Scientia Iranica A (2019) 26(4), 2133–2153

Sharif University of Technology
Scientia Iranica
Transactions A: Civil Engineering
http://scientiairanica.sharif.edu

Invited Paper

Toxicity evaluation of highway stormwater runoff

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Received 6 February 2019; received in revised form 2 March 2019; accepted 13 May 2019

KEYWORDS
Highway;
Stormwater runoff;
Toxicity;
Freshwater toxicity species;
Marine species toxicity;
First-flush toxicity;
Microtox^{TM};
Toxicity Identification Evaluation (TIE);
BMPs performance.

Abstract. This paper presents the results of two major studies evaluating the toxicity of stormwater runoff generated from the urban and non-urban highways in California. Two major toxicity studies were: (1) statewide highway runoff toxicity evaluation and (2) hydrographic (first-flush) toxicity evaluation of runoff from highly urbanized highways. Extensive grab and composite runoff samples were collected from numerous highway sites throughout the state of California for multiple storm events and multiple years. Wide ranges of toxicity testing, including the three U.S.EPA standard species, marine species, green algae growth, and Microtox^{TM} were performed on grab and composite samples. The results obtained revealed that the highway runoff was generally toxic, and the toxicity was mostly associated with heavy metals and organic compounds such as herbicides, pesticides, and surfactants. While outside the scope of this study, an independent performance evaluation of stormwater treatment showed that toxicity removal after Best Management Practice (BMP) was possible, even though some influent samples entering the BMP were toxic.

1. Introduction

When monitoring the characteristics of urban stormwater runoff quality, special emphasis may be given to the toxicity evaluation of discharged runoff as well as providing a plan for Toxicity Reduction Evaluation (TRE) of the receiving waters. In California, the water bodies that are impaired through toxicity or any other pollutants are updated every two years under a list known as 303(d) list. Listing a water body as impaired in California is governed by the water quality control policy for developing water quality data for receiving waters to determine if they contain pollutants at levels that exceed protective water quality criteria and standards. This biennial assessment is required under Section 303(d) of the Federal Clean Water Act (CWA).

In the current 303(d) list (based on the 2016 update), about 1274 water bodies in California are listed as impaired due to known or unknown toxicities (https://www.waterboards.ca.gov/water_issues/programs/tmdl/integrated2014_2016.shtml). In addition, there are numerous other water bodies in the 303(d) list that are categorized as impaired and their impairments are related to metals, pesticide, and other organic compounds that can directly or indirectly cause toxicity. Therefore, toxicity of discharged stormwater runoff is an important component of the overall objective of the clean water act. To reduce the toxicity in discharged runoff, CWA established an enforceable regulatory component known as the National Pollution Discharge Elimination System (NPDES). Under this regulatory requirement, Total Maximum Daily Load

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doi: 10.24200/sci.2019.21420

(TMDL) of specific pollutants should be established in order to reduce the toxicity or other pollutants adversely impacting on the environment. As part of CWA regulatory requirements, the U.S.EPA toxic rule was specifically established to numerically specify the Pollutants Of Concern (POC) in order to protect receiving waters for beneficial uses. Similarly, the state of California adopted the U.S.EPA toxic rule with some modifications known as the California Toxic Rule (CTR). Both the U.S.EPA toxic rule and CTR provide numerical concentration thresholds for specific organic and inorganic pollutants. Occasionally, runoff water may meet the numeric pollutant concentration threshold, but the water samples can still be toxic. This may be due to the combined toxic effect of multiple pollutants that can be evaluated only through standard toxicity testing.

In the past, several toxicity studies were performed to evaluate the toxicity of stormwater runoff from roadways. One comprehensive study in San Francisco Bay Area showed that the toxicity in over 90% of roadway runoff samples was from transportation-related activities and the cause of toxicity was found to be non-polar organics and metallo-organics [1]. In a separate toxicity study performed by Greenstein et al. [2], using the simulated runoff from parking lots showed that the primary cause of toxicity to purple sea urchin egg fertilization was related to dissolved zinc. Other toxicity studies conducted by Pitt et al. [3] and Marsalek et al. [4] on roadway runoff reached similar conclusions, finding greater toxicity in roadway runoff than in other land uses. The roadway runoff toxicity was hypothesized by Marsalek et al. [4] to be partially due to road salts used for deicing. More recently, Wu et al. [5] assessed the toxicity of urban storm water runoff samples in Shanghai using the zebrafish (*Danio rerio*) embryo test and the bacterial luminescence (*Vibrio qinghaiensis*) assay. Results showed that all the grab samples inhibited luminescence, while some of the composite samples promoted it, which indicated that different types of toxicants might affect the species. One of the limitations of the above toxicity studies was that the toxicity was evaluated for either a single site or a single season.

The importance of toxicity testing was recognized by the California Department of Transportation (Caltrans) and for this reason, they performed several important toxicity studies as part of their comprehensive stormwater runoff characterization project on their facilities including highways. Two of the most important toxicity studies were: (1) the statewide toxicity characterization study of urban and non-urbun highway runoffs, and (2) hydrographic (first-flush) toxicity evaluation of three major urban highways in Los Angeles. These studies were performed on multiple highway sites and during multiple rainy seasons, and they were designed to address many questions related to the stormwater runoff quality including the question "Is highway runoff toxic and if yes, what is (are) the cause(s) of toxicity?" The first study was performed by University of California at Davis (UCD)in cooperation with numerous environmental consulting firms and the UCD Center for Aquatic Biology and Aquaculture (CABA). The second toxicity study was jointly performed by the Department of Civil and Environmental Engineering at UCD and the University of California at Los Angeles (UCLA) in collaboration with the Southern California Coastal Water Research Projects (SCCWRP) and the Nautilus Environmental. The findings and conclusions drawn from the above two studies are the focus of this paper.

2. Statewide highway runoff toxicity evaluation

As stated above, the statewide highway runoff toxicity evaluation was performed in collaboration with numerous environmental consulting firms to assist in stormwater runoff monitoring and sample collections in the statewide highway runoff characterization study during 2000-2003 stormwater runoff monitoring seasons. Toxicity testing, Toxicity Identification Evaluation (TIE), statistical analysis, and interpretation of the results were performed by the UCD Center for Aquatic Biology and Aquaculture (CABA). A complete report related to this study was prepared by Johnson et al. [6] and further details can be obtained from their study. Major findings in addressing the objective of the study are presented in this section of the paper. Specific topics addressed include: (i) site and sample selection, (ii) toxicity testing, (iii) Toxicity Identification Evaluation (TIE), (iv) statistical analysis, and (v) results and discussion.

2.1. Site and sample selection

As part of the statewide highway runoff characterization study, 23 highway sites were selected throughout the state of California with wide ranges of Annual Average Daily Traffic (AADT) and land use coverage [7,8]. These sites were selected to monitor the comprehensive stormwater characterization including the toxicity evaluation on a statewide basis. The study was performed for three years during the 2000-2003 rainy seasons, with each year beginning in the fall and ending in the spring of the following year. Each site was sampled three times during each year for toxicity assessment–once during the first rainfall event of the year and then, two more times during randomly selected storm events. In the first year of the study, the majority of the sites were sampled only during the last storm event. During the second and third years of monitoring season, the storm events were sampled randomly. Due to an unexpectedly high

amount of rainfall over a majority of the state, a significantly large number of samples were received. Because of the unforeseen nature of this event, there were not enough reagents on stock to run all the samples twice. To attenuate this problem, the number of samples collected was reduced to 17 sites. These sites were selected based on the displayed high amounts of toxicity in the previous years.

2.2. Toxicity testing

All toxicity testing procedures followed those outlined for the short-term methods of estimating the chronic toxicity of effluents and receiving waters to freshwater organisms [9]. Toxicity was evaluated using whole-organism bioassays, as specified by the three U.S.EPA standard freshwater species including *Pimephales promelas* (fathead minnow), *Ceriodaphnia dubia*, and *Selenastrum capricornutum* (green algae). These three species were used to assess chronic or acute toxicity by comparing the biomass (*P. promelas*), mortality (*P. promelas, C. dubia*), reproduction (*C. dubia*), or growth (*S. capricornutum*) of ambient waters or effluent with control water. Toxicity was determined by the presence of a statistically significant difference in any of these parameters in the control and ambient waters.

Every sample was identified as either nontoxic or toxic for a specific toxicity bioassay. The combination of endpoints resulted in the potential of the sample to be toxic in any of the 5 ways, namely, *P. promelas* survival, *P. promelas* biomass, *C. dubia* biomass, *C. dubia* survival, and *S. capricornutum* growth. Some samples were toxic in only one of the 5 endpoints, while others were toxic in a combination of the endpoints. The results are reported as change in absorbance over time (slope). From these data, an inhibition percentage was calculated for each dilution in every sample, using the following equation:

$$\text{Percent (\%) Inhibition} = 1 - \frac{\text{Sample slope}}{\text{Control slope}} \times 100. \quad (1)$$

2.3. Toxicity Identification Evaluations (TIEs)

Toxicity Identification Evaluations (TIEs) consisted in a set of manipulations that were designed to identify a specific chemical or class of chemicals responsible for any observed toxicity. Phase I and Phase II TIEs were performed using the U.S.EPA protocols [10-12]. For this part of the study, modified Phase-I and Phase-II TIE were used to identify general class of the chemical causing the toxicity in the test sample. Six ml C8 solid phase extraction columns were used to remove non-polar organic chemicals from the ambient water samples. Control blank waters were first pumped through the columns prior to the ambient water samples. Settled ambient samples (1000-1800 ml) were then passed through the column. The first 200 ml of the C8 solid-phase extracted water for both the control blank and the ambient water was discarded to minimize potential artifactual column toxicity. HPLC-grade or OPTIMA-grade methanol (MeOH) was used for column activation and extraction. Eluates (methanol extractions) were obtained by running 3.0 ml MeOH through the loaded column at a rate of one ml/min. The concentration at which the eluate was added back in the Phase-I TIE ranged from 2 to 3 times the ambient concentration.

