# CONTAMINANTS IN URBAN RUNOFF AND THEIR IMPACT ON RECEIVING WATERS

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#### ABSTRACT

This paper describes a sampling program to determine the potential impact of contaminants in urban runoff introduced to Santa Monica Bay through low flow (dry weather) conditions. A sampling protocol was developed whereby four selected storm drains were sampled and analyzed for conventional water quality parameters (e.g., chemical oxygen demand, total suspended solids, etc.), and short-term chronic toxicity using three marine organisms (red abalone *Haliotus rufescens*, purple sea urchin *Strongylocentrotus purpuratus*, and giant kelp *Macrocystis pyrifera*). The obtained results show that some of water quality parameters (e.g., chemical oxygen demand) of the selected storm drains were comparable or worse than typical secondary effluents. Several storm drains also exhibited toxicity. However, the most likely source of toxicity varied from metals to organic contaminants.

#### **KEYWORDS**

EC50; solid phase extraction; storm drain; toxicity; urban runoff; water quality

# INTRODUCTION

In highly developed areas such as Los Angeles, most wastewaters are treated to secondary standards or beyond. Consequently there is very little opportunity to improve the quality of degraded receiving waters through additional wastewater treatment; other sources of contaminants to receiving waters, such as Santa Monica Bay, must be found and controlled. Originally urban runoff (stormwater runoff, low flow, nuisance flow from urban areas) was thought to be free from contaminants. Recent work has shown that it can be a significant source of contaminants, and is largely unregulated. To further improve the quality of receiving waters the United States has begun to better control urban runoff pollution through a series of permits (e.g., NPDES) and best management practices (e.g., porous pavement).

This manuscript describes a sampling program to determine the impact of contaminants in urban runoff introduced to Santa Monica Bay through low flow (dry weather) conditions. Four storm drains were selected as representative of the entire watershed. Sampling was conducted over 10 periods that involved compositing manually collected grab samples. The collected storm drain samples were analyzed for conventional water quality parameters (e.g., ammonia, chemical oxygen demand, total suspended solids, etc.), and short-term chronic toxicity using three marine organisms.

# EXPERIMENTAL PROCEDURES

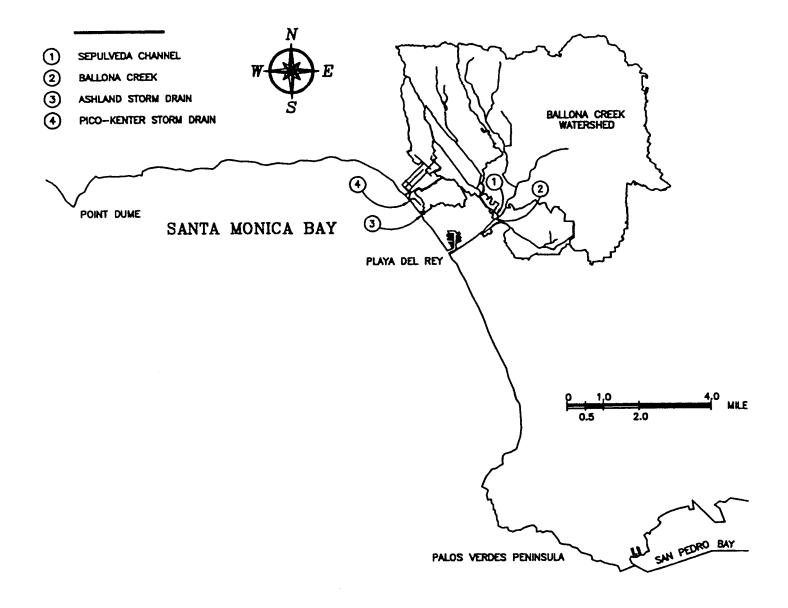
# Sampling Locations and Procedures

Four storm drains in the Santa Monica Watershed were selected for toxicity evaluation (Pico-Kenter, Ashland Avenue, Ballona Creek at Inglewood and Sepulveda Channel at Ballona Creek). The first two storm drains were named with reference to their neighboring street. Figure 1 shows the location of these four storm drains. Ten samples were collected between April 1992 and December 1992.

