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Toxicity Identification Evaluation of Wet and Dry Weather Runoff from the Tijuana River

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The Tijuana River Watershed, covering an area of 4,483 km^2, two-thirds of which is in Mexico, lies astride the California-Baja California section of the United States-Mexico Border. For decades, raw sewage from the city of Tijuana, Mexico has flowed into the Tijuana River and across the international border into the United States. Rapid population growth and urbanization, poor land-use practices, and inadequate sewage treatment and collection facilities (Ganster 1996) have caused pollution in Mexico. The fastest growing aspect of Tijuana industry has been the maquiladoras, which are companies that import components for assembly and re-export. The maquiladora industry, fueled primarily by foreign investment, has grown faster than the capability of the municipality to deliver services to them. Moreover, the lack of pollution prevention measures for industry and other businesses in Tijuana, coupled with a deficiency in proper enforcement of existing regulations, has generated non-point source pollution that contaminates the Tijuana River (SCCWRP 1992).

As part of a 1988 study of toxicity of surface runoff in a number of southern California rivers, the Southern California Coastal Water Research Project (SCCWRP) measured the toxicity of runoff from the Tijuana River using the Microtox™ test based on bacterial photoluminescence (SCCWRP 1990). Samples from the Tijuana River had the highest flow-weighted toxicity among all the rivers sampled, perhaps due to the raw sewage and industrial wastes discharged into the river south of the International Border. In light of the rapid increase in industrial development of the Tijuana region over the past decade, the objective of the present study was to evaluate the toxicity of both wet and dry weather runoff from the Tijuana River using the standard test organism *Ceriodaphnia dubia*. Toxicity identification evaluations (TIE) were also performed in order to determine the general category of chemicals that caused such toxicity.

MATERIALS AND METHODS

Toxicity tests of water samples from the Tijuana River were conducted during both dry and wet weather. For the purpose of this study, if it had not rained for 14 days, then the sample was considered to be a dry weather sample. Grab samples were taken from the Tijuana River at Via San Ysidro approximately 200m past the international border along the north bank of the river. Samples were transported in sealed amber bottles in coolers on ice and promptly stored at 4°C. Two dry weather samples were collected on March 14, 1997 and April 22, 1997, and acute and chronic toxicity tests were carried out. Two wet weather samples were collected on February 28, 1997 and April 2, 1997, and acute

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and chronic toxicity tests as well as toxicity identification evaluations were performed on these samples.

The toxicity test method used in this study was the *Ceriodaphnia dubia* survival and reproduction test. This measures the chronic toxicity of whole effluents and receiving water to *C. dubia,* during a three-brood seven-day static renewal exposure. The methods used for culturing, feeding and testing are based on the EPA manual, "Short Methods for Estimating the Chronic Toxicity of Effluents and the Receiving Waters to Freshwater Organisms" (U.S. EPA 1989). Modifications included a reduced test volume (20 ml), smaller number of replicates (4, 8, or 10), and different test concentrations ranging from 0-100%. The acute test lasted 48 hours using at least 5 concentrations, with organisms checked for mortality at 24 and 48 hours. The chronic test duration was 7 days and at least 5 concentrations were tested. These abbreviated test procedures have been approved and used by the EPA (U.S. EPA 1991). All samples were used within 48 hours of collection.

A colony of *Ceriodaphnia dubia* was cultured in the lab. The starter colony was ordered from Aquatic BioSystems, Inc. in Fort Collins, Colorado. The culture water consisted of 3 parts Sparkletts Drinking Water and 1 part Evian bottled water aerated, using pressurized air from the lab, for at least 24 hours before use. A 16/8-hour day/night photoperiod was maintained using a white fluorescent light controlled by a timer. The diet for *C. dubia* consisted of 0.1 ml each (daily) of *Selenestrum capricornutum* algae and yeast trout chow (YCT). Both were ordered pre-made from Aquatic BioSystems Fort Collins, Colorado.

Acute and chronic toxicity tests were conducted using the effluent samples. A range of concentrations from 0% to 100%, were used with at least four replicates per group. Culture water was used to dilute the effluent to the desired concentration. Tests were terminated when 60% or more of the control organisms had their third brood (U.S. EPA 1989). For the test to be statistically acceptable, the survival of the control organisms had to be at least 80% with an average of 15 (or more) young produced (U.S. EPA 1989). Physical chemistry measurements of each sample were taken at the start of each test using Hach Titration kits, a pH meter, and a DO meter. Each sample was analyzed for pH, hardness, alkalinity, and dissolved oxygen. During our experiments all values were in the physiologically tolerable range for *Ceriodaphnia dubia* testing according to EPA guidelines (U.S. EPA 1989).