When pesticides were suspected, piperonyl butoxide (PBO) was added to samples at 100 to 200 μg/L to help in identifying the class of pesticide present in the sample. PBO was used to determine whether the toxicant was a metabolically activated organophosphorous (OP) pesticide. OP pesticides, such as diazinon, chlorpyrifos, or malathion, are metabolically activated by the cytochrome P450 detoxification system through conversion of the parent compound to the more toxic -oxon form. PBO, by blocking the cytochrome P450 system, prevents oxon formation and toxicity. PBO has been shown to block the toxicity of up to 4 toxic units of metabolically activated OP pesticides [13].

EDTA and sodium thiosulfate, in concentrations ranging from 1.25 to 50 mg/L and from 7.5 to 60 mg/L, respectively, were added to ambient water when preliminary screening results suggested that toxicity was due to chemicals other than non-polar organic compounds.

Phase-II procedures utilized 6 ml C8 Solid-Phase Extraction (SPE) columns to remove non-polar organic chemicals from the ambient water. The chemicals were subsequently eluted by increasing concentrations of methanol. The 8 methanol:water fractions were 50:50, 70:30, 75:25, 80:20, 85:15, 90:10, and 100:0. Bioassays were performed on each fraction and on a methanol laboratory control blank to determine whether any fraction retained toxicity. Bailey et al. [13] and Crepeau et al. [14] determined the fraction by eluting several more common insecticides used in the California Central Valley. HPLC-grade or OPTIMA-grade methanol (MeOH) was used for column activation and extraction. Eluates (methanol extractions) were obtained by running 3 ml of MeOH through the loaded column at a rate of 1 ml/min. The concentration at which the eluate was added in the Phase-II TIEs was 2.5-3 times the sample concentration.

2.4. Statistical analysis

Toxicity is defined as a statistically significant difference ($p < 0.05$) between a sample and the laboratory control water. Acute toxicity in the *C. dubia* and *P. promelas* assays is defined as a statistically significant increase in mortality of a test sample compared to the laboratory control within 96 hrs. Chronic toxicity is

defined as a significant increase in mortality or a significant decrease in growth or reproduction compared to the laboratory control in more than 96 hrs.

CABA at UCD uses twice the number of control replicates in the *C. dubia* and *S. capricornutum* assays to increase the power of the statistical analysis. It is recommended that the optimal size of the control group (when a single control is to be compared with multiple treatments) should be approximately the square root of $k-1$ (where k is equal to the total number of treatments in the experiment) times the size of each separate group.

All *C. dubia* reproduction, *P. promelas* biomass and mortality, and *S. capricornutum* growth data were analyzed by Bartlett's Test for homogeneity of variance. When variances were homogeneous, data were analyzed using an Analysis of Variance and Dunnett's mean separation tests. When variances were heterogeneous, first, data were transformed into relative ranks and then, they underwent the same process as that for homogeneous variances. *C. dubia* mortality was analyzed with Fisher's Exact Test. No statistical analysis was performed on TIE results.

It is worthwhile to note that the statistical analyses used in this study are different from those outlined in U.S.EPA [11,12]. The U.S.EPA protocols were designed for the whole effluent toxicity testing in which all samples were tested in a dilution series, and the statistical analyses recommended by U.S. EPA were designed to analyze data from a dilution series.

2.5. Results and discussion

This section of the paper is organized to present and discuss the following topics: (i) general findings of toxicity results, (ii), Phase-I toxicity identification evaluation, and (iii) Phase-II toxicity identification evaluation.

2.5.1. General findings of toxicity results

Complete sets of toxicity tests consisted of *S. capricornutum* growth test, *C. dubia* mortality and reproduction, and *P. promelas* biomass and mortality tests. Complete sets of tests were performed on 23 site-event samples during the 3 years of the project. There were 138 samples tested for toxicity; 20 samples classified as the first storm events, 66 as the second events, and 52 as the third storm events. Every site necessarily produced toxicity at some time during the course of the first two years of the study. Most highway sites produced stormwater runoff that was toxic to more than one test organism (72%) during more than one storm event. Sites for the third year of the study were selected because they exhibited acute toxicity during the first two years. However, a few highway sites did not exhibit any toxicity during one event in the third year.

The most sensitive test in demonstrating toxicity was the *P. promelas* biomass assay (79%) followed by the *P. promelas* mortality assay (61%). The *C. dubia* reproduction test was the next most sensitive (58%) followed by the *C. dubia* mortality assay (37%) and the *S. capricornutum* assay (35%). It was generally true that if toxicity was to be exhibited in only a single test, it was the *P. promelas* biomass assay.

2.5.2. Phase-I Toxicity Identification Evaluation (TIE)

As presented in Table 1, Phase-I toxicity evaluation showed that non-polar organic compounds, heavy metals, and surfactants were the major contaminant groups causing toxicity. Of 35 stormwater runoff samples subject to Phase-I TIE during 2000-01 monitoring season, 51% non-polar organic chemicals fell into the primary toxicant category, 14% heavy metals, 17% organophosphorous (OP) pesticides, and 9% Dissolved Oxygen (DO) or low-pH. During the 2001-02 monitoring season, the primary toxicant groups were non-polar organics in 62%, heavy metals in 43%, surfactants in 26%, OP pesticides (mostly non-polar organics) in 12%, and DO/pH in 2% of the samples. The causes of toxicity for samples tested during the 2002-03 monitoring season were identified as non-polar organics in 58%, heavy metals in 28%, and surfactants in 47% of the samples.

When interpreting the data, it is important to consider the different sensitivities of the test organisms to certain groups of contaminants. For example, *C. dubia* is more sensitive than *P. promelas* and *S. capricornutum* to OP pesticides. *S. capricornutum* is more sensitive to heavy metals than both *P. promelas* and *C. dubia* are. This is illustrated in Table 2, in which TIE results are listed by test species. Of 53 water samples that underwent TIE testing with

Table 1. TIE results by chemical groups identified as Primary Toxicants (PTs) during 2000-03 monitoring seasons.

Monitoring season	Number of TIE samples	Metals	Non-polar organics	OP Pesticides	Surfactants	DO/pH
2000-2001	35	5 ($\sim$ 14%)	18 ($\sim$ 51%)	6 ($\sim$ 17%)	0	3 ($\sim$ 9%)
2001-2002	42	18 ($\sim$ 43%)	26 ($\sim$ 62%)	5 ($\sim$ 12%)	11 ($\sim$ 26%)	1 ($\sim$ 2%)
2002-2003	36	10 ($\sim$ 28%)	21 ($\sim$ 58%)	0	17 ($\sim$ 47%)	0
Total	113	33 ($\sim$ 29%)	65 ($\sim$ 58%)	11 ($\sim$ 10%)	28 ($\sim$ 25%)	4 ($\sim$ 4%)

Table 2. TIE results and primary toxicant groups by test species.

Test species	Number of TIE samples	Metals	Other non-polar organics	OP pesticides	Surfactants	Other/TL
P. promelas	53	13 (25%)	37 (70%)	0	21 (40%)	DO/pH: 4 (8%)
C. dubia	40	3 (8%)	25 (63%)	11 (28%)	6 (15%)	0
S. capricornutum	20	17 (85%)	5 (25%)	0	0	0

fish, 70% were identified as non-polar organics, 40% surfactants, and 25% metals as the primary toxicant groups. Physiological stress caused by low DO or pH of water was responsible for toxicity in 8% of the samples. Of 40 samples tested with *C. dubia*, 63% were identified as non-polar organics, 28% OP pesticides, 15% surfactants, and 8% metals as the causes of toxicity. Lastly, of 20 samples tested with *S. capricornutum*, 85% indicated that heavy metals were the cause of toxicity and only in 25% non-polar organic chemicals were identified as a toxicant.

2.5.3. Phase-II Toxicity Identification Evaluation (TIE)

During the second phase of toxicity identification evaluation, Lethal Concentration (LC) of a chemical in water that caused 50% mortality or inhibition, known as LC50, was determined. The results of the available LC50s for metals and organic contaminants are listed in Table 3. These values and the available chemical concentrations for individual constituents in water samples were used for calculating Toxic Units (TUs), where 1 TU = concentration/LC50. Heavy metals that were present at TU values > 0.5 were lead (Pb), copper (Cu), and zinc (Zn). Zn, in particular, was present at concentrations toxic to *P. promelas* and *S. capricornutum* in most samples with the highest TU values, as high as 13.1 (*P. promelas*) and 16.4 (*S. capricornutum*) for one highly urbanized highway site in Los Angeles. The sum of TUs was calculated for each water sample and the results indicated that metals significantly contributed to toxicity at most sampling sites.

Phase-II TIEs were performed for organic toxicant compounds during 2002-03 monitoring season. The qualitative identification of the primary organic toxicants present in stormwater samples is shown in Table 4. As shown, the vast majority of these compounds, present at concentrations that caused toxicity to either *P. promelas* or both *P. promelas* and *C. dubia*, were pesticides. The cause of toxicity in stormwater runoff to *C. dubia* due to pesticides was also verified by Carpenter et al. [15].

Anthracene-dione (sites 4-36 and 4-37) is a bird

Table 3. Available LC_{50}s for heavy metals and organic chemicals by test species.

Chemical constituent	48-hr LC_{50} (μg/L) *C. dubia*	96-hr LC_{50} (μg/L) *P. promelas*	72-hr EC_{50} (μg/L) *S. capricornutum*
Ag	10.233	9.500	
As	13350	12600	
Cd	27.300	3215	137
Cr	47650	52000	
Cu	96	66	396.500
Pb	248	2100	
Se		3840	
Zn	14433.330	282	225
Diazinon	0.470	0.178	
Diuron	17.900[a] mg/L	14.200 mg/L	
Endosulfan		1.320	
Malathion	1.600	8600*	
Naled	0.200-0.800[a]	3300	
Prowl	280[b,*]	138*	588.800
PBO	1000 μg/L		
Simazine		100*	1240[c]
Trifluran	500-600[b,*]	20-3400*	673[c]

[a] *Daphnia pulex* LC_{50}; [b] *Daphnia magna* LC_{50}; [c] 96-hr EC_{50}; * From Extension Toxicology Network (EXTOXNET).

Table 4. Results from chemical analysis of toxic organic chemicals identified in Phase-II TIEs with samples collected during the monitoring season 2002-03, which were toxic to both *C. dubia* and *P. promelas*.

