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 alga *Pseudokirchneriella subcapitatum*) 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 estimated mass of toxicity was associated with the first 20% of discharged runoff volume. Furthermore, on average, 90% of the toxic samples were collected 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.

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. Toxicity from roadways was also examined in a study of simulated runoff from parking lots; toxicity to the purple sea urchin egg fertilization was present 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.

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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.

Methods

Sample Collection

Stormwater runoff samples were collected from three highly urbanized highway sites in West Los Angeles (Figure 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 hours 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 alga *Pseudokirchneriella subcapitatum* and two marine species (the purple sea urchin *Strongylocentrotus purpuratus* and the luminescent bacterium *Photobacterium 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.

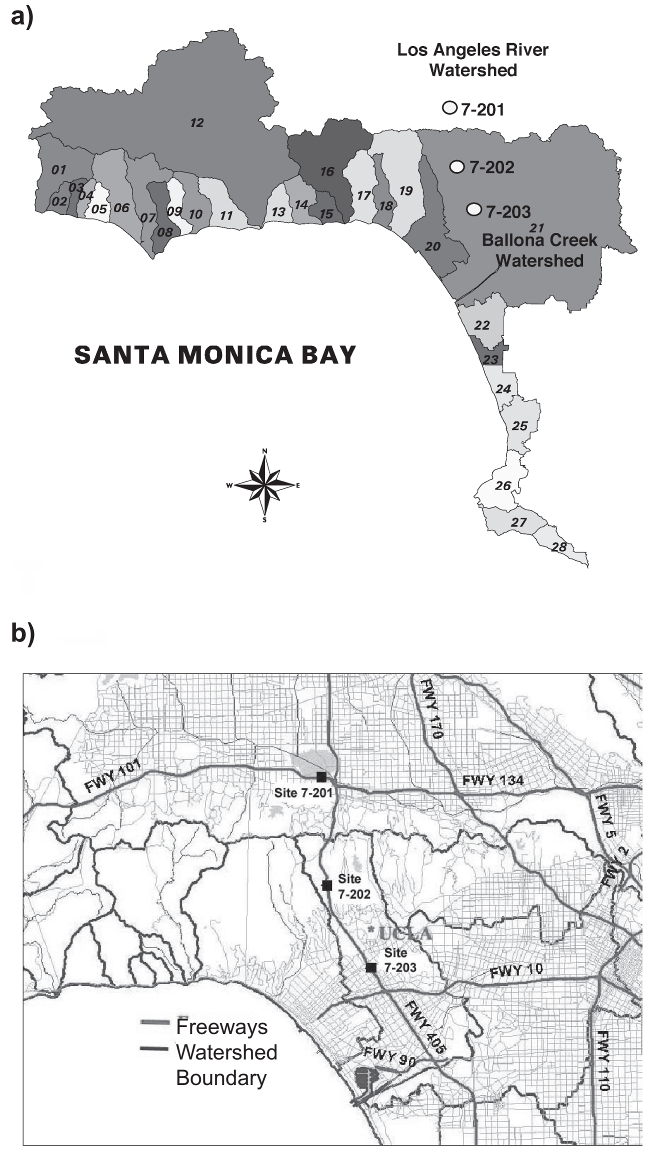

Figure 1. Monitoring site locations with respect to local watershed boundaries (a) and major highways (b).

Description of Toxicity Testing

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 exposure of sperm to the samples. Eggs were then added and given 20 minutes 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 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.

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 dubia*	Fathead Minnow	*Pseudokirchneriella subcapitatum*
2002-03 Monitoring Season							
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
2004-05 Monitoring Season							
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 Figure 1.

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.

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 hours 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.

Ceriodaphnia 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 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 *P. subcapitatum* alga daily according to EPA protocol guidance. Control and dilution water consisted of moderately hard synthetic water prepared with eight parts nanopure water and two 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.

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 USEPA (2002b) guidelines. The test design consisted of four 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 eight parts nanopure water and two 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 hours 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 hours. After drying, fish were weighed on a Mettler 240AE balance to the nearest 0.01 mg.

Pseudokirchneriella subcapitatum *algal growth inhibition*

The freshwater unicellular alga, *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 ±2ºC in darkness in the event that microscopic confirmation of cell density was needed (using an improved Neubauer hemacytometer at 400 x 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.

Toxicity Identification Evaluations (TIEs)

TIE methods generally followed those published by USEPA (1991, 1993a, 1993b). 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 modifications with regard to test chamber size, followed the procedures published in USEPA (2002c). TIEs for both species were performed in 30-ml glass scintillation vials with 15 to 20 ml of 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 hours. 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. *C. dubia* were fed a mixture of *Pseudokirchneriella* algae and YCT at 48 hours.

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 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 one pg (one part per trillion in less than one ml) for nearly all measured metals.

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, *C. dubia*, 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, *C. dubia* 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 (2002 a,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, Equation 1 below:

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

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.

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

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

The toxic response data from each grab sample was then applied to Equation (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.

Results

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.

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 Figure 2. The grab samples from the first 60 minutes of the storm discharge were usually toxic to sea urchins (Table 2) and often produced the greatest toxicity response (i.e., lowest fertilization percentage) recorded for the event. A few of the post 60-minute 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 Figure 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.