The physical and chemical characteristics of the toxicants were broadly defined using the Phase I TIE methods known as toxicity characterizations (U.S. EPA 1991). There were seven categories of toxicity tests conducted. The initial toxicity test measured acute toxicity over a 48-hour period. The baseline toxicity test measured chronic toxicity over a 7-day period. The filtration test used a 1.2 µm filter with pH adjustment to remove suspended solids. The aeration test is designed to determine how much effluent toxicity can be attributed to volatile substances (2-hr test with pH adjustment). The C-18 solid phase extraction (SPE) test is designed to remove organic compounds and metal chelates that are relatively non-polar. The C-18 extraction columns (3 ml) were obtained from J.T. Baker Inc., Phillipsburg, N.J. The EDTA addition test removes cationic metals. Concentrations of EDTA used were 3 mg/l and 8 mg/l. The sodium thiosulfate addition test is designed to remove oxidants that may be responsible for effluent toxicity.

Concentrations used were 100 mg/l and 250 mg/l. The pH was adjusted using HCl and NaOH and measured with a pH meter with a precision of 0.1 pH units. The pH was adjusted to values of 3 and 11 during test procedures (e.g. filtration, aeration, SPE) and then returned to pH_i (initial pH) before incubation of test organisms. The baseline chronic toxicity test was initiated on the same day as the TIE characterization tests for wet weather samples, and at the same time as the acute toxicity test for dry weather samples.

Statistical analysis was done in accordance with the U.S. EPA Hypothesis Testing Flowchart (U.S. EPA 1989). ToxCalc version 5.0 by Tidepool Software, McKinleyville, CA. Information Data Base Management System was used. This program has been designed specifically to analyze toxicity testing and TIE data.

RESULTS AND DISCUSSION

Acute toxicity tests were performed on all the wet and dry weather samples from the Tijuana River. All samples showed 100% survival after 48 hours, with the exception of the wet weather sample taken on April 2, 1997, which showed 0% survival after 48 hours. A comparison of the water chemistry parameters (pH, dissolved oxygen, alkalinity, and hardness) showed that the wet weather samples had slightly lower values for alkalinity and hardness than did the dry weather samples; however in all cases the measured values were within the acceptable limits for *Ceriodaphnia* testing. Values ranged from 7.4 to 8.4 for pH; 7.9 to 9.1 mg/l for dissolved oxygen; 46-69 mg/l for hardness; and 34-58 mg/l for alkalinity.

Chronic toxicity for each of the dry and wet weather samples is shown in Figure 1. Toxic Units (TU) are determined by calculating 100% / NOEC. The no observable effect concentration (NOEC) is the highest concentration an organism can be exposed to without an observed adverse effect. Dry weather samples from the Tijuana River had very low toxicity, with values of 1.0 and 1.25 TU's. However, wet weather samples were more toxic, with values of 4 and 10 TU's (Fig. 1). It has been documented that there is substantial within- and between-storm variability in toxicity in runoff (SCCWRP 1990). Such variability arises from differences in flow conditions of the river, temporal variability among source contributions, storage-discharge relationships in the watershed, and biological processes. SCCWRP (1990) showed that in runoff from the Los Angeles River, concentrations of suspended solids, metals, and chlorinated hydrocarbons all increased with increasing flow. However, they found that toxicity was inversely related to flow, and concluded that dissolved components probably cause the toxicity observed in the Microtox test of runoff samples.

Daily precipitation amounts (recorded at Lindbergh Field, San Diego, CA) for wet weather samples were 0.2 cm on Feb 27, 1997, and 0.5 cm on April 2, 1997. For the wet weather sampling days, mean daily flow of the Tijuana River (at the International Border) was $1.63 \times 10^5 m^3/d$ on February 27, 1997 and $1.13 \times 10^5 m^3/d$ on April 2, 1997. For the dry weather sampling days, mean daily flow was 5.03×10^4 m3/d on March 14, 1997, and much lower (less than $8 \times 10^1 m^3/d$) on April 22, 1997.

