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Technical Report

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II. Abstract

The Microtox™ toxicity analyzer, Beckman Inc., was investigated as a new tool for the assessment of sediment toxicity. The Microtox system employs the bioluminescent marine bacterium, Photobacterium phosphoreum, as the test organism, and uses the decrease in light output, relative to a reagent blank, as the measure of toxicity. Sediments from nine impoundments along the Ten Mile River were tested for toxicity using several different elutriation techniques. Elutriation with a 0.1 M HCl-KCl pH2.0 buffer was found to provide the most consistant results with the Microtox analyzer. A general pattern of increasing toxicity in the downstream reaches of the Ten Mile River was observed.

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IV. Introduction

Few tests are currently available to assess the potential toxic effects of contaminated sediments to aquatic organisms. Those that are available are time consuming and require test organisms which can be difficult to maintain. The disposal of dredged sediments into navigable waters is regulated by Section 103 of Public Law 92-532, the Marine Protection, Research, and Sanctuaries Act of 1972. Under this Act, dredged material must be evaluated to determine possible adverse environmental impacts prior to disposal. The Environmental Protection Agency (EPA) and the Corps of Engineers (COE) have jointly published the manual "Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters; Implementation Manual for Section 103 of Public Law 92-532 (Marine Protection, Research, and Sanctuaries Act of 1972)," to provide guidance in the assessment of the environmental impact of dredged material.[1] While this manual outlines specific protocol for conducting bioassays which may be used in the evaluation of dredged material, it " is not intended to establish standards or rigid criteria," and encourages research leading to the development of new procedures to augment those already available.

The Microtox toxicity analyzer (Beckman Instruments, Inc., Carlsbad, CA) is currently used to test the toxicity of aqueous samples. The system employs lyophilized marine bacteria (Photobacterium phosphoreum) which upon reconstitution, emit light. When exposed to a toxicant, the level of bioluminescence, relative to a reagent blank, is diminished in direct proportion to the toxicant concentration for concentrations above the threshold limit of detection. The Microtox system is quick and easy to use, requiring only a few milliliters of sample and 30 minutes to one hour testing time, compared to the minimum 96 hours needed in many other bioassays. Additionally no culturing of test organisms is required. The Microtox system has been compared to fish and invertebrate bioassays for the evaluation of the toxic effects of a wide variety of substances by numerous invesigators and has been found to have comparable precision and accuracy.[2,3,4,5] The costs of

Microtox testing are also significantly less than those of other conventional bioasssays (fish and daphnids). A 1983 cost estimate is $52 per test after capital costs, including technician salary.[6]

The objective of this investigation was to examine the Microtox analyzer as a new method for determining sediment toxicity and to recommend guidelines for a Microtox sediment toxicity protocol. Because the Microtox system is limited to the testing of aqueous solutions, the toxicity of sediment to the Microtox organism was determined by measuring the toxicity of the liquid phase of sediment samples elutriated under various experimental conditions. Specifically, in response to the needs of the Massachusetts Division of Water Pollution Control (MDWPC), the toxicity of sediments from several impoundments in the Ten Mile River Basin, located in and about North Attleborough, Massachusetts was studied. The results from these tests are reported in terms of the percent sample concentration which resulted in 50 percent light diminution after 30 minutes of exposure (30EC50). These values were calculated by linear regression analysis.

V. Background

Microtox testing :

The Microtox toxicity analyzer (Beckman Instruments, Inc.) is a bioassay system which employs the bioluminescent marine bacterium Photobacterium phosphoreum. The bacteria are available in lyophilized form from Beckman and may be stored refrigerated (4° C) twelve months or longer. Upon reconstitution the bacteria emit light, which is diminished in response to a toxicant challenge. The Microtox analyzer is equipped with a constant temperature reaction chamber and a precision photometer. The photometer may be connected directly to a strip chart recorder to provide a permanent record of test results.

When exposed to a toxicant the light emitted by the Microtox organism is diminished in direct proportion to the toxicant concentration for concentrations above the threshold limit of detection. During the testing procedure bacteria are exposed to toxicants at up to four different concentrations with two replicates of each concentration. After some predetermined exposure period, the light output of each replicate is measured and compared with initial light output and reagent blanks to determine the percent sample concentration which will produce 50 percent light diminution (EC50). The Microtox test has several advantages over other conventional acute toxicity bioassays including small sample requirements, low cost , and the use of a statistically large population of test organisms (more than 10^5 bacteria per test). More detailed information on the operating principles of the Microtox analyzer may be found in reports by Beckman Inc.[2] and by Sheehan, Sellers and Ram.[6]

Sediment Toxicity Methods :

No chemical procedures exist which can determine the adverse environmental effects a contaminated sediment may have. While bulk

sediment chemistry or liquid phase chemical analysis may provide useful information on the type and degree of contamination present, these tests cannot provide information on the environmental availability of contaminants, their ultimate fate or possible synergistic effects. Because of this, bioassays rather than chemical assays are used as the measure of a sediment's potential for creating adverse environmental effects. All bioassays for sediment toxicity consist of two main procedures:1) the bioassay itself, and 2) sediment preparation. Each of these activities involves a complex set of variables, and different investigators have addressed these in a number of ways.

1) Bioassays :

Sediment bioassays may be categorized by the type of organism employed, the method of sediment exposure, or the biological end-point measured during the test procedure. Organisms from many different trophic levels have been investigated for their suitability for sediment bioassays. The EPA/COE Implementation Manual suggests a battery of tests.[1] For liquid phase bioassays the EPA has specified that such tests be conducted with, " appropriate sensitive marine organisms, " and defines this as meaning at least three species consisting of one phytoplankton or zooplankton species, one crustacean or mollusc and one fish.[9] For solid phase bioassays the EPA specifies a minimum of three species, including one filter-feeding, one deposit feeding, and one burrowing species.[9] The EPA/COE Implementation Manual suggests that these be chosen to include a crustacean, an infaunal bivalve molusc and an infaunal polychaete worm. This manual further suggests that all sediment bioassays include a species of mysid shrimp of genus Mysidopsis or Neomysis to provide an internal standard. The phytoplankton bioassay is usually satisfied by a standard algal assay. In terms of classification, the Microtox comes most nearly under this category. It should be noted that the protocols developed by the EPA/COE apply to marine waters and no similar protocols have been established, as of yet, for fresh waters. Because the Microtox procedure includes a step for osmotic adjustment, the Microtox test may be used for either fresh water or marine samples.