Samples were bailed from the storm drains using a stainless steel bucket. Morning and afternoon grab samples were collected into a 2- or 4-L glass bottle, composited, and stored in ice chests with blue-ice packs before being transported to the laboratory. All samples were stored in a refrigerator at 4°C until the time of analysis. The time between sample collection and analysis was within the recommended holding time by US EPA (1974).

#### **Reagents and Materials**

Analytical grade or better chemicals and HPLC grade solvents (e.g., methanol and methylene chloride) were used for the chemical analyses and solid phase extraction. The 1000 mg C18 solid phase extraction columns were obtained from Burdick and Jackson (Muskegon, MI).



# Fig. 1. Sampling location of the selected storm drains.

# TABLE 1. CONVENTIONAL WATER QUALITY PARAMETERS

# **Conventional Chemical Analysis**

Conventional water quality analyses were performed on the collected storm drains samples. The analyzed water quality parameters are listed in Table 1. All the parameters, except the uv absorbance, were analyzed according to the *Standard Methods* (1989) procedures. The uv-absorbance of the collected storm drain samples (filtered) was measured at a wavelength of 254 nm, using Hewlett-Packard HP 8452A Diode Array Spectrophotometer. The measured uv-absorbance is a qualitative measure of the amount of organic compounds in the samples.

# Solid Phase Extraction

The C18 SPE method described by Mount and Anderson-Carnahan (1989) was the basis of our fractionation procedures to isolate nonpolar organic compounds from the collected storm drain samples. The following procedure was used to fractionate nonpolar organic compounds from the collected samples.

Filter blank. A 1  $\mu$ m glass fiber filter (Whatman GF/B) was prepared by first acid washed with 10% HNO<sub>3</sub> acid and then rinsed thoroughly with deionized water. Subsequently, approximately 200 ml of deionized water was passed through the filter and, the last 30-50 ml of filtrate were collected for the filter toxicity blank. The storm drain sample was then filtered.

Column blank. The 1000 mg C18 SPE columns were conditioned by pumping through 25 ml of HPLC grade methanol through the column at a flow rate of 5 ml/min. Before the sorbent dried, approximately 50 ml of deionized water was pumped through the column. The last 25-30 ml deionized water was collected for a column blank toxicity test. Pumping continued until no water emerged from the column.

*Elution blank.* Three elution blanks were collected from the prepared column by passing  $2 \times 1.0$  ml of each of the following solvent: 50% (v/v) methanol in water, 100% methanol, and 50% (v/v) methylene chloride in methanol. The eluates were collected in a clean glass vial as the SPE elution blanks. The column was allowed to dry between each elution.

SPE fractionation. The same C18 SPE column was again conditioned with 25 ml of methanol and 25 ml of deionized water. Before the sorbent dried, 1000 ml of filtered storm drain sample was pumped through the column at a rate of 5 ml/min. Two 30-40 ml samples of the post C18 column were collected after 500 ml of the sample passes through the column. The sorbent was dried by continuing the pumping after the whole 1000 ml sample passed through. Then 2 x 1.0 ml of methanol/water, methanol and methylene chloride/methanol were added sequentially into the column. Each fraction was collected into clean glass vials. The column was allowed to dry prior addition of each elution solvent mixture.

Toxicity testing was performed on the filtered sample, post C18 sample, the SPE eluates and all the blanks (e.g., filter blank, column blank and elution blank).

# **Toxicity Procedures**

Three marine test methods described in the California Ocean Plan (SWRCB, 1990) were used in this study: the echinoderm fertilization test, red abalone embryo development test, and giant kelp germination/germ tube growth test. Storm drain samples were stored under refrigeration in sealed 4-L glass bottles until the day of testing. Samples were thoroughly mixed before a 2.5-L subsample was removed and filtered through 1  $\mu$ m glass fiber filter (Whatman GF/B).