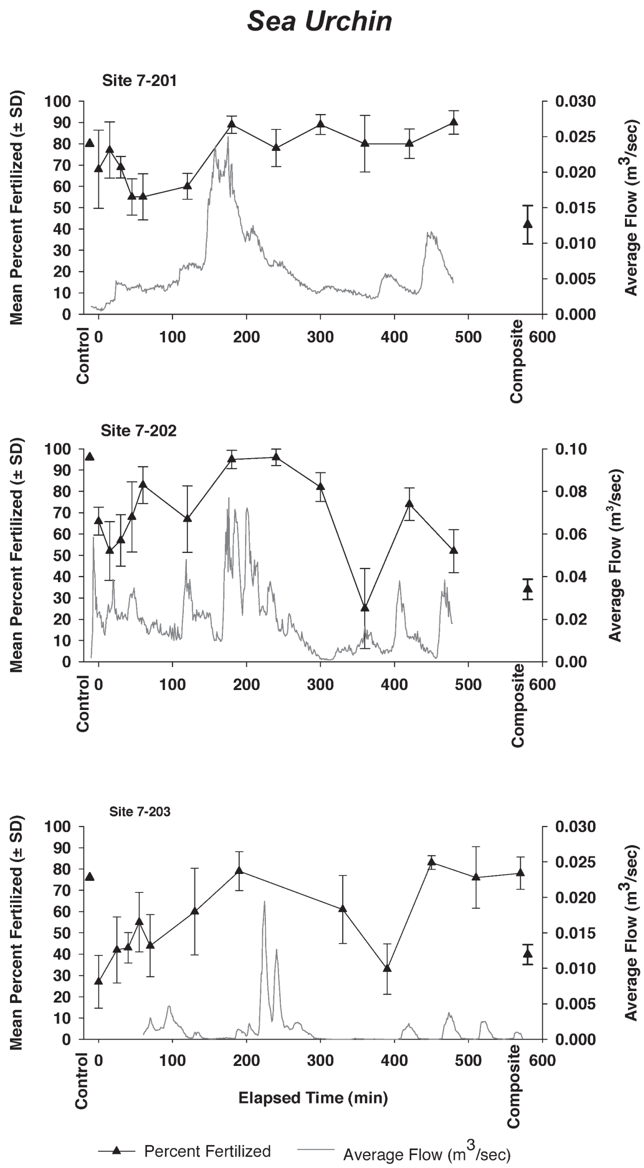

Figure 2. Sea urchin egg fertilization test response to runoff samples (February 11, 2003 storm event). Grab and composite samples were tested at a 12.5% concentration of stormwater; near the EC50 value determined in prior tests.

Toxicity to freshwater species

Representative toxic responses in undiluted stormwater grab samples for *C. dubia* and fathead minnows are summarized in Figures 4 - 5 for a storm event that occurred on March 15, 2003. In summary, toxicity to both *C. dubia* and fathead minnows was frequently observed, and generally greatest in grab samples collected early during the storm event (Table 2; Figures 4 - 5). *C. dubia* 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 minutes 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 minutes 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 *C. dubia* for the three highway sites during a storm event in October 2004 are shown in Figures 6 and 7. The toxicity of the composite sample is also presented on the same plot. In 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.

Toxicity Evaluation of Composite Samples

The runoff composite samples were always toxic to sea urchins and Microtox™, rarely toxic to *C. dubia* or fathead minnows, and never toxic to algae (Table 2). As shown in Figure 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 (Figure 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 (Figures 6 and 7). This observation may be due to the fact that only a few of the early runoff samples were toxic and this effect was, therefore, diluted by mixing with other runoff samples.

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

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

	Grabs 1 - 5		Composite			
Toxicity Test	N	% Toxic	N	% Toxic	EC50/LC50	NOEC
2002-2003 Samples						
Sea Urchin Fertilization	35	74	5	100	18	7
Microtox™	35	20	5	80	na	37
Ceriodaphnia Survival	34	66	2	50	>100	≥100
Ceriodaphnia 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
2004-2005 Samples						
Ceriodaphnia Survival	80	40	6	0	>100	≥100
Ceriodaphnia 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

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 *C. dubia* 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 *C. dubia* survival, fathead minnow survival, and algal growth. The 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; Figures 2 - 5).

The cause of the differences in toxicity response among test methods was not determined in this study, but it is likely that a combination of factors including differential sensitivity and matrix interactions played a role. The presence of significant correlations among the marine test methods and among the freshwater methods, but not between freshwater and marine species, suggests that there may have been an interaction with changes in sample ionic composition due to salinity adjustment for the marine tests. The greater responsiveness of the sea urchin and Microtox™ tests may have also been related to the short exposure duration of these, which would limit the loss of toxicity due to degradation, changes in speciation, or sorption onto test chamber surfaces.

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.

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

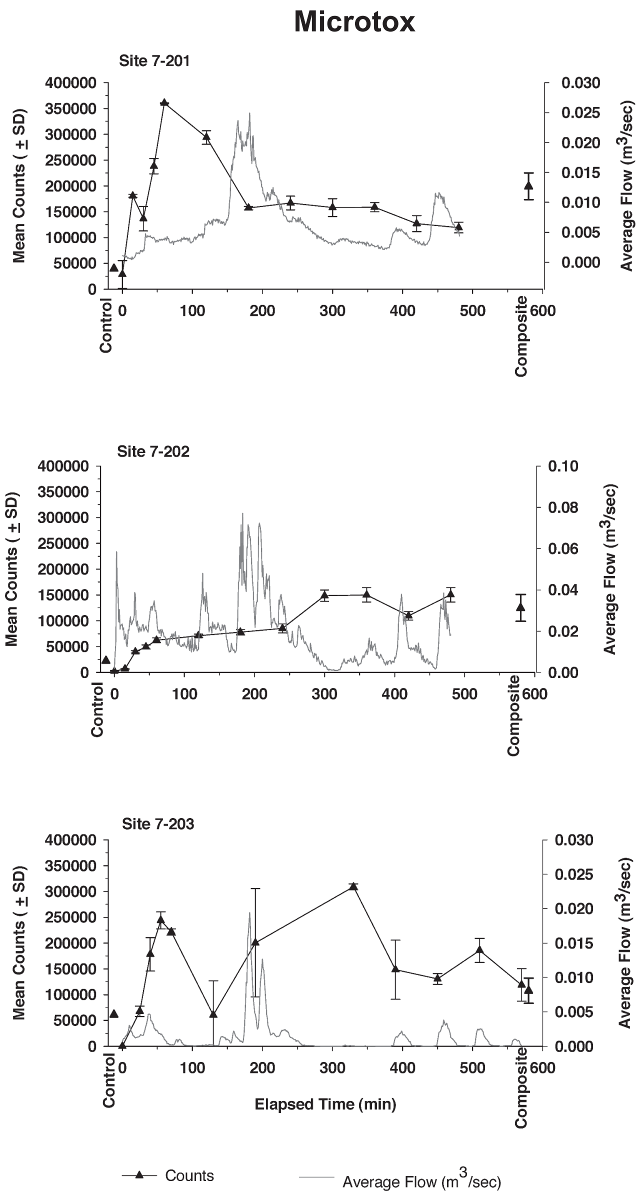

Figure 3. Microtox™ test response to runoff samples (February 11, 2003 storm event). Grab and composite samples were tested at a 9.4% concentration of stormwater; near the EC50 value determined in prior tests.

