declined from NOEC <12% to NOEC=12%. This

Table 4.

Toxicity identification evaluation (TIE) of Ballona Creek filtered dry-weather samples. The C18 solid phase extraction column removed hydrophobic compounds. EDTA was added to chelate divalent metals. Oxidants were neutralized by adding sodium thiosulfate. To adjust concentrations to 50% and 100% runoff, column elutriates were diluted to 0.1% and 0.2% v/v with seawater. Only results from the 56% effluent treatment are presented for pre-column filtrates, post-column filtrates, EDTA additions, and thiosulfate additions. The pre-C18 column filtrates are dry-weather flow samples before TIE manipulation. MeOH=methanol; $MeCl_2$=methylene chloride; np=not performed.

TREATMENT	SEA URCHIN FERTILIZATION (%) December 1992	January 1993
Pre-C18 column filtrate (reference)	15	16
Post-C18 column filtrate	76	20
C18 column eluates		
50% MeOH (0.1%)	100	88
50% MeOH (0.2%)	100	100
100% MeOH (0.1%)	94	52
100% MeOH (0.2%)	7	56
50% MeOH-50% $MeCl_2$ (0.1%)	100	93
50% MeOH-50% $MeCl_2$ (0.2%)	63	100
EDTA addition		
3 mg/L	44	92
8 mg/L	12	96
30 mg/L	np	92
Thiosulfate addition		
10 mg/L	99	10
25 mg/L	98	12

could have also resulted from sensitivity differences between test organisms. Because the reference toxicant series was omitted, the relative sensitivities were unknown.

Ashland dry-weather flow had the highest toxicity and the most degraded water quality (high ammonia, low dissolved oxygen, and high salinity; Table 3). Ashland dry-weather flow was frequently blocked from reaching the ocean creating a pool of brackish, stagnant water. Water quality in the field did not correspond exactly to water quality in the laboratory during testing. Effluents were filtered and diluted to 56% or less with seawater or brine for the bioassays.

Results of TIE experiments suggest that the composition of dry-weather urban runoff was variable. The first TIE experiment indicated the presence of toxicants with properties of metals, oxidants, and nonpolar organics; but additions of thiosulfate completely removed toxicity. The data suggested either a synergistic effect by several contaminants, or the presence of a single compound with properties of metals, nonpolar organics, and oxidants. In the second TIE experiment, toxicity was completely removed by the 3 mg/L EDTA addition while other manipulations did not alter it, indicating that toxicity was due to contaminants resembling metals.
The test organisms used in this study are environmentally relevant; they occur in Santa Monica Bay and could be exposed to the contaminants in dry-weather flows. However, we did not consider the fate of the contaminants, the routes of exposure, or the intensity, frequency, and duration of exposure.

Conclusions

The study demonstrated spatial and temporal variability in toxicity for four significant sources of urban dry-weather flow to Santa Monica Bay. Further research is needed to identify the contaminants responsible for the toxicity. The responses of the test species indicate that no single test organism can predict the relative toxicity of dry-weather effluents among storm drains. Once we understand which contaminants have the greatest impact on marine organisms and which storm drains are the primary sources of these contaminants, management of urban runoff will be more successful.

References

- Anderson, B.S., J.W. Hunt, S.L. Turpen, H.R. Barber-McNutly, M. Martin, D.L. McKeown, and F.H. Palmer. 1991. Marine Bioassay Project sixth report; Interlaboratory comparison and protocol development with four marine species. Report No. 91-21WQ. California Water Resources Control Board, Sacramento. 204 pp.
- Dinnel, P.A., J.M. Link, and Q.J. Stober. 1987. Improved methodology for a sea urchin sperm cell bioassay for marine waters. *Arch. Environ. Contam. Toxicol*. 16:23-32.
- Mount, D.I. and L. Anderson-Carnahan. 1989. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures. EPA/600/3-88/035. US EPA, Duluth, MN.
- Norberg-King, T.J., D.I. Mount, J.R. Amato, D.A. Jensen, and J.A. Thompson. 1992. Toxicity identification evaluation: Characterization of chronically toxic effluents, phase I. EPA/600/6-91/005F, US EPA. Duluth, MN.
- SCCWRP see *Southern California Coastal Water Research Project*
- Santa Monica Bay Restoration Project. 1993. Reduce pollution at the source. Pp. 14-99, *In*: Santa Monica Bay Restoration Project Action Plan, 1993. Santa Monica Bay Restoration Project, Monterey Park, CA.
- SMBRP see *Santa Monica Bay Restoration Project*
- SWRCB see *State Water Resources Control Board*
- Southern California Coastal Water Research Project. 1989. Toxicity of stormwater runoff in Los Angeles County. Pp. 66-71, *In*: Annual Report, 1988-89. Southern California Coastal Water Research Project, Long Beach.
- Southern California Coastal Water Research Project. 1992. Surface runoff to the Southern California Bight. Pp. 19-28, *In:* Annual Report, 1990-91 and 1991-1992. Southern California Coastal Water Research Project, Long Beach.
- State Water Resources Control. 1990. Water Quality Control Plan, Ocean Waters of California. California Ocean Plan, State Water Resources Control Board, Sacramento. 23 pp.
- Zar, J.H. 1984. Biostatistical analysis, second edition. Prentice-Hall, Inc., Englewood Cliffs, NJ. 718 pp.

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Pipetting a sample.