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Toxicity of urban highway runoff with respect to storm duration

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ABSTRACT

The toxicity of stormwater runoff during various time-based stages was measured in both grab and composite samples collected from three highly urbanized highway sites in Los Angeles, California between 2002 and 2005. Stormwater runoff samples were tested for toxicity using three freshwater species (the water flea *Ceriodaphnia dubia*, the fathead minnow *Pimephales promelas*, and the green algae *Pseudokirchneriella subcapitata*) and two marine species (the purple sea urchin *Strongylocentrotus purpuratus*, and the luminescent bacteria *Photobacterium phosphoreum* using Microtox™). Toxicity results varied substantially throughout the storm events for both freshwater and marine species toxicity tests. In general, however, the first few samples were found to be more toxic compared with those collected during later stages of each storm event. In most cases, more than 40% of the toxicity was associated with the first 20% of discharged runoff volume. Furthermore, on average, 90% of the toxicity was observed during the first 30% of storm duration. Toxicity identification evaluation results found copper and zinc to be the primary cause of toxicity in about 90% of the samples evaluated with these procedures. Surfactants were also found to be the cause of toxicity in less than 10% of the samples.

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1. Introduction

Stormwater runoff generated from roadway and other land uses has been increasingly found to be the major source of non-point source pollution to receiving waters. In order to meet regulatory water quality requirements, wide ranges of constructive best management practices (BMPs) are being implemented to remove organic and inorganic pollutants in order to protect marine and wildlife species in receiving waters. The performance of these BMPs are usually measured based on removal of pollutant concentrations or mass, and therefore, less attention has been made to evaluate toxicity. Knowledge of roadway

runoff toxicity is essential to accurately evaluate BMP effectiveness with regard to removal of the toxic fraction of pollutants, which is dependent on a variety of complex factors, including, but not limited to, chemical forms and interactions with other chemicals and physical water quality parameters.

Only limited studies have been conducted on runoff that is predominantly or exclusively from roadways (Buckler and Granato, 1999). One comprehensive study in the San Francisco Bay Area detected toxicity in over 90 percent of roadway runoff samples from transportation-related activities (BASMAA, 1996). The cause of toxicity for roadway runoff in the BASMAA study was found to be non-polar organics and metallo-organics. A study

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Nomenclature

English symbols

BMP	Best Management Practice
CASQA	California Stormwater Quality Association
CEL	Calscience Environmental Laboratories, Inc.
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EC50	Median Effect Concentration
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
HCl	Hydrochloric Acid
LAS	Linear Alkylbenzene Sulfonates
LC50	Median Lethal Concentration
LOEC	Lowest Observed Effect Concentration
MBAS	Methylene Blue Activated Substances
NaOH	Sodium Hydroxide
NOEC	No Observed Effect Concentration
QA/QC	Quality Assurance/Quality Control
SCCWRP	Southern California Coastal Water Research Project
STS	Sodium Thiosulfate
SWRCB	State Water Resources Control Board
TIE	Toxicity Identification Evaluation
UCD	University of California Davis
UCLA	University of California Los Angeles
YCT	Yeast, Cerophyll, Trout Chow
x	Concentration of runoff, %
y	Toxic response, %
a	Maximum toxic response
b	Slope
x ₀	Runoff concentration at 50% response

of simulated runoff from parking lots found toxicity to the purple sea urchin egg fertilization test in all samples tested, with evidence suggesting dissolved zinc as the primary cause of toxicity (Greenstein et al., 2004).

Two other toxicity studies conducted by Pitt et al. (1995) and Marsalek et al. (1999) on roadway runoff reached similar conclusions, finding greater toxicity in roadway runoff compared to the other land uses. The cause of roadway runoff toxicity was hypothesized by Marsalek et al. to be partially due to road salts used for deicing. In general, toxicity studies performed to date on highway runoff have been based on a single organism, a few storm events, and a single wet season. The toxicity of roadway runoff throughout the hydrograph for multiple storm events and seasons has not been evaluated, and is the primary focus of this paper.

2. Methods

2.1. Sample collection

Stormwater runoff samples were collected from three highly urbanized highway sites in west Los Angeles (Fig. 1). These three sites were part of a four-year first flush characterization study that was commissioned by the California Department of Transportation (Kayhanian and Stenstrom, 2005; Lee et al.,

2004; Han et al., 2006). Both composite and grab samples were collected to evaluate the toxicity of urban highway runoff throughout the storm duration. A series of grab samples were collected throughout the storm events. In general, five grab samples were collected during the first hour of each storm event, followed by one grab sample every hour until the end of the storm.

Table 1 summarizes the samples collected and toxicity tests performed. Toxicity tests were all initiated within 36 h of sample collection. Three storm events were monitored on February 11, March 15, and April 14 of 2003; six storm events were monitored on October 27, December 6, January 8, February 12, March 19, and April 29 during the 2004–05 wet season. There was some variation in the number of composite and grab samples collected and tested due to a combination of insufficient discharge volumes and malfunctions of the automatic composite sampling equipment. Most samples from both wet seasons were tested using two freshwater species (the water flea *Ceriodaphnia dubia*, and the fathead minnow *Pimephales promelas*). Samples collected during the 2002–03 wet season, were also tested using the freshwater unicellular green algae *Pseudokirchneriella subcapitata* and two marine species (the purple sea urchin *S. purpuratus* and the luminescent bacteria *P. phosphoreum* using Microtox™). A suite of freshwater and marine species were tested to determine the relative sensitivity among species and to explore possible correlations between the marine and freshwater toxicity tests.

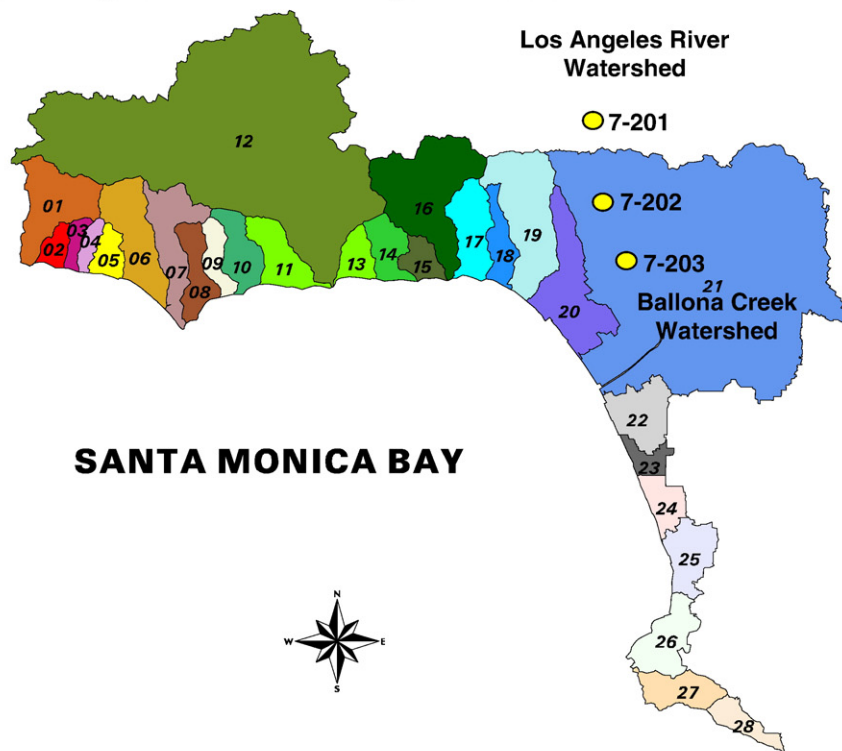
2.2. Description of toxicity testing

2.2.1. Purple sea urchin egg fertilization test

This test measures toxic effects on sea urchin sperm, which are expressed as a reduction in their ability to fertilize eggs. All samples of runoff collected during the 2002–03 wet season were

evaluated for toxicity using the purple sea urchin fertilization test (USEPA, 2002a). The test consisted of a 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 are expressed as a reduction

(a) Monitoring sites within Santa Monica Bay sub-watersheds



(b) Monitoring sites along major highways in Los Angeles

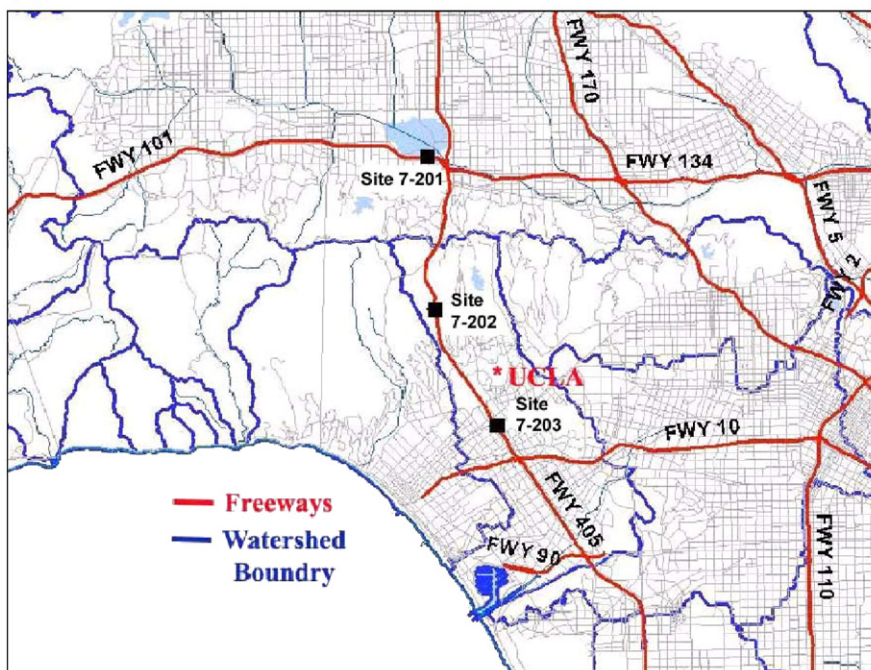


Fig. 1 – Monitoring site locations with respect to (a) local watershed boundaries and (b) major highways.

Table 1 – Summary of toxicity tests performed on composite and grab samples collected from three highway sites

Storm event/site	Sample type		Toxicity test				
	Composite	Grab	Sea urchin	Microtox™	Ceriodaphnia	Fathead minnow	<i>P. subcapitatum</i>
<i>2002–03 monitoring season</i>							
SE1/7-201	x	x	x	x	x	x	x
SE1/7-202	x	x	x	x	x	x	x
SE1/7-203	x	x	x	x	x	x	x
SE2/7-201	x	x	x	x			
SE2/7-202							
SE2/7-203		x	x	x	x	x	x
SE3/7-201							
SE3/7-202	x	x	x	x	x	x	x
SE3/7-203		x	x	x	x	x	x
<i>2004–05 monitoring season</i>							
SE2/7-201	x	x			x	x	
SE2/7-202	x	x			x	x	
SE2/7-203	x	x			x	x	
SE4/7-201		x			x	x	
SE4/7-202		x			x	x	
SE4/7-203		x			x	x	
SE6/7-201		x			x	x	
SE6/7-202		x			x	x	
SE6/7-203		x			x	x	
SE7/7-201		x			x	x	
SE7/7-202	x	x			x	x	
SE7/7-203	x	x			x	x	
SE8/7-202	x	x			x	x	
SE8/7-203		x			x	x	
SE9/7-202		x			x	x	
SE9/7-203		x			x	x	

Note: SE1/7-201 denotes storm event/site ID number (e.g., SE1/7-201=storm event 1, monitoring Site 7-201). Sites 7-201, 7-202, and 7-203 are located on HWY 101, HWY 405, and HWY 405, respectively, shown in Fig. 1.

in fertilization percentage. Purple sea urchins (*S. 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 solution at a temperature of 15 °C.

