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16. Abstract

The Cross Lake Bridge in Shreveport, Louisiana, spans Cross Lake that serves as the city's water supply. Concern about accidents on the bridge contaminating the lake prompted the Louisiana Department of Transportation and Development (LADOTD) to construct a closed drainage system on the bridge to convey precipitation falling on the bridge deck to a concrete lined holding pond on the east side of the lake. Once there, bridge runoff is tested for oil and grease, pH, and lead before discharge to 12-mile bayou.

This report describes the methodology and results of two studies dealing with runoff from the Cross Lake Bridge. The first examined the degree to which repairs to the Cross Lake Bridge's closed drainage system were successful in capturing runoff from the bridge. An earlier study found that, because of leakage, only about one half of the precipitation falling on the bridge actually ended up in the holding pond. This is substantially less than would be expected from a catchment that is essentially impervious. To test the success of repairs to the drainage system, volume balances for 22 precipitation events were conducted by comparing the rainfall on the bridge to the subsequent flow into the pond. The ratio of the volume of runoff to the volume of precipitation was found to average 0.86, which when compared to values from the previous study as well as published values, suggested a successful repair of the drainage system.

The second study examined the use of the Microtox toxicity analysis system manufactured by Azur Environmental for use as a screening tool to assess toxicity of the pond contents. Microtox protocols rely on measurement of light output from a specific strain of luminescent bacteria. Toxicity is indicated when light output from the bacteria decreases. Tests can be run in time periods ranging from 5 to 30 minutes. The standard endpoint of the tests occurs once light output has dropped 50 percent and is termed the EC50. Samples from the water column and sediment were collected and analyzed for toxicity using the basic test (liquid) and the basic solid phase test (sediment).

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Each research project has an advisory committee appointed by the LTRC Director. The Project Review Committee is responsible for assisting the LTRC Administrator or Manager in the development of acceptable research problem statements, requests for proposals, review of research proposals, oversight of approved research projects, and implementation of findings.

LTRC appreciates the dedication of the following Project Review Committee Members in guiding this research study to fruition.

LTRC Administrator/Manager
Chester G. Wilmot
Special Studies Research Manager

Members
Ed Bodker
Gregory du Cote
Curtis Fletcher
Vince Russo

Directorate Implementation Sponsor
William B. Temple

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by

D.M. Griffin, Jr., Ph.D., P.E., D.WRE

Civil Engineering Program
Louisiana Tech University
Ruston, LA 71270

LTRC Project No. 01-3ENV
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ACKNOWLEDGMENTS

The principal investigator wishes to express sincere appreciation to Curtis A. Fletcher, Rest Area Manager of LADOTD, Dr. Chester Wilmot of LTRC, and members of the Project Review Committee for their patience and assistance with this project.

IMPLEMENTATION STATEMENT

With regard to the development and assessment of a series of volume balances for rainfall and runoff for the Cross Lake Bridge, the results indicate that repairs to the bridge were successful in stopping leakage, therefore no implementation is necessary. With regard to the second objective, a toxicity analysis of the water column and sediment within the Cross Lake holding pond, study results suggest that material that settles in the holding pond exhibits toxicity as measured by Microtox test protocols, while the water column exhibits minimal toxicity. A previous study has shown that pond sediment is scoured away when the pond is drained. Thus, the results may be implemented by developing procedures for minimizing the discharge of sediment when the pond is drained.

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INTRODUCTION

The Cross Lake Bridge is approximately 10,000 feet long and spans Cross Lake. It is a part of Interstate 220 which bypasses Shreveport, Louisiana from Interstate 20. Interstate 20 is the longest interstate highway in the country and is heavily traveled. Cross Lake, however, serves as the potable water supply for the city of Shreveport. During construction of the bridge, concern was expressed over the possibility of an accident on the bridge contaminating the city's water supply. As a result of this concern, LADOTD agreed to modify the bridge to include a "closed" drainage system and to construct a concrete lined holding pond on the east bank of the lake to collect and hold runoff resulting from precipitation events prior to testing and discharge. Runoff is collected through approximately 90 curb inlets located along the length of the bridge. Once testing of the pond contents is complete, the pond is drained. A schematic of the pond and the location of sampler/flow meters used in this and previous studies is shown in Figure 1.

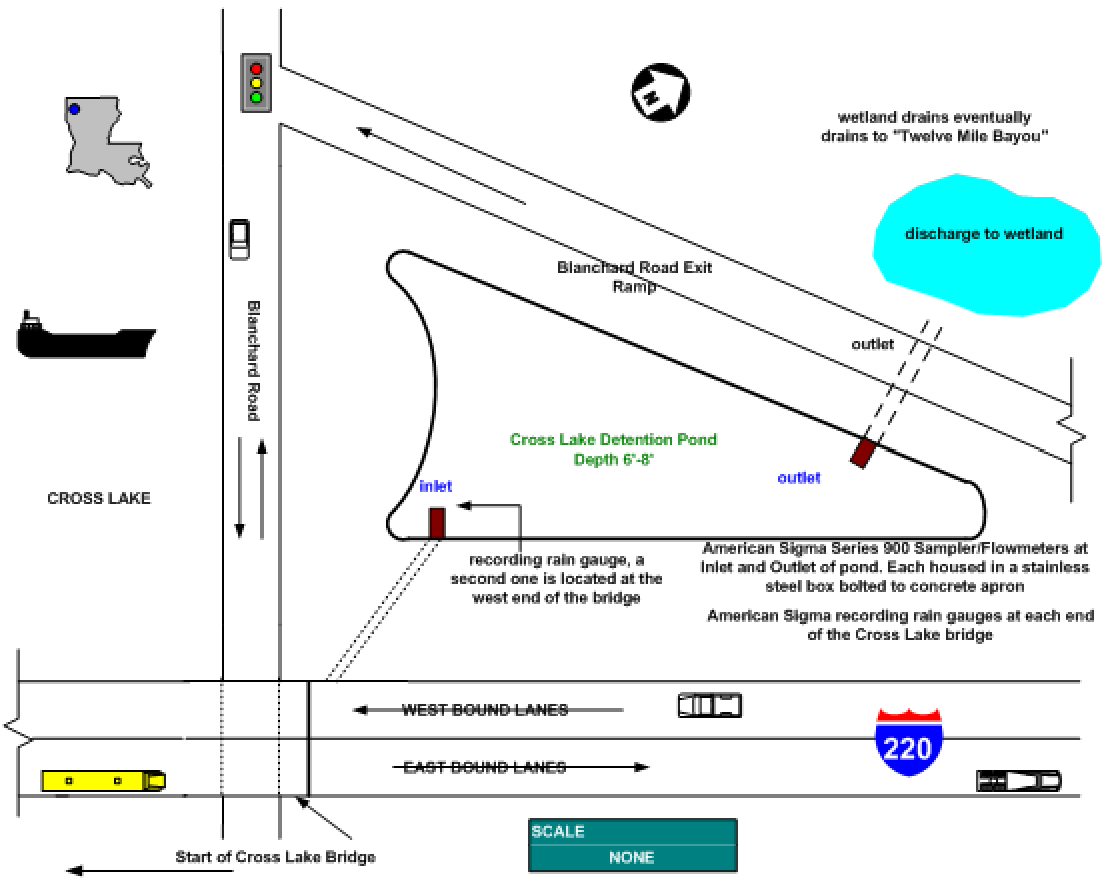

Figure 1

Schematic of the Cross Lake pond and bridge

Previous Work

In June 2003, the Louisiana Transportation Research Center (LTRC) published Final Report 346 entitled "Determination and Treatment of Substances in Runoff in a Controlled Highway System (Cross Lake)" *[1]*. The report dealt with the dynamics of contaminant transport from the Cross Lake Bridge into the holding pond on the east bank of Cross Lake. The findings of that report were as follows:

Runoff coefficients (the ratio of measured runoff to measured rainfall) were found to have symmetric distribution with an average value of 0.5. This implies that only 50 percent of the rainfall that falls on the surface of the Cross Lake Bridge is transported as runoff to the detention pond.

Laboratory tests on runoff samples for Chemical Oxygen Demand (COD) and filtered COD resulted in a geometric mean of 62 mg/liter COD and 19 mg/liter for filtered COD. Thus, 70 percent of the COD in the runoff results from suspended or settleable material rather than dissolved materials.

The concentration of total suspended solids (TSS) in the runoff was found to have a geometric mean of 59 mg/liter, while volatile suspended solids have a geometric mean of 23 mg/liter. This implies that approximately 60 percent of the solids in runoff from the bridge are made up of inert materials.

In addition, data collected in previous work show that sediment from the holding pond contains high concentration of heavy metals such as zinc (589,350 ppb), copper (98,320 ppb), and lead (92,400 ppb) *[1]*. A first flush phenomenon was observed during runoff events. In the study, "first flush" was defined as a condition where the percentage of pollutant mass transported is greater than the corresponding percentage of runoff volume transporting it. On average 35 percent of the mass of contaminants was transported in the first 25 percent of the runoff volume, 60 percent in the first 50 percent of the runoff volume, and 80 percent in the first 75 percent of the runoff volume. Data collected during pond releases indicated that a substantial amount of the settleable and suspended contaminants deposited on the pond bottom are scoured from the pond bottom and released when the pond is emptied in the routine fashion.

The fact that data indicated only 50 percent of the measured rainfall showed up as runoff caused concern. Given that the catchment, the Cross Lake Bridge deck, is entirely impervious; this value was quite low when compared to published values of 0.7- 0.95 *[2]*, *[3]*, *[4]*. According to McCuen, runoff coefficients ranging from 0.7 to 0.95 adequately reflect the runoff expected from 5 to 10 year event frequencies *[3]*. Higher intensity, less frequent events require the use of higher coefficients because the initial abstraction has a proportionately smaller effect on the runoff. Subsequent to publication, it was determined that the closed runoff collection system, designed as part of the bridge, exhibited substantial leakage. A repair effort was initiated in 2004–2005. One major objective of this study was to determine how effective the repairs were in eliminating leakage from the bridge.

Measurement of Acute Toxicity Using Microtox

Metcalf and Eddy note that until recently pollution control measures centered on measurement of conventional pollutants, which have been identified as degrading water quality in receiving streams *[5]*. However Section 101(a) (3) of the Federal Clean Water Act prohibits the discharge of toxic pollutants. As a result, a variety of toxicity protocols have been developed to determine the toxicity of effluents to a variety of target organisms using test endpoints such as growth rate, survival, and reproduction. Early test protocols examined the toxicity of one specific chemical. These were found to be lacking in that they did not account for synergistic effects between multiple compounds. More recently whole effluent toxicity protocols measure the aggregate toxicity of unaltered effluents. Earlier studies on the Cross Lake drainage system examined the concentrations and mass transport of traditional contaminants as well as metals. These are of concern because they have been shown to be toxic to higher life forms. However the toxicity of the discharge from the holding pond was never quantitatively examined. Therefore a second major objective of this study was to examine the toxicity of the discharge from the Cross Lake holding pond. A variety of test protocols are available for determining the toxicity of liquids and solids to various organisms. Because the pond is drained rather frequently, a rapid screening test for acute toxicity was desired. The Microtox toxicity testing protocols utilize a luminescent strain of bacteria as the test organism. Acute toxicity protocols can be run in 30 minutes. Results are based on the decrease in luminescence of the bacteria tested. For these reasons, the Microtox toxicity test was chosen. At present, the contents of the Cross Lake Pond must meet discharge limits for pH, oil, grease, and lead before being discharged. The author suggested that a simple, easy-to-use, and reliable toxicity testing procedure might provide a rapid screening of the discharge for toxicity as well.

The Microtox Toxicity protocol is purchased as a self-contained system and is recognized as a rapid and relatively easy-to-use test for both whole effluent as well as solid-phase toxicity testing. The procedure uses a specific strain of luminescent bacteria, Vibrio fischeri (formerly known as Photobacterium phosphoreum). These organisms are exposed to either whole effluent or sediments, and the reduction in light output by the test organisms over time is measured. The standard endpoint is a 50 percent reduction in light output, termed the EC50. Results can be obtained in time of 5 to 30 minutes depending on the chemicals to which the organism is exposed. The procedure is based on the parameter Gamma, defined as the ratio of light lost at time *t* to the light remaining at time *t* for a given sample concentration when compared to a control sample (blank).

$$G_t = [(R_t \times I_o) \div I_t] - 1 \qquad (1)$$

The effect of the liquid or solid phase on the organisms is defined as:

$$\%Effect\ t = [G_t \div (1 + G_t)]100 \qquad (2)$$

Rate theory for biological inhibition suggests a simple mathematical relationship between the concentration of a toxic substance and the response of the susceptible organism when the response is measured in terms of G values

$$G = kC^p \qquad (3)$$

In this equation, *C* is the concentration, *p* is the number of toxic molecules per target site, and *k* is a composite factor relating to free energy changes and volume changes during the reaction. This equation may be recast in a linear form, equation (4), which allows for the prediction of concentration from Gamma values.

$$Log\ C = b \times \mathrm{Log}\ G + Log\ a \qquad (4)$$

Output from the Microtox program provides the initial light output from the sample and control, the light output at time *t*, the corresponding value of Gamma and the %Effect. Results of the analyses are evaluated statistically by evaluating the fit of the data to equation (4), and computing a value of the regression coefficient R^2. The software included in the Microtox “package” contains a number of statistical procedures for trend analysis. In addition, there are a number of software “wizards” to assist the analyst in choosing the correct procedure, analyzing data, and sample preparation. This makes the procedure relatively easy to use by inexperienced personnel.