Sediment bioassays may be characterized by the method of presentation of the sediment to the organism. Aquatic organisms may either be exposed to some liquid phase of the sediment or to the solid phase with some overlying water. Additionally, field studies of organisms living in waters associated with the sediment in question may be used. The 96 hour liquid phase test is a commonly used test. [1, 10] In this test organisms are exposed only to the liquid with no underlying sediment substrate. As such it is not appropriate for infaunal organisms. A number of solid phase tests may be found described in the available literature. The most common duration of exposure is 96 hours, [1, 10, 11] although solid phase bioassay studies have been performed for 10 days [12] and even as long as 28 days [13]. Flow condition is another variable to be considered in both liquid and solid phase bioassays. Flow regimes range from no flow to nearly continuous flow. For the purposes of this study, however, the method of presentation is largely dictated by the Microtox organism. As mentioned previously the Microtox test is limited to liquid samples. The test is also designed for no flow and for exposure periods from 5 minutes up to an hour or more.

The biological endpoint measured in a bioassay may vary from test to test. Death is perhaps the most common endpoint, having the advantages of being easily quantified, definable and having definite ecological significance.[14] Other endpoints that may be used included physiological and behavioral changes. Cough frequency has been used as the measured endpoint in fish sediment bioassays. [10] Bioaccumulation of known toxicants in either fish or invertebrates is another parameter often measured.[15, 16, 17] However, unless specific levels of accumulation constituting toxicity have been previously defined, the significance of bioaccumulation as it relates to environmental impacts may be unclear. This is especially so for compounds whose toxic effects may not be known and for cases in which synergistic effects may be significant. Some organisms may be more susceptible to toxicants at various developmental stages, and some work has been done which attempts to correlate developmental abnormalities with sediment toxicity using oyster larvae. [18] Whichever biological endpoint is employed, it is important to keep in mind that the functional definition of toxicity for the test will be bound to that endpoint, and

as such it must be chosen carefully. The Microtox toxicity test uses a decrease in the unique characteristic of bioluminescence as the endpoint. The biochemical pathways in the light emitting reactions are such that a block in electron transport will cause a decrease in bioluminescence.[2] Blocks in electron transport are the result of toxicants and have detrimental effects on organisms,[19] and it is this functional definition of toxicity which makes Microtox a good indicator for general toxicity.

2) Sediment Preparation :

The methods used to collect and prepare a sediment sample prior to toxicity testing are as important as the methods used during the actual test procedures. There are many variables to consider, and the development of any protocol entails many choices. Much of the lack of standardization in the field of sediment toxicity testing arises from the fact that many decisions must necessarily be made on an arbitrary basis. The first step in sample preparation is collection. After collection storage conditions must be considered. If elutriation procedures are used, the sediment-water ratio, pH conditions, time and temperature of elutriation are among the factors which must be considered in the development of a protocol. Each step in the process should be considered in terms of practicality, expense, time and availability of equipment.

A) Sampling Methods :

There are three sampling methods predominantly used for the collection of aquatic sediments: Ponar dredge, Ekman dredge, and bored core sampling. The EPA/COE Implementation Manual states either grab or core samples may be used for bioassays without indicating a preference for either.[1] They do note, however, that sediment samples should be mixed to ensure homogeneity. Different sampling techniques may be more

or less appropriate for different types of sediment studies. Cores may not be practical when large volumes of sediment are needed. On the other hand, when sediment samples will be used for the extraction of interstitial waters, cores may be necessary.

All sample containers should be chosen to minimize both the possibility of toxicants leaching from the container into the sample or loss of toxicant from the sample by adsorption onto the container wall. Polyethylene or polyethylene-lined containers are most frequently used for sediment samples. However, glass containers may be preferable for samples for which suspected organic toxins are of primary concern.[1]

B) Sample Storage :

Generally sediment samples, which are biologically active, should be stored cold. Some investigators freeze sediment samples,[11, 20, 21] while others store sediment at 2-4 ° C.[1, 10, 22] Freezing of samples may be more useful for studies in which changes in chemical speciation due to reduction are to be avoided.[21] However, the EPA/COE Implementation Manual states emphatically that samples should never be frozen.[1] If freezing is the method of choice, it should be kept in mind that sediment containers must be able to withstand expansion of the sediment due to ice formation.

Duration of storage is another consideration. Sediment should be tested for toxicity as soon as possible. Two weeks is considered the longest sediment should be stored before testing.[1] A study on the effect of storage conditions on the extractability of several metals and organic material found that detectable changes had ocurred after 15 days at 4 ° C.[21] The best results were obtained by extraction within 24 hrs.

The atmospheric conditions under which sediment is to be stored should also be considered. Three approaches have been found in the literature. One approach is to fill the container completely with sample.[1] Another is to fill the free space in the container with overlying water from the sampling site.[10] The third approach is to store the sediment under a nitrogen atmosphere.[10] A fourth approach,

used in this study, involves sealing the container with ambient atmosphere in the free space and allowing oxygen depletion to occur naturally. Storage under overlying water may not be appropriate if interstitial water is to be extracted, and unless an excess of sediment is available, it may not be practical to fill containers completely with sediment. If coring tubes are to be used to transport the sediment, it is also unlikely that they will be filled completely. Additionally, it may not be possible to place sediment under a nitrogen atmosphere for some hours after collection.

C) Elutriation Techniques :

The extraction of interstitial water from a sediment is a fairly straightforward process. Centrifugation is the easiest method of extraction. The variables to consider in this procedure are the time, speed, and temperature of centrifugation, and the treatment of the supernatant prior to toxicity testing. The protocol used by Bahnick et al. was to centrifuge in stainless steel centrifuge bottles, sealed under a nitrogen atmosphere, for 12 minutes at 10,000 rpm and 4 ° C. The supernatant was then recentrifuged at 14,000 rpm for 15 min. This supernatant was then used for testing without filtration.[10] In order to obtain any significant quantity of liquid a fairly large weight of sediment must be centrifuged, and this can lead to excess wear on centrifuge equipment. This method is also less practical for sediments with low moisture content.

Elutriate procedures are more varied than procedures for interstitial water. The specific water used, the sediment-water ratio, the pH, the type of acid and/or buffer used, if any, oxygen saturation, time, temperature and treatment after elutriation are all among the many variables which must be considered in an elutriation procedure. Moreover, different elutriation procedures may exhibit preferential extraction for certain types of toxicants. Procedures which may be well adapted for the extraction of toxic metals may not extract organic toxicants as well. It is also important to ascertain that the method used will not produce toxicity in and of itself.

The sediment-water ratios reported in the literature range from 1:4 to 1:20 parts sediment to water. There seems to be no clear basis for choosing a particular ratio, and of the literature reviewed, there was no discussion included on how this parameter might affect quality of results. For this investigation a sediment weight to final weight ratio of 1:20 was used at the request of MDWPC personnel.

The water to be used in an elutriation procedure may be chosen from several sources. Possibilities include overlying water from the site, water from the proposed dispersal site if the sediment is to be disposed of in another body of water, or water of known purity such as laboratory distilled water. The EPA/COE Implementation Manual states that the liquid phase for chemical analysis may be prepared with dredging site water, while the liquid phase for bioassay tests should be prepared with disposal site water.[1] The use of disposal site water may be necessary when marine disposal is planned and marine organisms are used for toxicity testing. The Microtox testing procedure has the advantage that any of the above waters may be used for sample preparation. Using water of known purity can avoid introduction of further unknown variables.