The stormwater samples were adjusted to a salinity of approximately 34 g/kg using hypersaline brine. The brine was prepared by freezing and partially thawing seawater. Since the addition of brine dilutes the sample, the highest stormwater concentration that could be tested for the sperm cell test was 50%. The salinity-adjusted samples were diluted with seawater to produce test concentrations ranging from 50% to 3%.

Composite stormwater samples were tested prior to testing of the grabs using the sea urchin egg fertilization test. Each composite sample was tested at four or five concentrations. The vials from the composite exposures were quickly scanned on a microscope and an EC50 (concentration producing a 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 EC50. In all cases, five replicates of each concentration were tested.

Seawater control (0.45 µm and 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. Water quality parameters of temperature, dissolved oxygen (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.

2.2.2. Microtox™ chronic test

A modified version of the Microtox™ chronic test method was used in this study. The photoluminescent bacteria, *P. phosphoreum* were exposed to a concentration series of runoff for 22 h and toxic effects are 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 five concentrations: 75%, 37.5%, 18.7%, 9.4%, and 4.7%. The grab samples were tested at concentrations ranging from 50% to 9%, based on the estimated EC50. Samples were salinity adjusted prior to testing with a brine solution provided by Azur. Temperature, dissolved oxygen, pH, ammonia, and salinity were measured in all test samples prior to test initiation.

2.2.3. *C. dubia* 7-day chronic survival and reproduction

Toxicity tests using *C. dubia* were performed following USEPA (2002b) guidance. Test animals were obtained from internal laboratory cultures. The tests were carried out in 30 ml

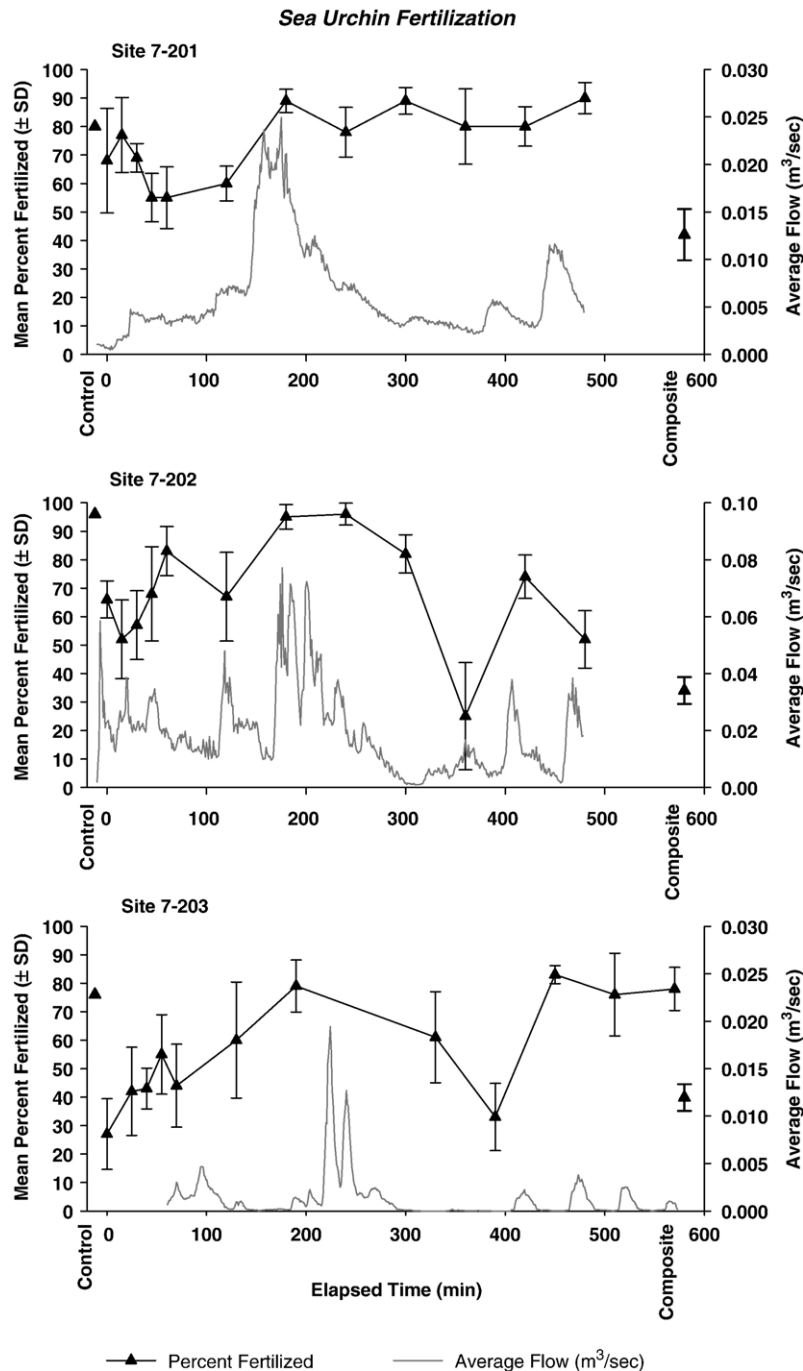


Fig. 2 – Sea urchin egg fertilization test response to runoff samples (February 11, 2003 storm event). Grab and composite samples were tested at a 12.5% concentration of stormwater; near the EC50 value determined in prior tests.

polystyrene cups with 15 ml of test solution. Each concentration had 10 replicate chambers. At test initiation, a single neonate was transferred to each test chamber. Test chambers were then covered with clear Plexiglas™ covers and placed in a temperature-controlled room maintained at 25 ± 1 °C. Light was provided with cool-white fluorescent bulbs and maintained on a 16:8 hour light:dark cycle. Organisms were fed a mixture of YCT (yeast-Cerophyll®-trout chow) and a suspension of *Pseudokirchneriella* algae daily according to EPA protocol guidance. Control and dilution water consisted of moderately hard

synthetic water prepared with 8 parts nanopure water and 2 parts Perrier®. Water quality parameters of pH, DO, ammonia, conductivity, and temperature were measured and recorded, and test solutions were renewed daily. As the daphnids were transferred to fresh test solution each day, observations of survival and reproduction were recorded.

2.2.4. Fathead minnow 7-day chronic survival and growth
Survival and growth toxicity tests using the fathead minnow (*P. promelas*) were performed over a 7-day period according to the

Table 2 – Mean incidence of toxicity in the first 5 runoff grab samples and mean composite EC50/LC50 and NOEC values

Toxicity test	Grabs 1–5		Composite			
	N	% Toxic	N	% Toxic	EC50/LC50	NOEC
<i>2002–2003 samples</i>						
Sea urchin fertilization	35	74	5	100	18	7
Microtox™	35	20	5	80	na	37
<i>Ceriodaphnia</i> survival	34	66	2	50	>100	≥100
<i>Ceriodaphnia</i> reproduction	34	83	2	0	>100	75
Fathead minnow survival	34	80	2	50	>100	≥100
Fathead minnow growth	20	48	2	50	>100	≥100
Algal growth	33	49	1	0	>100	≥100
<i>2004–2005 samples</i>						
<i>Ceriodaphnia</i> survival	80	40	6	0	>100	≥100
<i>Ceriodaphnia</i> reproduction	80	80	6	17	>100	≥100
Fathead minnow survival	65	85	6	33	>100	≥100
Fathead minnow growth	65	86	6	33	>100	≥100

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 test solution. Control and dilution water consisted of moderately hard synthetic water prepared with 8 parts nanopure water and 2 parts Perrier®. Following initiation, test chambers were placed in an environmental room maintained at 25 ± 1 °C and covered with clear Plexiglas™ covers. A 16:8 hour light:dark illumination cycle was provided for the duration of the test. Test solutions were renewed once per day, and fish were fed *Artemia nauplii* three times daily. Water quality parameters of pH, DO, ammonia, conductivity, and temperature were measured and recorded, and test solutions were renewed daily. A number of test chambers were aerated at 24 h and thereafter due to a rapid drop in DO to values less than a threshold value of 4.0 mg/L. Fish weights were determined at the end of the test by placing fish from each test chamber on individual tared aluminum pans and drying them in an oven at 60 °C for 24 h. After drying, fish were weighed on a Mettler 240AE balance to the nearest 0.01 mg.

2.2.5. *P. subcapitatum* algal growth inhibition

The freshwater unicellular algae, *P. subcapitatum* (formerly known as *Selenastrum capricornutum*) 96-hour growth inhibition toxicity test was also performed according to the USEPA (2002b) guidelines. 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. The stock culture was purchased from Aquatic Biosystems of Fort Collins, Colorado. Test chambers consisted of four 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 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. Protocol-specific light levels (400 ± 40 foot-candles) were verified prior to test initiation. Test chambers were arranged randomly on shelves in the environmental chamber based on assigned numbers and covered with a clear Plexiglas™ sheet to prevent cross-contamination.

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. 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 in the event that microscopic confirmation of cell density was needed (using an improved Neubauer hemacytometer at 400× magnification). 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.

2.3. Toxicity identification evaluations (TIEs)

TIE methods generally followed those published by USEPA (1991, 1993a,b). The following TIE treatments with targeted toxicants were performed for this study: (1) Baseline (none-unmanipulated sample); (2) EDTA addition (divalent cationic trace metals); (3) Sodium thiosulfate addition (oxidizable compounds, some trace metals); (4) C18 solid-phase extraction (non-polar organics); (5) C18 methanol elution (non-polar organic confirmation); (6) pH adjustment (pH-dependant toxicants); (7) Aeration (surfactants and volatile compounds); (8) Zeolite extraction and post-zeolite ammonia spikes (ammonia); and (9) EDTA addition to post-zeolite-treated sample (ammonia versus cationic trace metals). TIE methods were applied in a step-wise approach with identification and confirmation steps dependant upon results obtained during Phase I characterization tests. The number and type of treatments applied for any given sample was dependant upon Phase I results, known water quality parameters of concern (i.e. trace metals and ammonia), visual foaming suggesting surfactants, and volume of sample available.

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 remaining Grab 1 through 5 samples. Treatments were performed on full-strength sample, and in some instances, 50% dilutions. Appropriate method controls were employed for all TIE procedures for comparison purposes.