There are three types of Microtox tests: the Basic Test, the 100% Test, and the Basic Solid Phase Test. The Basic Test can detect contaminants that are soluble in water or an organic solvent. Bennet and Cubbage reviewed and summarized evaluations of Microtox for a variety of sediments and elutriates by a number of state and federal agencies as well as universities *[6]*. Based on their findings, they recommended the use of the Microtox Basic Test for gathering data in support of freshwater sediment criteria development. Bulich et al.

determined that 5-minute EC Microtox values for both pure compounds and complex industrial and municipal effluents were similar to published 24 to 96 hour fish bioassay data *[7]*. Curtis et al. came to similar conclusions when they compared Microtox EC50 data to published LC50 values for Pimephales promelas *[8]*. Ankley and Qureshi et al. found that Microtox is a poor indicator of ammonia toxicity *[9]*, *[10]*. Giesy and Hoke found that Microtox is as sensitive to many compounds as are most insect, crustacean, mollusk, and fish bioassay species *[11]*. Burton concluded that for a single chemical Microtox is more sensitive than other microbial tests *[12]*. In this study, the Basic test was used to examine the toxicity of contaminants in the water column while the Basic Solid Phase Test was used to examine the sediments for toxicity.

OBJECTIVE

There were two objectives in this study. The first was to assess the degree to which repairs to the Cross Lake Bridge have eliminated the discharge of contaminated runoff from the bridge deck into Cross Lake. The second was to examine the use of Microtox protocols as a screening tool for determining acute toxicity effects in the water column and the sediment components of the Cross Lake holding pond discharge.

SCOPE

In order to complete the first objective of the study, rainfall onto the bridge and subsequent runoff into the holding pond was measured for as many rainfall events as possible. The ratio of runoff to rainfall was computed for each event and compared to published values for catchments with similar characteristics as well as to the results of previous studies on the Cross Lake Bridge. Because substantial work has already been carried out on contaminant transport from the bridge, determination of contaminant concentrations was not a primary activity in accomplishing this objective.

The second objective was accomplished by sampling the pond contents, both the water column and the sediment deposited within the pond, and carrying out acute toxicity tests using Microtox protocols. The standard parameter by which toxicity levels were assessed using this procedure was the EC50, the liquid or sediment concentration at which bacterial luminescence was reduced by 50 percent. This parameter was computed for each sample.

METHODOLOGY

The methodology used to obtain the data necessary for each of the project objectives is described in the following sections.

Objective 1–Volume Balance from Cross Lake Bridge

The intent of this portion of the project was to determine to whether leakage from the closed drainage system on the Cross Lake Bridge had been reduced. This was accomplished by measuring and comparing the volume of rainfall hitting the bridge to the volume of runoff measured entering the pond. In order to measure the rainfall, American Sigma Tipping bucket rain gages were installed at each end of the bridge. These recorded rainfall in increments of 0.01 in. The data was stored in the unit until downloaded onto a laptop by a graduate student. The area of the bridge was obtained from LADOTD as 880,000 ft^2, multiplying the total rainfall by the area results in the volume of water hitting the bridge. Estimating the volume of runoff entering the pond was more difficult. Precipitation falling on the bridge is conveyed to the holding by a concrete culvert 36-in. in diameter. This pipe rarely flows full, thus in order to estimate the flow, independent measurements of flow depth and velocity must be obtained each time the flow is to be measured. This was accomplished using an American Sigma programmable flow meter. A probe capable of measuring both depth and velocity was first installed on the pipe invert approximately 15 ft. upstream from the exit. This was necessary in order to minimize the effects of turbulence at the exit. A schematic of the probe installation is shown in Figure 2. Water depth above the bottom of the probe is obtained from a pressure sensor installed on the bottom of the probe. Liquid velocity is measured using Doppler techniques. A signal is sent out upstream in the "active Doppler region," the signal is reflected off of bubbles or particles in the flow. The flow velocity is computed based on the time it takes for the signal to return to the probe. At this point the instrument provides independent measurements of flow depth and velocity. The flow meter was programmed with the size and shape of the conduit, a 36-in. circular pipe. The computer in the flow meter then computed the area of flow from the flow depth using a pre-programmed equation. Multiplying the area of flow times the velocity results in the flow rate. The flow meter was programmed to collect depth and velocity measurements at 10-min. intervals and compute the corresponding flow rate. These were then downloaded to a laptop at regular intervals. A plot of flow rate into the pond versus time results in what is termed an "inflow hydrograph." The volume of runoff entering the pond for a storm event was obtained by numerically integrating the area under the inflow hydrograph using Mathcad. This could then be compared to the volume of rainfall on the bridge deck. Rainfall volumes, runoff volumes, and the resulting runoff coefficients for all measured events are provided in Table 1 of the Appendix.