The toxicity tests were conducted in two phases: Phase I - Relative Toxicity of Storm Drains and Phase II - Examination of Toxic Components. Seawater dilutions of each sample were prepared by adding appropriate amounts of seawater and brine solutions to create the desired dilutions and maintain a salinity of 32-35 mg/g. Dilutions containing 5.6, 10, 18, 32, and 56% (v/v) storm drain samples in seawater were prepared for each location. Toxicity test organisms were added to each sample within three hours of dilution.

Echinoderm fertilization test. The echinoderm fertilization test was conducted according to methods described by Dinnel et al. (1987). Purple sea urchins Strongylocentrotus purpuratus were collected from the intertidal in northern Santa Monica Bay and held at SCCWRP until used in the tests. Ten ml of each sample dilution was added to replicate glass test tubes and equilibrated to 15°C in a water bath. Sea urchins were then induced to spawn through injection of potassium chloride. The gametes were collected and diluted with seawater to produce stock solutions of the density recommended by the protocol. The test was conducted by adding sperm to each test tube. After 60 min of sperm exposure, eggs were added to each tube for a 20 min fertilization period. The sample was then preserved for microscopic examination. Toxic effects were indicated by a reduction in the percentage of fertilized eggs from that observed in a control sample (seawater).

Abalone development test. The abalone development test, using embryos of the red abalone Haliotus rufescens, was conducted according to methods described by Anderson *et al.* (1990). Sexually mature abalone were obtained from a commercial aquaculture facility and held at SCCWRP until used in the tests. Two hundred mls of each sample dilution were added to replicate 250 ml glass beakers and placed in a 15°C water bath. Abalone were induced to spawn by exposure to uv-irradiated seawater. The eggs were then fertilized, adjusted in density, and added to the exposure beakers. The developing embryos were exposed for 48 hours and preserved for microscopic examination. Toxic effects were indicated by an increased incidence of larvae with abnormally developed shells.

Giant kelp test. Tests with giant kelp were conducted according to the procedures described by Anderson *et al.* (1990). Kelp blades containing reproductive spores (sporophyll) were obtained from offshore, uncontaminated kelp beds located near Santa Barbara and used within 24 hours. The toxicity test was conducted in 250 ml beakers containing 200 ml of the sample dilution. A glass microscope slide was placed on the bottom of each beaker to provide a surface for settlement of the kelp spores. Zoospore release from the sporophyll blades were induced by desiccation followed by immersion in seawater. The density of the released spores was adjusted and the appropriate number of spores were added to each beaker. The spores were exposed to the sample dilutions for 48 hours at 15°C and a controlled light level ( $50\mu \text{Em}^{-2}\text{sec}^{-1}$ ). During this period of 48 hours, the spores germinated and formed gamethphyte plants. The slides were then removed from each beaker and preserved for microscopic examination. Two endpoints were assessed: percentage spore germination and gametophyte length. Toxic effects were indicated by reductions in germination and gametophyte length, relative to a control group.

#### **EDTA and Sodium Thiosulfate Addition Tests**

EDTA and sodium thiosulfate addition tests described by Norberg-King et al. (1992) were conducted during the second phase of the toxicity test. The unfiltered storm drain samples with EDTA or sodium thiosulfate were analyzed for toxicity using the echinoderm fertilization test.

EDTA addition test. A stock solution of EDTA was prepared and added into 30 ml unfiltered storm drain samples. The final concentrations of EDTA in the samples were 3, 8, and 30 mg/L. Three different dilutions, 12%, 25% and 56%, were prepared from these EDTA-added samples and used for the toxicity test.

Sodium thiosulfate dddition test. A stock solution of sodium thiosulfate was prepared and added into 30 ml of unfiltered storm drain samples. The final concentrations of sodium thiosulfate in the samples were 10 and 25 mg/L. Similar to the EDTA addition test, three dilutions, 12%, 25% and 56%, were prepared and used for the toxicity test.