February 11, 2003 is shown in Figure (9a). 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 *C. dubia* 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 Figure 9b. As shown, approximately 50% of the estimated toxicity to fathead minnows was associated with the first 20% of discharged runoff volume.

Hydro-toxicity graphs

Two representative types of hydro-toxicity graphs were shown previously in Figures 2 through 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.

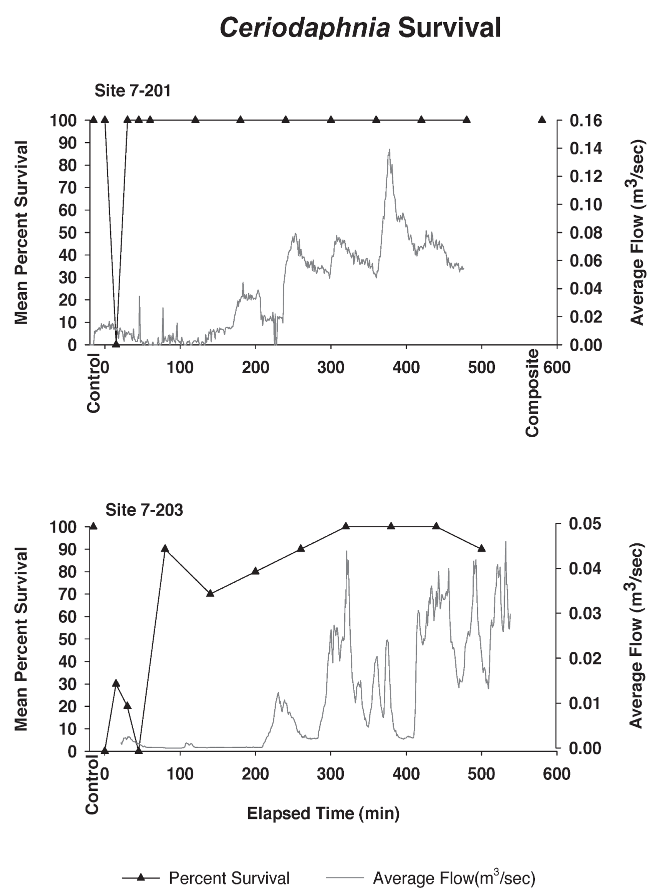

Figure 4. *Ceriodaphnia* survival test response to runoff samples (March 15, 2003 storm event).

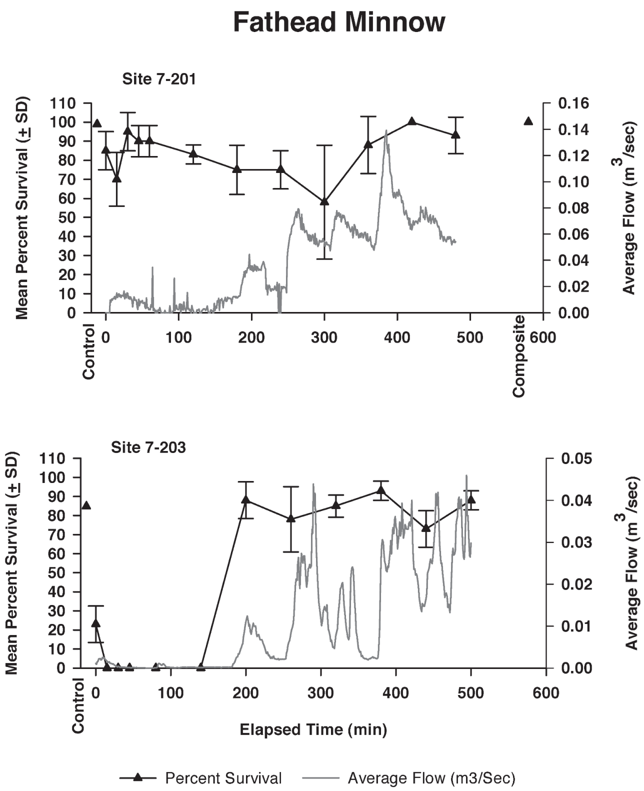

Figure 5. Fathead minnow survival test toxicity response to runoff samples (March 15, 2003 storm event).

An example pollutograph for total and dissolved Cu and Zn for Site 7-201 during the February 11, 2003 storm event is shown in Figure 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. 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 Kahanian 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, 1993b; Ruesink and Smith 1955, Sandrin and Maier 2002) and Phase III TIE analyses with fathead minnows and *C. dubia* 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 (Figures 2 - 7). Toxicity, however, was occasionally not related to the first flush samples and occurred in grab samples collected later during a storm (Figures 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 *C. dubia*.