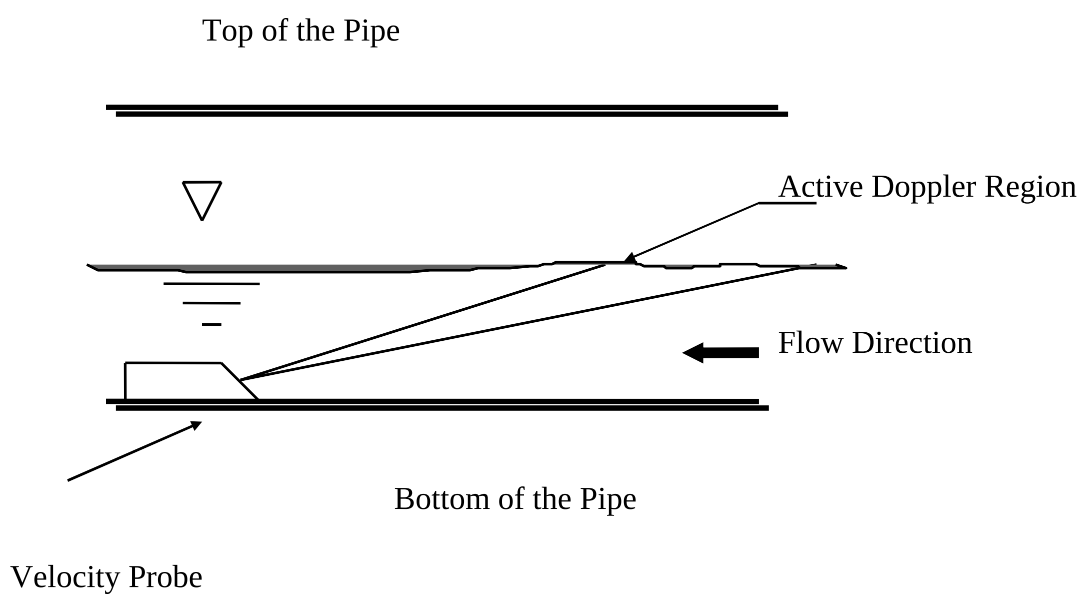

Figure 2
Schematic of depth/velocity probe installation

Objective 2–Toxicity of Pond Components using Microtox

Microtox is a proprietary system manufactured by Azur Environmental. It is a rapid (5-30 min. depending on procedure) screening method for determination of acute toxicity. It uses a specialized strain of luminescent bacteria. Toxicity is assumed to occur when the light output of the bacteria in contact with the sample drops below that of a control. The endpoint of the test used here occurred when the light output dropped to 50 percent of that of the control. The fraction of liquid sample or the concentration of sediment causing this drop is then referred to as the EC50. Samples of the pond liquid and sediment were collected and returned to the Folk Lab for analysis. Liquid samples were analyzed using the Basic Test as prescribed by Microtox. Initially four samples ranging in value from 5.6 percent to 45 percent pond contents were prepared. For each, the values of Gamma and the %Effect were determined at the end of a specified time period, usually 15 min. These values were then fitted to a equation relating concentration and Gamma [equation (4)], and a value of the regression coefficient was determined to evaluate the fit. In all cases, the fit of the data was evaluated visually as well. Sediment samples were analyzed using the basic solid phase test as prescribed by Microtox. Nine sediment concentrations ranging from 387 mg/liter to 99,000 mg/liter were prepared. Values of Gamma and the %Effect were obtained for each. These were fitted to the linear model relating concentration to Gamma [equation (4)]. The calibrated equations were then used to predict the EC50. For the liquid samples, the EC50 is expressed as the percentage of effluent in distilled water required to reduce the light output by 50 percent. For sediment samples, the EC50 is the concentration of sediment in the sample required to drop the light output by 50 percent. Results of all test performed are provided in Tables 2 and 3 in the Appendix.

DISCUSSION OF RESULTS

Objective 1–Volume Balance from Cross Lake Bridge

Data for a total of 24 storm events were collected. Additional events were monitored and data were collected, but because of equipment and/or human error, these results could not be used. The average rainfall event was 0.61 in., corresponding to a volume of 334,800 gallons; the median rainfall was 0.509 in., corresponding to a volume of 279,000 gallons, and the standard deviation of the rainfall was 0.516 in., corresponding to 283,200 gallons. A histogram of measured rainfall events, Figure 3, shows that most were small, 0.25 in. or less.

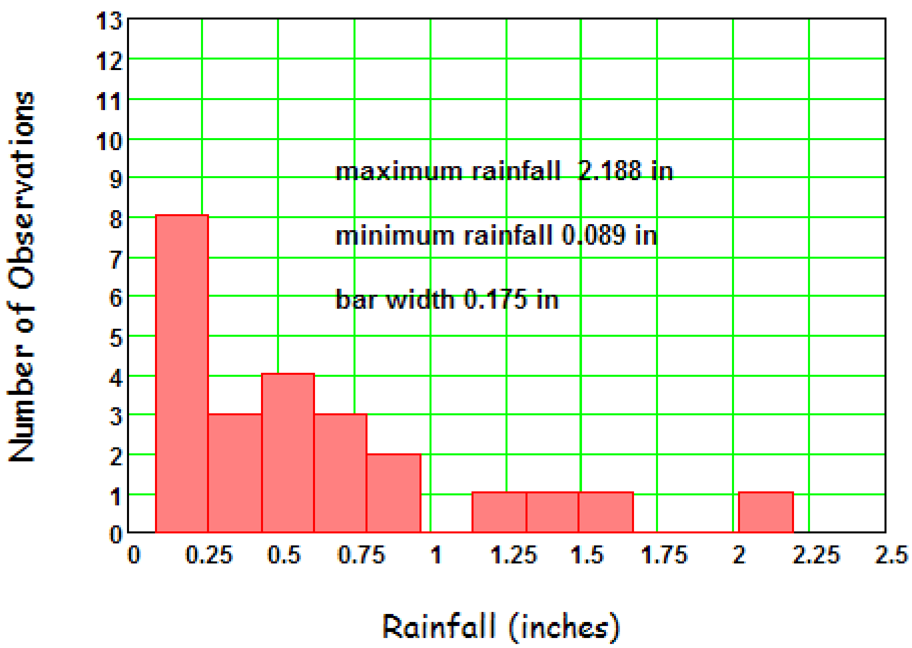

Figure 3
Histogram of rainfall events

[Note: For an explanation of how histograms in this report were constructed see "Histogram Construction" located in the Appendix]

A similar histogram for measured runoff volumes is shown in Figure 4. Runoff volumes ranged from less than 100,000 gallons to nearly 1 million gallons. Most were in the 100,000 to 200,000 gallon range.

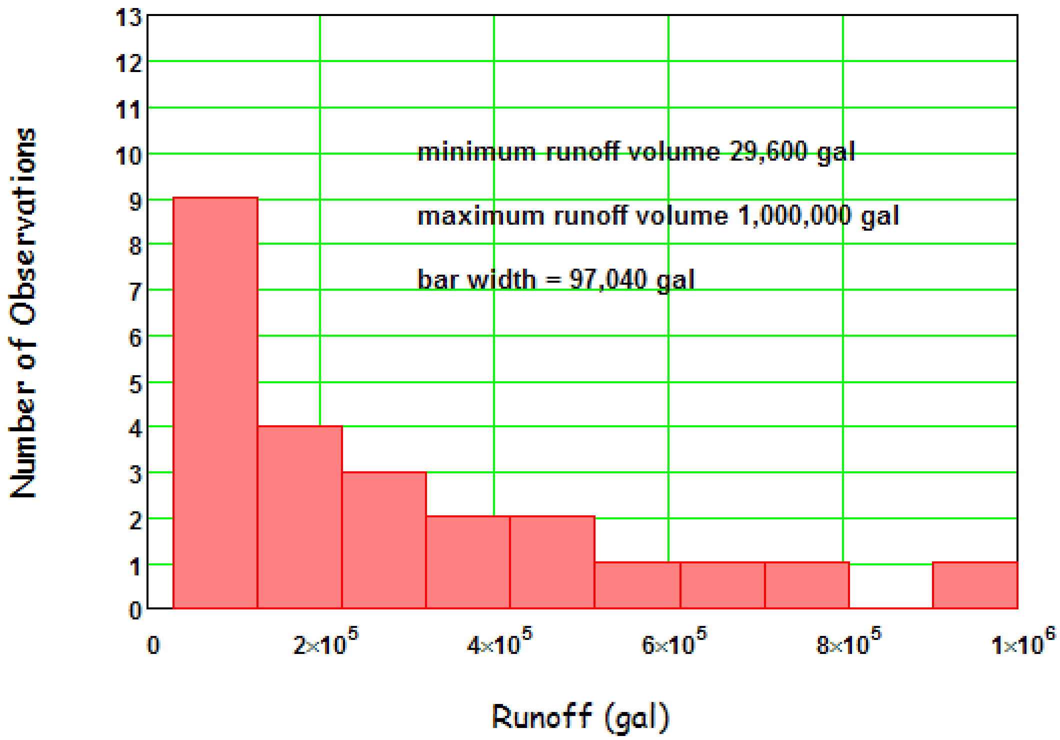

Figure 4
Histogram of runoff volumes

The ratio of runoff to rainfall for a specific event is termed the runoff coefficient (C). This value can range from near zero on highly pervious surfaces, such as agricultural land, to near one on highly impervious surfaces, such as the Cross Lake Bridge deck. For this study, the average runoff coefficient was 0.83. A histogram of measured runoff coefficients is shown in Figure 5, 18 of 24 measured C values were greater than 0.7. Unless an equipment malfunction or other reason for removing values could be identified, events were not deleted from the data set.