#### RESULTS AND DISCUSSION

#### Summary of Water Quality Data

Table 2 summarizes the water quality data for the storm drain samples. The mean and standard deviation of each water quality parameter over 10 sampling periods are given. The results show that water quality of the storm drain at Ashland Avenue is the worst among the four selected storm drains. The Ashland Avenue storm drain is stagnant during low flow periods. During high tides, sea water may enter the drain, which was detected by high total dissolved solids (TDS) concentration. Ashland Avenue is the only drain that has a tidal interaction. Table 2 also shows that samples from the Sepulveda Channel have high total dissolved solids (TDS) and hardness. The high TDS concentration results from ion exchange regeneration waters released by permit to this storm drain. The dissolved oxygen (DO) concentration in both Ballona Creek and the Sepulveda Channel were greater than the saturation concentration because of photosynthesis; both drains are open channels and have abundant algae.

Parameters	Pico-Kenter	Ashland Avenue	Ballona Creek	Sepulveda Channel
Alk (mg/L as CaCO3)	$266 \pm 36$	$316 \pm 64$	$233 \pm 40$	$176 \pm 49$
Hardness (mg/L as CaCO3)	287 ± 90	$1290 \pm 1122$	675 ± 349	$1513 \pm 792$
Conductivity (µmho/cm)	1795 ± 927	$7560 \pm 6702$	$2052 \pm 919$	$4852 \pm 1411$
TDS (mg/L)	$1050 \pm 510$	$4618 \pm 4323$	$1445 \pm 795$	3346 ± 3346
TSS (mg/L)	49 ± 55	365 ± 475	47 ± 65	$24 \pm 32$
VSS (mg/L)	$21 \pm 25$	86 ± 101	9±9	9±6
COD (mg/L)	66 ± 35	$249 \pm 61$	$41 \pm 18$	$70 \pm 16$
DOC (mg/L)	$31 \pm 32$	$46 \pm 18$	$28 \pm 33$	29 ± 27
Turbidity (NTU)	$15.5 \pm 13$	$145.4 \pm 208.2$	$23.3 \pm 43.9$	$7.3 \pm 12.2$
DO (mg/L)**	$7 \pm 1.3$	$3.3 \pm 2.6$	$13.7 \pm 1.1$	$14.5 \pm 0.5$
рН	8 ± 0	$7.6 \pm 0$	$8.6 \pm 0.5$	$9.2 \pm 0.3$
Ammonia (mg/L as NH3-N)	$0.18 \pm 0.22$	$0.84 \pm 0.96$	$0.28 \pm 0.33$	$0.22 \pm 0.49$
Nitrite (mg/L as NO <sub>2</sub> -N)	$0.10 \pm 0.05$	$0.12 \pm 0.18$	$0.10 \pm 0.08$	$0.16 \pm 0.15$
uv absorbance (at 254 nm)	$0.407 \pm 0.102$	$0.870 \pm 0.339$	$0.172 \pm 0.051$	$0.173 \pm 0.053$

TABLE 2. SUMMARY OF WATER QUALITY DATA FOR THE SELECTED STORM DRAINS.

Note: **\*\*** measured in the field

Parameter	Location					
	Pico-Kenter	Ashland	Ballona Creek	Sepulveda Ch.	Effluent	
COD (mg/L)	72	249	41	70	~50-100	
TSS (mg/L)	49	365	47	24	< 30	
Turbidity (NTU)	15.5	145.5	23.3	7.3	< 2.2	
DO (mg/L)	7	3.3	13.7	14.5	> 2	
pH	8	7.6	8.6	9.2	~ 6-9	
Ammonia (mg/L as NH3-N)	0.18	0.84	0.28	0.22	< 2	

TABLE 3. COMPARISON OF WATER QUALITY OF STORM DRAIN SAMPLES AND SECONDARY EFFLUENT.

At various sampling times, the water quality of some of the storm drains was comparable or worse than typical secondary effluents. Table 3 show the selected water quality parameters comparison between the storm drain samples and typical secondary effluent. The obtained results show that the chemical oxygen demand (COD) of water samples from Ashland Avenue is much greater than the value of typical secondary effluents prior discharge to the receiving waters. Similar observation was made on the total suspended solids (TSS) of the analyzed storm drain samples. Therefore, urban runoff controls should be evaluated in programs for improving the quality of receiving waters.