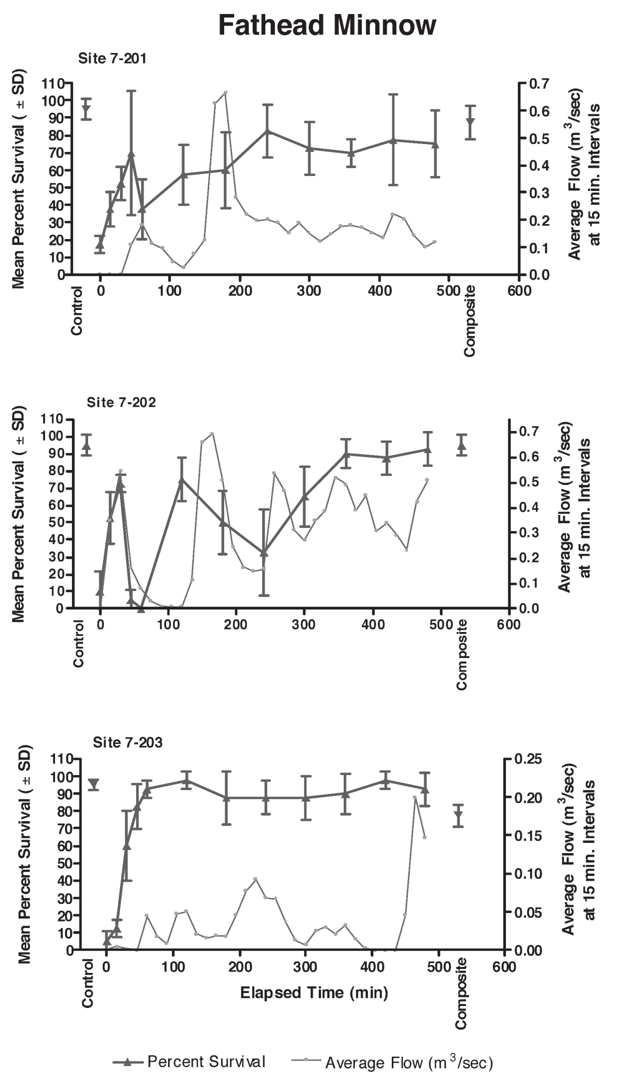

Figure 6. Fathead minnow survival test toxicity response to runoff samples (October 26 - 27, 2004 storm event).

Discussion

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. Surfactant toxicity in these samples was suggested by a reduction in toxicity in the C18, Zeolite, and aeration treatments, but not with EDTA.

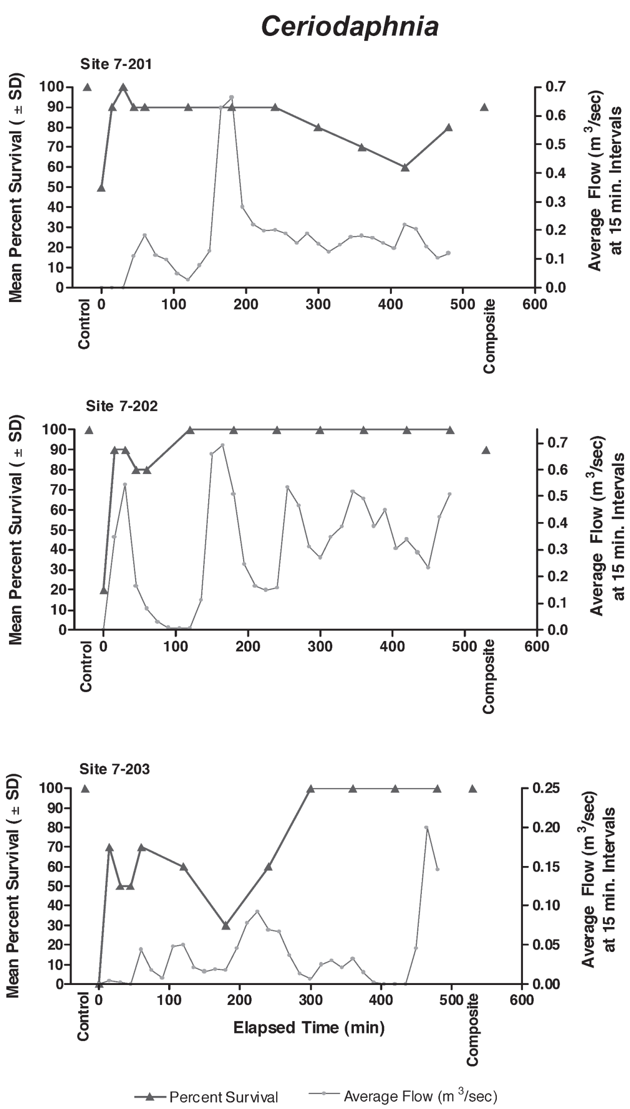

Figure 7. *Ceriodaphnia* survival test toxicity response to runoff samples (October 26 - 27, 2004 storm event).

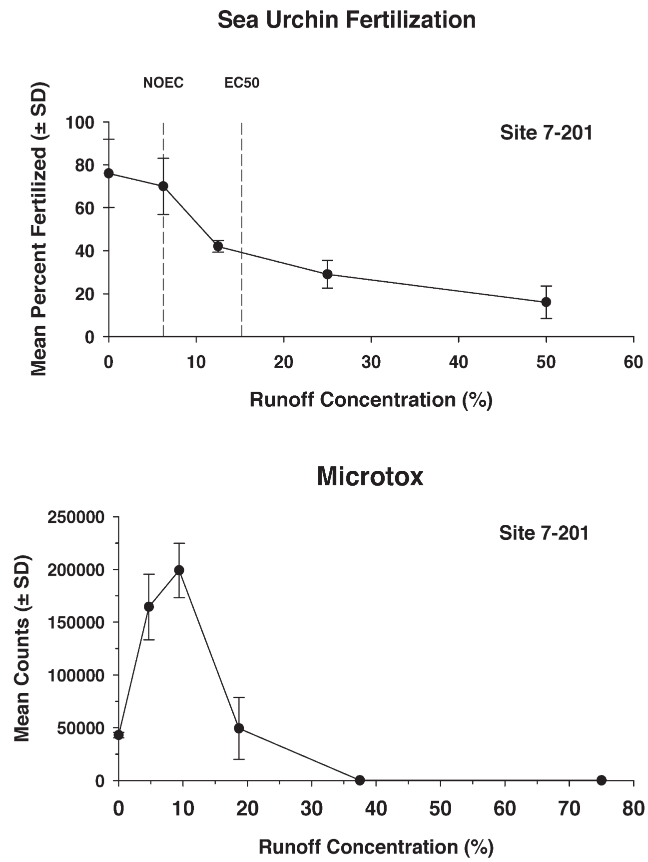

Figure 8. Dose-response plots for the sea urchin egg fertilization and Microtox™ tests for the February 11, 2003 stormwater composite sample from Site 7-201.