Effectiveness of the TIE procedures was evaluated using 96-hour acute survival exposures using both fathead minnows and *Ceriodaphnia*. Acute test methodology, with minor

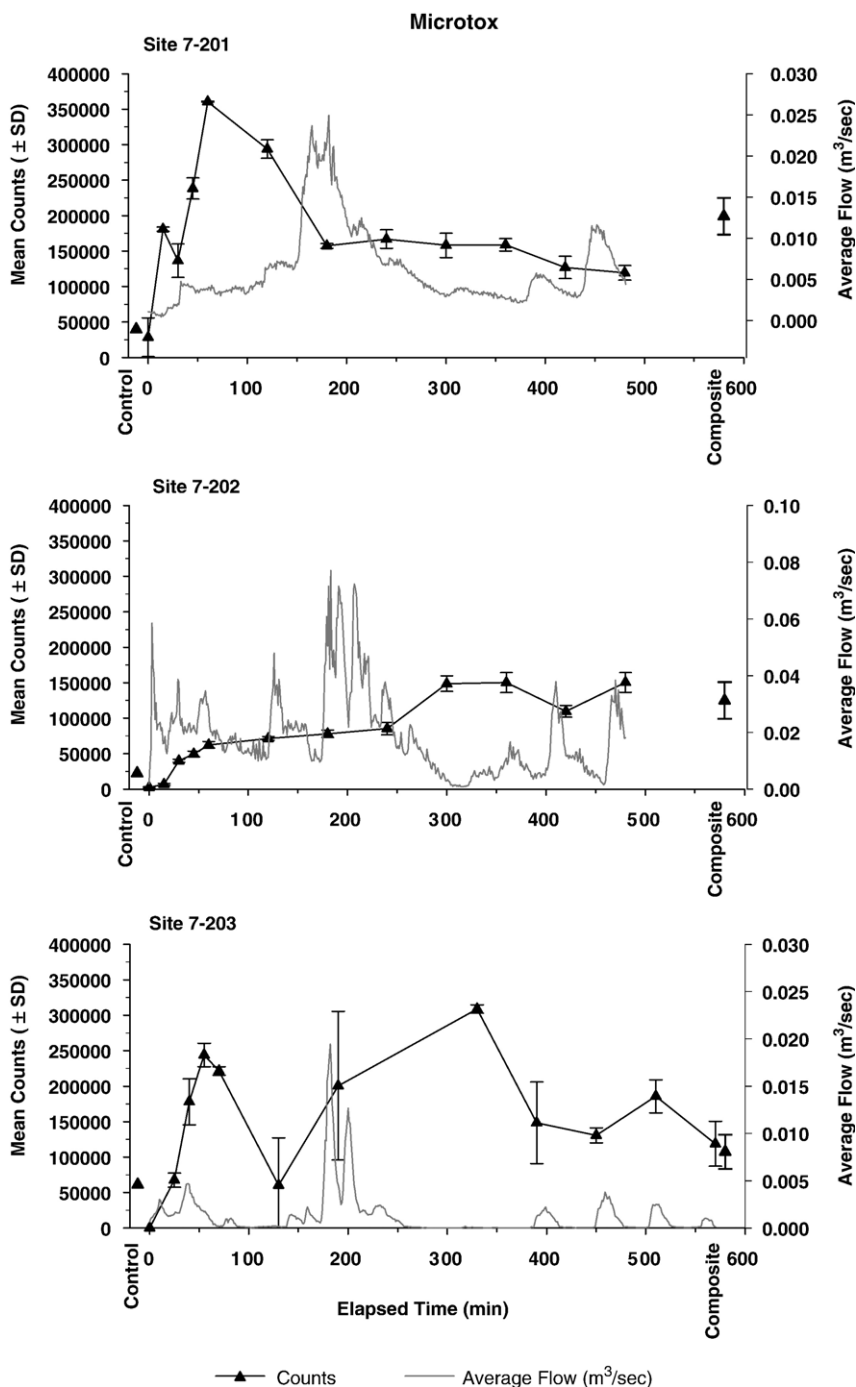


Fig. 3—Microtox™ test response to runoff samples (February 11, 2003 storm event). Grab and composite samples were tested at a 9.4% concentration of stormwater; near the EC50 value determined in prior tests.

modifications with regard to test chamber size, followed the procedures published in USEPA (2002d). TIEs for both species were performed in 30-ml glass scintillation vials with 15 to 20-ml test solution in each of 4 replicates. Five organisms were exposed in each replicate test chamber. Water quality parameters of pH, DO, conductivity, and temperature were measured and recorded daily in a surrogate vial for each treatment, and prior to and following test solution renewal at 48 h. Total ammonia was measured upon sample receipt, and the unionized fractions calculated daily throughout the

duration of the tests based on fluctuations in pH. Fathead minnows were not fed during the test. *Ceriodaphnia* were fed a mixture of *Pseudokirchneriella* algae and YCT at 48 h.

2.4. Analytical methods to measure metals concentration

This investigation focused on analytical measurements of major trace metals (Cu, Pb, Ni, and Zn). Samples for metals analysis were analyzed using the Agilent 7500i Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). These

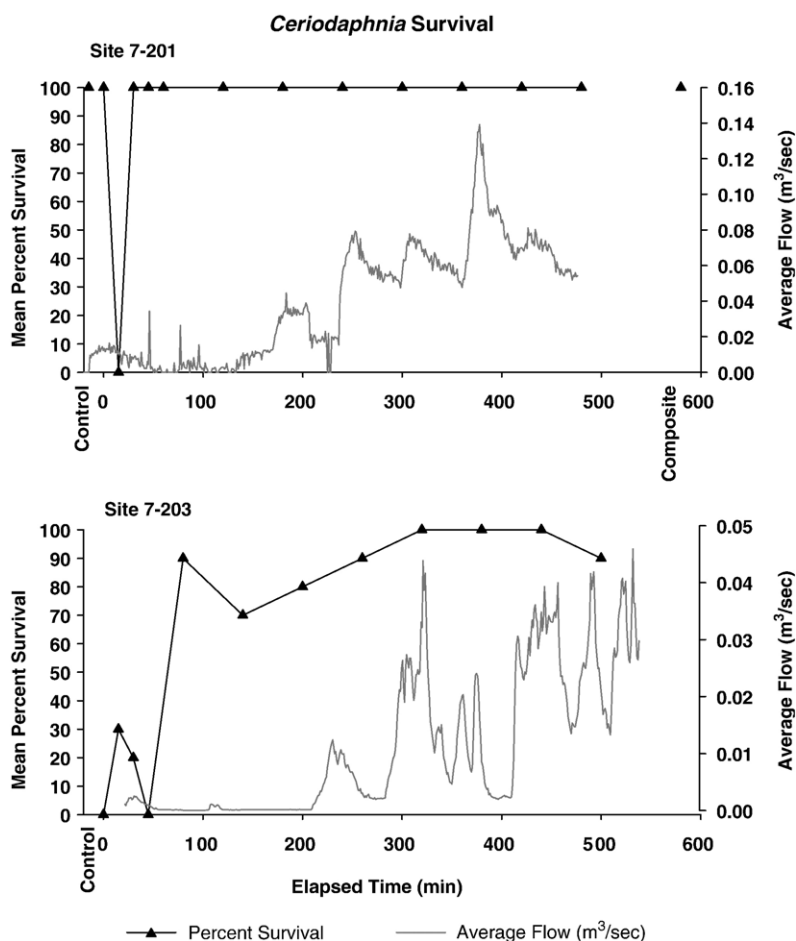


Fig. 4–*Ceriodaphnia* survival test response to runoff samples (March 15, 2003 storm event).

constituents were analyzed using the modified USEPA method 200.8. All standard protocols were followed and a proper standard solution was also prepared for these analyses. The modification was mainly employed to reduce the method detection limit. Ultimate detection limits were about 1 pg (1 part per trillion in less than 1 mL) for nearly all measured metals.

2.5. Statistical methods and data evaluation

The highest test concentration not producing a statistically significant reduction in response (NOEC) for the composite samples was calculated by comparing the response in each concentration to the dilution water control. Various statistical tests were used to make this comparison, depending on the characteristics of the data. Water flea survival and reproduction data were usually tested against the control using Fisher's Exact and Steel's Many-One Rank test, respectively. Sea urchin fertilization, *Ceriodaphnia*, fathead minnow survival, and algae growth data were evaluated for significant differences using Dunnett's multiple comparison test, provided that the data met criteria for homogeneity of variance and normal distribution. Data that did not meet these criteria were analyzed by the non-parametric Steel's Many-One Rank or Wilcoxon's tests.

Measures of median effect for each test were calculated as the LC50 (concentration producing a 50% reduction in survival) for

minnow and water flea survival, and the EC50 (concentration producing a 50% reduction in sea urchin egg fertilization, bacterial luminescence, fathead minnow growth, *Ceriodaphnia* reproduction, and algae growth). Effects concentration values were calculated using Probit, Trimmed-Spearman Karber, or Linear Interpolation analyses, depending on specific assumptions met by the data. Proportion data was arcsine square-root transformed prior to all statistical analyses to normalize the distribution of the data. No other transformations were applied. All procedures for calculation of median effects followed USEPA (2002a,b,c) guidelines.

To evaluate the presence of a first flush effect on a mass basis, toxicity was compared with runoff volume. This evaluation removes 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 relative to the normalized volume fraction throughout the duration of the runoff event. 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 concentration 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 relationship was fitted to a logistic regression equation shown below:

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

where,

x	Concentration of runoff, %
y	Toxic response, % fertilized, survival, or light output
a	Maximum toxic response
b	Slope; and
x_0	Runoff concentration at 50% response

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

$$x = \sqrt[b]{\frac{ax_0^b - yx_0^b}{y}} \quad (2)$$

The toxic response data from each grab sample was then applied to Eq. (2) to calculate a predicted concentration of runoff. The predicted concentrations were then integrated over the runoff hydrograph to determine the “mass” of

toxicity and then normalized to determine the fraction discharged at various volume fractions. For the combinations of site and storm for which there were no composite samples, a logistic equation for an earlier storm for that site was used to make the predicted concentration calculations.

3. Results and discussion

3.1. Toxicity evaluation of grab samples

The frequency and magnitude of the toxicity of grab samples was visually summarized through the creation of “hydro-toxicity-graphs.” A hydro-toxicity-graph is a plot that presents the degree of toxicity during the entire storm event while showing the hydrograph (flow rate vs. storm duration) on the same plot. The variability of toxicity for grab samples is further discussed below for both marine and freshwater species tested.