Several studies have shown that the availability of toxicants to aquatic organisms is more directly related to sediment concentrations than to concentrations found in waters equilibrated under ambient conditions[10, 17, 23]. For tests such as the Microtox, which cannot rely on direct exposure to the solid phase, various extraction procedures are available which can be used to liberate toxicants from the sediment to the liquid. The goal of such procedures is to simulate the true availability of toxicants to an aquatic ecosystem.

Acidification is the method most commonly used to extract toxicants from the solid phase of a sediment sample. Many toxicants, especially heavy metals, are more soluble at low pH. Several authors have concluded that HCl extraction should be the preferred method for an operational definition of extractable metal concentration in sediments[24, 25]. When choosing a particular acid extraction method several parameters must be considered: the type of acid, the resulting pH, and the buffering capacity, especially if extraction is to be of long duration. Other than acid and/or buffer additions to the elutriating water, samples may be processed in the same manner as other

elutriates.

The degree of oxygen saturation during elutriation and storage procedures may or may not affect sample toxicity. Release of different toxic compounds may be either increased or decreased under anaerobic conditions. Conflicting reports exist on the solubility of individual toxicants, with one investigator finding an apparent increase and another finding a decrease under anaerobic conditions.[26, 27] Banhick et al. studied the toxicity of sediment elutriates prepared under both oxic and anoxic conditions and found no significant difference in toxicity. Different sediments may well behave differently in this respect, due to differing sediment chemical constituants and microbial populations.

The time and temperature of elutriation can also affect toxicity results. The EPA/COE Implementation Manual suggests elutriating at 18-24 ° C. for 30 minutes on a shaker table at 100 oscillations per minute.[1] In an analysis of heavy metal solubility Bahnick et al. found that less than one percent of the total sediment concentration of several key metals was solubilized by the end of 21.5 days while maintaining original sediment moisture content under a nitrogen atmosphere at 4 ° C.[10] Further analysis of their data indicates that the incremental change in solubility decreases over time, and that nearly half of the total extraction occured within two days. On the basis of this information, two days' extraction time was chosen for this investigation.

VI. Site Description

The Ten Mile River originates in Plainville, Massachusetts, and flows southwest through North Attleborough and Attleboro to Pawtucket and East Providence, Rhode Island, where it empties into the Seekonk River (Figure 1). The river is 35.4 kilometers long and has an average elevation drop of 1.9 meters/kilometer. Fifteen impoundments along the river comprise almost half of the length of the river. The Bungay and the Sevenmile Rivers flow into the Ten Mile River at Attleboro, MA. and at the state line respectively. Additionally a smaller tributary, Speedway Brook, flows into the Ten Mile River in the city of Attleboro. Urban development in the Ten Mile River Basin is densest along the river. Three wasterwater treatment plants discharge into the river. Additionally, industrial discharge permits have been have been granted to numerous manufacturing companies. Jewelry, electroplating, metal finishing and dye operations are all local industries which discharge into the river. A more complete description of the river basin may be found in "The Ten Mile River Basin: 1981 Water Quality Data," [7] and further information on industrial permits in "The Ten Mile River: 1975 Part D." [8] The specific impoundments studied in this invesigation are listed in Table 1 with their locations along the river system and dates of sample collection.