An example of Phase I TIE characterization results for two grab samples is provided in Figure 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 (EPA 1991, 1993a, 1993b) 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 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).

Table 3. Spearman correlations between toxicity test methods.

Method	Spearman r (*p* value)					
	Microtox™	*Ceriodaphnia* *Survival*	*Ceriodaphnia* *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)
Ceriodaphnia Survival			0.84(0.00)	0.54(0.00)	0.52(0.02)	0.66(0.00)
Ceriodaphnia Reproduction				0.63(0.00)	0.23(0.36)	0.29(0.17)
Fathead Minnow Survival					0.44(0.06)	0.48(0.00)
Fathead Minnow Growth						0.04(0.86)

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

The toxicity of some metals including cadmium, nickel, and zinc, have been found to increase in toxicity to *C. dubia* 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 examples shown in Figure 11. The pH/toxicity relationships 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.

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 mg/L) was approximately 59 times greater than the lowest acute LC50 value reported for *C. dubia* (9.5 µg/L), and 37 times the lowest chronic LC50 value reported for fathead minnows (15 µg/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 µg/L) and Site 7-203 (70 µg/L) were very similar to chronic LC50 values derived for both species (Nautilus Environmental unpublished data 2005). The highest concentration of dissolved zinc (4,490 mg/L) was up to 47 times greater than the lowest acute LC50 value reported for *C. dubia* (95 µg/L), and 14 times the lowest acute LC50 value reported for fathead minnows (330 µg/L) at a similar pH range (Schubauer-Berigan *et al*. 1993a).

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	*Ceriodaphnia* Survival	0.44	0.45	0.26	0.26
7-203	Fathead Minnow Survival			0.27	0.31

The overall mean concentrations of zinc at all three sites, ranging from 166 to 290 µg/L, were greater than the lowest LC50 values reported for *C. dubia* in the literature (Bitton *et al*. 1996; Schubauer-Berigan *et al*. 1993a; and Nautilus Environmental unpublished data 2006), but less than median effect concentrations reported for fathead minnows. The concentration of nickel also exceeded values found to cause toxicity to *C. dubia* at a pH >8.0 (Schubauer-Berigan *et al*. 1993a); however, the pH of the test samples was generally well below 8.

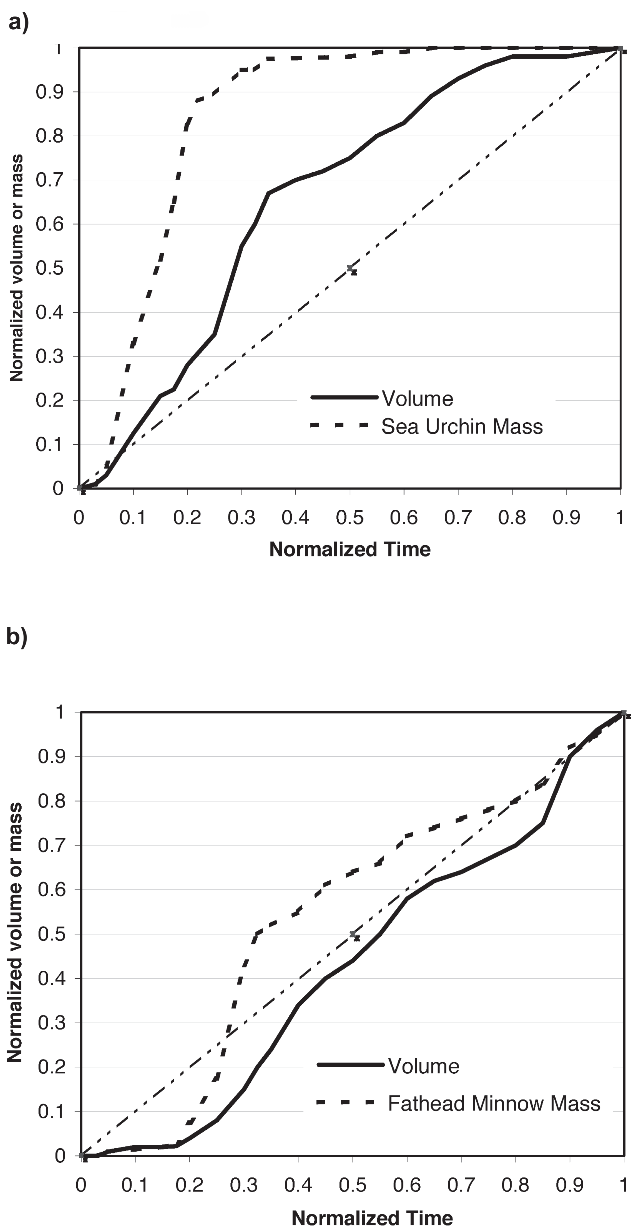

Figure 9. First flush effect of toxicity based on normalized time and mass rate for sea urchin (a) and fathead minnow (b), exposed to grab runoff samples.