# Toxicity

*Phase I - Relative toxicity of storm drains.* The objectives of this phase of toxicity testing were to determine the most toxic storm drain among the four selected sampling locations, and also to determine the most sensitive test organism among the three test species. Initially, samples were collected from the selected storm drains and tested for toxicity according to the previously described procedures. For each toxicity test, except kelp germ tube test, the percentage response of the organisms at each tested dilution/concentration of the collected storm drain samples was calculated; for the kelp germ tube test, the mean length of the kelp germ tube was measured instead. These dose-response results were then plotted versus the various concentration of the samples used in the toxicity tests. Figure 2 shows examples of dose-response plots for abalone, sea urchin, giant kelp germination and germ tube length tests.

From the dose-response plots, EC50 values, i.e., effective concentration that caused 50% toxic effect on the test organisms, can be obtained. The obtained EC50 values are used as the indicator of relative toxicity; lower EC50 values indicate greater toxicity. Table 4 shows the obtained EC50 values from Phase I of the toxicity testings for the four selected storm drains. The EC50 values for Ashland Avenue are the lowest among the four selected storm drains, followed by Pico-Kenter and Ballona Creek. Table 4 also shows that most of the samples from Ballona Creek and Sepulveda Channel have an EC50 greater than 56%, suggesting very little toxicity for our test conditions. An inconsistent pattern of toxicity was found in the urchin test of the Sepulveda Channel sample collected on Sept. 8 and thus the EC50 value was not able to be determined (see Figure 2a). For samples collected on Sept. 29, the EC50 of the abalone and kelp tests was not determined due to technical difficulties which prevented the measurement of toxicity.

The Ashland Avenue storm drain was the most toxic to each test organism and consistently produced the greatest toxicity in all tests conducted. No clear distinction between the relative toxicity of the Ballona Creek and Pico-Kenter storm drains was observed. The abalone test was more sensitive to Pico-Kenter samples, with kelp test being the least sensitive. Ballona Creek samples produced the greatest toxic effects on sea urchin sperm while the abalone and kelp tests were unaffected by samples from this storm drain.

*Phase II - Examination of toxic components.* The objective of this phase of toxicity testing was to determine the type of compounds (e.g., organics or metals) that caused the toxicity in the selected storm drain. Based on the toxicity results from Phase I, Ballona Creek storm drain was selected for this phase and the organism selected for toxicity testing was the sea urchin. Even though the relative toxicity of this location is not as great as Ashland Avenue, the annual input of runoff from Ballona Creek to the Santa Monica Bay is much greater than the other storm drains.

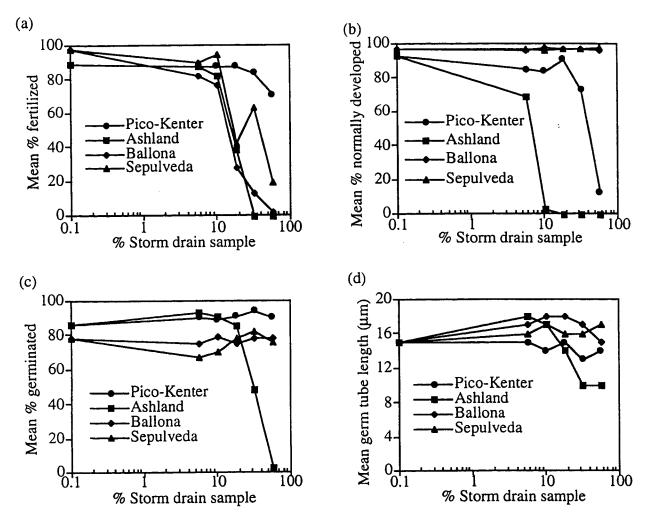


Fig. 2. Example dose response plot of various toxicity tests. (a) purple sea urchin development test; (b) red abalone development test; (c) kelp germination test; (d) kelp germ tube test. Control values are those plotted at a concentration of 0.1%. Pico-Kenter and Ashland samples were collected on Aug. 24 '92 and Ballona and Sepulveda samples were collected on Sept. 8 '92.