3.1.1. Toxicity to marine species

Results of sea urchin fertilization tests for the February 11, 2003 grab samples, tested at a concentration of 12.5% (near the EC50 determined in the composites), are shown in Fig. 2. The grab samples from the first 60 min. of the storm discharge were usually toxic to sea urchins (Table 2) and

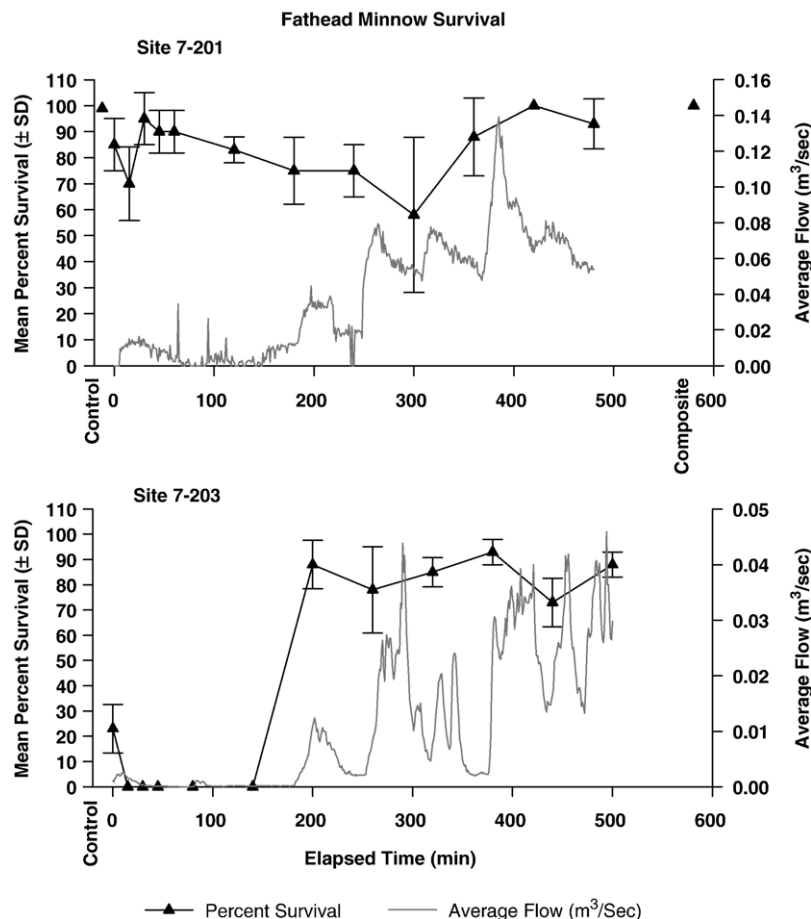


Fig. 5–Fathead minnow survival test toxicity response to runoff samples (March 15, 2003 storm event).

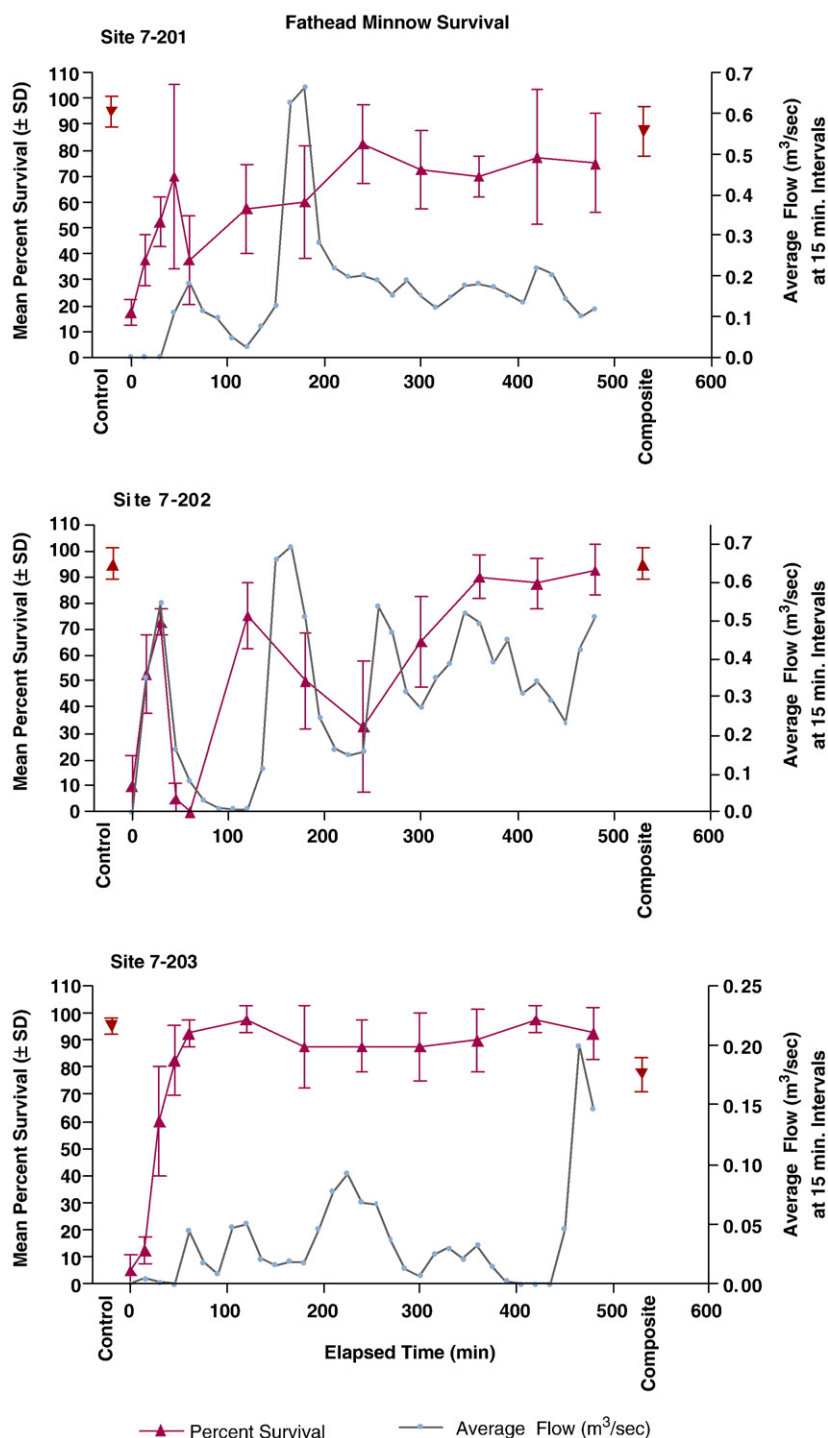


Fig. 6–Fathead minnow survival test toxicity response to runoff samples (October 26–27 2004 storm event).

often produced the greatest toxicity response (i.e., lowest fertilization percentage) recorded for the event. A few of the post 60-min. grab samples were substantially toxic to sea urchins. Toxic samples were most often associated with periods of relatively low flow velocity.

Toxicity results for Microtox™ for the storm event of February 11, 2003 are shown in Fig. 3. In summary, only the first one or two grab samples were toxic to Microtox™ at the selected test concentration of 9.4%. All other samples showed

enhancement, which was characterized by a higher level of luminescence (light counts) compared to the controls.

3.1.2. Toxicity to freshwater species

Representative toxic responses in undiluted stormwater grab samples for *Ceriodaphnia* and fathead minnows are summarized in Figs. 4 and 5 for a storm event that occurred on March 15, 2003. In summary, toxicity to both *Ceriodaphnia* and fathead minnows was frequently observed, and generally greatest in grab samples

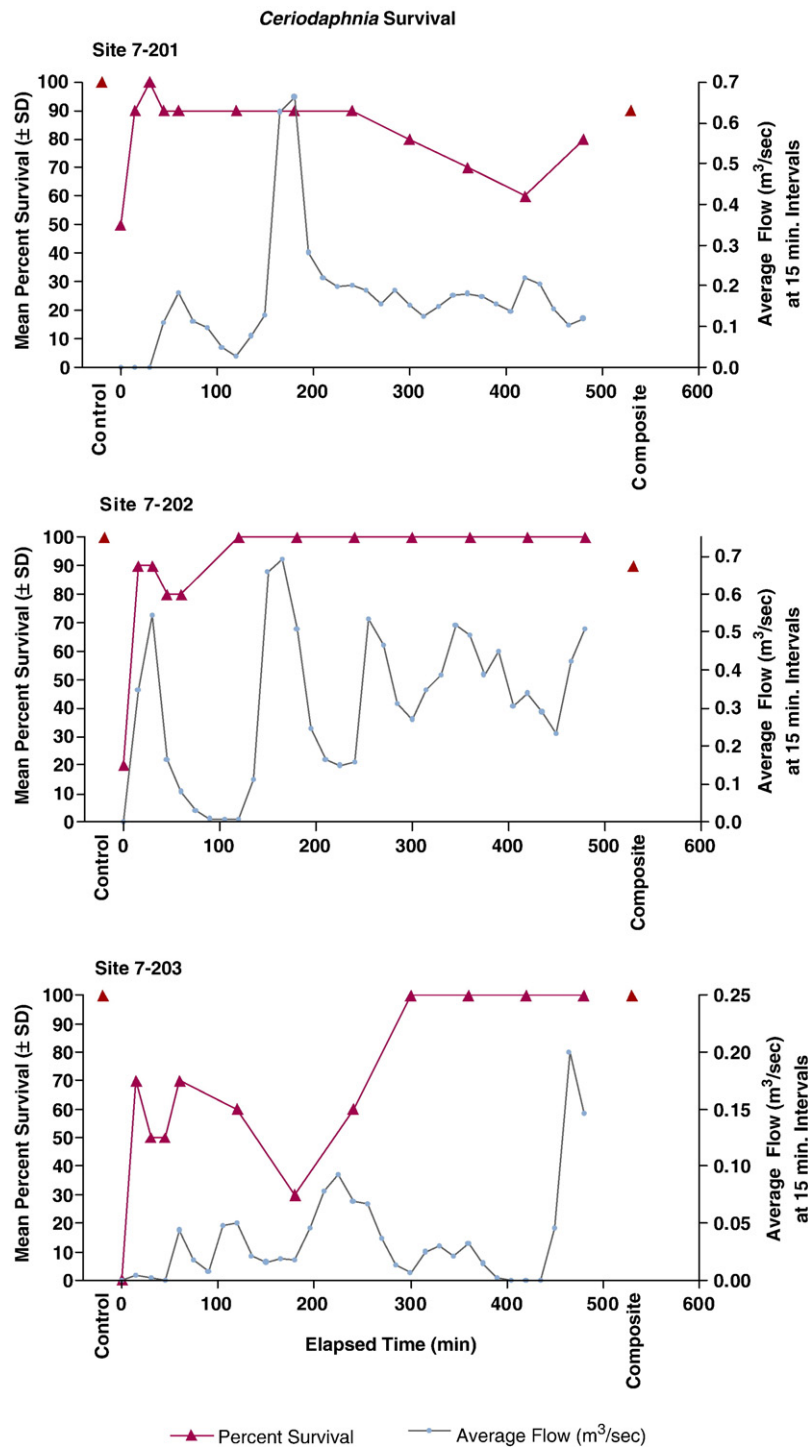


Fig. 7—*Ceriodaphnia* survival test toxicity response to runoff samples (October 26–27 2004 storm event).

collected early during the storm event (Table 2, Figs. 4 and 5). *Ceriodaphnia* reproduction and fathead minnow growth endpoints for the same event (not shown here) exhibited a similar temporal pattern in toxicity at both sites, with the greatest effects present in samples collected during the first 60 min. of discharge.

The response of green algae to the stormwater grab samples was more variable than that observed for the other test methods (not shown here). A similar temporal pattern was evident, however, with the greatest toxic response usually occurring in a

few of the samples collected during the first 60 min. of discharge. Algal growth was enhanced in many of the grab samples, possibly due to additional available nutrients.