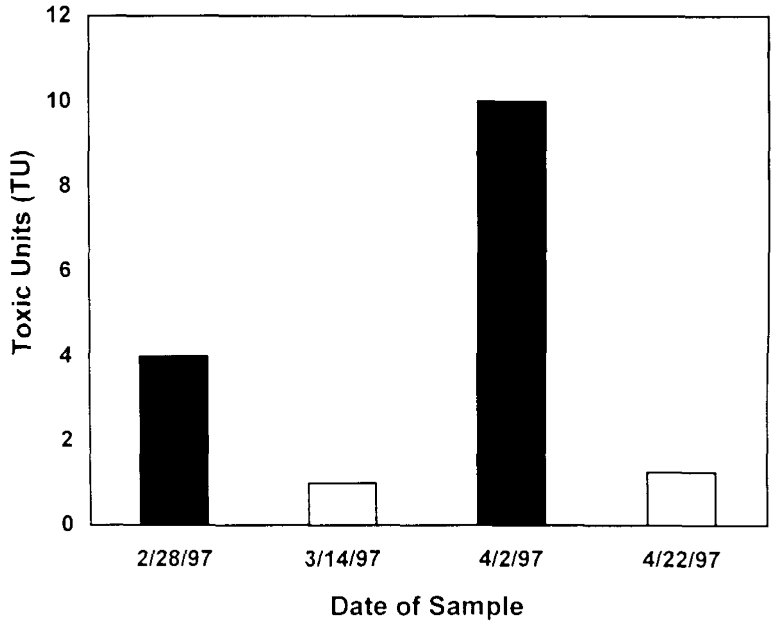

Figure 1. Chronic toxicity of wet (2/28/97 and 4/2/97) and dry (3/14/97 and 4/22/97) weather runoff from the Tijuana River

Although it appears that toxicity in the Tijuana River seems to increase during wet weather, high flow storm events, our data is currently too limited to conclude that wet weather runoff is characteristically more toxic than dry weather flow.

Toxicity identification evaluations were performed on the wet weather samples from the Tijuana River that showed chronic toxicity. Filtration of the sample taken on February 28, 1997 did not show significant removal of *Ceriodaphnia* toxicity, indicating that dissolved components must be responsible for the observed toxicity. Moreover, treatment by sodium thiosulfate, aeration, and EDTA showed no significant effect. The lack of toxicity removal by EDTA is indicative of the fact that metals do not play a major role in the toxicity observed. However, the solid phase extraction (SPE) treatment did show significant removal of toxicity (Fig.2). Survival in all SPE treatments was not significantly ($p<0.05$) different from the control (dilution water). Considering the high toxicity of the initial effluent (4 TU's), the SPE treatment rather efficiently removed toxicity; at pH_3 and pH_{11} survival was 100%, and at pH_i (8.4) survival was 75% (Fig. 2). With regard to reproductive effects, there was no significant ($p<0.05$) difference from the control in the number of neonates after SPE treatment at both pH_i and pH_{11} (Fig.3). However, at pH_3, reproduction was still decreased as compared to the control, indicating that some toxicity remained after SPE treatment at low pH. Apparently at the lower pH, there was less binding of non-polar functional groups to the silica sorbent of the C-18 SPE cartridge resulting in the observed reproductive outcomes.

A second TIE was performed on the wet weather runoff sample collected on April 2. 1997. No removal of toxicity was evidenced after treatment by sodium thiosulfate,

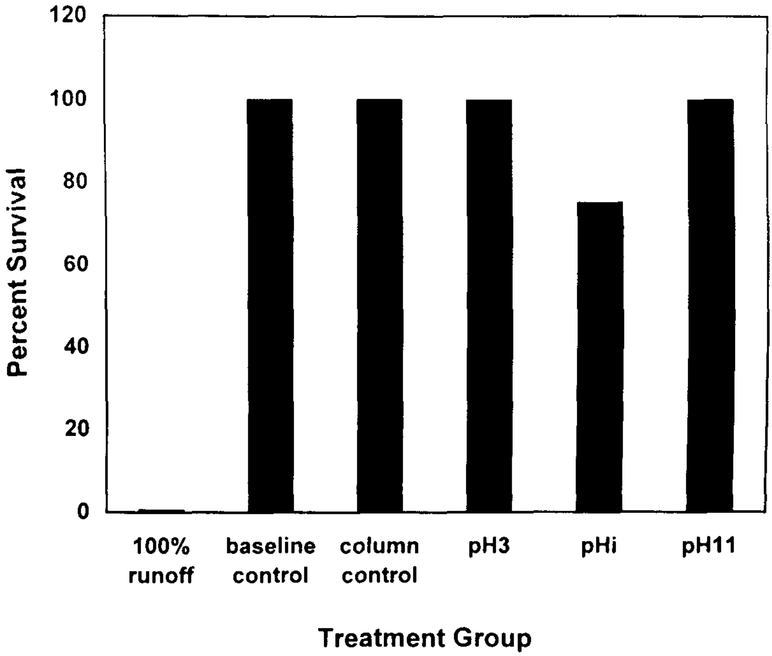

Figure 2. TIE of wet weather sample (2/28/97) from the Tijuana River; survival of C. *dubia* after treatment by solid phase extraction at pH_3, pH_i, and pH_{11}. The value for the 100% runoff treatment group as shown represents 0% survival.