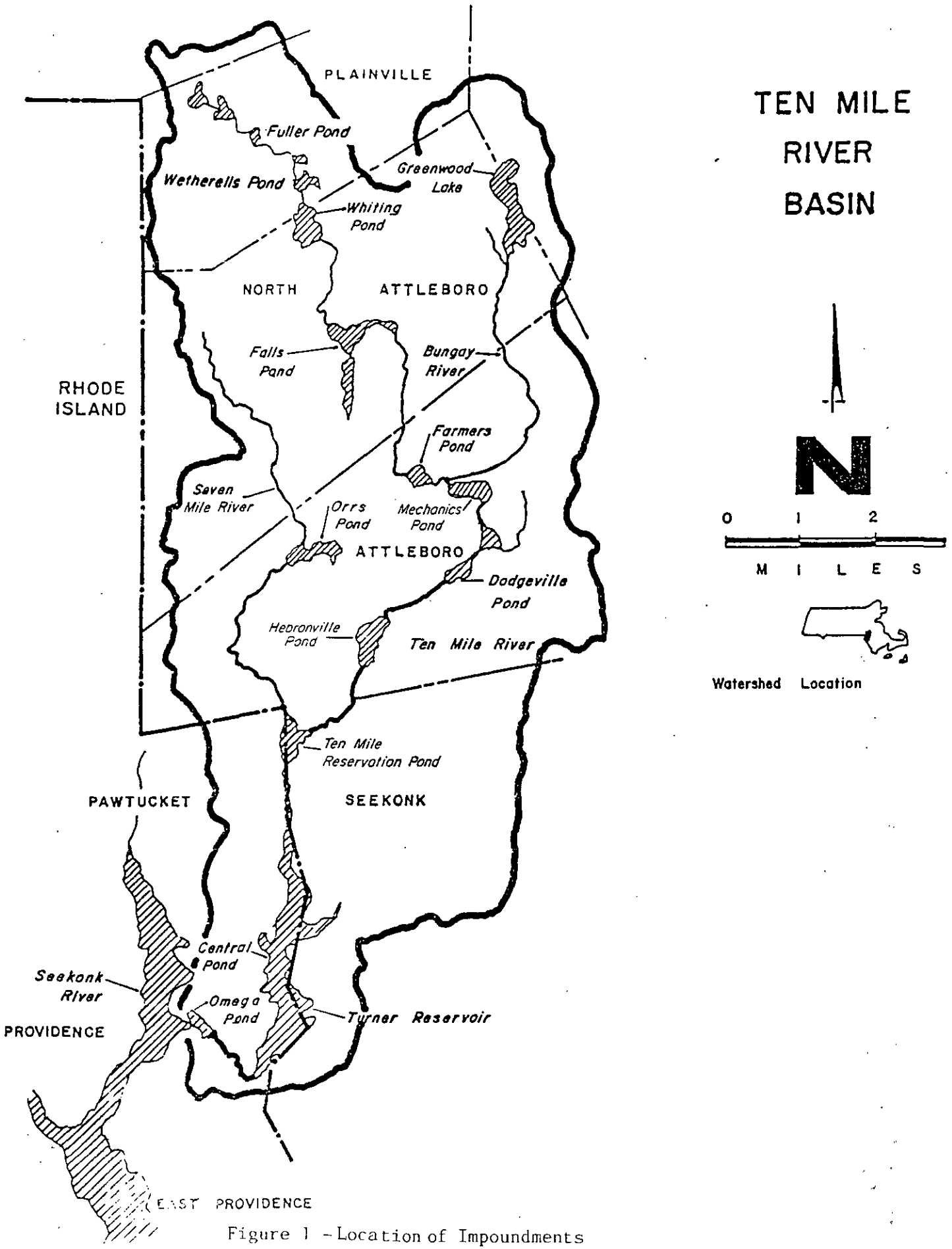

Figure 1 - Location of Impoundments

TABLE 1 - IMPOUNDMENTS STUDIED FOR SEDIMENT TOXICITY

IMPOUNDMENT	DATE	DISTANCE FROM MOUTH		MUNICIPALITY
		MILES	KILOMETERS	
FULLER	7-6-84	20.5	33.0	PLAINVILLE, MA.
WETHERELLS	8-7-84	19.5	31.4	PLAINVILLE
FALLS	7-6-84	17.0	27.4	NORTH ATTLEBOROUGH, MA.
FARMERS	8-7-84 10-4-84	15.0	24.1	ATTLEBORO, MA.
MECHANICS	7-6-84	14.0	22.5	ATTLEBORO
DODGEVILLE	6-19-84 8-7-84	12.5	20.1	ATTLEBORO
HEBRONVILLE	8-7-84 10-4-84	10.5	16.9	ATTLEBORO
RESERVATION	8-28-84	6.0	9.7	EAST PROVIDENCE, RI.
CENTRAL	8-28-84 10-4-84	5.0	8.0	PAWTUCKET, RI.

VII. Experimental Methods

Sediment Collection and Handling:

Sediment cores from each of nine impoundments along the Ten Mile River in southeastern Massachsetts and Rhode Island were collected by Massachusetts Division of Water Pollution Control personnel (see Table 1 for locations and dates). Four of these impoundments were sampled twice. The cores ranged in length from approximately 15 to 50 centimeters. The depth at which the cores were taken was generally between one and two meters. The overlying water was removed from the cores immediately after collection. The cores were stored in closed plastic coring tubes and refrigerated until transport to the University of Massachusetts, Amherst. Samples arrived within two days of collection. Laboratory preparation of sediment for Microtox testing was begun within twelve hours after sample arrival.

The top 20 centimeters of sediment were removed from each core and placed in large,clean (acid washed, distilled-water rinsed) glass beakers. The sediment was then mixed by hand with a stainless steel spatula , and any large debris removed. The lower portion of the sediment core was discarded. Where more than one core from a site was available, the top 20 centimeters of each core were combined into one sample. Subsamples of the mixed sediment were weighed out for each of the elutriate procedures described below: interstitial water, RO:DI H_2O elutriate, 0.1 M HCl-KCl pH 2.0 elutriate, anoxic elutriates, and nitrogen purged elutriates. Portions were also taken for density and dry weight analysis. Sediments were exposed to open air conditions the minimum time necessary to carry out mixing and weighing opperations (about 15 min.). Additionally, all samples were analyzed for Microtox toxicity within two weeks of sample preparation.

Interstitial Water :

After subsamples had been taken for elutriate procedures, the

remaining sediment was centrifuged to obtain interstitial water. Sediment was placed into 250 ml polyethylene centrifuge bottles. Samples were centrifuged for 15 minutes at 7,000 rpm and 4 ° C. The supernatant was then decanted into 50 ml centrifuge tubes and recentrifuged (10 min., 7,000 rpm, 4 ° C). The interstitial water was decanted into clean glass sample bottles and stored capped and refrigerated until Microtox analysis could be performed (within two weeks).

RO : DI H_2O Elutriate :

Sediment was mixed with reverse-osmosis: deionized water (RO:DI) at a one to twenty wet weight to final weight ratio (e.g. 4 g sediment/ 76 g RO:DI H_2O) in clean 125 ml flasks. The sediment slurry thus formed was placed on a shaker table oscillating at 100 cycles/minute for 48 hours at 22-24 ° C with constant light conditions. The flasks were stoppered with foam plugs to facilatate diffusion of air into and gas out of the sediment mixture. After the elutriation period, the sediment - water mixture was allowed to settle for a short period of time (15 to 30 min.) and decanted into clean 50 ml centrifuge tubes. The liquid was centrifuged 10 min. at 7,000 rpm and 4 ° C. The supernatant was then decanted into clean sample bottles and stored capped and refrigerated until Microtox analysis was performed.

0.1 M HCl-KCl pH 2.0 Elutriate :

Sediment was elutriated with a 0.1 M HCl-KCl pH 2.0 buffer in the same manner as for the RO:DI H_2O elutriate. The HCl-KCl buffer was prepared by the method found in Perrin and Dempsey.[28] 435 ml of 0.2 M KCl and 65 ml 0.2 M HCl were added and brought to one liter final volume with RO:DI water. After the elutriation period, the HCl-KCl elutriate was centrifuged and stored similarly to the RO:DI elutriate. The pH was checked both before and after elutriation.

Anoxic Elutriates :

RO:DI H_2O and 0.1 M HCl-KCl sediment slurries were prepared as above. These were placed in stoppered 100 ml serum bottles under a N_2 atmosphere. It should be noted that the water used in the preparation of these samples was not purged of dissolved oxygen. The sediment slurries were then elutriated under otherwise similar conditions as outlined above. After the elutriation period, the serum bottles were opened, and the supernatant decanted and centrifuged. After centrifugation, the supernatant was decanted into clean serum bottles, placed under a nitrogen atmosphere, and stoppered. These samples were also stored refrigerated until Microtox tests were performed.

Nitrogen Purge :

For elutriate samples, oxic and anoxic, which demonstrated toxicity (i.e sample 30EC50 less than or equal to 110% sample concentration), further tests were made to evaluate the effect of nitrogen purging on the sample toxicity. The purpose of the nitrogen purge was to estimate the contribution of ammonia to the total toxicity. The pH of a small portion of elutriate was raised to 10.0 or above with 1N NaOH. Nitrogen gas, as an inert gas, was then bubbled through the elutriate for five to ten minutes. Theoretically the increase in pH should shift ammonium, NH_4^+, to ammonia NH_3, which is then purged from the sample by the nitrogen gas. The pH of the sample was readjusted down to 7.0 with 1N HCl before retesting for Microtox toxicity.

Microtox Testing :

Interstitial water and RO:DI H_2O elutriated samples were tested for Microtox toxicity following the procedures described by the manufacturers and modified by Sheehan, Sellers and Ram.[6] The pH of the samples was adjusted to 7.0 +/- 0.1 with 0.1 N HCl or 0.1 N NaOH immediately prior to Microtox analysis. Sample osmolality was then adjusted to 2% NaCl (by weight) with Microtox Osmotic Adjusting Solution (22% NaCl). Serial dilutions of the osmotically adjusted

samples were made using Microtox Diluent (2% NaCl). These were allowed to equilibrate to 15 °C in the Microtox analyzer incubation chamber before testing. The lyophilized bacteria (Microtox Reagent) were reconstituted and diluted according to the manufacturer's specifications. The reconstituted reagent was allowed to equilibrate for 15 to 20 minutes before initial light readings were taken. Light output was recorded at 5, 15, and 30 minutes after exposure, and the EC50 values calculated by regression analysis.