Sampling date	Monitoring site	Organic toxicant	Concentration (ppb)
11/7/2002	4-36	Anthracene-dione	7.2
"	4-37	Anthracene-dione (also two phthalates)	4.9
"	10-03*	Malathion	4.7
11/8/2002	11-97	Two phthalates	Contaminated
"	11-98*	Permethrin**, piperonyl butoxide	7.7, 0.37
"	11-100*	Diazinon, malathion	4.6, 1.8
"	11-101	DEHP	Contaminated
"	12-10	Two phthalates	Contaminated
2/11/2003	11-100	Malathion	0.6
"	11-97	Propyzamide	0.125
2/12/2003	10-02	Propyzamide	0.41
"	10-03*	Diazinon, trifluralin	3.8, 1.35
3/14/2003	6-06*	Propyzamide and thiabendazole	0.68, 2.8

*Done single as well as stacked (for which analytes generally agreed with the same fraction or an adjacent one);

**Permethrin concentration is the sum of cis- and trans-isomers.

repellant. Thiabendazole (site 6-06 with agricultural land use) is a systemic benzimidazole fungicide used to control fruit and vegetable diseases such as mold, rot, blight, and stain. Propyzamide (pronamide) is used as an herbicide on lettuce and alfalfa (sites 10-02, 11-97, 11-15, and 6-06 with agricultural land use). Trifluralin (highway site 10-03) is another herbicide. Samples that were toxic to both species often contained organophosphate or pyrethroid insecticides (diazinon, malathion, and permethrin) at toxic concentrations. Malathion (sites 10-03 and 11-100) and diazinon (sites 11-100 and 10-03) are organophosphate insecticides applied in agricultural as well as in urban areas. Permethrin (site 11-98, San Diego Co.) is a pyrethroid insecticide and Piperonyl butoxide (PBO) (11-98) is a synergist, applied with some pyrethroids to enhance toxicity. In addition, two phthalates were responsible for, or contributed to, *P. promelas* and *C. dubia* toxicity at sites 4-37 (Maintenance Station, Contra Costa Co.), 11-97 (I-15, San Diego Co.), and 12-10 (Maintenance station, Orange Co.). DEHP is a plasticizer used in a wide variety of products. Animal studies indicate very low toxicity and DEHP is used to contain blood products for transfusion.

Because Caltrans does not apply any pesticide, the question arises as to how these organic compounds reached highway transportation right of way. It is unlikely that spills during transport were responsible. The other scenarios that these organic compounds might reach highway right of way include: (1) drift from applications immediately adjacent to the highway sites, (2) dry deposition, or (3) wet deposition. Although drift from adjacent applications cannot be ruled out, given the range of sites at which the organics are found, dry and wet depositions may be significant contributors to the organic compound loads. Studies performed by the United States Geological Survey (USGS) in the Mustang Creek watershed in Merced County, California, indicate that wet and dry depositions can account for a significant portion of the organics observed at a large range of sites in an agricultural watershed [16]. In addition, the studies performed by Ma et al. [17] and Burnel et al. [18] found that anthropogenic activities exerted influence on increase in trace metals and Polycyclic Aromatic Hydrocarbons (PAHs), especially in high traffic areas. According to the above studies, it is reasonable to assume that dry and wet depositions were the most likely mechanisms through which the organic compounds reached the wide range of highway sites across urban and rural areas.

One of the contributing factors to TIE evaluation is the impact of land use on toxicity, particularly when downstream runoff is impacted by upstream agricultural and urban settings. Results of this investigation are summarized in Table 5. As indicated, based on the TIE results, the primary causes of toxicity were associated with surface type, categorized as residential, agricultural, transportation, and mixed land use areas, and for most sites, the toxicity was metabolically activated pesticides disproportionately responsible for the TIE results. Generally, the highway paved surfaces acted as the catalyst for deposition of pesticide during the dry period, which was then measured in runoff samples during rain events.

Spearman rank correlations were calculated between several site-specific variables and the results of the toxicity assays for the 2002-03 sampling season. For this statistical analysis, the correlation of the results of

Table 5. Distribution of TIE results organized by land use classification for the major sources of contamination.

Contaminant	Monitoring year	Land use[a]							
		R	C	I	A	F	O	T	M
Metals	2000	2	1					1	
	2001	4	1				1	2	1
	2002							1	1
Surfactants	2000								
	2001	1			1				3
	2002							2	
Metabolically activated pesticide	2001	1							2
	2002				1		2		1
Non-polar organic	2000				1			2	
Total		8	2	0	3	0	3	8	8

[a]Land use identification: R = Residential, C = Commercial, I = Industrial, A = Agricultural, F = Forest, O = Open, T = Transportation, and M = Mixed (combination of any of the above).

Table 6. Spearman rank correlation coefficients for all correlations with $p < 0.10$.

Impact variable	*C. dubia* reproduction	*C. dubia* mortality	*P. promelas* biomass	*P. promelas* mortality
Rainfall event				0.303 (0.086, 33)
Antecedent dry period	–0.395 (0.076, 21)	0.436 (0.048, 21)	–0.395 (0.076, 21)	0.322 (0.072, 32)
Cumulative precipitation				–0.318 (0.076, 32)
Impervious fraction	–0.444 (0.039, 22)	0.410 (0.059, 22)	–0.444 (0.039, 22)	0.362 (0.038, 33)
AADT	–0.509 (0.037, 17)	0.441 (0.077, 17)	–0.509 (0.037, 17)	0.496 (0.014, 24)

Note: Numbers in parentheses are significance values and the degrees of freedom for the correlations, respectively. A blank cell indicates a correlation was present with a $p > 0.10$. AADT = Annual Average Daily Traffic.

all the 5 toxicity tests (using actual numerical values for all test outcomes, e.g., reproduction was provided in a number of neonates counted during the test) with multiple variables, namely rainfall events (amount), maximum rainfall intensity, antecedent dry period, the antecedent rainfall event, total runoff flow volume during the storm event, peak flow rate during the storm event, cumulative precipitation during the storm event, catchment area, impervious fraction of catchment area, and AADT, was investigated. The results of this analysis are summarized in Table 6. As shown, there was no correlation between the toxicity test results and the measures of maximum intensity, antecedent rainfall event, total flow volume, peak flow volume, or catchment area. Nor was there any correlation between *S. capricornutum* toxicity and any of the environmental variables.

Generally, it appears that long antecedent dry periods result in lower reproduction of and higher toxicity to the *C. dubia* and *P. promelas*. A higher percentage of impervious fraction in the catchment also results in higher toxicity, as does a higher traffic volume. The greater the cumulative precipitation, the lower the *P. promelas* mortality, but the correlation was not quite significant at $p = 0.05$ and no other toxicity assay exhibited a nearly significant relationship (although all had negative signs for the correlations indicating that higher precipitation would result in lower toxicity). The only somewhat counterintuitive result was that the greater the rainfall event, the higher the *P. promelas* mortality, although again, the relationship was not significant at the level of $p = 0.05$.

Overall, over the course of this study, for most samples, at least one toxicity test gave a positive result (i.e., every sample could be considered toxic by at least one toxicity test). Therefore, based on the data and

analysis presented above, it is safe to say that the urban and some non-urban highways within specific land use areas are generally toxic. However, the toxicity may diminish through stormwater runoff treatment by the available BMPs. For example, a separate toxicity study was performed by UC Davis for Caltrans in which the toxicity of both the influent and effluent of a detention basin was assessed for one rainy season. One aspect of the study was to find out if the entering water to this BMP was toxic or not, and if toxic, whether the toxicity would be diminished after treatment. The detention basin was located at the intersection of Highway 605 and Interstate 91 in southern California and designed to treat highway stormwater runoff with a WQV of 70 m^3. Five storm events were monitored during the 2005-06 wet season for both influent and effluent of the detention basin. The performance of the detention basin in toxicity removal was assessed. Toxicity testing was done based on the U.S.EPA standard toxicity testing. Results obtained from this BMP performance evaluation study indicated no toxicity to the effluent of detention basin, even though a number of influent grab samples exhibited moderate toxicity to *P. promelas*. Providing complete results and discussion of this study is beyond the scope of this paper; however, the limited data gathered from the above-mentioned study showed that toxicity of the highway stormwater runoff could be substantially reduced or removed completely by treating through the available BMPs (see Section 4.2).

3. Hydrographic (first-flush) toxicity evaluation of the urban highway runoff

This toxicity study was performed during the 2002-03 monitoring season as part of the first-flush highway runoff characterization study performed from 2001 through 2003. The study was conducted in three highly urbanized highway sites in West Los Angeles in cooperation with the Department of Civil and Environmental Engineering at UCLA. The actual toxicity testing was performed in collaboration with Southern California Coastal Water Research Project (SCCWRP) located in Costa Mesa, CA, and Nautilus Environmental located in San Diego, CA. These two organizations in Southern California were chosen for several reasons, most notably: (a) extensive experience and expert staff with state-of-the-art toxicity testing capabilities, (b) capability to perform the marine water toxicity testing, and (c) close location to the monitoring sites for meeting the required 36 hrs of holding time for toxicity testing. A complete report was prepared for this study and further information can be obtained from Kayhanian and Stenstrom [19]. Major findings of this part of the study with respect to addressing the objective of the study are presented in this section. The presented and discussed topics include: (i) monitoring site description, (ii) sample collection method, (iii) toxicity testing methods, (iv) Toxicity Identification Evaluation (TIE), (v) statistical analysis, and (vi) results and discussion.

3.1. Monitoring sites description

Three highway monitoring sites were used for the hydrographic toxicity evaluation of runoff. These highway sites were the same sites previously used for the first-flush characterization study [20]. All three sites were located in west Los Angeles in a highly urbanized area with large average daily traffic volume. The selective characteristics of the sites are summarized in Table 7. All the sites were virtually impervious and the runoff coefficient was usually 0.9 to 0.95. Each site was equipped with an American Sigma rain gage and flow meter. The flow rate and the amount of rainfall were recorded automatically at one-minute intervals. Data from each site were downloaded into a Windows-based laptop computer after the end of each storm.