filtration, or EDTA. However, the SPE test again showed excellent removal of toxicity. For survival, there was no significant ($p<0.05$) difference between the different pH groups and the control. Survival was 100% at pH_i (7.4) and 60% at pH_3 and pH_{11} (Fig. 4). The methanol elution test showed that the column did remove the toxicity. With regard to reproductive effects, the SPE treatment for the pH_3 group was the only one to show significant difference from the control, indicating no removal of reproductive toxicity ($p<0.05$) (Fig. 5). This pH-related effect on toxicity removal by SPE treatment was similar to that obtained for the first wet-weather sample (Fig. 3).

This study provides the first data for the Tijuana River watershed showing that a particular class of toxicants is responsible for toxicity in urban runoff. SPE treatment efficiently removed toxicity from the wet weather samples pointing to the important role that nonpolar organics play in causing the observed toxicity to *Ceriodaphnia* (U.S. EPA 1991). These pollutants may include detergents and surfactants, petroleum hydrocarbons, and pesticides. It is of interest to note that nonpolar organics, most probably carbofuran and methyl parathion, were identified by TIE procedures in an agricultural watershed in California (Norberg-King et al. 1991). These results contrast somewhat with those from TIE testing in Ballona Creek in Los Angeles County, CA where SCCWRP (1997) using marine species (sea urchin fertilization and abalone larval development tests) found that EDTA treatment was the sole TIE method that removed toxicity from wet weather runoff, implying metals were the source of such toxicity. It is important to note here that

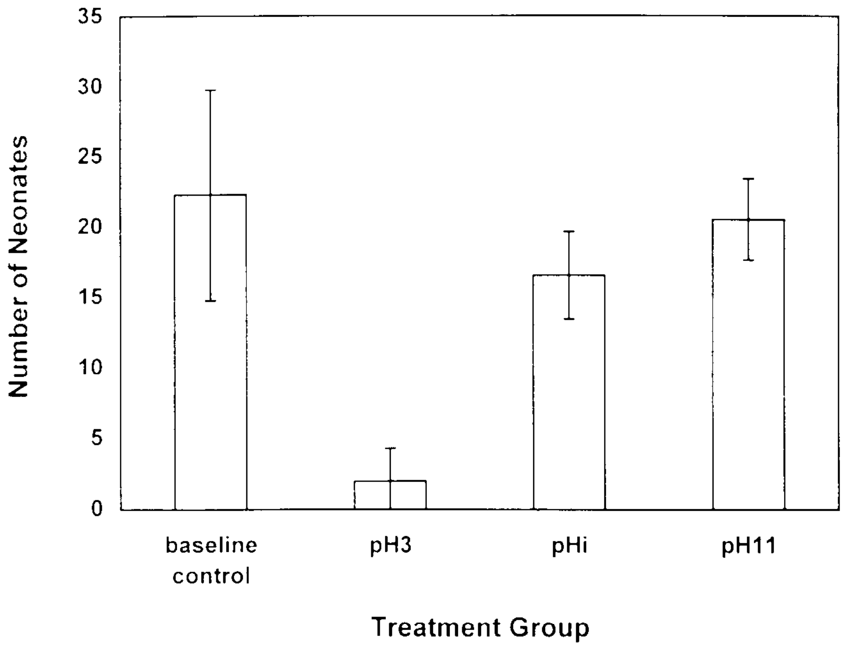

Figure 3. TIE of wet weather sample (2/28/97) from the Tijuana River; reproductive success of C. *dubia* after treatment by solid phase extraction at pH_3, pH_i and pH_{11}.

since SCCWRP (1997) used different species for toxicity testing than in our study, the different TIE may be influenced by the differing sensitivity to pollutants between species.

Urbanization has had a profound impact on the arid landscape of southern California during the past century. As urbanization increases, pollutant loads entering rivers and streams also increase. The type and concentrations of pollutants are dependent on various factors including: the extent of urbanization, land-use, the type and degree of vehicular traffic, municipal street cleaning, atmospheric quality, animal populations, and specific storm and watershed characteristics.