The 0.1 M HCl-KCl elutriate samples were treated as above with a modification in the adjustment of osmolality. When calculating the amount of Microtox Osmotic Adjusting Solution (MOAS) needed to produce a final 2% saline solution, an allowance was made for the contribution of the KCl to the total osmotic balance of the solution. Instead of adding 10% by volume of MOAS to the sample, 6.25% was added. When the HCl-KCl elutriate was adjusted to pH 7.0, many of the samples formed a precipitating floc. When this occured, the floc was allowed to settle by gravity for 10 to 20 minutes, and a portion of the clear overlying supernatant carefully pipetted off for Microtox analysis. Similar precipitation occured in some of the nitrogen purged samples. These samples were also allowed to settle before taking a portion for Microtox testing. Figure 2 provides a procedural flowchart of the experimental methods used in this investigation.

Chemical Analysis:

Aliquots of the various elutriate samples tested for Microtox toxicity were acidified to pH 2.0 with concentrated hydrochloric acid. These aliquots were refrigerated and returned to MDWPC personnel. The MDWPC then arranged to have metals analysis performed on each of the samples. The following metals determinations were made: cadmium, chromium, copper, iron, lead, nickel, silver, and zinc. The values for each of these metals were compared to Microtox results by regression analysis.

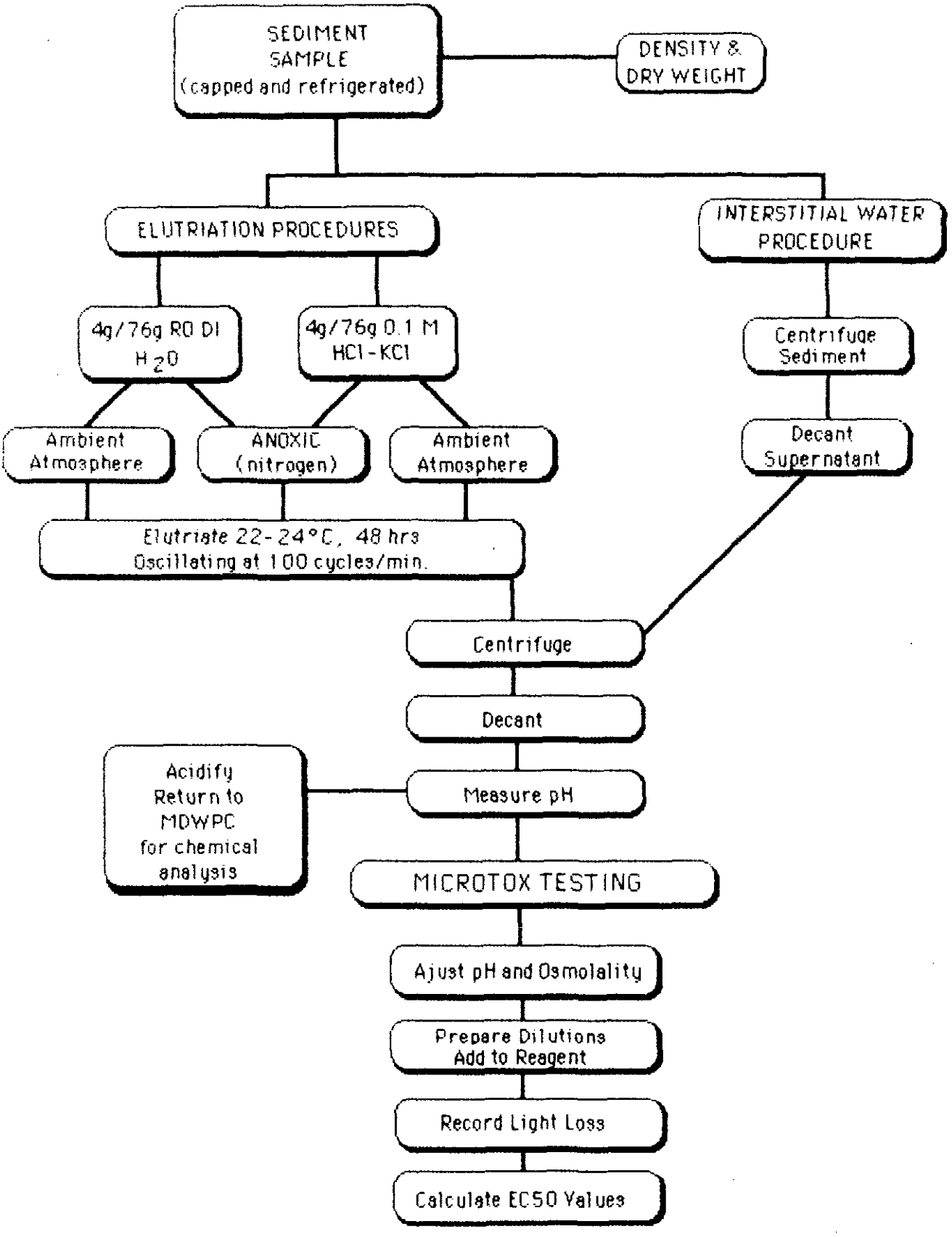

Figure 2 - PROCEDURAL FLOWCHART

VIII. Results

Appendix I, "Microtox Data", contains detailed information on test results for this investigation. The initial pH, the concentrations tested, and the highest percent light loss achieved are reported. Also reported in this appendix are linear regression statistics for each of the Microtox tests (slope and Pearson product-moment correlation coefficient). The concentrations calculated to exhibit 50 and 25 % light loss (EC50 and EC25) are tabulated in Appendix I as well as the expected light loss due to 50% sample concentration. The results of this study are reported as the percent sample concentration which results in a 50 percent reduction in light output at 30 minutes exposure time (30EC50). Reporting of EC50 values greater than 100% sample concentration may seem confusing at first. These numbers represent extrapolated values. Rather than list samples which did not cause 50 % light diminution as being non-toxic, the extrapolated values are given. Additionally, where samples exhibited a stimulatory influence on the light output of the Microtox test organisms, the EC50 is reported as a negative number. A large negative EC50 indicates that the sample was slightly stimulatory, while a small negative EC50 indicates greater stimulation.