Similar toxicity responses and variability were observed in grab samples collected during the 2004–05 wet season. Representative toxicity results for fathead minnows and *Ceriodaphnia* for the three highway sites during a storm event in October 2004 are shown in Figs. 6 and 7. The toxicity of the composite sample is also presented on the same plot. In

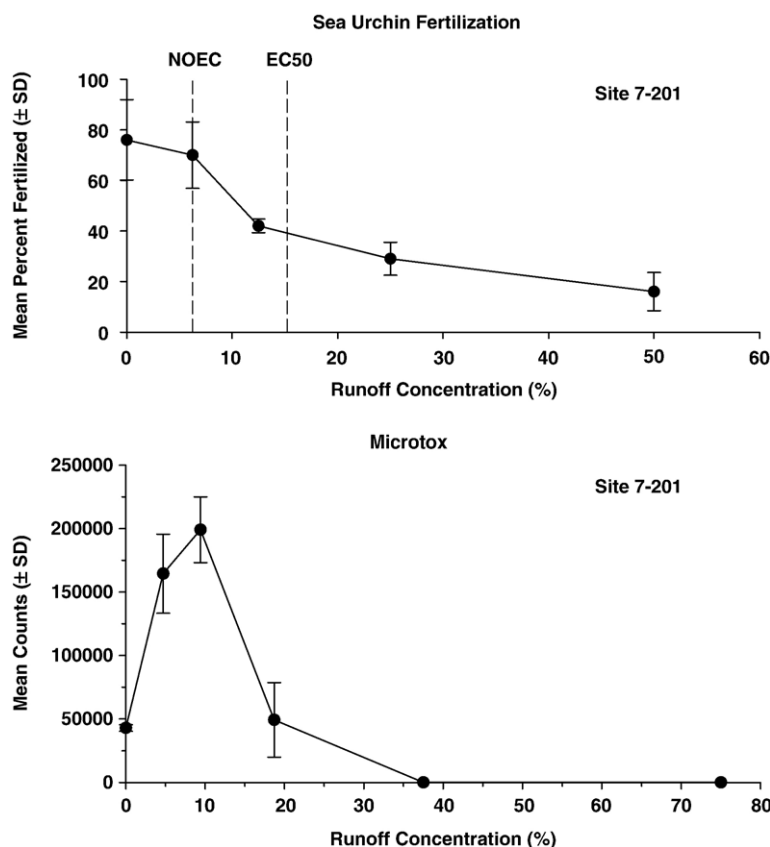


Fig. 8—Dose-response plots for the sea urchin egg fertilization and Microtox™ tests for the February 11, 2003 stormwater composite sample from Site 7-201.

summary, once again the early runoff samples were generally more toxic compared with those collected later during the storm event; however, toxicity was also observed to increase substantially later during a storm in several cases.

3.2. Toxicity evaluation of composite samples

The runoff composite samples were always toxic to sea urchins and Microtox™, rarely toxic to *Ceriodaphnia* or fathead minnows, and never toxic to algae (Table 2). As shown in Fig. 8, a strong dose-response was present for sea urchins in each

composite sample, with EC50s ranging from 10.3% to 15.2%. Toxicity was detected at runoff concentrations as low as 6%. A different dose-response relationship was present for the Microtox™ test; each of the composite samples produced a stimulatory effect on the bacteria in some test dilutions, resulting in higher luminescence than in the controls and confounding calculation of the EC50 (Fig. 8).

Composite sample toxicity was less frequent and of lesser magnitude with the freshwater tests, despite the presence of a few grab samples for the same storm event that were toxic individually (Figs. 6 and 7). This observation may be due to the

Table 3 – Spearman correlations between toxicity test methods

Method	Spearman r (p value)					
	Microtox™	<i>Ceriodaphnia</i> survival	<i>Ceriodaphnia</i> reproduction	Minnow survival	Minnow growth	Algae growth
Sea urchin fertilization	0.40(0.02)	−0.22(0.20)	0.12(0.56)	0.02(0.88)	0.39(0.10)	0.12(0.52)
Microtox™		0.20(0.24)	0.04(0.86)	0.13(0.46)	0.06(0.80)	0.20(0.27)
<i>Ceriodaphnia</i> survival			0.84(0.00)	0.54(0.00)	0.52(0.02)	0.66(0.00)
<i>Ceriodaphnia</i> reproduction				0.63(0.0)	0.23(0.36)	0.29(0.17)
Minnow survival					0.44(0.06)	0.48(0.00)
Minnow growth						0.04(0.86)

Note: Analysis based on first five grabs for 2003 runoff samples; and N varies from 17 to 35.

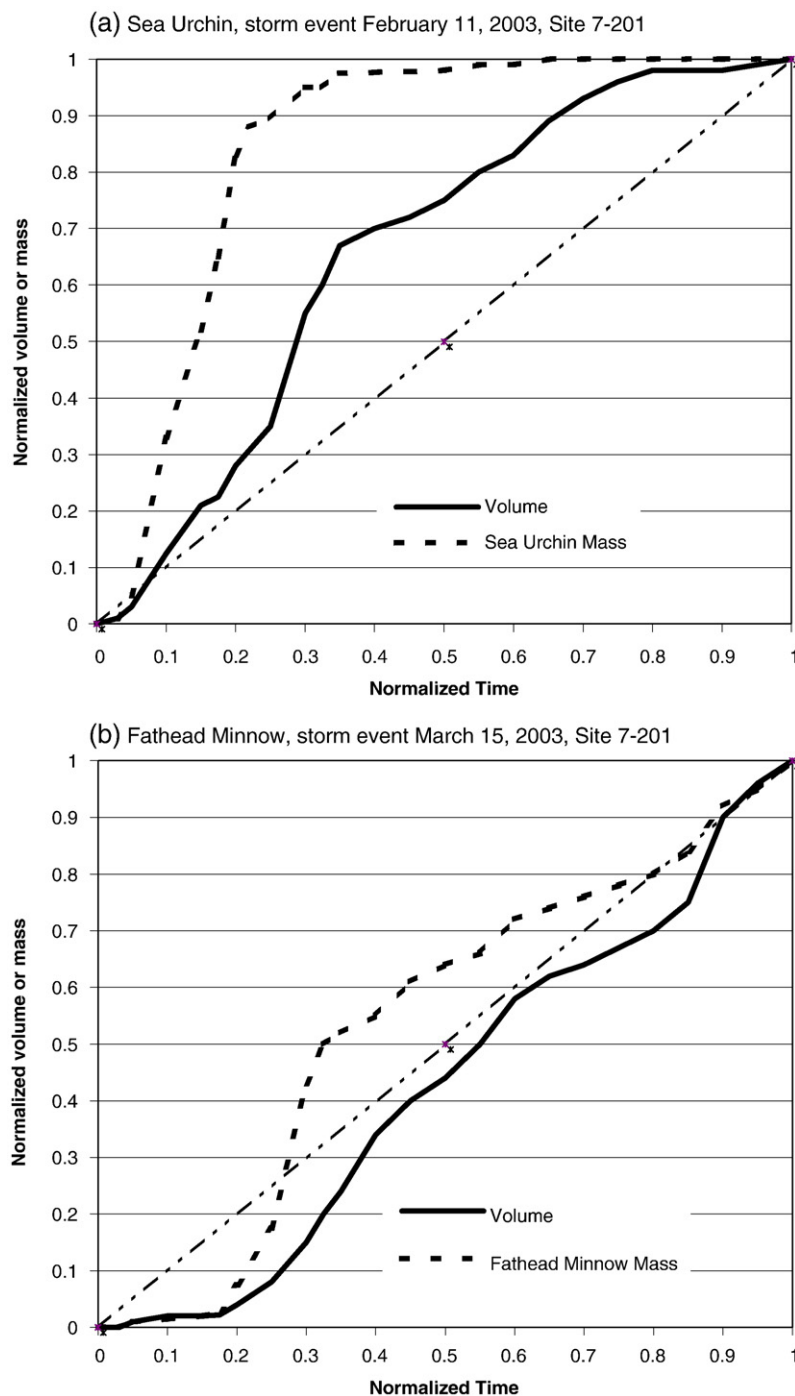


Fig. 9—First flush effect of toxicity based on normalized time and toxicity mass for (a) sea urchin and (b) fathead minnow, exposed to grab runoff samples.

fact that only a few of the early runoff samples were toxic and this effect was, therefore, diluted by mixing with other runoff samples.

3.3. Comparative toxicity responses between test methods

Substantial differences were observed among the results for the different toxicity tests. The sea urchin fertilization test was usually the most responsive of the five methods and it was the only method that reliably detected toxicity in the composite samples (Table 2). The Microtox™ and green algae

tests had limited utility in this study because enhancement of luminescence or cell growth was evident in many of the samples, potentially confounding the identification of toxicity patterns. The degree of toxicity observed using *Ceriodaphnia* and fathead minnow tests was typically much less in both grab and composite samples than that observed using the two marine species (Table 2).

The magnitude of toxicity responses for the sea urchin and Microtox™ tests were significantly correlated with each other (Table 3). Correlations were also present between *Ceriodaphnia*

Table 4 – Estimated proportion of toxicity discharged at normalized storm volumes of 0.2 and 0.3

Site	Toxicity test	February 11, 2003		March 15, 2003	
		0.2	0.3	0.2	0.3
7-201	Sea urchin fertilization	0.42	0.43		
7-201	Microtox™			0.20	0.30
7-201	Fathead minnow survival			0.51	0.57
7-202	Sea urchin fertilization	0.48	0.88		
7-203	Sea urchin fertilization	0.19	0.20		
7-203	<i>Ceriodaphnia</i> survival	0.44	0.45	0.26	0.26
7-203	Fathead minnow survival			0.27	0.31

marine tests did not have a statistically significant correlation with the freshwater test responses, suggesting that different types of toxicants may have been affecting some of the species (Schiff et al., 2002). There was a general correspondence between marine and freshwater methods in the temporal pattern and relative severity of toxicity among the different sites, however (i.e. a first flush effect is apparent and runoff from Site 7-201 is less toxic than that from 7-203 (Figs. 2–5)).

3.4. First flush effect on toxicity results

First flush effects on runoff toxicity were evaluated by two different methods: (1) a toxicity mass loading approach over time, and (2) hydro-toxicity-graphs that show the magnitude of toxicity relative to flow over time.

3.4.1. Toxicity mass loading

The first method to evaluate the effect of first flush was the creation of visual plots showing relative toxicity on a mass basis normalized to flow volume over time. This evaluation removes the effect of flow rate on the interpretation of toxicity results. As an example, a first flush toxicity effect on sea urchins for samples collected from a storm event on February 11, 2003 is shown in Fig. 9(a). A first flush effect for toxicity was evident in samples from both Sites 7-201 and 7-202 as the normalized proportion of toxicity to discharged runoff volume is greater for the initial stage of the storm event. For example, the proportion of sea urchin toxicity discharged during the first 20% of storm duration is 48% for Site 7-202 (Table 4).