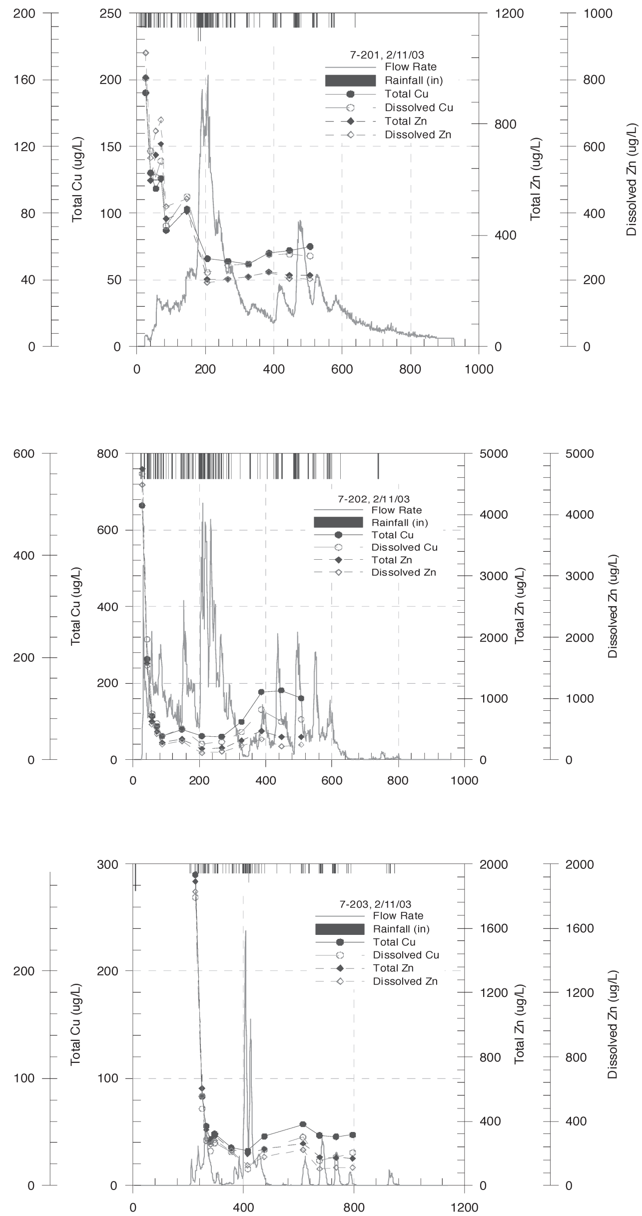

Figure 10. Pollutographs showing the variability of dissolved Cu and Zn over time for three highway sites during the storm event of February 11, 2003.

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 *C. dubia*, 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 between concentrations of dissolved copper and zinc in highway runoff and fathead minnow and *C. dubia* survival are summarized in Figure 12, which com-

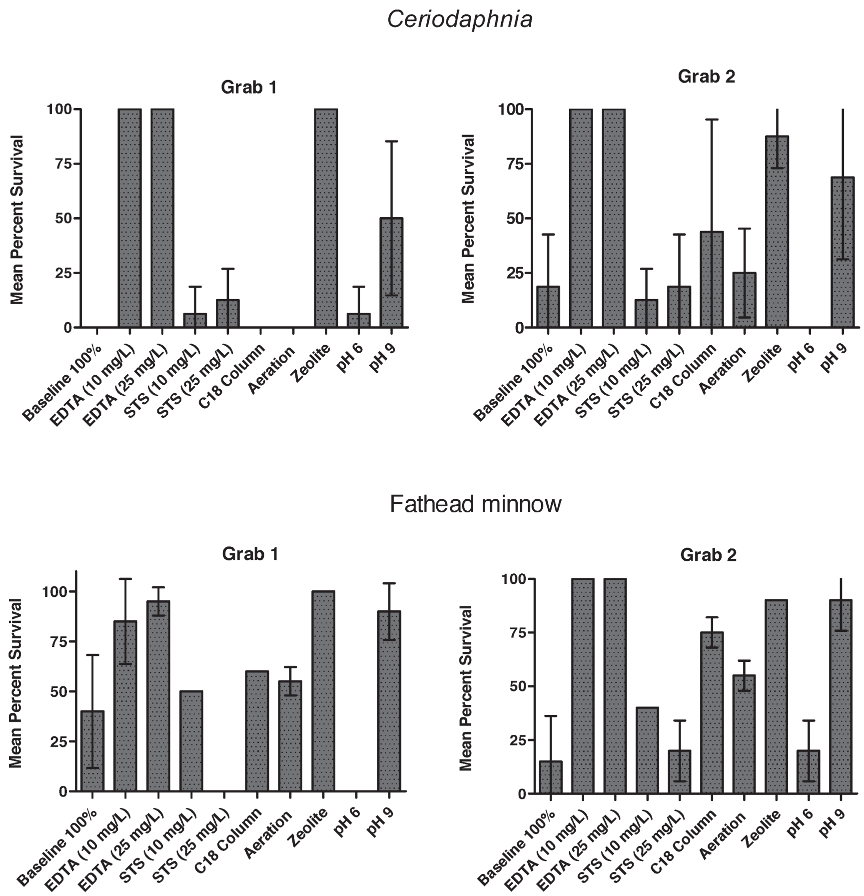

Figure 11. Phase I TIE results for *Ceriodaphnia* and fathead minnows (April 28, 2005 storm event site 7-202, mean ±1SD, n = 4).

bines the available data collected at all three locations from four to five 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 Figure 12. These data, however, fail to provide strong evidence for cause and effect relationships for the following reasons:

- Chemical data often co-vary
- Only a select group of potentially toxic chemicals were analyzed and reported
- 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)
- 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)
- 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)
- 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 (Figure 12). There are a few instances,