Appendix II presents the results of chemical analysis of the elutriates tested for Microtox toxicity in this study. Metals results are compared to the 30EC50's by regression analysis, and the regression coefficients listed. Regression analyses were performed using both the linear concentration of metals detected and the log-concentration of metals. In general, greater correlation was found using the log-concentration of metal, although this was not true in all cases. The Interstitial 30EC50 values showed no correlation with any of the metals tested. The RO:DI H_2O 30EC50's showed some correlation with copper, nickel and total metal concentrations (r^2 = .60, .65, and .66 respectively). The 0.1 M HCl-KCl elutriate samples showed the greatest correlation with the metals analysis. The regression coefficient (r^2) for chromium, copper, lead, nickel, zinc and total metals were all above 0.5. The highest r^2 value was for copper (0.93). Neither the

RO:DI H_2O Anoxic samples nor the HCl-KCl anoxic samples showed significant correlation with the metals analysis data.

An intital study using sediment from Dodgeville Pond indicated that reproducibility of results was fairly good. Microtox tests were run on four interstitial water samples. Two of the samples were taken from the top 20 centimeters of sediment of one core, one from the bottom of the same core, and one from the top of a second core from the same sampling site. Taken together the samples had an average 30EC50 of 57.9% with a standard deviation of 5.4%. Interstitial water from the lower portion of the core had only slightly lower toxicity than for upper portions of the core.

Table 2 provides a summary of 30EC50 values expressed as percent concentration for each site. This information is also presented graphically for the nonpurged samples in figures 3-7. The results are arranged by site location starting at the headwaters.

Figure 3 shows the 30EC50 values for interstitial water. There is a slight trend towards increasing toxicity (lower EC50 values) downstream. However, two of the four sites which were sampled twice showed markedly different toxicities.

Figure 4 shows the 30EC50's for the RO:DI H_2O elutriate. This set of samples showed a more clearly defined trend towards increased toxicity downstream. Repeat samples also showed better correlation than the interstitial water samples.

The 0.1 M HCl-KCl elutriate samples (figure 5) showed both the most clearly defined trend towards increasing toxicity with sample location and the greatest correlation between repeat samples.

The results from the RO:DI H_2O anoxic tests (figure 6) show no clear trend relating toxicity to location. Most samples showed very low toxicity, and in all but one of twelve cases RO:DI H_2O anoxic samples showed less toxicity than their oxic counterparts.

The 0.1 M HCl-KCl anoxic samples (figure 7) did not show the clearly defined trend in toxicity levels that the oxic 0.1 M HCl-KCl samples did. Again in all but one of twelve cases the anoxic samples demonstrated less toxicity than their oxic counterparts. The correlation between repeat 0.1 M HCl-KCl anoxic samples was the same as that of the HCl-KCl oxic samples (r=.89).

Nitrogen purge tests were performed only for those samples which

had shown 30EC50's of less than or equal to 110% and were not performed for repeated sampling sites. In 30 out of 34 tests purged samples showed less toxicity than non-purged samples. Overall there was weak correlation between purged and non-purged samples (r=.46). The best correlation for any one elutriate method was for RO:DI H_2O oxic samples (r=.95). HCl-KCl oxic samples showed the weakest correlation between purged and non-purged samples (r=-.38).

Dry weight and sediment specific gravities are presented in Table 3.

TABLE 2 - 30EC50 VALUES (Percent Sample Concentration)

	NON-PURGED					NITROGEN-PURGED				
	INTERSTITIAL	RO:DI H_2O	0.1 M HCl-KCl	RO:DI H_2O ANOXIC	0.1 M HCl-KCl ANOXIC	INTERSTITIAL	RO:DI H_2O	0.1 M HCl-KCl	RO:DI H_2O ANOXIC	0.1 M HCl-KCl ANOXIC
FULLER (7-6-84)	108	2210	133	264	148	157	---	---	---	---
WETHERELLS (8-7-84)	87	76	33	173	59	161	132	40	---	281
FALLS (7-6-84)	204	880	40	232	41	---	---	28	---	18
FARMERS (8-7-84)	35	36	6	43	58	47	47	122	95	160
FARMERS (10-4-84)	70	32	16	731	81	---	---	---	---	---
MECHANICS (7-6-84)	24	81	25	75	10	125	109	191	43	22
DODGEVILLE (6-19-84)	56	44	3	---	---	---	---	---	---	---
DODGEVILLE (8-7-84)	35	23	9	26	17	63	22	147	57	61
HEBRONVILLE (8-7-84)	56	11	6	202	110	170	23	92	---	1036
HEBRONVILLE (10-4-84)	1291	78	19	221	92	---	---	---	---	---
RESERVATION (8-28-84)	33	37	14	438	31	60	40	114	---	98
CENTRAL (8-28-84)	33	18	4	350	21	76	41	65	---	122
CENTRAL (10-4-84)	202	41	7	1595	23	---	---	---	---	---