Our results suggest that toxicity will continue to enter the Tijuana River and Estuary, especially during wet weather. Some data suggest that runoff from lower socioeconomic neighborhoods contain significantly higher concentrations of pollutants than does runoff from more affluent parts of an urban area in the United States (U.S. EPA 1983). It is probable that urbanization and land-use in Mexico offers similar challenges for managing runoff and non-point source pollution loading. Identifying the water quality problem, targeting resources to the worst pollution sources, providing adequate information and education. and offering inducements to participants are several of the more important factors in controlling non-point source pollution in developing countries such as Mexico (Line et al. 1996).

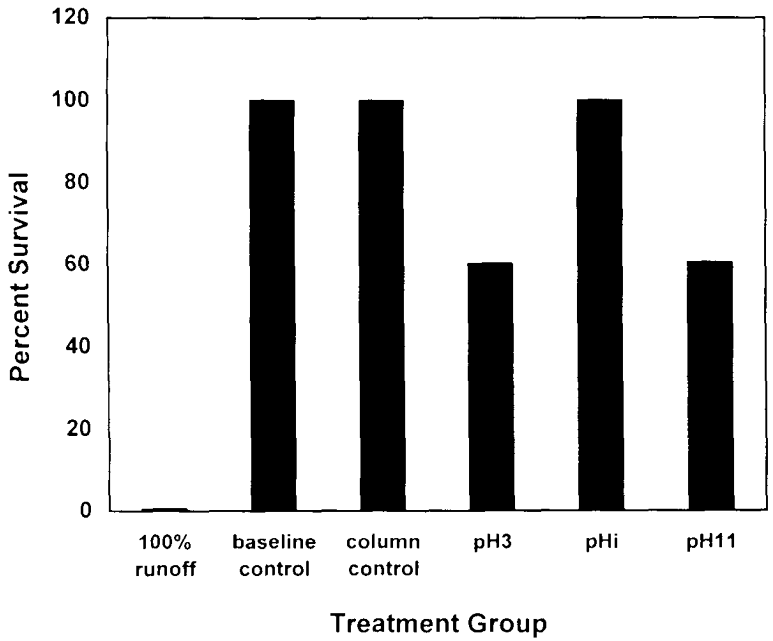

Figure 4. TIE of wet weather sample (4/2/97) from the Tijuana River; survival of *C. dubia* after treatment by solid phase extraction at pH_3, pH_i and pH_{11}. The value for the 100% runoff treatment group as shown represents 0% survival.

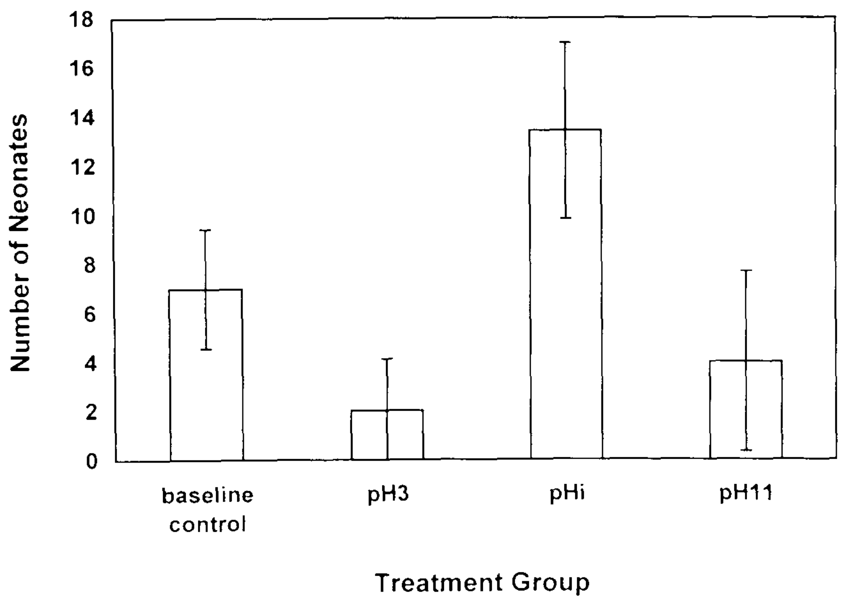

Figure 5. TIE of wet weather sample (4/2/97) from the Tijuana River; reproductive success of C. *dubia* after treatment by solid phase extraction at pH_3, pH_i and pH_{11}.

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