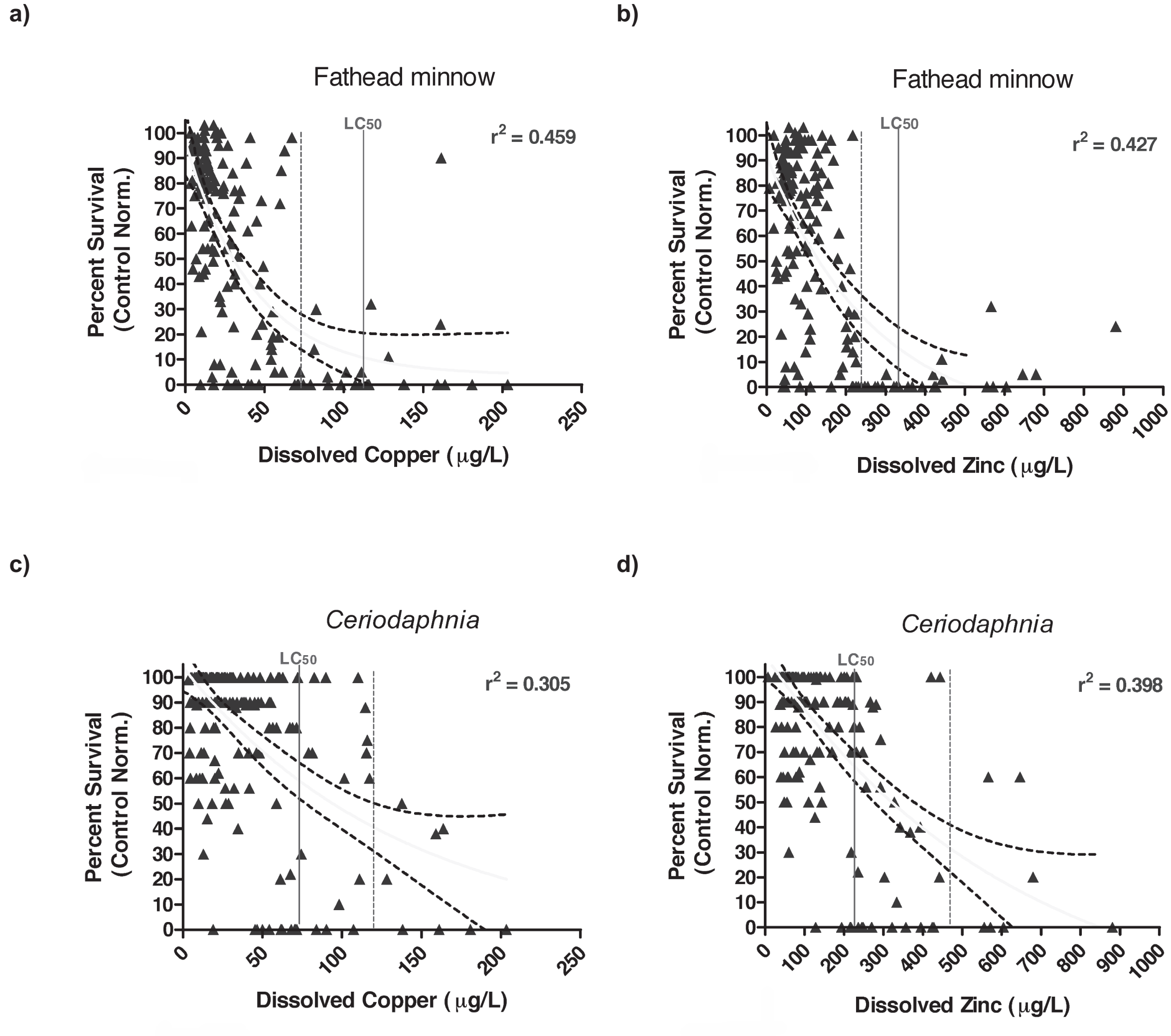

Figure 12. Distribution of dissolved and copper and zinc data and relation to 7-day survival of fathead minnows and *Ceriodaphnia* in highway runoff samples from all samples collected between February 2003 and March 2005. Relative threshold concentrations equivalent to a 20% or greater response relative 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.

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 *C. dubia* 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 *C. dubia* was less than expected in many samples, based on copper and zinc concentrations alone).

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

Highway Site	Sample Size/ Sample Type	Statistical Value	Dissolved Trace Metal Concentration (mg/L)			
			Cu	Pb	Ni	Zn
7-201	47/All grabs	Range	8 - 161	0.3 - 1.9	1 - 35	7 - 880
		Median	21	0.8	5	102
		Mean	38	0.9	7	166
		SD[b]	38	0.4	7	189
	20/Grabs 1-5	Range	14 - 161	0.3 - 1.6	4 - 35	7 - 880
		Median	30	0.8	6	121
		Mean	55	1.0	11	241
		SD[b]	49	0.4	8	252
7-202	60/All grabs	Range	4 - 560	0.2 - 13.0	<1 - 100	18 - 4490
		Median	28	0.8	6	116
		Mean	71	1.3	13	290
		SD[b]	107	1.8	19	617
	25/Grabs 1-5	Range	4 - 560	0.5 - 12.5	5 - 100	86 - 4490
		Median	107	1.6	19	321
		Mean	136	2.3	25	563
		SD[b]	141	2.5	25	890
7-203	53/All grabs	Range	5 - 269	1.0 - 72.0	1 - 59	25 - 1830
		Median	29	3.2	5	96
		Mean	45	9.8	8	168
		SD[b]	54	16	12	261
	19/Grabs 1-5	Range	10 - 269	1.0 - 22.0	2 - 59	46 - 1830
		Median	41	3.9	7	157
		Mean	70	5.5	14	261
		SD[b]	70	4.8	15	366

[a] Data provided 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

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 (Figures 13 and 14). 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 *C. dubia*, 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 the observed toxicity as reflected by r^2 values provided in Figures 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