		EC50					
Location	Sampling	Abalone	K	Urchin			
	Date	Development	Germ.	Length	Fertilization		
Pico-Kenter	Aug. 24 '92	42	> 56	> 56	> 56		
	Sept. 29 '92	nd	nd	nd	> 56		
	Oct. 12 '92	21	> 56	> 56	41		
Ashland Avenue	Aug. 24 '92	6.8	32	> 56	17		
	Sept. 29 '92	nd	nd	nd	14		
	Oct. 12 '92	10	22	50	< 5.6		
Ballona Creek	Sept. 8 '92	> 56	> 56	> 56	14		
	Sept. 29 '92	nd	nd	nd	> 56		
	Oct. 12 '92	> 56	> 56	> 56	> 56		
Sepulveda Channel	Sept. 8 '92	> 56	> 56	> 56	nt		

TABLE 4. EC50 VALUES FOR STORM DRAINS SAMPLES (PHASE I).

Note: All values are in % storm drain samples. EC = effective concentration to cause 50% toxic effect; nd = not determined as technical difficulties prevented measurement of toxicity; nt = toxicity found but data not amenable to testing for EC50 (see Fig. 2a).

Two samplings were performed during this phase and the sampling procedures were slightly different than previous samplings. Grab samples from morning and afternoon were collected separately. Preliminary toxicity tests were performed on these two grab samples in order to determine which grab sample had a higher level of toxicity. Solid phase extraction (SPE) was then performed on the grab sample which exhibited greater toxicity. Samples collected from the extraction (e.g., SPE eluates, post C18, column blanks, etc.) were tested for toxicity.

Currently, most of the methods used for toxicity-based (bioassay-directed) fractionations required the extraction of the sample with an organic solvent and after some preliminary clean-up, and subsequent fractionation of the extract using normal-phase chromatography. The solvent systems used are extremely toxic to aquatic organisms (Burkhard *et al.*, 1991) and when these methods are used, solvent exchange and/or evaporation procedures are required before toxicity testing can be done. Losses of volatile toxicants can occur during these steps which may bias results

The octadecyl (C18) solid phase extraction procedure used in this project was based on the method developed by Mount and Anderson-Carnahan (1989). This toxicity-based method has been successfully used to extract and fractionate nonpolar toxicants from the effluents for toxicity tests using cladocerans (water fleas) and fishes. In addition, low artifactual toxicity and excellent detection limits for gas chromatography/mass spectrometry (GC/MS) for toxicants identification can be obtained from this method. However, a preliminary recovery study showed that highly hydrophobic compounds such as chrysene and benzo(a)pyrene cannot be eluted from the C18 sorbent by the elution solvents used by Mount and Anderson-Carnahan. Therefore a modified elution solvent system which consists of three fractions were used, i.e., 50% (v/v) of methanol in water, 100% of methanol, and 50% (v/v) of methylene chloride in methanol. These elution solvents fractionate the organic compounds in the samples into three fractions based on their polarity. The most polar compounds are eluted into the first fraction (i.e., the 50% methanol fraction) and most nonpolar compounds are eluted into the third fraction (i.e., the 50% methylene chloride fraction).

The three SPE eluates were tested for toxicity using the urchin fertilization test. Two dilutions were used for the test, i.e., 0.1% and 0.2%, which corresponds to 50% and 100% of storm drain sample after including the 500 fold increase obtained through the SPE procedures (the concentration factor of 500x was obtained based on a sample volume of 1000 ml and elution volume of 2 ml). Table 5 shows the percentage fertilization of the SPE eluates (which has been normalized for blank response), post C18 effluents and the filtrates (pre-C18) of the Ballona Creek samples collected during this phase. The results show that the 100% methanol fraction was the most toxic among the three eluates for both sampling periods. Little or no toxicity was present in the first and third eluate. The results suggest that most of the toxicants were present in the 100% methanol fraction for both sampling periods. Table 5 also shows the toxicity results of pre- and post C18 samples at the highest concentration tested. For the Dec. 14 afternoon sample, an improvement of toxicity was observed after the sample passed through the C18 column. The post C18 sample showed greater percentage fertilization (76%) than the untreated (pre-C18) sample (which only has 15% fertilization). This observation suggests that the C18 column removed toxicity and organic toxicants were most likely present in the sample. For the Jan. 19 morning sample, no reduction of toxicity was observed in the post C18 sample and only moderate toxicity was observed in the 100% methanol fraction. Normally this results would suggest the presence of metals, which are not removed by the C18 column; however, in this case it is not conclusive due to poor fertilization in the column blank. The presence of metals and other toxicants such as oxidative compounds in the samples can be confirmed by the EDTA and sodium thiosulfate addition tests.