3.2. Sample collection method

The sampling method for first-flush toxicity evaluation of highway runoff followed the procedure described by Kayhanian and Stenstrom [21] and, in general, the sample collection regimes shown in Figure 1. All samples were collected by grab method. Typically, 5 grab samples were collected in the first hour with the first grab sample being collected as soon as an adequate runoff volume reached the sampling point. The additional 4 samples were collected in 15-min intervals. After the first hour, one grab sample was collected per hour for the next 7 hrs, providing a total of 12 grab samples. For storm events lasting less than 8 hrs, fewer grab samples were collected. For storm events lasting longer than 8 hrs, an additional one or two grab samples could be collected in the period from 8 hrs to the end of the storm. The runoff volume was

Table 7. Summary of the descriptions of monitoring sites.

Site no.	Monitoring location	Freeway/ post mile	Area (m^2)	Type	Annual average daily traffic
7-201	Eastbound US 101	US 101/PM 17	12802	Grade	328,000
7-202	IS 405 Freeway and Sepulveda	IS 405/PM 34.8	16918	Fill	260,000
7-203	Santa Monica Blvd. North Bound Exit on IS 405	IS 405/PM 30.8	3917	Cut	322,000

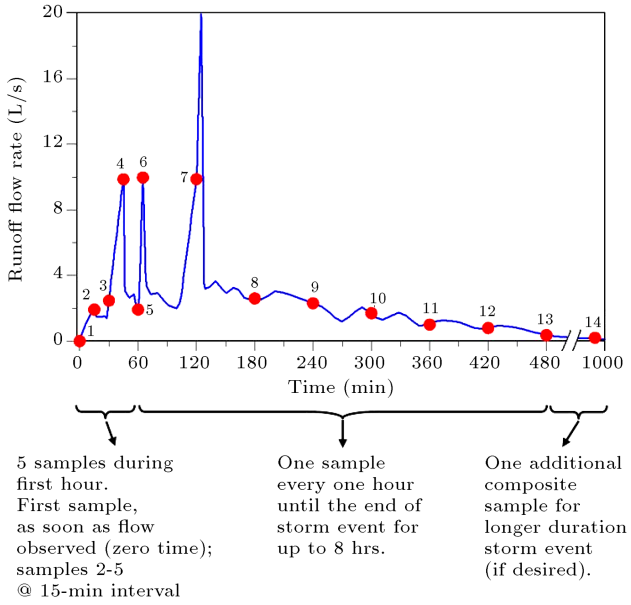

Figure 1. Sample collection method for first-flush toxicity evaluation.

continuously monitored and recorded during the entire event of each storm.

Each sample collected was identified with a storm number, a site number, and a grab number. Samples from 6 storm events were submitted to Nautilus Lab and SCCWRP for toxicity tests (storm events 2, 4, 6, 7, 8, and 9). Overall, a total of 178 grab samples and 6 composite samples were tested during this study. Immediately upon the arrival of a sample to the toxicity lab, an aliquot of it was drawn and the following water quality characteristics were measured and recorded in the laboratory sample check-in log sheet: arrival temperature, alkalinity, DO, hardness, and pH. Temperature and conductivity were measured using an Orion model 130 meter. DO was measured using a YSI model 55 meter. An Orion model 250A+ meter was used to measure pH. Alkalinity and hardness were determined using Hach titrimetric test kits. Immediately after subsampling for the above measurements, the samples were placed in a cold room maintained at $4 \pm 2^{\circ}$C.

3.3. Toxicity testing methods

Multiple toxicity testing methods were employed, including: (i) *P. promelas* 7-day survival and growth test, (ii) *C. dubia* 7-day survival and reproduction test, (iii) Selenastrum algal growth inhibition, (iv) purple sea urchin egg fertilization test, and (v) Microtox™ chronic test.

3.3.1. P. promelas 7-day survival and growth test

C. dubia 7-day survival and growth toxicity tests were performed following US.EPA guidance [22]. *P. promelas* 7-day survival and growth test estimates chronic toxicity by evaluating the survival and growth of larval fathead minnows over time. Larval fish (one day old at test initiation) were exposed to the samples for a period of 7 days. Ten fish larvae were arbitrarily added to each test chamber. A second technician verified counts and conditions of all test organisms before and after addition of the larvae to test chambers. A 16:8 hrs light:dark illumination cycle was considered as the duration of the test. Organisms were fed a mixture of YCT (yeast-Cerophyll®-trout chow) and a suspension of algae daily according to EPA protocol guidance. Test chambers were covered with a clear plexiglass sheet to prevent test solution contamination.

A number of grab sample test chambers were aerated for 24 hrs and thereafter, a rapid drop occurred in DO towards values less than 4.0 mg/L. Aeration was performed at a rate of approximately one to two bubbles per second through Tygon® microbore plastic tubing. Samples that required aeration were identified on the raw bench datasheets. Test solutions were renewed once per day and organisms were fed *Artemia* nauplii three times per day. Temperature, pH, DO, and conductivity were measured daily in both freshly prepared test renewal solution and the test solution collected from the test chambers for each concentration and control. Survival status was recorded for organisms in each test chamber once per day. At test termination, final observations were made and test animals were prepared for weight determination. Fish weights were determined by placing fish from each test chamber on individual tared aluminum pans and drying them in an oven at 60°C for 24 hrs. After drying, the fish were weighed on a Mettler 240AE balance to the nearest 0.01 mg.

Concurrent positive control reference toxicant tests were conducted as a measure of consistent organism sensitivity as well as continuing laboratory proficiency with the method. Nominal copper (II) chloride (as copper) concentrations of 240, 120, 60, 30, 15, and 0 μg/L were prepared and tested. The LC_{50}/EC_{50} values were compared with the historical values obtained at Nautilus Lab.

3.3.2. C. dubia 7-day survival and reproduction test

Survival and growth toxicity tests using the fathead minnow (*P. promelas*) were performed over a 7-day period according to the U.S.EPA guidelines [22]. The test design consisted of 4 replicate test chambers with 10 fish each. The fish, supplied by Aquatic Biosystems Inc. of Fort Collins, CO, were one-day-old post-hatch upon initiation. Replicates consisted of 400-ml plastic cups containing 250 ml of the test solution. Control and dilution water consisted of moderately hard synthetic water prepared with 8 parts nano-pure water and 2 parts Perrier®. Following initiation, test chambers were placed in an environmental room maintained at $25 \pm 1^{\circ}$C and covered with clear Plexiglas™ covers. A diet of yeast, cerophyll, trout chow (YCT), and

Selenastrum suspension was added to each test sample and control prior to distribution to test chambers. A 16:8 hrs light:dark illumination cycle was considered as the duration of the test. Test solutions were renewed once per day and the fish were fed *Artemia* nauplii three times daily. Water quality parameters of pH, DO, ammonia, conductivity, and temperature were measured and recorded daily. Fish weights were determined at the end of the test by placing the fish from each test chamber on individual tared aluminum pans and drying them in an oven at 60°C for 24 hrs. After drying, the fish were weighed on a Mettler 240AE balance to the nearest 0.01 mg.

A number of grab sample test chambers were aerated at 24 hrs and thereafter, a rapid drop in DO occurred towards values less than a threshold value of 4.0 mg/L. Aeration, when needed, was performed at a rate of approximately one to two bubbles per second through Tygon® microbore plastic tubing to maintain DO levels above 4.0 mg/L throughout the duration of the test [22].

3.3.3. Pseudokirchneriella subcapitatum algal growth inhibition

The freshwater unicellular algae, *Pseudokirchneriella subcapitatum* (formerly known as *Selenastrum capricornutum*) 96-hr growth inhibition toxicity test was also performed according to the U.S.EPA guidelines [22]. The stock culture used to inoculate each treatment was between 4 and 7 days old and in log-phase growth at the time of test initiation. It was purchased from Aquatic Biosystems of Fort Collins, Colorado. Test chambers consisted of 4 replicate 125-ml Erlenmeyer flasks per sample. Test solutions were warmed to 25 ± 1°C and measurements of temperature, pH, DO, and conductivity were recorded. Fifty ml of the prepared test solution was then distributed to each exposure chamber. Nutrient-enriched control water was prepared according to EPA protocol specifications on the day of test initiation. Each test chamber was aseptically inoculated with the algal stock solution to an initial concentration of 10,000 cells per ml. Illumination was provided by a cool-white fluorescent light source suspended above the test vessels. Test chambers were arranged randomly on shelves in the environmental chamber based on the assigned numbers and covered with a clear Plexiglas™ sheet to prevent cross-contamination [23].

For the duration of the test period, each test chamber was manually swirled twice each day and positions rotated under the light source (once in the morning and once in the evening). Temperature was monitored daily. At test termination, chlorophyll-a fluorescence was measured in an aliquot drawn from each test chamber using a Turner model TD-700 fluorometer [23]. Fluorescence was automatically converted to cell density by comparison to an internal calibration curve. An additional subsample of each replicate was preserved with Lugol's iodine solution and held at 4 ± 2°C in darkness when microscopic confirmation of cell density was needed (using an improved Neubauer hemacytometer at 400 × magnification) [23]. The remaining volume in each replicate was then composited and measurements of pH, DO, temperature, and conductivity were recorded for each test treatment and control.

3.3.4. Purple sea urchin egg fertilization test

All samples of runoff collected during the 2002-03 wet season were evaluated for toxicity using the purple sea urchin fertilization test [24]. This test measures toxic effects on sea urchin sperm, which are expressed as a reduction in their ability to fertilize eggs. The test comprised 20-minute (min) exposure of sperm to the samples. Eggs were then added and given 20 min for fertilization to occur. The eggs were then preserved and examined later with a microscope to assess the percentage of successful fertilization. Toxic effects were expressed as a reduction in fertilization percentage. Purple sea urchins (*Strongylocentrotus purpuratus*) used in the tests were collected from the intertidal zone in northern Santa Monica Bay. The tests were conducted in glass shell vials containing 10 ml of the solution at a temperature of 15°C.