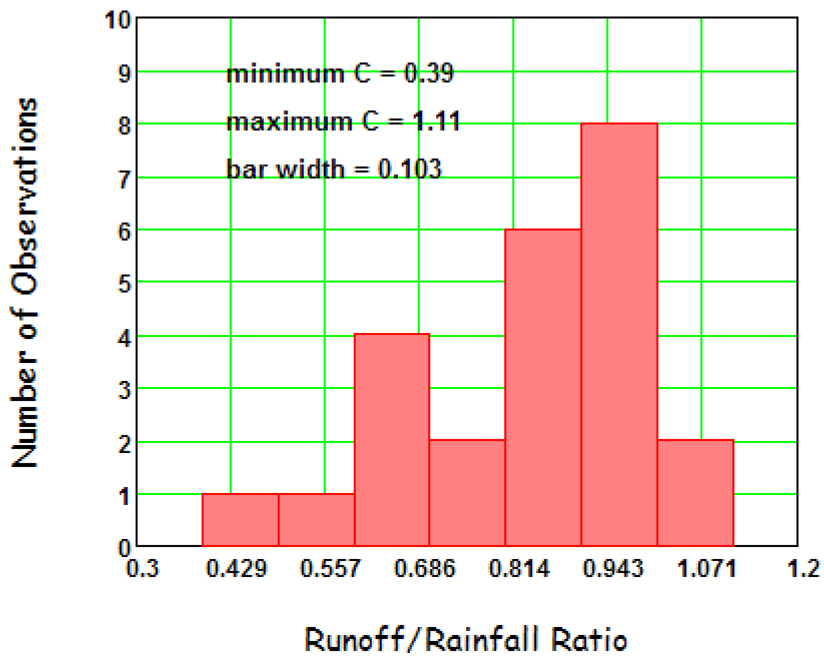

Figure 5
Histogram of measured runoff coefficients

Two runoff coefficient values were greater than 1.0. This suggests more runoff occurred than could be accounted for by the measured rainfall, which is not possible. Neither value was substantially greater than one, and this was most likely caused by the inherent inaccuracies in the measuring and totalizing process. Figure 6 shows the linear relationship (R^2 = 0.972) between measured rainfall and runoff for this study. Since the fraction of rainfall that runs off is expected to remain approximately constant (0.7 – 0.9), this relationship seems reasonable. The slope of the line of best fit corresponds to approximately 473,600 gallons of runoff per inch of rainfall, which translates to a runoff coefficient of 0.86 (very close to the average of the individual events).

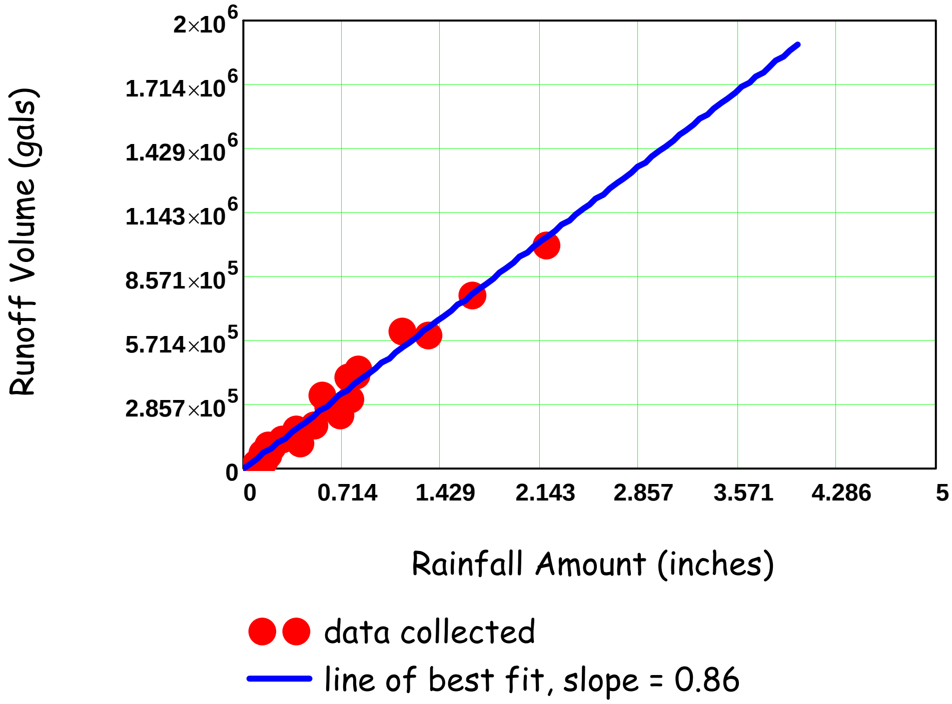

Figure 6
Relationship between rainfall and runoff

Comparison with Previous Data

As described in the Introduction, a previous study measured the rainfall and runoff for a total of 72 events between November 1996 and August 1997 *[1]*. The mean rainfall amount was similar to that measured in the current study, 0.5 in., corresponding to 274,200 gallons. The mean runoff volume was 139,800 gallons resulting in an average runoff coefficient of 0.49, substantially lower than would be expected from an impervious area. The distribution of rainfall and runoff amounts are shown in Figure 7 and Figure 8 and are similar to those shown for the current data set, most events totaling less than 0.4 in. The relationship between rainfall and runoff in the 2003 study was also linear (R^2 = 0.919) with a slope of 263,700 gallons/inch of rainfall, approximately half of that for the current study, corresponding to a value for the runoff coefficient of 0.481. The distribution of measured runoff coefficients for the 2003 study is shown in Figure 9 and has a significantly different shape than that of the current study. The distribution of C values in the previous study appears approximately normal in shape, suggesting some degree of randomness, as would be expected if the drainage system were experiencing substantial leakage.