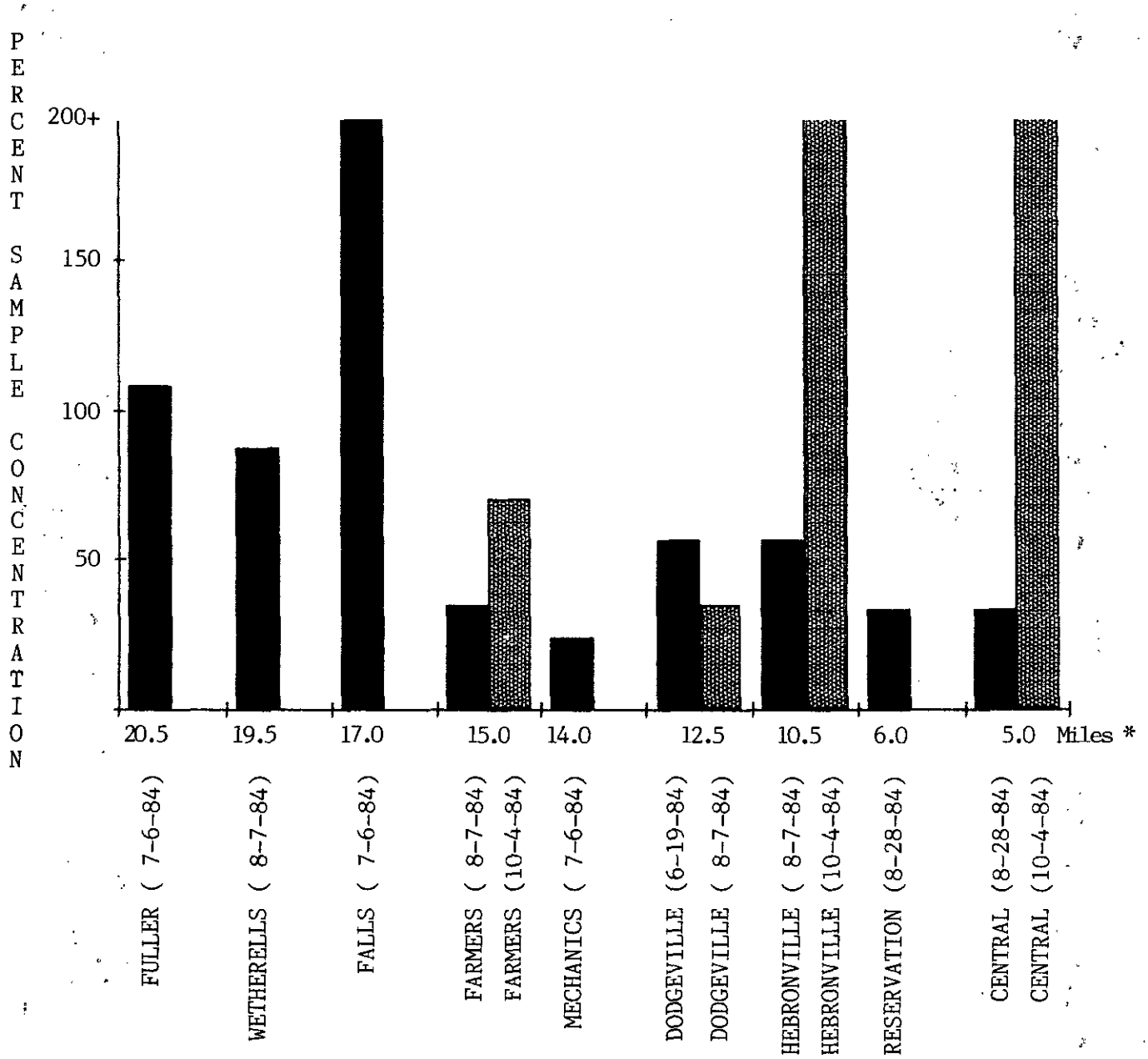

FIGURE 3 - INTERSTITIAL WATER 30EC50's (Percent Sample Concentration)
* miles from mouth