Composite stormwater samples were tested prior to testing of the grabs using the sea urchin egg fertilization test. Each composite sample was tested at 4 or 5 concentrations. The vials from the composite exposures were quickly scanned on a microscope and an EC_{50} (concentration leading to 50% reduction in fertilization) for the composite was estimated. For the first storm, all of the grab samples were then tested at the concentration of the estimated EC_{50}. For the second and third storms, in addition to testing at the EC_{50} concentration, the first 5 grabs from each site were also tested at 1/4 this concentration [23]. In all cases, 5 replicates of each concentration were tested.

Seawater control (0.45 μm activated carbon filtered natural seawater from Redondo Beach) and brine control samples (50% deionized water and 50% brine) were included in each test series for quality control purposes [23]. Water quality parameters of temperature, DO, pH, ammonia, and salinity were measured in the test samples to assure that the experimental conditions were within desired ranges and did not create unintended stress on the test organisms.

3.3.5. Microtox™ chronic test

A modified version of the Microtox™ chronic test method was used in this study as presented in [23] and briefly described here. The photoluminescent bacteria, *Photobacterium phosphoreum*, were exposed

to a concentration series of runoff for 22 hrs and toxic effects were expressed as a decrease in light output relative to controls. All reagents and the dehydrated bacteria were obtained from Azur (Carlsbad, CA). The modifications to the procedure involved using a more concentrated bacterial solution and buffer/salt solution so that more samples could be tested per batch of bacteria. Bacteria luminescence was measured using the photon sensing system of a Liquid Scintillation Counter (LSC). The composite samples were tested at 5 concentrations: 75%, 37.5%, 18.7%, 9.4%, and 4.7%. The grab samples were tested at concentrations ranging from 50% to 3%. Samples were salinity-adjusted prior to testing with a brine solution provided by Azur. Temperature, DO, pH, ammonia, and salinity were measured in all test samples prior to test initiation.

3.4. Toxicity Identification Evaluation (TIE)

TIE evaluation generally follows U.S. EPA published methods including: (1) Methods for aquatic toxicity identification evaluation - Phase-I toxicity characterization procedures, second edition (EPA/600/6-91/003)" [10], (2) Methods for aquatic toxicity identification evaluations - Phase-II toxicity identification procedures for samples exhibiting acute and chronic toxicity (EPA/600/R-92/080) [11]; and (3) Methods for aquatic toxicity identification evaluations - Phase-III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity (EPA/600/R-92/081) [12]. Descriptions of each TIE treatment used in this study are summarized in Table 8. Grab samples that exhibited acute toxicity during the first hour of the storm in the screening tests (grabs 1-5) were selected for TIEs. Due to limited sample volumes, TIE procedures were performed on select individual grab samples as well as equal-volume composite samples created from the remaining samples of grabs 1 through 5. Treatments were performed on the full-strength sample and, in some instances, on samples 50% diluted. A complete summary of stormwater samples tested and TIE methodologies applied is provided in Table 8. Effectiveness of the TIE procedures was evaluated using 96-hr *P. promelas* and *C. dubia* acute survival exposure as described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (821-R-02-12) [22].

Table 8. Summary of TIE treatments performed and target toxicants.

Treatment	Target toxicant
Baseline (100% sample)	None - Serves as the basis for comparison to determine treatment effectiveness
EDTA addition (10, 25, and 50 mg/L)	Divalent cationic trace metals
Sodium thiosulfate (STS) (10 and 25 mg/L)	Oxidizable compounds, some trace metals
C18 extraction	Non-polar organic compounds
C18 methanol elution	Recovers non-polar organic toxicants and surfactants removed by the C18 column
pH adjustment (pH 6 & 9)	Contaminants whose toxicity is pH-dependent
Aeration	Surfactants and volatile compounds
Zeolite extraction	Ammonia
Ammonia addition to zeolite-treated sample	Recovery of toxicity due to ammonia once removed by zeolite
EDTA addition to zeolite-treated sample	Removal of toxicity due to a combination of ammonia and cationic trace metals

3.5. Statistical analysis

Stormwater and reference toxicant data were analyzed using Tidepool Environmental Software Comprehensive Environmental Toxicity Information System (CETIS™), Version 1.025B [25]. Statistical differences from the control and No Observed Effect Concentrations (NOEC) were determined for each test using Dunnett's, Wilcoxon rank sum, Steel's many-one rank, or Fisher's exact multiple comparisons tests. Median lethal concentration (LC_{50}) or median effect concentration (EC_{50}) values were calculated for freshwater reference toxicant bioassays using maximum likelihood probit, trimmed Spearman-Karber, or linear interpolation analyses. The choice of statistical method was dependent upon specific model assumptions met or not met by the data as addressed in U.S.EPA [22,24].

The relationship between toxicological endpoints determined by Nautilus and various analytical chemical data provided by UC Davis was evaluated by performing Spearman rank correlation analysis. Prior to this analysis, proportion data were arcsine square root transformed and chemistry data were log transformed to normalize data distributions. These analyses were performed using Microsoft® Excel 2000. However, it is important to note that trace metal concentrations for only the first 5 storms were included, as concentrations for storm 9 were not available at the time of preparation of this report.

Best-fit regressions using a one-phase exponential decay model were used to graphically display relationships between toxicity endpoints and several chemical parameters. Regression analyses were performed using GraphPad Statistical Software Version 4.02 [26].

To evaluate the presence of a first-flush effect on a mass basis, toxicity was compared with runoff volume [23]. This evaluation removed the effect of flow rate on the interpretation of toxicity results. The first-flush toxicity mass was evaluated by comparing the normalized mass fraction of toxicity with the normalized volume fraction throughout the duration of the runoff event [23]. Because many of the grab samples were tested using a limited range of dilution, the effective concentration of runoff associated with toxicity discharged at discrete time points was not always known. The following procedure was used to estimate the predicted concentrations of runoff in all of the sea urchin fertilization data and a subset of the Microtox™ and fathead minnow data. These concentrations were used as surrogates for the toxicity concentration. For each site and storm for which there was a composite sample, the dose-response plot was fitted to a logistic regression equation shown below [23]:

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}, \tag{2}$$

where x represents concentration of runoff in percent; y is toxic response and represents fertilized, survival, or light output percentage; and a, b, and x_0 are constants.

Eq. (1) can be rearranged to calculate the concentration of runoff:

$$x = \sqrt[b]{\frac{a{x_0}^b - y{x_0}^b}{y}}. \tag{3}$$

The toxic response data from each grab were then applied to Eq. (2) to calculate a predicted concentration of runoff. Thereafter, the predicted concentrations were integrated over the runoff hydrograph to determine the "mass" of toxicity and then, normalized to determine the fraction discharged at various volume fractions [23]. Regarding the combinations of site and storm for which there were no composite samples, a logistic equation for an earlier storm in that site was used to make the predicted concentration calculations.

3.6. Results and discussion

Specific topics presented and discussed in this section of the paper include: (i) toxicity evaluation of grab samples, (ii) first-flush evaluation of composite samples, (iii) correlation between toxicity of freshwater and marine species, (iv) cause of toxicity to highway runoff, and (v) relationship between elevated metals concentration and toxicity.

3.6.1. Toxicity evaluation of grab samples

The frequency and magnitude of the toxicity of grab samples were graphically presented through the "hydro-toxicity-graphs" [23]. A hydro-toxicity-graph is a plot that presents the variability of toxicity during the entire storm event (i.e., first-flush versus the rest of the storm event) while showing the hydrograph (flow rate versus storm duration) on the background within the same graph. The results of hydrographic toxicity for grab samples related to *marine water* and *fresh water* species are discussed below.

Results of sea urchin fertilization tests for the February 11 grab samples tested at a concentration of 12.5% (near the EC_{50} determined for the composites) for all three highway sites are shown in Figure 2. From this plot, it can be noted that the first 120 min of the stormwater runoff samples usually contained the greatest toxicities to sea urchins (i.e., lowest fertilization percentage) recorded for the events. Some post-120-min grab samples from Sites 7-202 and 7-203 also contained substantial toxicities; however, toxicity to sea urchins was not present in any of the subsequent grab samples for Site 7-201. Toxic samples were most often associated with periods of relatively low flow velocity.

In addition, hydrographic toxicity results of Microtox™ for the storm event of February 11, 2003 are shown in Figure 3. As can be noted, generally,

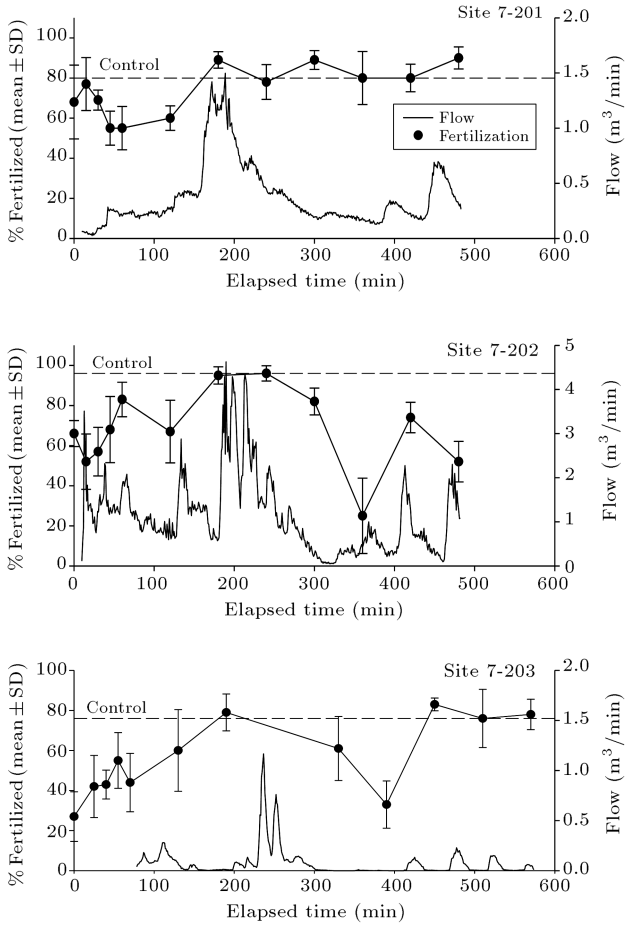

Figure 2. Sea urchin egg fertilization toxicity response to grab runoff samples (February 11, 2003 storm event). Exposures were performed at 12.5% concentration of stormwater, near the EC_{50} value determined in the prior composite sample tests (adapted from [23]).