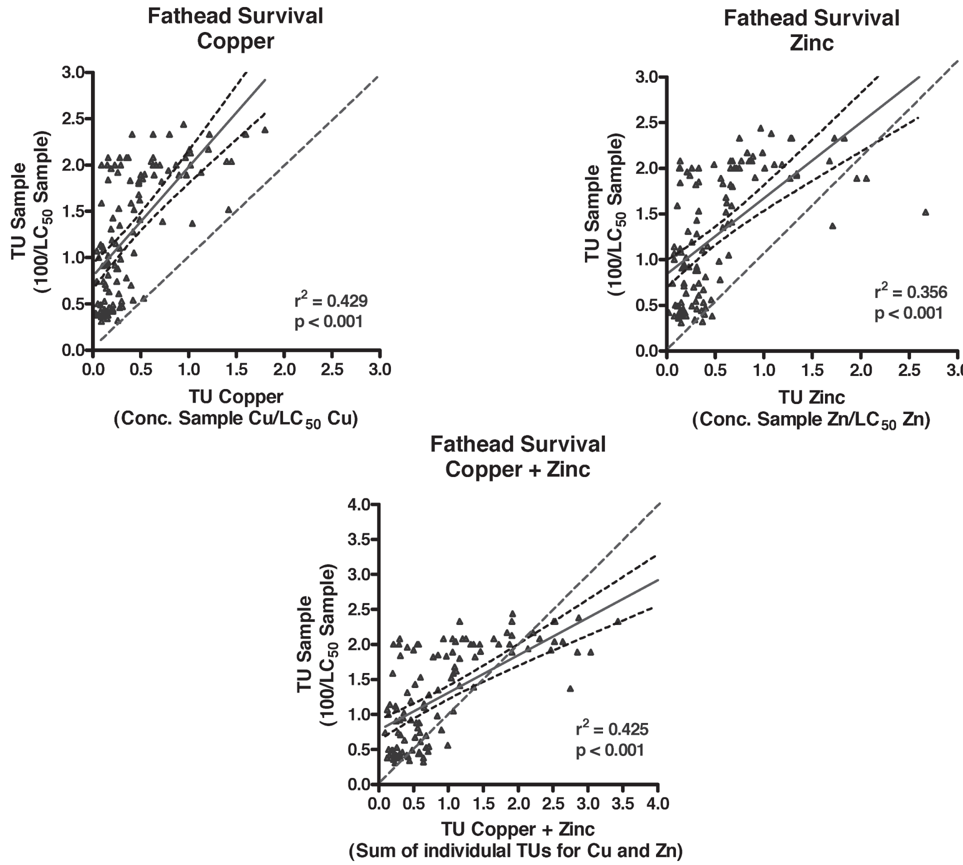

Figure 13. Correlation (linear regression) between stormwater toxic units for fathead minnows (100/LC50) and predicted TUs due to copper alone, zinc alone, and zinc + copper (metals concentration/ laboratory-derived LC50 values of 114 μg/L for copper and 330 μg/L for zinc). Analyses include all toxic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

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.

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 in this study. However, the relative sensitivity of these tests may vary in other locations if different toxicants are present.

(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 sometime 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 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 stormwa-

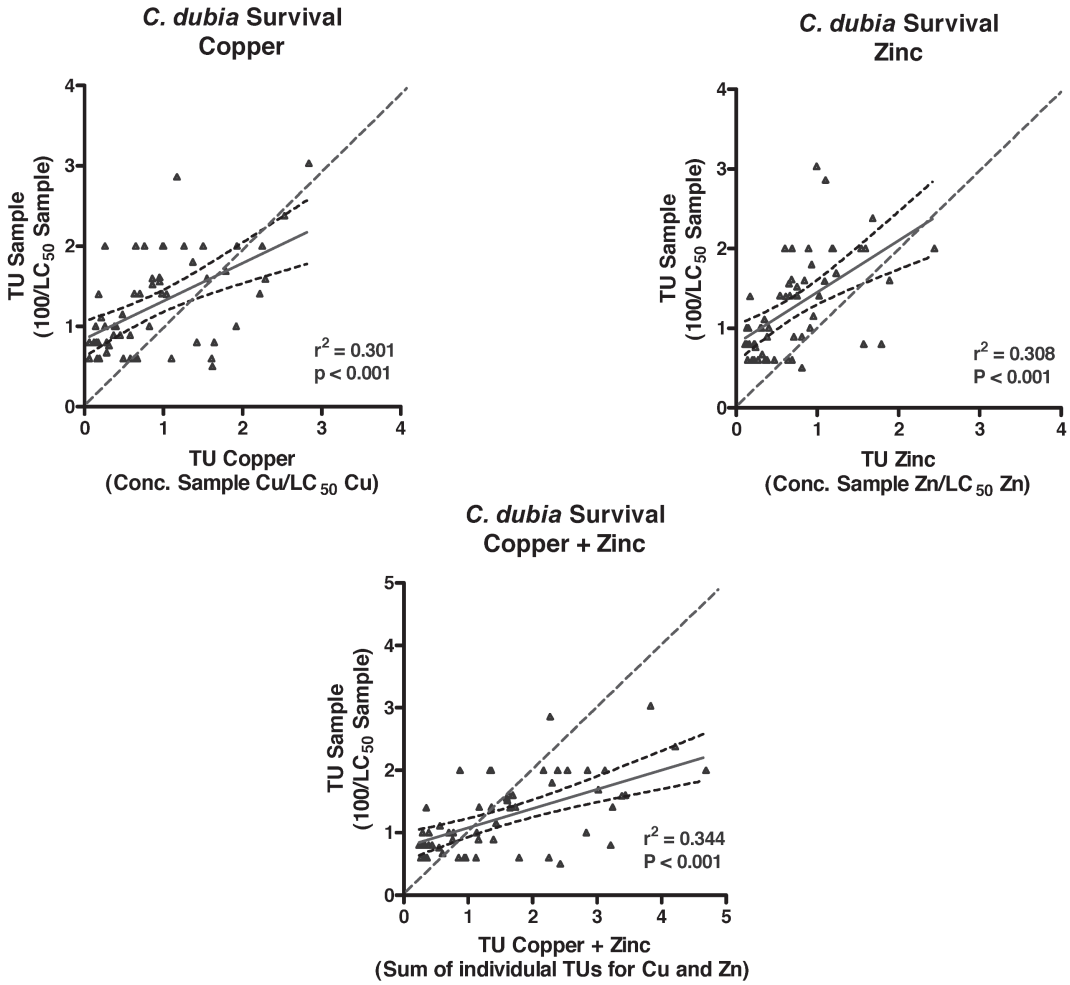

Figure 14. Correlation (linear regression) between stormwater toxic units for *Ceriodaphnia* (100/LC50) and predicted TUs due to copper alone, zinc alone, and zinc + copper (metals concentration/ laboratory-derived chronic LC50 values of 72 µg/L for copper and 210 µg/L for zinc). Analysis includes all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

ter 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. The testing of both composite and grab samples is necessary in order to evaluate the significance of first flush effects on water quality.

5) The cause of toxicity 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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