Evidence of a first flush effect on fathead minnow or *Ceriodaphnia* toxicity was also observed in most cases. The response of fathead minnows, however, was less pronounced than that for sea urchins. An example of mass first flush for fathead minnows from Site 7-201 during the March 2003 storm event is shown in Fig. 9(b). As shown, approximately 50% of the estimated toxicity to fathead minnows was associated with the first 20% of discharged runoff volume.

3.4.2. Hydro-toxicity-graphs

Two representative types of hydro-toxicity-graphs were shown previously in Figs. 2–7. As shown in these examples, effects are generally greatest in the first five grab samples collected during the first hour of runoff. Pollutographs (pollutant concentration during the entire storm duration) enable us to evaluate the visual relationships between runoff volume, rainfall, and chemical pollutant concentrations. The

pollutograph can be related to the hydro-toxicity-graph to further examine the influence of pollutant concentrations on toxic effects. An example pollutograph for total and dissolved Cu and Zn for Site 7-201 during the February 11, 2003 storm event is shown in Fig. 10. Similar trends in copper and zinc concentrations were observed for most other storm events at this site, as well as at the other two sites. As shown, the concentrations of dissolved and total copper and zinc are substantially higher during the early portion of the runoff, which correlates well with the observed first flush toxicity effects attributed to these metals based on TIE results (Section 3.5). Pollutographs for other metals such as As, Cd, Cr, Pb and Ni were also generated, and similar trends were observed with metal concentrations generally greater during the early stage of a storm event compared with the end of a storm event (Stenstrom and Kayhanian, 2005). Nevertheless, the concentration of these metals was below that expected to cause toxicity to all of the species evaluated based on laboratory water spiking studies (Bitton et al., 1996; Schubauer-Berigan et al., 1993a,b; Ruesink and Smith, 1955; Sandrin and Maier, 2002) and Phase III TIE analyses with fathead minnows and *Ceriodaphnia* further suggest copper and zinc were primary trace metals of concern.

The results presented in these figures, and similar results obtained during other storm events, clearly indicate a first flush effect. In most cases the greatest degree of toxicity was observed during the early stages of a storm event when lower runoff volume was discharged (see Figs. 2–7). Toxicity, however, was occasionally not related to the first flush samples and occurred in grab samples collected later during a storm (see Figs. 2, 6, and 7). It is important to note that, even when a strong first flush effect was observed, the majority of composite samples were non-toxic to both fathead minnows and *Ceriodaphnia*.

3.5. Cause 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 TIE procedures specified in the method section. TIE studies performed to date suggest 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 and zinc. Successful reduction or complete removal of toxicity following addition of a cationic chelating agent, EDTA, and subsequent metal spiking studies provided multiple lines of evidence identifying copper and zinc as the primary toxicants of concern. The cause of toxicity in the highway runoff for nearly 10% of the samples also appeared to be related to anionic surfactants.

An example of Phase I TIE characterization results for two few grab samples is provided in Fig. 11. As shown, EDTA successfully removed all observed toxicity. The specificity of this treatment indicates that cationic trace metals are responsible for observed toxicity in all of these samples. Zeolite, and to some extent, the C18 procedure reduced toxicity as well, however, these treatments are also capable of removing trace metals (USEPA, 1991, 1993a,b) and this was verified analytically. The pH 9 treatment also eliminated or substantially reduced toxicity in the examples shown. The pH 6 treatment, on the

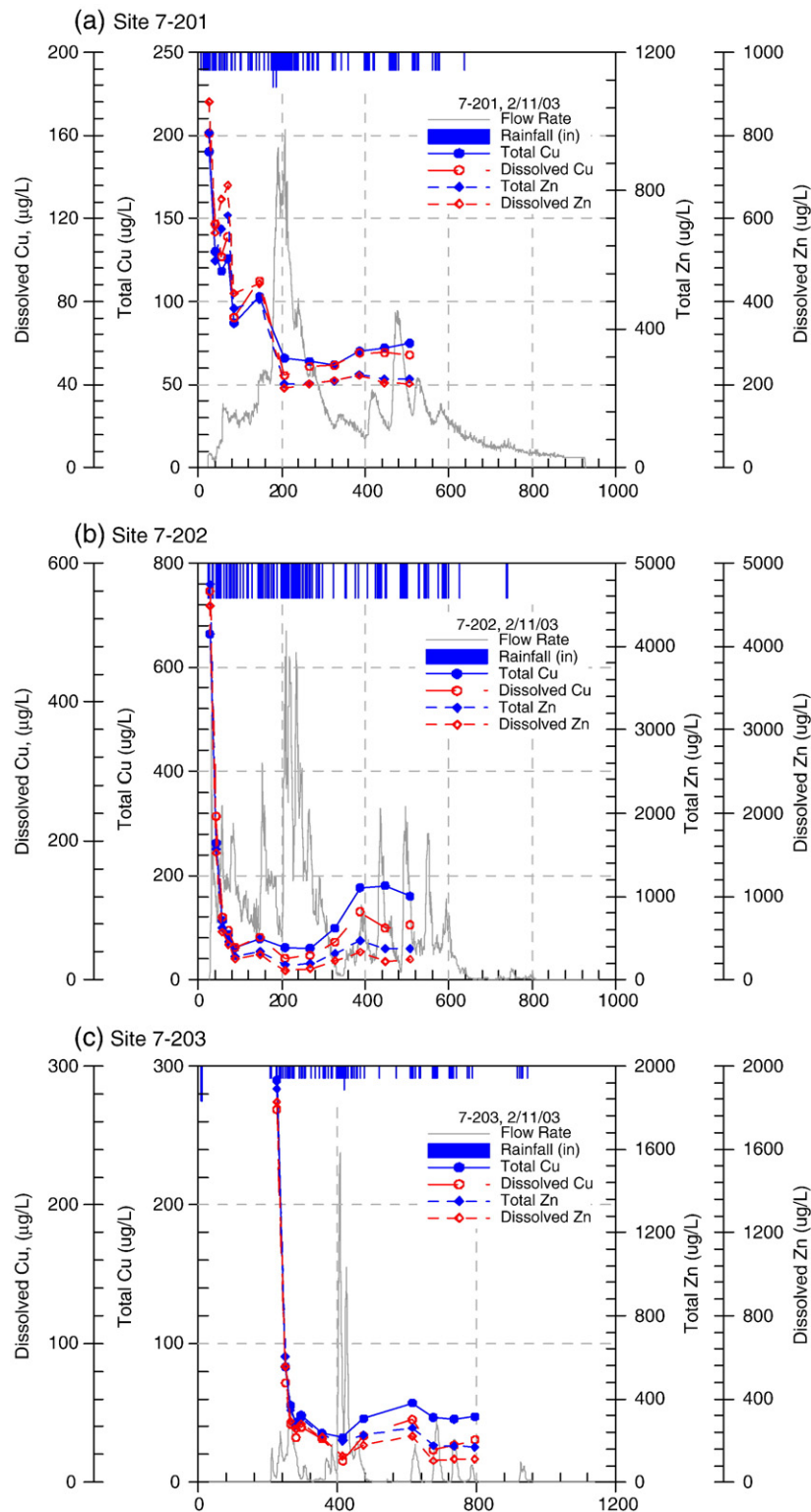


Fig. 10 – Pollutographs showing the variability of dissolved Cu and Zn over time for three highway sites during the storm event of February 11, 2003.

other hand, increased toxicity in several cases. These results indicate that the cationic trace metals of concern in these grab samples likely include metals that increase in toxicity as pH decreases, such as copper and lead (Schubauer-Berigan et al., 1993a). The toxicity of some metals including cadmium, nickel,

and zinc, have been found to increase in toxicity to *Ceriodaphnia* with increased pH. Based on toxicity and pH relationships observed during these tests, it does not appear that these three metals are primarily responsible for the observed toxicity in the samples shown in Fig. 11. The pH/toxicity relationships

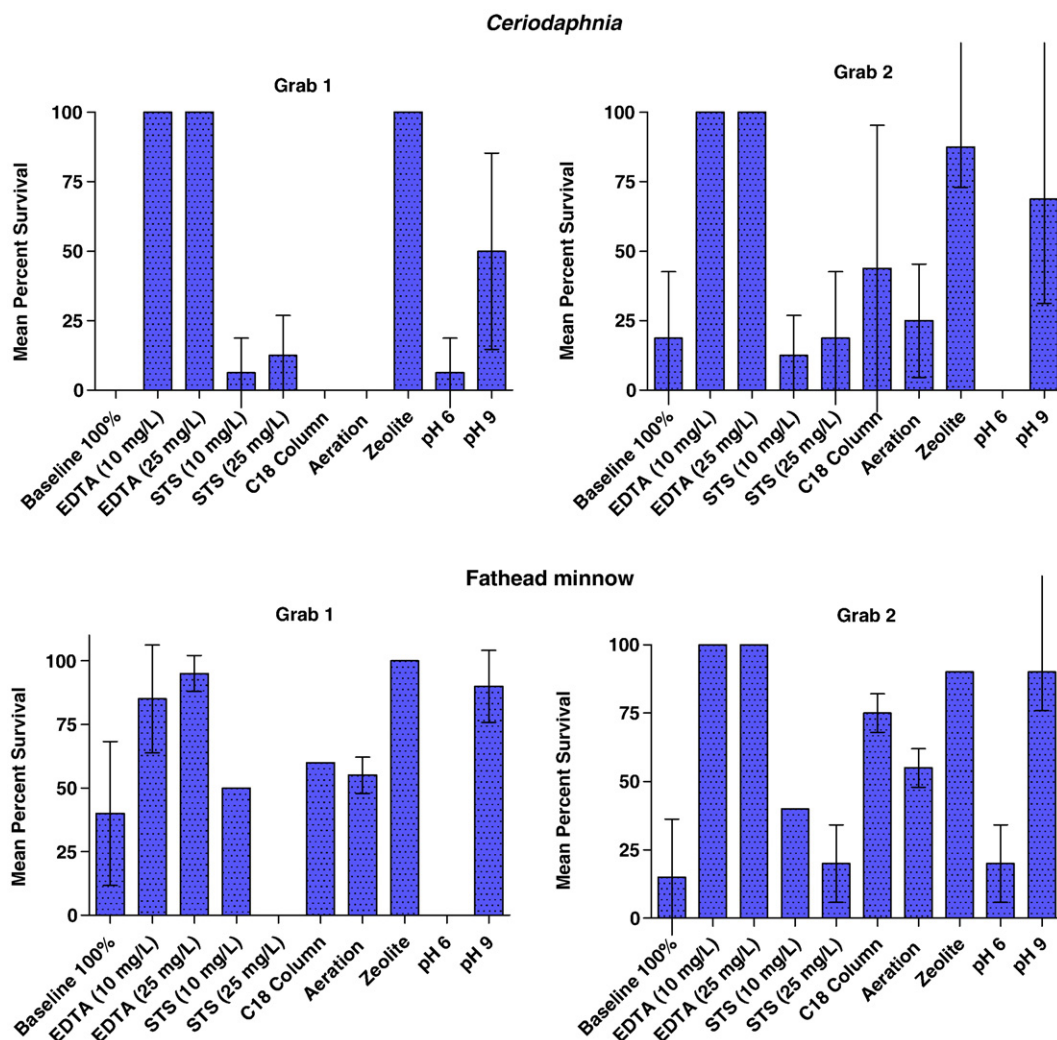


Fig. 11 – Phase I TIE results for *Ceriodaphnia* and fathead minnows (April 28, 2005 storm event Site 7-202, mean $\pm$ 1SD, $n=4$).

varied among samples; in some samples the opposite trend was observed (toxicity increased at pH 9 and was reduced at pH 6) suggesting toxicity due to metals such as zinc or nickel, as opposed to copper or lead, in samples where EDTA successfully removed toxicity.