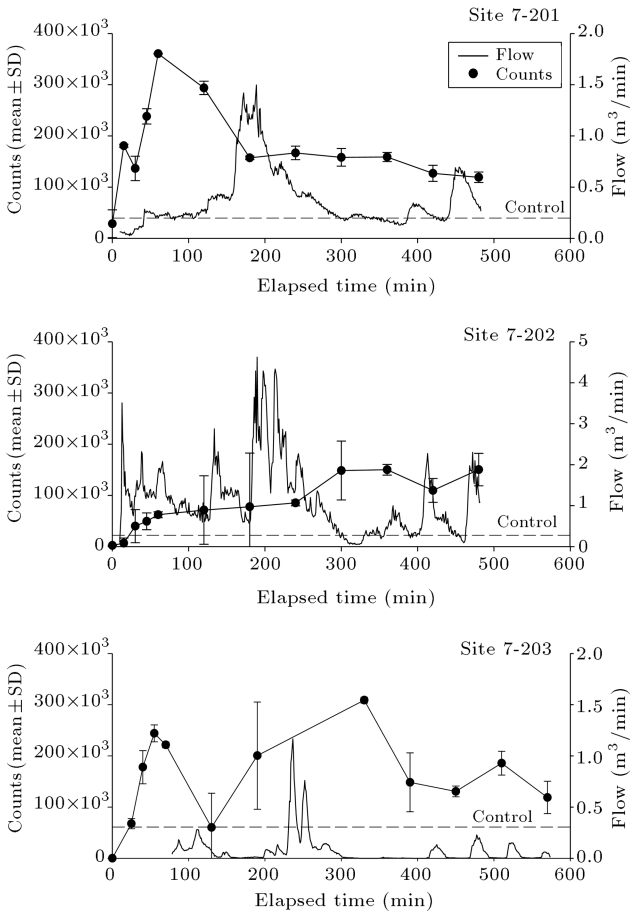

Figure 3. Microtox^{TM} toxicity response to grab runoff samples (February 11, 2003 storm event). Exposures were performed at 9.4% concentration of stormwater, near the EC_{50} value determined in the prior composite sample tests (adapted from [23]).

only the first or second grab samples were toxic to Microtox^{TM} at the selected test concentration of 9.4%. All other samples showed enhancement, which were characterized by a high level of luminescence (light counts) compared to the controls.

Representative hydrographic toxicity responses in 25 and 100% stormwater grab samples of the 3 *freshwater species* tested for most storm events showed that survival rates of both *Ceriodaphnia* and fathead minnows were generally the lowest in grab samples collected early during the storm event. *Ceriodaphnia* reproduction and fathead minnow growth endpoints in the same event also exhibited a temporal pattern in toxicity at all three sites, with the greatest effects present in samples collected during the first 60 min. The response of green algae to the March 15, 2003 grab samples was more variable than those in the fathead minnow and *Ceriodaphnia* test methods. A similar temporal pattern was evident, however, with the greatest toxic response usually occurring in samples collected during the first 60 min. of discharge.

3.6.2. First-flush toxicity evaluation of composite samples

Examples of toxicity response in composite samples compared with that in grab samples for fathead minnows and *Ceriodaphnia* are shown in Figures 4 and 5. As evident in these figures, composite samples were not toxic despite containing a few grab samples that were toxic individually. This observation might be due to the fact that only a few of the early runoff samples were toxic and toxicity was later diluted by mixing with other runoff samples. The cause of greater toxicity during the early portion of highway runoff is likely related to a higher pollutant concentration and hence, the first-flush effect, as will be further discussed in proceeding section. The higher toxicity with 100% mortality rate of *C. dubia* in the early portion of stormwater runoff for 6 storm events (e.g., first-flush samples) has also been confirmed by McIntyre et al. [27].

The toxicity of composite samples relative to runoff concentration was also evaluated through logistic

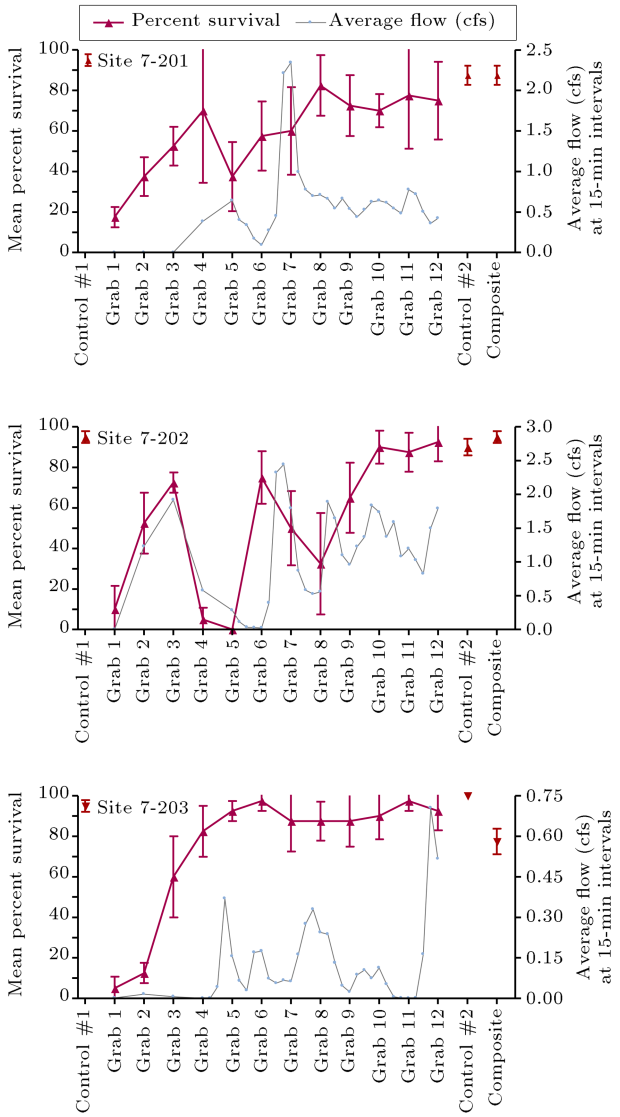

Figure 4. Toxicity screening results for undiluted samples and average flow data in 15-min intervals for fathead minnow survival for stormwater samples collected from 3 highway sites during October 26-27, 2004 storm event. (Note: Control #1 was batched with individual grab tests and Control #2 with the composite tests) (adapted from [23].)

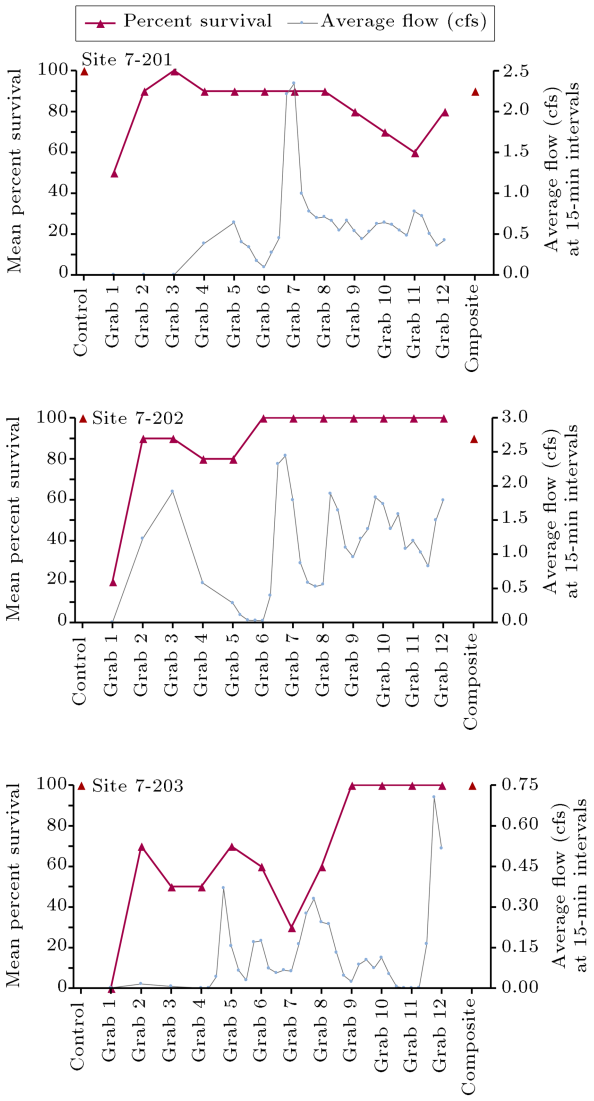

Figure 5. Toxicity screening results for undiluted samples and average flow data in 15-min intervals for Ceriodaphnia survival for stormwater samples collected from three sites during October 26-27, 2004 storm event. (Note: Control #1 was batched with individual grab tests) (adapted from [23].)

regression analysis for samples tested using both sea urchin egg fertilization and Microtox™ tests. A good fit between the data was observed and the R^2 values for the regressions were always greater than 0.96. The high R^2 values demonstrated that the regression approach was a feasible method to estimate the effective toxic concentration of runoff in the grab samples. Regression dose-response results for the two composite samples collected from highway Site 7-201 were very similar. Greater differences in response were present between the two composite samples obtained from highway Site 7-202. The results showed that slopes of the regression curves for Site 7-202 were much greater than those for Site 7-201, possibly indicating different toxicants of concern between the two sites.

Unlike to the freshwater species, composite samples were toxic to both marine test species evaluated. A strong dose-response was present in each composite sample, with EC_{50}s ranging from 10.3% to 15.2%. Toxicity was detected at runoff concentrations as low as 6%.

Although a dose-response relationship was observed in samples collected from each site using both marine species, the Microtox™ test was less sensitive than the sea urchin fertilization test to the composite samples [23]. Each of the three composite samples also produced a stimulatory effect on the bacteria in some

test cases of dilution, resulting in higher luminescence than that in the controls [23].