Sampling date and	Filtrate		SPE Eluates				Post	
grab sample	(pre-C18)	50% 1	MeOH	100%	MeOH	50% N	MeCl <sub>2</sub>	C18
analyzed	56%	0.1%	0.2%	0.1%	0.2%	0.1%	0.2%	56%
Dec. 14 ' 92 PM	15	100	100	94	7	100	63	76
Jan. 19'93 AM	16	88	100	52	56	93	100	20

# TABLE 5. TOXICITY RESULTS OF SOLID PHASE EXTRACTION (SPE) SAMPLES.

Note: All values are the mean of two replicates.

The objective of EDTA addition test is to detect toxicity caused by certain cationic metals. Non-toxic complexes will be formed after EDTA addition to the collected storm drain samples. Loss of toxicity with EDTA addition suggests that cationic metals are causing toxicity. The sodium thiosulfate addition test can detect toxicity caused by oxidative compounds (such as chlorine) and other compounds (such as copper and manganese). Toxicity from bromine, iodine, ozone, and chlorine dioxide is also reduced by the addition of sodium thiosulfate (Norberg-King *et al.*, 1992). The toxicity results of EDTA and sodium thiosulfate addition tests are shown in Table 6. For the sample collected on Dec. 14, sodium thiosulfate reduced toxicity while EDTA only partially reduced the toxicity. This indicates that oxidative compounds may have caused toxicity in the Dec. 14 sample. Reverse results were obtained for the sample collected on Jan. 19, 1993. High percentage fertilization (Table 6) was observed in the samples with added EDTA while low percentage fertilization was observed in the thiosulfate had no effect on the sample toxicity. Therefore, cationic metals may be present in the Jan. 19 sample and thus causing the toxicity.

The toxicity results obtained from this phase were variable and not conclusive due to the insufficient number of samples tested. For example, for Dec. 14 sample, toxicity was found in the raw sample (pre-C18 sample), the 100% methanol eluate and EDTA addition test whereas no toxicity was found in the thiosulfate addition test and post C18 sample. It is not clear what might cause this type of toxicity, but an organic oxidant is possible; it would be reduced by the thiosulfate and through adsorption onto the C18 column. Other possibilities also exist. More toxicity tests should be performed in order to determine these variabilities.

# TABLE 6. TOXICITY RESULTS OF THE EDTA AND SODIUM THIOSULFATE ADDITION TESTS.

Sampling	E	EDTA Addition			Thiosulfate Addition		
Date	3 mg/L	8 mg/L	30 mg/L	10 mg/L	25 mg/L		
Dec. 14 ' 92	44	12	-	99	98		
Jan. 19 ' 93	92	96	92	10	12		

Note: All values are the mean value of percentage fertilization at a concentration of 56%.

#### CONCLUSIONS

Water quality and relative toxicity of four storm drains in Santa Monica Watershed during the low flow conditions were analyzed. The water quality of the selected storm drains varied during the sampling periods and was often comparable or worse than typical secondary effluents. This indicates the need to control and regulate runoff into the storm drains in order to improve the water quality of the receiving waters. Short- term chronic toxicity tests also show that significant toxicity was present in the selected storm drains. Probable sources of the toxicity varied from metals to organic contaminants. More samplings are needed to determine the variability of the toxicity. Toxicity testing should also be included in monitoring programs of urban runoff. Further work to identify the toxic components through quantitative chemical analysis (such as gas chromatography/mass spectrometry for organics) are also needed.

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