3.6. Use of analytical chemistry data to estimate toxic effects

Since copper and zinc were the dominant toxicants identified based on a combination of TIE results and measured concentrations relative to documented effect levels, most of the discussion presented in this section is devoted to these two metals. Dissolved concentrations of these metals, in addition to lead and nickel, and summary statistics for four to five storm events monitored between February 2003 and March 2005 at the three highway sites are reported in Table 5. The highest concentration of dissolved copper measured (560 $\mu\text{g/L}$) was approximately 59 times greater than the lowest acute LC50 value reported for *Ceriodaphnia* (9.5 $\mu\text{g/L}$), and 37 times the lowest chronic LC50 value reported for fathead minnows (15 $\mu\text{g/L}$) at a similar pH range (Schubauer-Berigan et al., 1993a). The mean concentrations of copper in the first five grab samples

from Site 7-202 (136 $\mu\text{g/L}$) and Site 7-203 (70 $\mu\text{g/L}$) were very similar to chronic LC50 values derived for both species (Nautilus Environmental, 2005). The highest concentration of dissolved zinc (4,490 $\mu\text{g/L}$) was up to 47 times greater than the lowest acute LC50 value reported for *Ceriodaphnia* (95 $\mu\text{g/L}$), and 14 times the lowest acute LC50 value reported for fathead minnows (330 $\mu\text{g/L}$) at a similar pH range (Schubauer-Berigan et al., 1993a).

The overall mean concentrations of zinc at all three sites, ranging from 166 to 290 $\mu\text{g/L}$, were greater than the lowest LC50 values reported for *Ceriodaphnia* in the literature (Bitton et al., 1996; Schubauer-Berigan et al., 1993a; Nautilus Environmental, 2006), but less than median effect concentrations reported for fathead minnows. The concentration of nickel also exceeded values found to cause toxicity to *Ceriodaphnia* at a pH > 8.0 (Schubauer-Berigan et al., 1993a); however, the pH of the test samples was generally well below 8. TIE pH-adjustment tests further suggested that nickel, which has been found to increase in toxicity at elevated pH, was not responsible for observed effects to *Ceriodaphnia*, as toxicity of stormwater to this species generally decreased at an elevated pH. These combined observations appear to limit toxicological concern due to this particular trace metal. Relationships

Table 5 – Statistical summary of dissolved trace metal concentrations for storm events monitored from the three highway sites between February 2003 and March 2005

Highway site	Sample size/ sample type	Statistical value	Dissolved trace metal concentration (µg/L)					
			Cu	Pb	Ni	Zn		
7-201	47/All grabs	Range	8.0–161	0.25–1.9	0.99–35	6.8–880		
		Median	21	0.76	5.3	102		
		Mean	38	0.86	6.8	166		
		SD ^b	38	0.40	6.8	189		
	20/Grabs 1–5	Range	14.3–161	0.25–1.63	4.4–35.3	6.8–880		
		Median	30	0.80	6.2	121		
		Mean	55	0.99	11	241		
		SD ^b	49	0.43	8.3	252		
		7-202	60/All grabs	Range	3.5–560	0.19–13	0.42–100	18–4490
				Median	28	0.8	6.0	116
Mean	71			1.3	13	290		
SD ^b	107			1.8	19	617		
25/Grabs 1–5	Range		3.5–560	0.50–12.5	5.1–100	86–4490		
	Median		107	1.6	19	321		
7-203	53/All grabs	Mean	136	2.3	25	563		
		SD ^b	141	2.5	25	890		
		Range	4.6–269	0.95–72	0.90–58.6	25–1830		
		Median	29	3.2	4.7	96		
	19/Grabs 1–5	Mean	45	9.8	8.5	168		
		SD ^b	54	16	12	261		
		Range	9.7–269	0.95–22	1.95–59	46–1830		
		Median	41	3.9	7.0	157		
		Mean	70	5.5	14	261		
		SD ^b	70	4.8	15	366		

^a Data provided here includes a total of four storms monitored at Site 7-201 and five storms monitored at Sites 7-202 and 7-203.

^b SD=standard deviation.

between concentrations of dissolved copper and zinc in highway runoff and fathead minnow and *Ceriodaphnia* survival are summarized in Fig. 12. Fig. 12 combines the available data collected at all three locations from 4 to 5 storm events between February 2003 and March 2005. Relative threshold levels for toxicity in this study are depicted as vertical dashed lines on each figure. Toxicity, for the purpose of this comparison, is defined as a minimum 20% effect relative to the control. Based on the data collected to date, it is likely (>99% chance) that toxicity will occur when concentrations of dissolved copper and zinc exceed the threshold values depicted in Fig. 12. These data, however, fail to provide strong evidence for cause and effect relationships for the following reasons:

- (1) Chemical data often co-vary;
- (2) Only a select group of potentially toxic chemicals were analyzed and reported;
- (3) A number of environmental parameters may affect the toxicity of any given compound (e.g., pH, hardness, particulates, total and dissolved organics) (Paquin et al., 2003);
- (4) Speciation of the chemical may strongly affect toxicity (e.g., dissolved metals may consist of a variety of ionic

and nonionic forms that may differ greatly in their toxicity (Sandrin and Maier, 2002);

- (5) Stormwater consists of a complex mixture of compounds that may interact in a variety of ways to affect the toxicity of any given constituent (e.g., some chemicals, such as trace metals, organophosphorous pesticides, and ammonia are known to have additive or synergistic toxicity (Forget et al., 1999; Bailey et al., 1997, 2001); and mixtures of other chemicals may also result in antagonistic or inhibitory effects (Ankley et al., 1991); and
- (6) Combining all data for all storms may dilute relationships that may be clear for certain groups of samples.

Above a specific minimal level of both Cu and Zn, a substantial toxic effect is nearly always observed (Fig. 12). There are a few instances, however, where there is little or no effect despite elevated metal concentrations, presumably due to factors affecting bioavailability (Paquin et al., 2003; Schubauer-Berigan et al., 1993b). Interestingly, however, we have also observed that a relatively large number of samples with dissolved Cu and Zn concentrations below threshold values were still toxic, suggesting that either environmental parameters (i.e., pH, low hardness, and low DOC) may be enhancing the toxicity of these two metals relative to that in standard laboratory exposures, or toxicity may be due to some other chemical or chemicals in some samples. Another interesting observation was the relationship between laboratory-derived LC50 values and threshold toxicity effects for the two species evaluated. Laboratory-derived LC50 values for fathead minnows were greater than observed threshold effects concentrations, whereas laboratory-derived LC50 values for *Ceriodaphnia* were less than observed threshold effects concentrations for both copper and zinc (e.g., toxicity to fathead minnows was greater than expected in many samples and toxicity to *Ceriodaphnia* was less than expected in many samples, based on copper and zinc concentrations alone).

3.7. Assessment of toxic effects based on toxic units

The relationships between the suspected primary toxicants of concern identified during both TIE procedures and a review of analytical chemistry data was further evaluated by comparing predicted toxicity of copper and zinc based on their concentration in toxic samples to observed toxicity (Figs. 12 and 13). Data with non-toxic responses were excluded from analyses in order to focus on only those samples exhibiting toxicity. These relationships were evaluated by comparing toxic unit values (TUs) for the stormwater sample (100/stormwater LC50) and TUs predicted for each metal (metal concentration in the sample/laboratory-derived LC50 values). To be conservative, the lowest available laboratory-derived LC50 values at pH values similar to that typically observed in highway stormwater runoff were used for all TU calculations.

TU comparisons found statistically significant observed versus predicted relationships for both copper and zinc alone ($p < 0.001$). The relationship for fathead minnows was slightly stronger for copper than for zinc (r^2 values of 0.43 and 0.36, respectively). The relationship for *Ceriodaphnia*, however, was not different between the two metals ($r^2 = 0.30$ to 0.31). These relationships, in general, were able to explain only 30 to 40% of

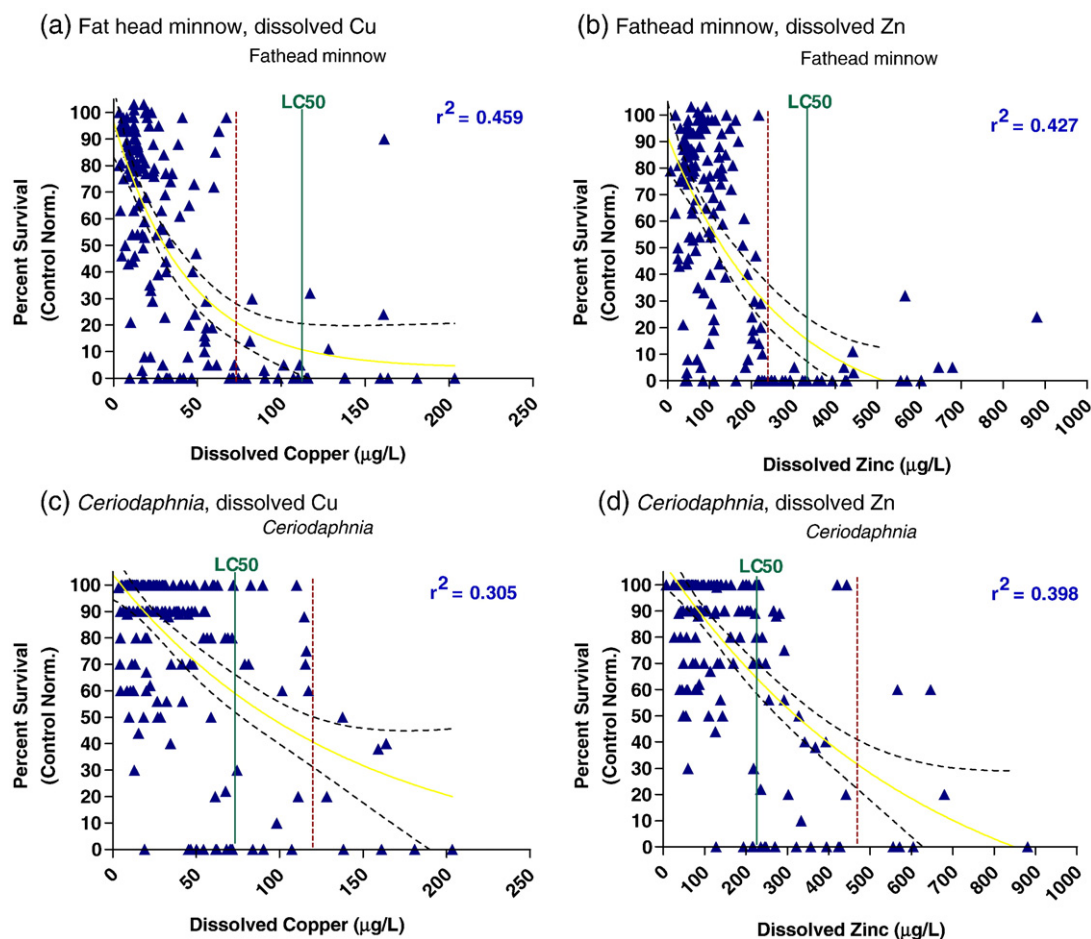


Fig. 12—Distribution of dissolved and copper and zinc data and relation to 7-day survival of fathead minnows and *Ceriodaphnia* in highway runoff samples from all samples collected between February 2003 and March 2005. Relative threshold concentrations equivalent to a 20% or greater response relative to the control are shown by the vertical dashed lines. Mean laboratory-derived LC50 values are shown by the vertical solid lines for comparison. Seven-day chronic LC50 values are shown with the exception of zinc for fathead minnows depicting a 96-LC50 value. Toxicity and trace metal relationships were evaluated using non-linear regression using one-phase exponential decay.

the observed toxicity as reflected by r^2 values provided in Figs. 13 and 14.