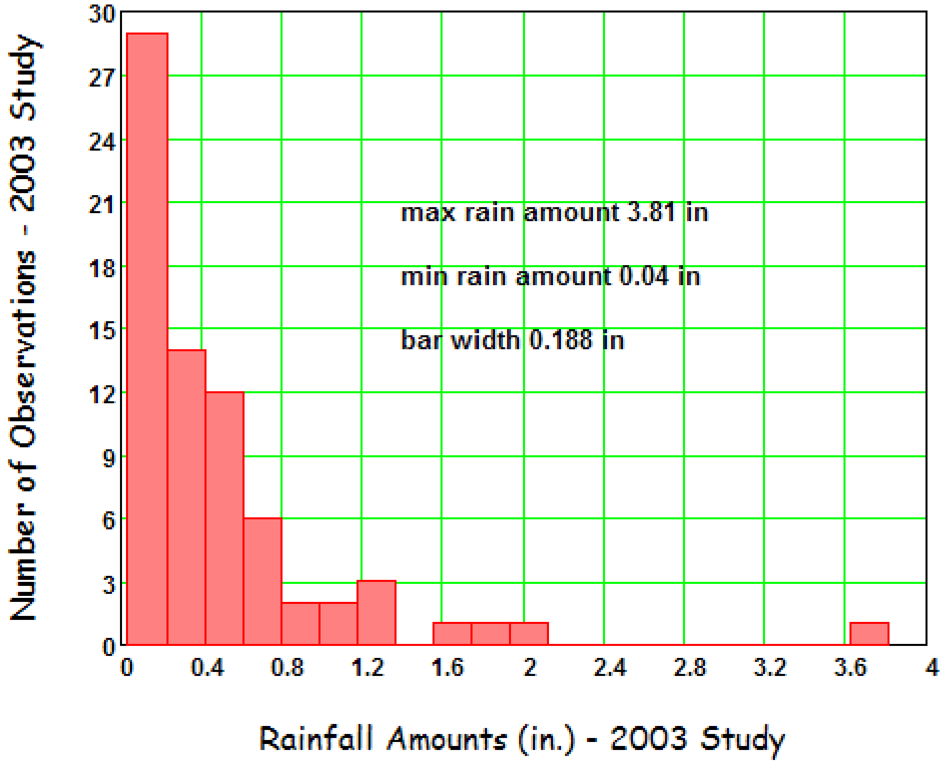

Figure 7
Distribution of rainfall amounts–2003 study

A comparison of data from both studies is presented in Figure 10. As shown, both studies resulted in linear relationships between rainfall and runoff; however, the average volume of runoff per inch of rainfall from the 2003 study was only about 56 percent of that for this study.