3.6.3. Correlations between toxicity of freshwater and marine species

In general, the sea urchin fertilization test was the most responsive one among the 5 toxicity test methods and the sensitivity to specific toxicants varied dramatically depending on both the species and toxicity endpoints evaluated. The relationship between *P. promelas* 7-day survival and *C. dubia* 7-day survival was relatively weak with an R^2 value of only 0.32. Relationships among *P. promelas* endpoints alone (acute and chronic survival, and growth) were all strong with R^2 values greater than or equal to 0.8. On the other hand, relationships among the various endpoints for *C. dubia* were much weaker with R^2 values ranging from 0.43 to 0.65. No correlation was observed between toxicity of freshwater and marine species.

3.6.4. Cause(s) of toxicity in runoff samples

Specific causes of toxicity were evaluated for two storm events occurring on March 18 and April 28, 2005 by applying a series of Toxicity Identification Evaluation (TIE) procedures as described in the section on methods. The TIE results for these two storm events showed that the primary cause of toxicity of highway runoff to both *Ceriodaphnia* and fathead minnows in most samples tested was related to cationic metals, primarily copper (Cu) and zinc (Zn). Successful reduction in or complete removal of toxicity was confirmed by the addition of a cationic chelating agent, EDTA, and the subsequent metal spiking studies, providing ample evidence identifying Cu and Zn as the primary toxicants of concern. The cause of toxicity to the highway runoff for nearly 10% of the samples also appeared to be related to anionic surfactants. The contribution of zinc, manganese, copper, and PAHs to the toxicity of stormwater runoff samples has also been confirmed by Wu et al. [5]. In a separate study, pesticide, insecticides, fungicides, and herbicides as the causes of toxicity to stormwater runoff were verified by Carpenter et al. [15].

3.6.5. Relationship between elevated metals concentration and toxicity

The selected published metal concentrations for toxicity tests related to *Ceriodaphnia* and fathead minnows are reported in Table 9. Above a specific minimal level of both Cu and Zn, a substantial toxic effect is almost always observed. There are some instances, however, in which there is little or no effect despite elevated metal concentrations (presumably due to factors affecting bioavailability) [32,33]. In this study, however, we observed that a relatively large number of samples with dissolved Cu and Zn concentrations below the threshold were toxic, suggesting that environmental parameters (i.e., pH, hardness, low DOC) might contribute to enhancement of the toxicity of these two metals in comparison with standard laboratory exposures. On the other hand, toxicity might be due to the presence of some other chemical(s) in some samples [23]. Another interesting observation was the relationship between laboratory-derived LC_{50} values and toxicity threshold effects in the two species evaluated. Laboratory-derived LC_{50} values for fathead minnows were greater than the observed concentrations for threshold effects, whereas laboratory-derived LC_{50} values for *Ceriodaphnia* were lower than the observed concentrations for threshold effects for both Cu and Zn (e.g., toxicity to fatheads was greater than expected and toxicity to *Ceriodaphnia* was lower than expected in many samples, based on Cu and Zn concentrations alone) [23].

4. Implications of toxicity results for stormwater runoff treatment

This section of the paper discusses the use of toxicity results for evaluating the overall quality of stormwater runoff as well as their application to the performance evaluation of stormwater BMPs. Specific topics addressed include: (i) the utility of toxicity results in stormwater runoff treatment and (ii) performance evaluation of BMPs based on toxicity results.

4.1. Utility of toxicity results in stormwater treatment

The cost and effectiveness of structural or treatment control BMPs are becoming the subject of increasing interest as stormwater dischargers face regulatory permit requirements that include TMDL waste load allocations. Collecting a high volume of stormwater during a short time, intermittent nature of the task, and variable quality of stormwater make treatment a tremendous challenge. The ultimate goal of BMPs is treating the stormwater runoff to a degree to achieve beneficial use of downstream receiving waters. Protection of aquatic life is considered to be a primary beneficial use in almost all receiving water environments. Estimating aquatic life toxicity based on chemical parameters alone, however, proves to be difficult based on a number of observations and points outlined below:

- Chemical data often co-vary, as observed in this study;
- Only a select group of potentially toxic chemicals are analyzed and reported;
- A number of environmental parameters may affect the toxicity of any given compound (e.g., pH, hardness, particulates, and total and dissolved organics);
- Speciation of the chemicals (i.e., dissolved versus particulate) may affect toxicity;

Table 9. Selected published metals toxicity data for *Ceriodaphnia* and fathead minnows (adapted from [23]).

Metal	Test duration	Endpoint	NOEC (μg/L)	LOEC (μg/L)	LC50 (μg/L)	Reference
Ceriodaphnia dubia:						
Cu	48 hr	Survival	nr[a]	nr	11	[28]
	48 hr	Survival	nr	nr	9.5 (pH 6-6.5), 28 (pH 7-7.5), 200 (pH 8-8.5)	[29]
	7 d	Survival	48.6	97.2	71.5 (52.3-90.7 ± 1 SD)	[30]*
	7 d	Reproduction	46.5	93.1	71.8 (45.2-98.4 ± 1 SD)	[30]*
Ni	48 hr	Survival	nr	nr	>200 (pH 6-6.5), 140 (pH 7-7.5), 13 (pH 8-8.5)	[29]
Pb	48 hr	Survival	nr	nr	120	[28]
	48 hr	Survival	nr	nr	280 (pH 6-6.5), $>2,700$ (pH 7-7.5), $>2,700$ (pH 8-8.5)	[29]
Zn	48 hr	Survival	nr	nr	60	[28]
	48 hr	Survival	nr	nr	>530 (pH 6-6.5), 360 (pH 7-7.5), 95 (pH 8-8.5)	[29]
	7 d	Survival	160	330	210 (pH 7.0-7.5)	[31]*
	7 d	Reproduction	<160	160	150 (pH 7.0-7.5)	[31]*
Fathead minnow (Pimephales promelas):						
Cu	96 hr	Survival	nr	nr	15 (pH 6-6.5), 44 (pH 7-7.5), >200 (pH 8-8.5)	[29]
	7 d	Survival	31.2	56.0	114 (63.3-165 ± 1 SD)	[30]*
	7 d	Growth	25.7	49.0	96.6 (58.2-135 ± 1 SD)	[30]*
Pb	96 hr	Survival	nr	nr	810 (pH 6-6.5), $>5,400$ (pH 7-7.5), $>5,400$ (pH 8-8.5)	[29]
Ni	96 hr	Survival	nr	nr	$>4,000$ (pH 6-6.5), 3,400 (pH 7-7.5), 3,100 (pH 8-8.5)	[29]
Zn	96 hr	Survival	nr	nr	780 (pH 6-6.5), 330 (pH 7-7.5), 500 (pH 8-8.5)	[29]
	7 d	Survival	330	690	500 (pH 7.0-7.5)	[31]*
	7 d	Growth	330	690	530 (pH 7.0-7.5)	[31]*

[a]nr: not reported.

*Based on unpublished laboratory data of the Nautilus Environmental.

- Stormwater consists of a complex mixture of compounds that may interact in a variety of ways that affect toxicity of any given constituent;
- Variability between storm events, sites, grab samples, and species makes it difficult to draw conclusions about the potential effectiveness of BMPs for any given storm event.

Any toxicity index or numerical evaluation based on chemical performance alone must be viewed as a first-step management tool for evaluating the performance of BMPs before investing in comprehensive toxicity testing. Another issue of importance is whether exceedances of established water quality standards always constitute environmental impairment. For example, a review of the analytical data collected during this study indicates that a large number of non-toxic grab samples exceeded the recommended maximum water quality criteria for both dissolved Cu (13 μg/L) and Zn (120 μg/L) in freshwater with regard to the hardness of 100 mg/L $CaCO_3$. Water quality exceedances, especially for trace metals, often fail to elicit toxicity in environmental samples since the criteria have been developed using clean laboratory water without natural organics and particulates [34]. Toxicity tests directly address the issue of bioavailability.

In summary, a toxicity result is a directly pertinent measure of the success of BMPs in protecting the receiving water quality as it integrates all interactions between various water quality variables. The complex nature and variability of the chemistry of stormwater runoff may make it difficult to evaluate whether or not aquatic life is impaired for a given storm event based on these measurements alone. Chemistry data are undoubtedly very helpful in understanding trends, dynamics, and potential effects. Identification of primary toxicants of concern through the use of TIEs can help in prioritizing which chemical parameters should be evaluated during future sampling events and identifying BMPs methods most appropriate for the removal of primary chemical(s) of concern.

4.2. Performance evaluation of BMPs based on effluent quality (e.g., toxicity removal)

Up to now, most stormwater construction treatment BMPs have been designed and operated for stormwater runoff collection and retaining the runoff volume for a specified time (i.e., 48 to 72 hrs.). However, in the future, the design and performance evaluation of BMPs may be based on a desired effluent water quality, including the complete removal of toxicity [35]. For instance, as part of a separate toxicity study, the performance of a detention basin was evaluated based on toxicity removal [36]. The complete results of this study are beyond the scope of this paper, but interested readers can obtain further information from Kayhanian et al. [36]. For the purpose of discussion, a summary of mean toxicity screening results for 3 storm events is presented in this section. As an example, the performance of detention basin based on survival and growth of *P. promelas* for 3 storm events evaluated on February 27, 2006; March 17, 2006; and March 28, 2006 are shown in Figures 6 and 7, respectively. In these figures, total rainfall data between samples are