Many of the samples were more toxic than would be predicted by copper or zinc concentrations alone. Summing the toxic units for copper and zinc, however, failed to enhance the observed versus predicted relationship relative to either of these two metals alone. These results, in addition to TIE results, suggest that both copper and zinc are likely toxicants of concern; however, other environmental parameters (i.e., pH, hardness, and DOC) or other toxicants not included in this evaluation may be contributing to unaccounted-for differences in observed versus predicted toxicity.

4. Conclusions

Major conclusions drawn from this research study are summarized below:

- (1) Toxicity to both freshwater and marine species was frequently observed, but varied between storm events,

test species, and monitoring locations. The sea urchin fertilization test was the most sensitive of the five methods evaluated.

- (2) Samples from all storms exhibited toxicity to all test species, but there was little correlation between the marine and freshwater tests. Fathead minnows were more sensitive than *Ceriodaphnia* for most of the stormwater samples tested. The toxicity responses for green algae and Microtox™ were less consistent, and occasionally showed stimulation or atypical dose response relationships. The availability and concentration of nutrients in stormwater likely contributed to the additional variability observed with these two species.
- (3) A first flush effect was almost always observed with both freshwater and marine species. In general, a majority of the toxicity was attributable to early storm duration. However, toxicity was occasionally not related to the first flush samples and occurred in grab samples collected later during a storm. While all species responded to the first flush, toxicity in samples collected

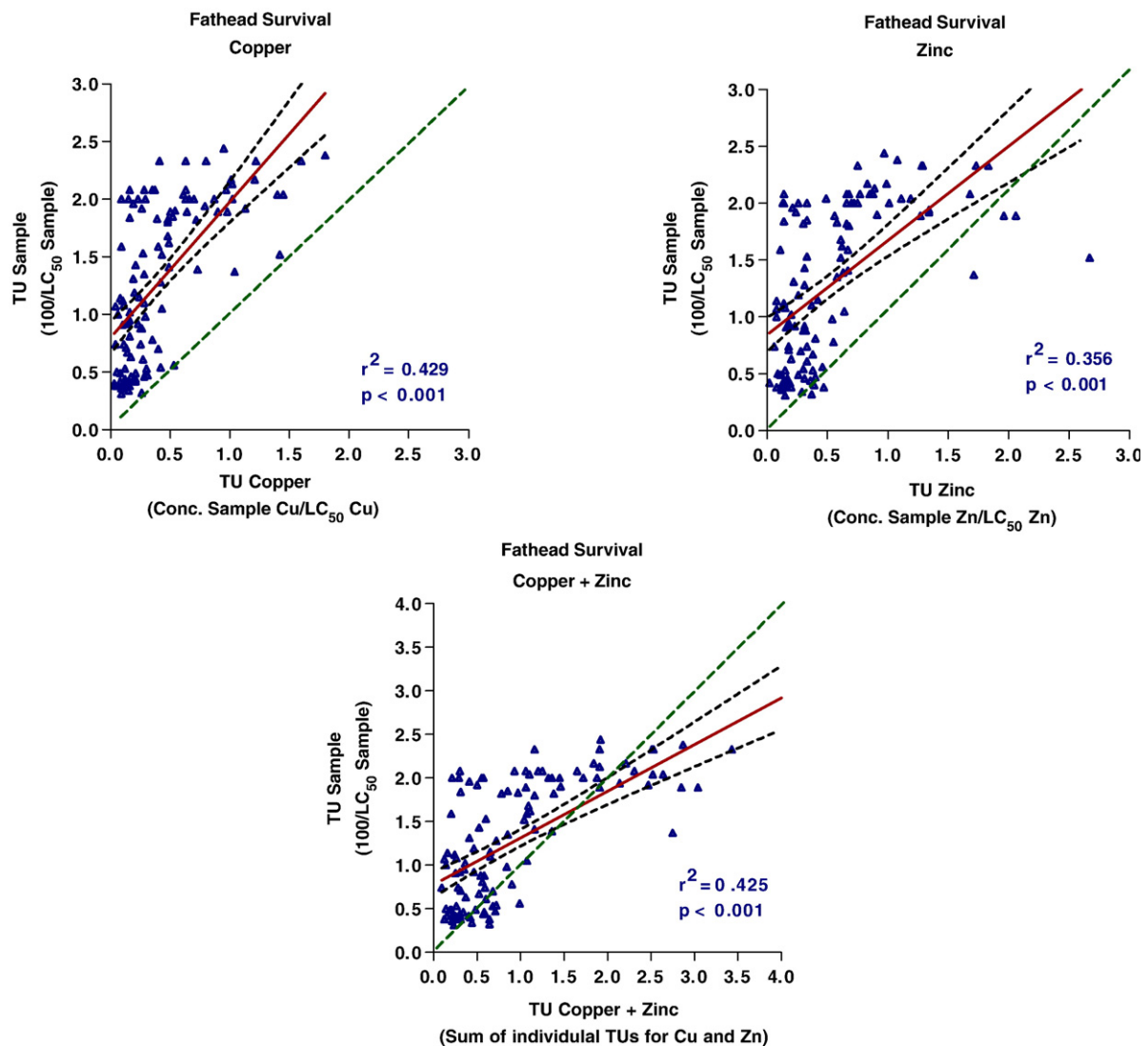


Fig. 13 – Correlation (linear regression) between stormwater toxic units for fathead minnows (100/LC₅₀) and predicted TUs due to copper alone, zinc alone, and zinc + copper (metals concentration/laboratory-derived LC₅₀ values of 114 µg/L for copper and 330 µg/L for zinc). Analyses include all toxic samples collected between February 2003 and March 2005.

after the first hour of each storm often differed between species. The proportion of toxicity attributable to the first flush suggests that efforts focused on treating the first portion of stormwater runoff volume may be more beneficial in protecting the environment while also being more cost effective.

- (4) A majority of the composite samples were non-toxic to freshwater test species, even when a strong first flush effect was observed in individual grab samples. A toxic response in composite samples was more prevalent with the marine species tested.
- (5) A primary cause of toxicity to *Ceriodaphnia* and fathead minnows for the most toxic runoff samples at the three highway sites evaluated was identified as copper and zinc. Results also suggest that anionic surfactants were also a cause of toxicity in a few samples.
- (6) A review of the analytical data collected during this study indicated that observed toxicity to fathead

minnows and *Ceriodaphnia* was not all accounted for by the measured concentrations of dissolved copper and zinc alone, the constituents identified as the probable cause of toxicity to these two species in several samples. Greater sensitivity of fathead minnows in many samples relative to *Ceriodaphnia* further suggests that some other toxicant, in addition to copper or zinc, is causing toxicity to the minnow.

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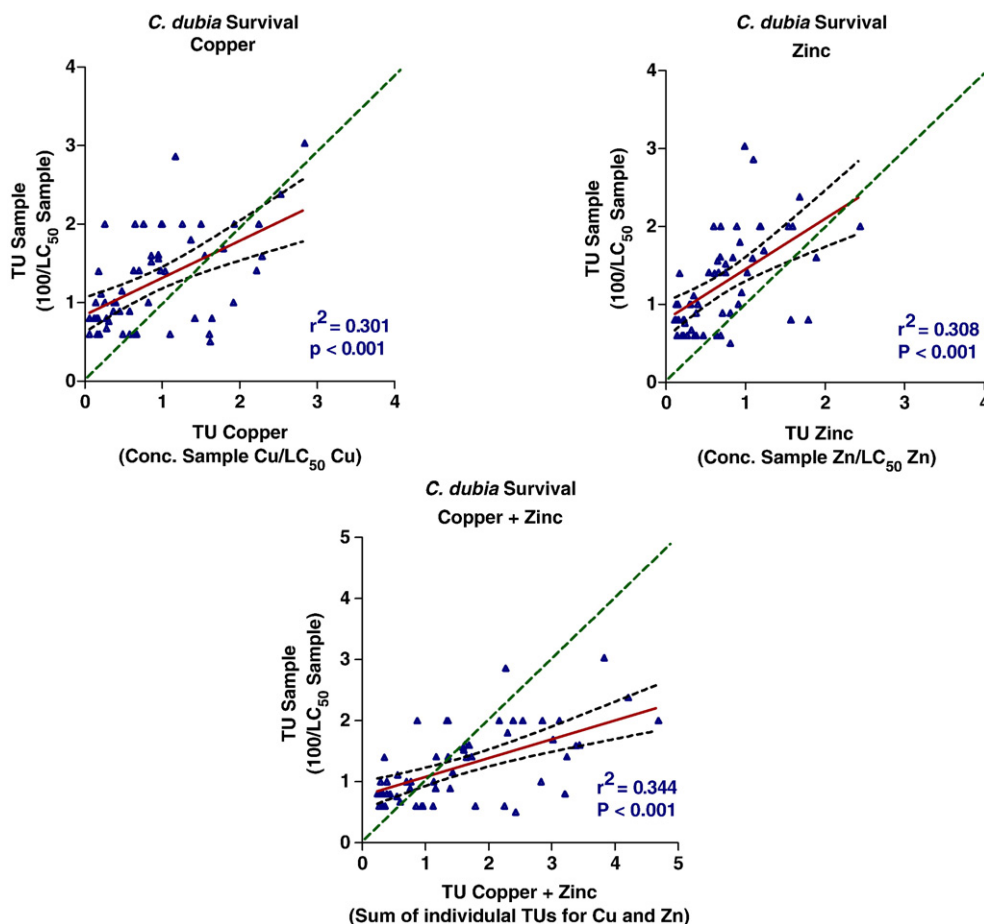


Fig. 14—Correlation (linear regression) between stormwater toxic units for *Ceriodaphnia* (100/LC₅₀) and predicted TUs due to copper alone, zinc alone, and zinc+copper (metals concentration/laboratory-derived chronic LC₅₀ values of 72 µg/L for copper and 210 µg/L for zinc). Analysis includes all toxic samples collected between February 2003 and March 2005.

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