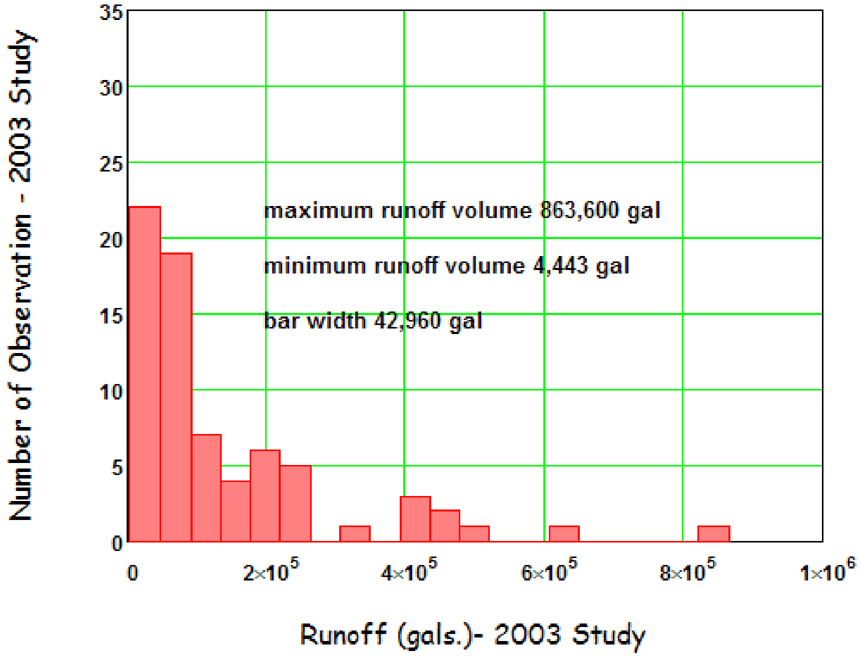

Figure 8
Distribution of runoff volumes–2003 study

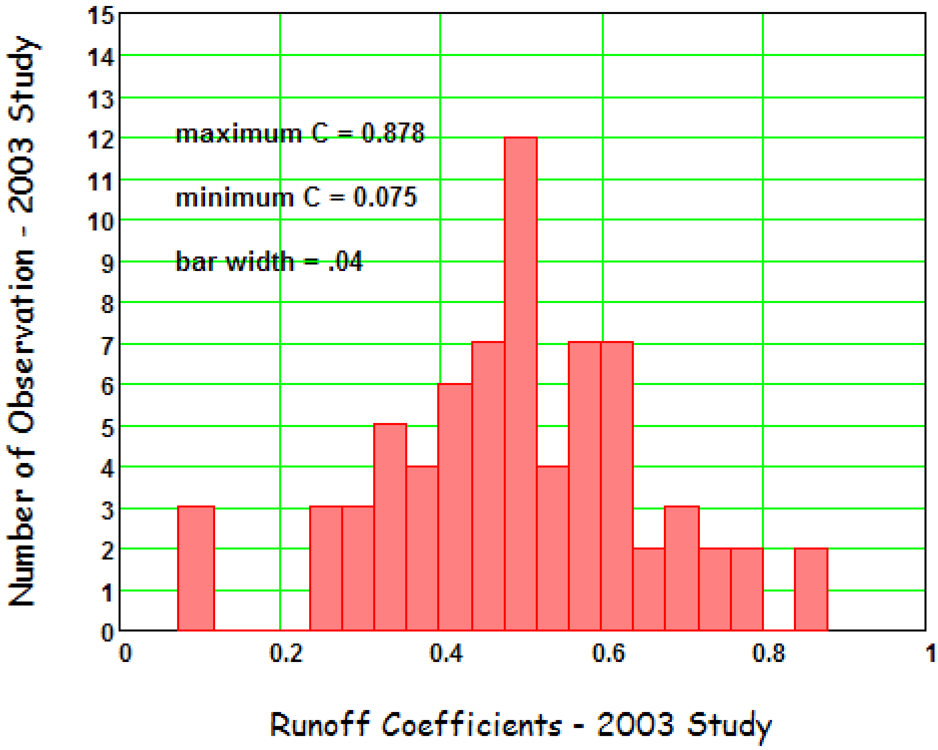

Figure 9
Distribution of runoff coefficients, C–2003 study

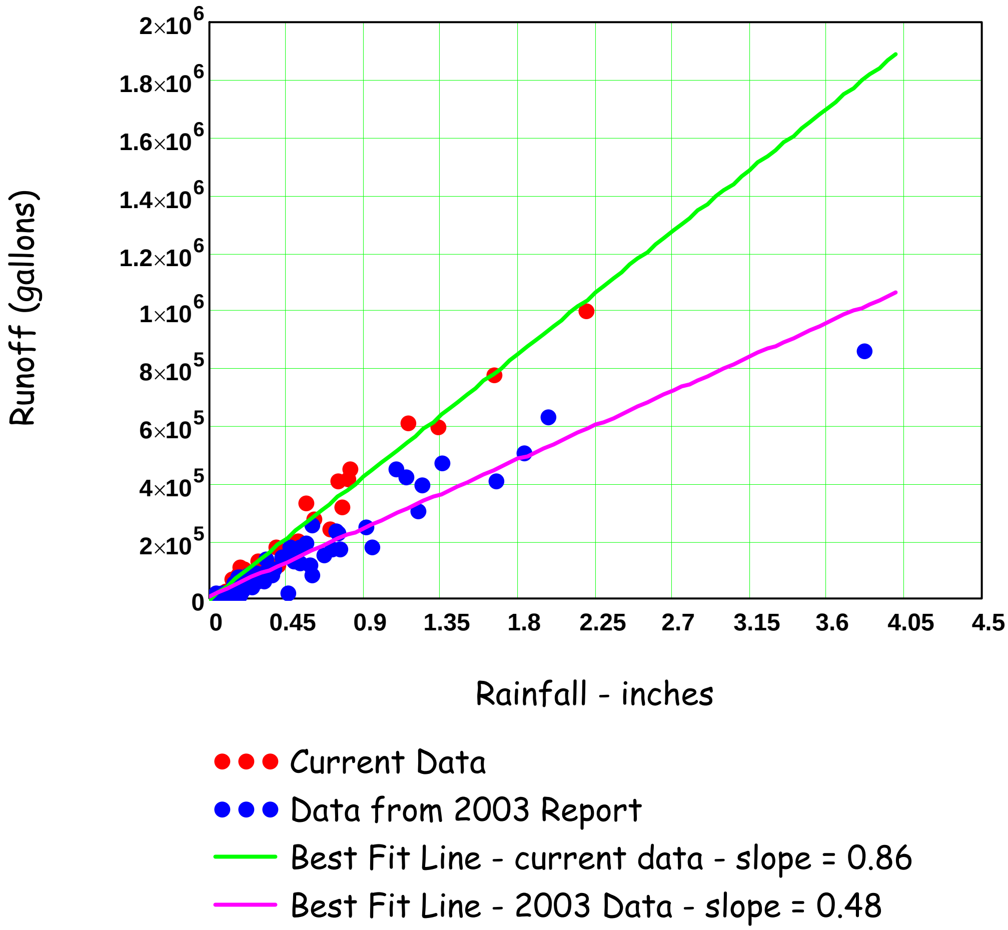

Figure 10

Comparison of data from current study and 2003 study

Objective 2–Toxicity of Pond Components using Microtox

Pond Liquid

Table 3 in the Appendix is a summary of the Microtox analyses conducted on the liquid contents of the pond. Recall that the EC50 is the contaminant concentration that reduces light output by 50 percent. A value of 8 percent means that distilled water diluted to 8 percent by volume with pond liquid produced enough of a toxic effect to drop the light output by 50 percent. The lower the proportion of effluent needed to reach EC50, the higher the toxic effect is assumed to be. In only 3 of 16 tests conducted, toxic effects were exhibited by the pond water. The largest proportion of sample used to calibrate the basic test is 45 percent, thus the values of 125 percent and 199 percent were obtained by extrapolating the linearized equation. Values greater than 100 percent indicate that more pond effluent than distilled water is required to produce an EC50 endpoint and suggest lower toxicity effects. This may be important, however, if the pond discharges into a dry ditch, as sometimes occurs.

Pond Sediment

Table 2 in the Appendix summarizes the toxicity results of 13 sediment samples collected from settled material in the Cross Lake holding pond. The EC50 is the sediment concentration that reduces the light output from the bacteria by 50 percent. The lower the sediment concentration required for this to occur, the higher the assumed sediment toxicity. Visual inspection of the data, as well as the regression coefficients, indicate that the data points fit the linearized equation well. The regression coefficient was less than 0.8 in only two cases. The upper limit on the sediment concentration used in the basic sediment test is 99,000 mg/liter, thus any EC50 greater than 99,000 mg/liter was estimated by extrapolation of the linearized curve. EC50 values for sediment ranged from 4,253 mg/liter to 258,900 mg/liter. There were no instances where a “no effect” reading was obtained for pond sediment.

CONCLUSIONS

Rainfall and runoff from 24 storm events were measured between November 2004 and April 2005. Runoff coefficients were computed for each. The average value obtained for all events was 0.83, which compares favorably with published values ranging from 0.7 to 0.95 for event frequencies of 5 to 10 years. Data from this study were compared to the results of 72 events monitored between September 1996 and August 1997. The average runoff coefficient for these events was found to be approximately 0.49. Comparison of the data sets showed that runoff per inch of precipitation increased by a factor of approximately 1.8 after completion of repairs to the drainage system. Based on these results, it appears that repairs to the drainage system on the Cross Lake Bridge were successful at eliminating the vast majority of the leakage occurring.

Sixteen samples from the water column of the Cross Lake pond were collected between March 2004 and February 2005. Only three of the samples indicated any toxic effects as measured by the EC50, while the remainder indicated “no effect.” In two of the results obtained, the values had to be extrapolated beyond the limits of the actual data. Taken as a whole these data suggest little toxicity is associated with the water column in the Cross Lake holding pond.

Thirteen samples of pond sediment were collected between September 2004 and July 2005. EC50 values ranged from 4,253 mg/liter to 259,000 mg/liter. EC50 values greater than 99,000 mg/liter were estimated by extrapolating beyond measured data points. None of the samples indicated a “no effect” response. Taken as a whole, the data suggest some degree of toxicity is associated with the sediment fraction of the pond contents.

RECOMMENDATIONS

Results suggest that there is toxicity associated with the sediment fraction of the pond discharge. This is significant because, as shown below, flow and contaminant measurements suggest that when the pond is drained high velocities at the pond outlet scour settled sediment, creating high concentrations in the trailing end of the discharge. Such high concentrations may create or exacerbate toxic conditions to higher life forms in receiving streams. It is therefore recommended that LADOTD examine possible procedures for keeping sediment within the pond during times when it is being drained.