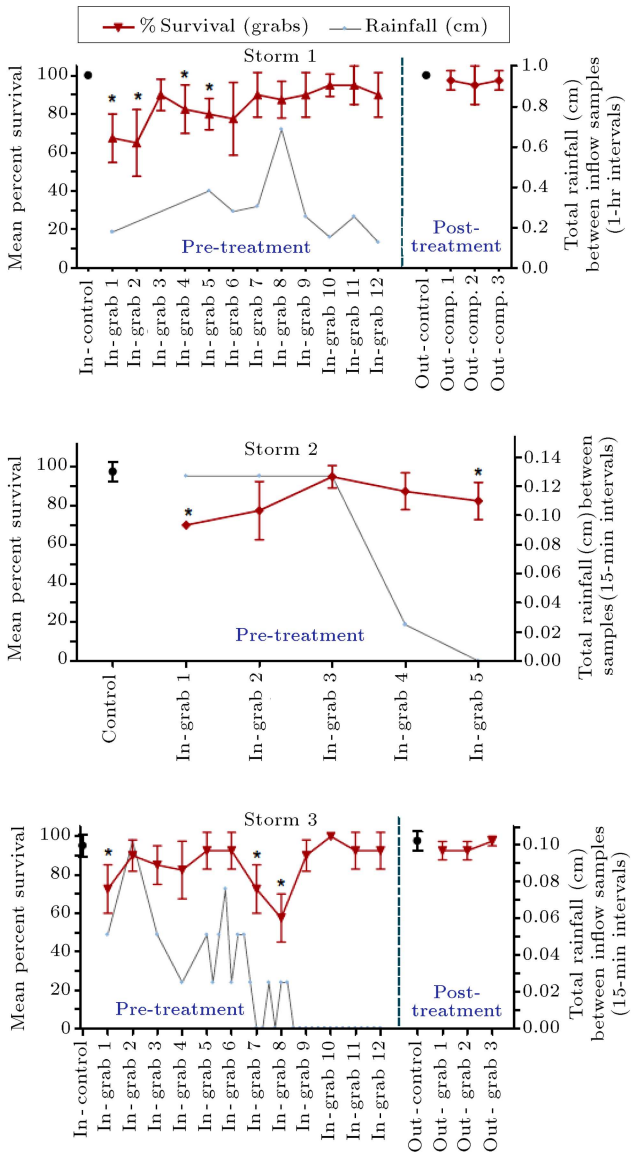

Figure 6. Summary of toxicity screening results for *P. promelas* survival in undiluted samples with pre- (in)- and post(out)-BMP samples collected during storm events on February 27, 2006 (storm 1), March 17, 2006 (storm 2), and March 20, 2006 (storm 3). (Note: The pre-treatment control growth for storm event 2 was exceptionally high relative to all concurrent samples and another concurrent control tested with the post-treatment samples. Due to this apparent anomaly and lack of any observable trends, all statistical comparisons were performed using the post-treatment control for this storm event.)

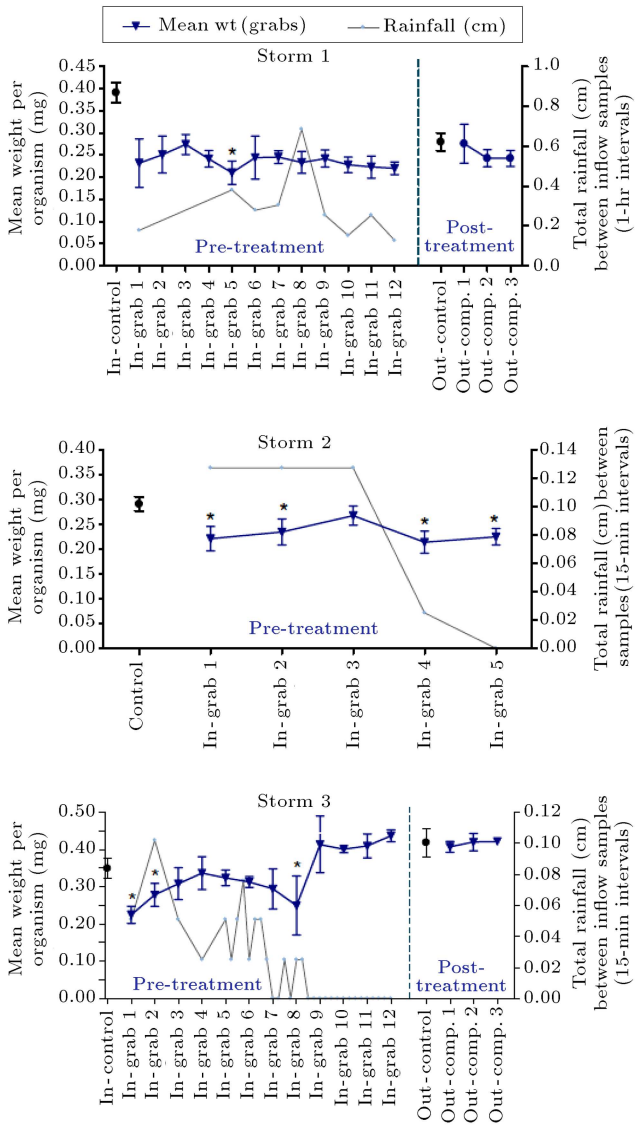

Figure 7. Summary of toxicity screening results for *P. promelas* growth in undiluted samples with pre (in)- and post (out)-BMP samples collected during storm events on February 27, 2006 (storm 1), March 17, 2006 (storm 2), and March 20, 2006 (storm 3). (Note: The pre-treatment control growth for storm event 2 was exceptionally high relative to all concurrent samples and another concurrent control tested with the post-treatment samples. Due to this apparent anomaly and lack of any observable trends, all statistical comparisons were performed using the post-treatment control for this storm event.)

also included for comparison. As shown, during these storm events, a number of BMPs influent grab samples exhibited toxicity to *P. promelas*. None of the 6 effluent samples tested for post-BMPs treatment were toxic, suggesting that the BMPs were effective in completely removing toxicity during the monitored storm events. In addition, a study performed by McIntyre et al. [27] showed that while untreated highway runoff was generally lethal to salmon and invertebrates, this acute mortality was eliminated when the runoff was filtered through soil media in bioretention columns. A separate study performed by Anderson et al. [37] demonstrated that the use of bio-swales would promote filtering of stormwater runoff pollutants and showed no toxicity in effluent while the influent was toxic. In summary, the results obtained by these studies showed that treatment of highway stormwater runoff by construction BMPs (e.g., detention basin, bioretention, and bio-swale) was capable to remove toxicity while the influent runoff showed to be toxic.

5. Summary and conclusions

The results presented in this paper showed that the highway runoff was generally toxic. In addition, the results obtained from the two major toxicity studies verified other conclusions summarized below.

5.1. Statewide toxicity evaluation study

Major conclusions drawn from the statewide highway runoff toxicity evaluation study were:

- Toxicity in at least one assay occurred at every site during the three years of the study;
- Results showed that nearly 92% of the samples exhibited toxicity in at least one of the bioassays. In general, samples tested were toxic in 58% of the *C. dubia* reproduction tests, 37% in the *C. dubia* mortality tests, 72% in the *P. promelas* biomass tests, and 61% in the *P. promelas* mortality tests;
- Some highway sites exhibited significantly higher toxicity than others and analyses indicated that multiple factors contributed to a higher level of toxicity. The major contributing factors were parameters such as wide impervious surfaces, long dry periods between storm events, and high average daily traffic volume;
- Results of the TIEs indicated a range of potential causes of toxicity, including several sources such as metabolically activated pesticides, which were not the result of transportation-related activities, but contributed through the surrounding agricultural land use activities;
- There were significant differences between storm events in the proportion of tests that indicated toxicity, most probably due to the high proportion of toxic events in the first storm events on highways.

5.2. First-flush toxicity evaluation study

Major conclusions drawn from the first-flush toxicity evaluation study were:

- Toxicity to both *C. dubia* and *P. promelas* was frequently observed, but varied between storms, species, and locations. Samples from all storms exhibited toxicity to one or both species;

- *P. promelas* was more sensitive than *C. dubia* to most of the stormwater samples tested. Sublethal endpoints for both test species were more sensitive than survival ones;
- Site-specific differences were apparent, with one site consistently exhibiting greater toxicity than the other two sites;
- A first-flush effect was almost always observed with both species at lethal and sublethal endpoints. The frequency and magnitude of toxicity in the first several grab samples collected during each storm event were typically greater than those observed in samples collected later during the storm. However, toxicity was not related to the first-flush samples occasionally and occurred in grab samples collected later during a storm;
- In most cases, both species responded to first-flush; however, toxicity in samples collected after the first hour of each storm often varied between the two species;
- Survival at the end of the 7-day exposure period in the first grab sample of each storm series was near zero in most cases for both species tested;
- A majority of the composite samples were non-toxic to both test species, even when a strong first-flush effect was observed;
- TIE tests identified copper (Cu), zinc (Zn), and surfactants as toxic constituents of primary concern in toxic first-flush samples;
- Results illustrated the importance of species/ endpoint selection and performing site-specific studies. In other words, results obtained for a given site and storm could not be broadly applied for infer causes of toxicity in other sites and storms;
- A review of analytical data collected during this part of the study indicated that a large number of non-toxic grab samples exceeded the recommended maximum water quality criteria for both dissolved copper and zinc in freshwater. These results showed that concentration data alone should not be used to infer effects.

5.3. Overall assessment and concluding remarks

Overall, the results of both toxicity evaluation studies revealed that, generally, highway runoff in urban areas was toxic. While, not always may be true, generally the first storm event of season and the early portion of the stormwater runoff during each storm event (i.e., first-flush samples) exhibited more toxicity than the rest of the samples collected during stormwater events. Therefore, collecting and treating the first portion of the stormwater runoff volume can be far more beneficial in terms of improving the stormwater runoff discharge effluent quality. As part of a separate toxicity study (results not shown in this paper), it was determined that when the stormwater runoff volume was treated through a common BMP treatment, such as using a detention basin, the treated effluent was free of toxicity. The removal of toxicity by common BMP treatments (i.e., detention basin, sand filters, infiltration basin, bioretention, etc.) can even be more pronounced, especially when the cause of toxicity is associated with heavy metals from transportation facilities. The removal of these heavy metals can more easily be achieved than the removal of some organic compounds by these BMPs.

Acknowledgments

The funding for both toxicity studies was provided by the California Department of Transportation (Caltrans), Division of Environmental Analysis. The authors gratefully acknowledge the assistance of many staff and researchers who contributed to these studies, especially Dr. Inge Werner and Ms. Linda Deanovic from the UCD Center for Aquatic Biology and Aquaculture (CABA), Dr. Steve Bay from SCCWRP, Mr. Chris Stransky from Nautilus Environmental, and Dr. Stenstrom from UCLA as well as numerous graduate students from both UCD and UCLA and numerous environmental consultants. Mention of the names of equipment, products, or supplies in this paper shall not be construed as an endorsement. Opinions, findings, and conclusions or recommendations expressed in this paper are those of the authors and do not necessarily reflect the views of the California Department of Transportation or The Regents of the University of California.

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