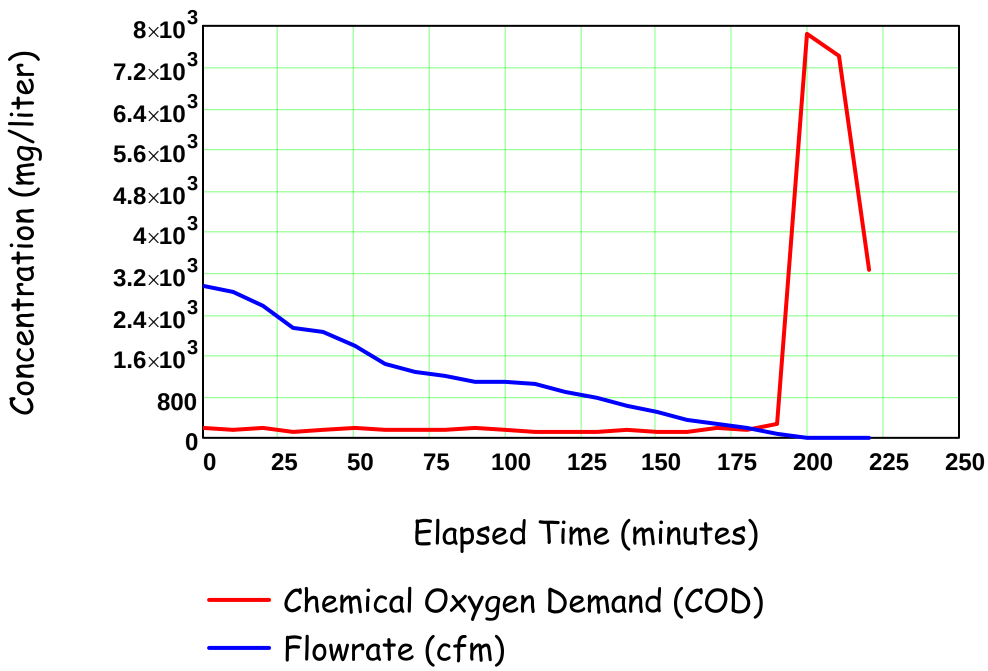

Figure 11
Chemical oxygen demand and flow rate during pond discharge

ACRONYMS, ABBREVIATIONS, & SYMBOLS

a	(appears as log a)—Intercept of the linearized Microtox Equation
b	slope of the linearized Microtox equation
C	Runoff coefficient, the ratio of measured runoff volume to measured precipitation volume, also used to represent sediment concentration in the Basic Sediment Test.
COD	Chemical Oxygen Demand
EC50	Solids concentration or liquid volume fraction at which light output is reduced by 50 percent in the Microtox test protocols—generally considered the test endpoint
G_t	Ratio of light expected from a nontoxic sample to that observed, minus 1
I_o	Initial light output of Microtox control sample
I_t	Light output of Microtox control sample after time, *t*
k	A composite correction factor relating to free energy and volume changes during reaction, used in Microtox computations
LADOTD	Louisiana Department of Transportation and Development
LTRC	Louisiana Transportation Research Center
Microtox	A standard test for aquatic toxicity testing.
P	Number of toxic molecules per target site
R_t	Ratio of light output of Microtox control sample (blank) after time t to the initial light output of the control (blank)
TSS	Technical Safety Services

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APPENDIX

Table 1
Runoff/rainfall data

Date	Runoff volume (gal)	Rainfall volume (gal)	Runoff coefficient
11/29/2004	1,000,000	1,200,000	0.83
12/5/2004	200,000	280,000	0.71
12/6/2004	600,000	730,000	0.82
12/8/2004	84,000	87,000	0.97
12/21/2004	62,000	93,000	0.67
12/22/2004	450,000	450,000	1.00
12/30/2004	89,800	93,000	0.97
1/1/2005	69,000	71,000	0.97
1/2/2005	29,600	49,000	0.60
1/5/2005	130,000	154,000	0.84
1/6/2005	330,000	310,000	1.06
1/12/2005	420,000	440,000	0.95
1/27/2005	614,000	630,000	0.97
1/30/2005	410,000	410,000	1.00
2/2/2005	280,000	330,000	0.85
2/7/2005	780,000	905,000	0.86
2/23/2005	316,000	420,000	0.75
2/24/2005	120,000	220,000	0.55
3/21/2005	180,000	210,000	0.86
3/27/2005	193,000	278,000	0.69
3/31/2005	242,000	384,000	0.63
4/5/2005	320,000	820,000	0.39
4/25/2005	102,000	109,000	0.94
4/26/2005	110,000	99,000	1.11

Table 2

Microtox results for sediment phase

Date	Test Performed	Sample	EC50 (mg/liter)	Coefficient of Determination (R^2)
9/22/2004	solid phase	Submerged sediment	6095	0.97
10/23/2004	solid phase	Submerged sediment	122600	0.96
12/9/2004	solid phase	Submerged sediment	74340	0.93
12/19/2004	solid phase	Submerged sediment	85590	0.95
12/23/2004	solid phase	Submerged sediment	207000	0.85
1/4/2005	solid phase	Submerged sediment	258900	0.88
1/14/2005	solid phase	Submerged sediment	71520	0.96
2/3/2005	solid phase	Submerged sediment	125900	0.97
2/4/2005	solid phase	Submerged sediment	116100	0.99
6/28/2005	solid phase	Damp sediment	7355	0.82
7/3/2005	solid phase	Dry sediment	38840	0.52
7/7/2005	solid phase	Submerged sediment	4253	0.73
7/8/2005	solid phase	Sediment in pond discharge	13250	0.97

Table 3

Microtox results for pond water column

Date	**Test Performed**	**Sample**	**EC50**	**R^2**
3/27/2004	Basic Test	Pond liquid	No effect	
3/31/2004	Basic Test	Pond liquid	No effect	
9/3/2004	Basic Test	Pond liquid	No effect	
9/13/2004	Basic Test	Pond liquid	8.0%	0.95
9/28/2004	Basic Test	Pond liquid	199%	0.99
11/4/2004	Basic Test	Pond liquid	No effect	
11/24/2004*	Basic Test	Pond liquid	No effect	
11/24/2004*	Basic Test	Pond liquid	125%	0.98
12/1/2004	Basic Test	Pond liquid	No effect	
12/9/2004	Basic Test	Pond liquid	No effect	
12/10/2004	Basic Test	Pond liquid	No effect	
12/19/2004	Basic Test	Pond liquid	No effect	
12/24/2004	Basic Test	Pond liquid	No effect	
1/14/2005	Basic Test	Pond liquid	No effect	
2/2/2005	Basic Test	Pond liquid	No effect	
2/4/2005	Basic Test	Pond liquid	No effect	

* Different locations in pond

Histogram Construction

Histograms shown in this report were initially constructed using Mathcad, version 14 and then transferred to this document. They were constructed using the Mathcad algorithm initiated by the statement “plot = histogram (7, data_set).” In this statement, 7 is a user specified number of “bins” into which the data values will be separated, “data_set” is a vector of values. The bin size is determined by dividing the range of the data vector by the number of bins specified by the user. The output “plot” consists of a two column matrix. The first column is the midpoint of each bin; the second is the number of observations in that bin. The midpoint values are treated as the independent variable and plotted on the x-axis, while the number of observations corresponding to each midpoint is plotted as the dependent variable on the y-axis. The resulting plot is then transferred to the report. The numbers on the x-axis refer to the plot grid lines, not the bars.