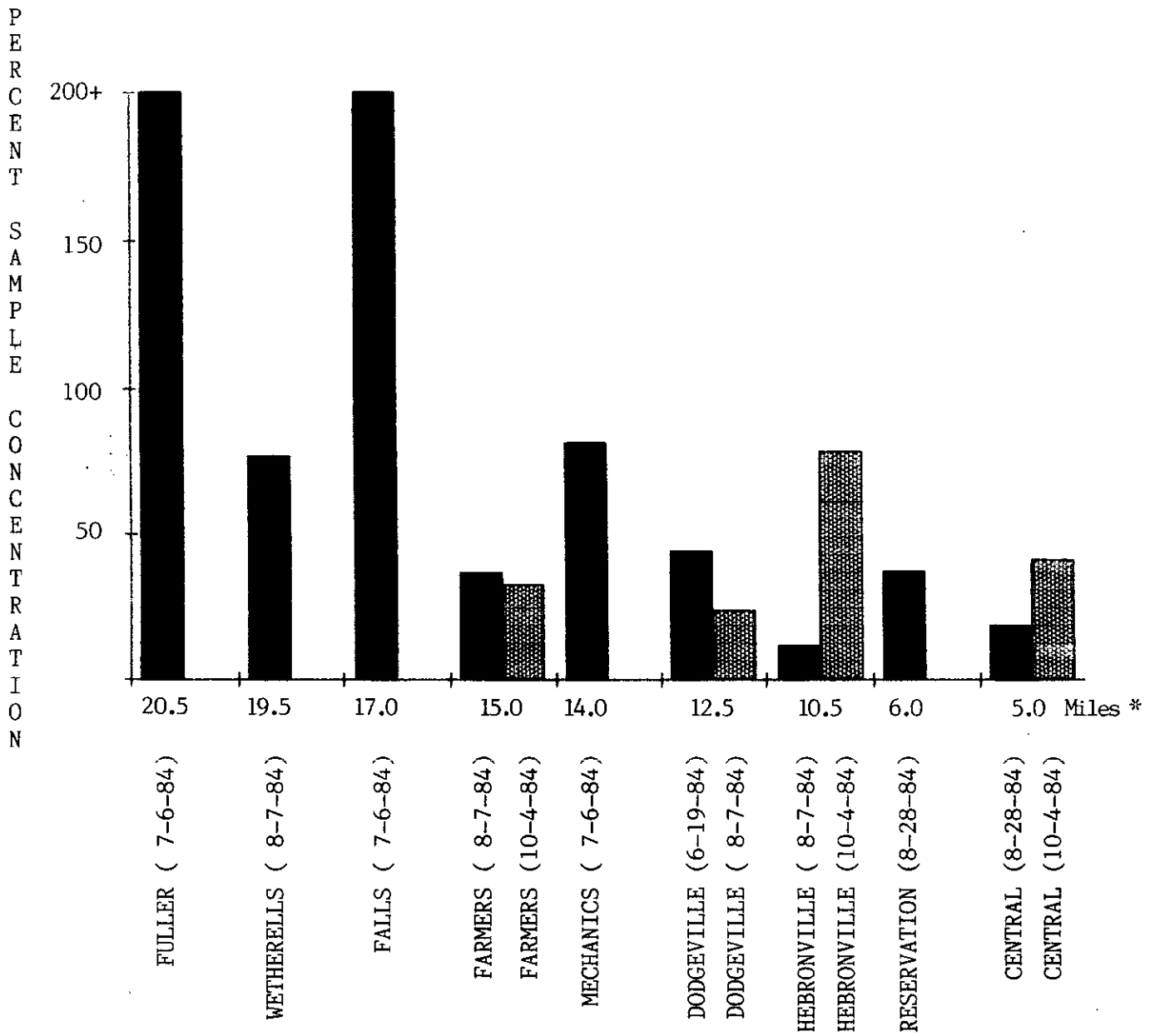

FIGURE 4 - RO:DI H_2O 30EC50's (Percent Sample Concentration)
* miles from mouth

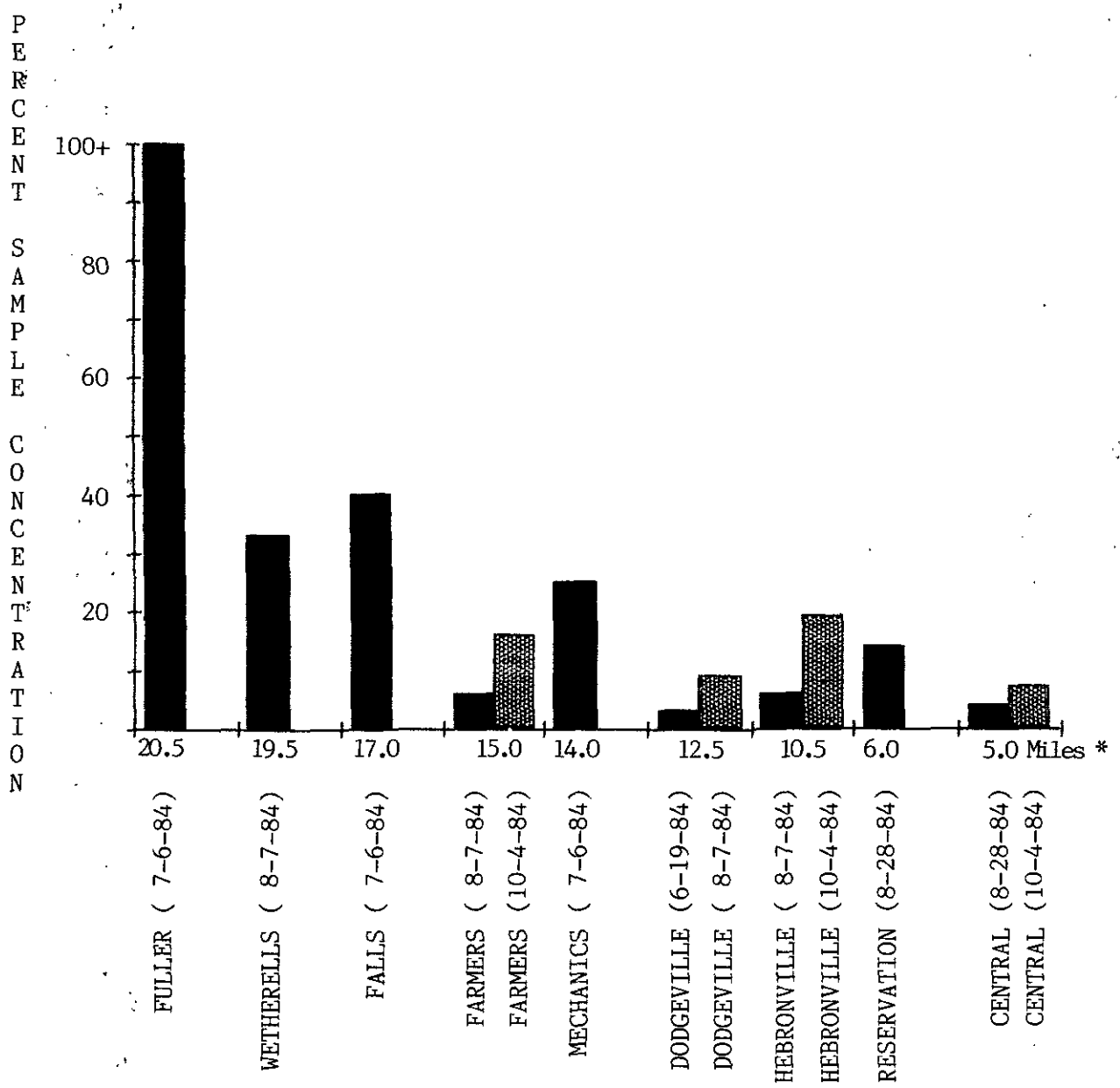

FIGURE 5 - 0.1 M HC1-KC1 30EC50's (Percent Sample Concentration)

* miles from mouth

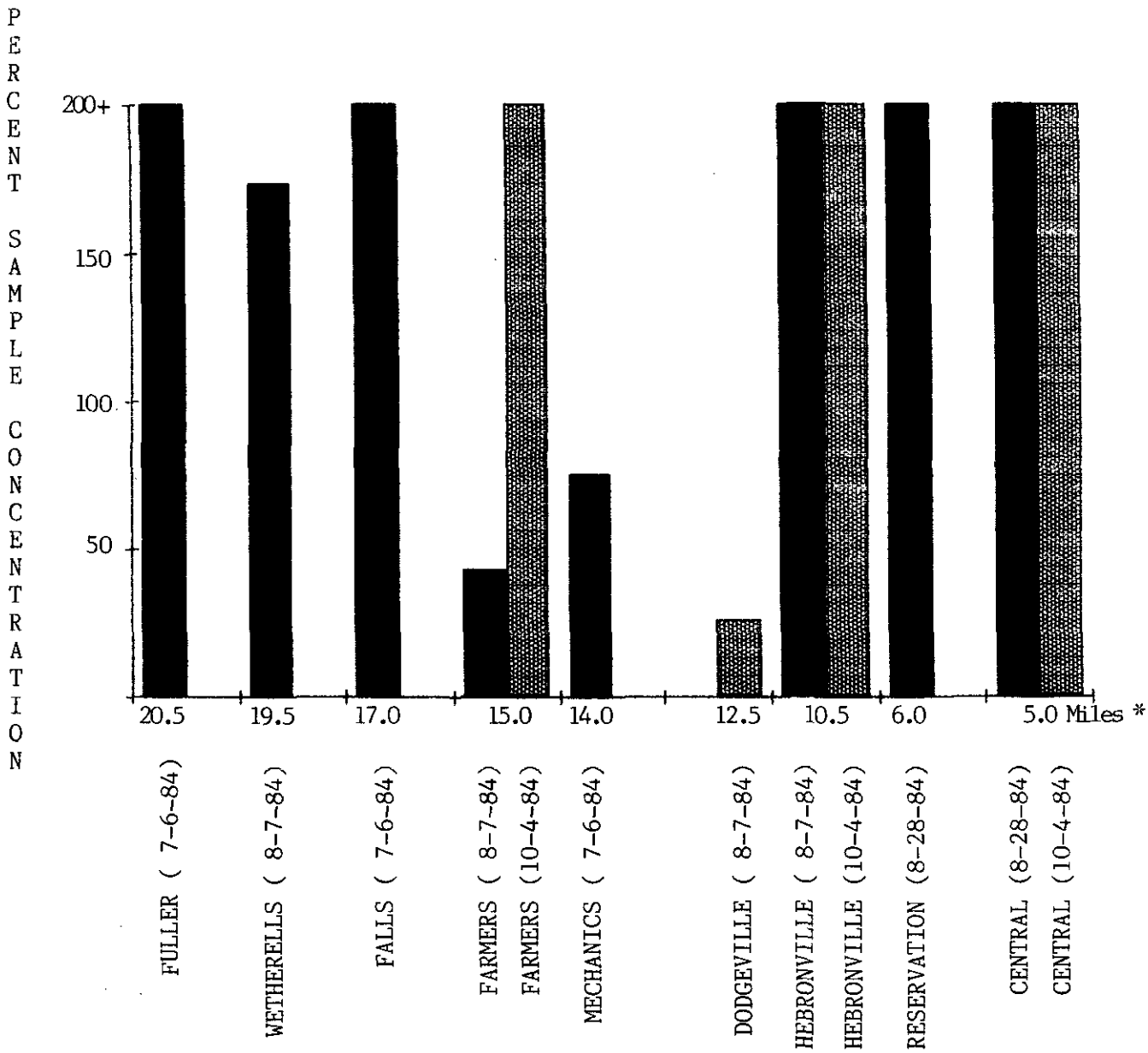

FIGURE 6 - RO:DI H_2O (Anoxic) 30EC50's (Percent Sample Concentration)

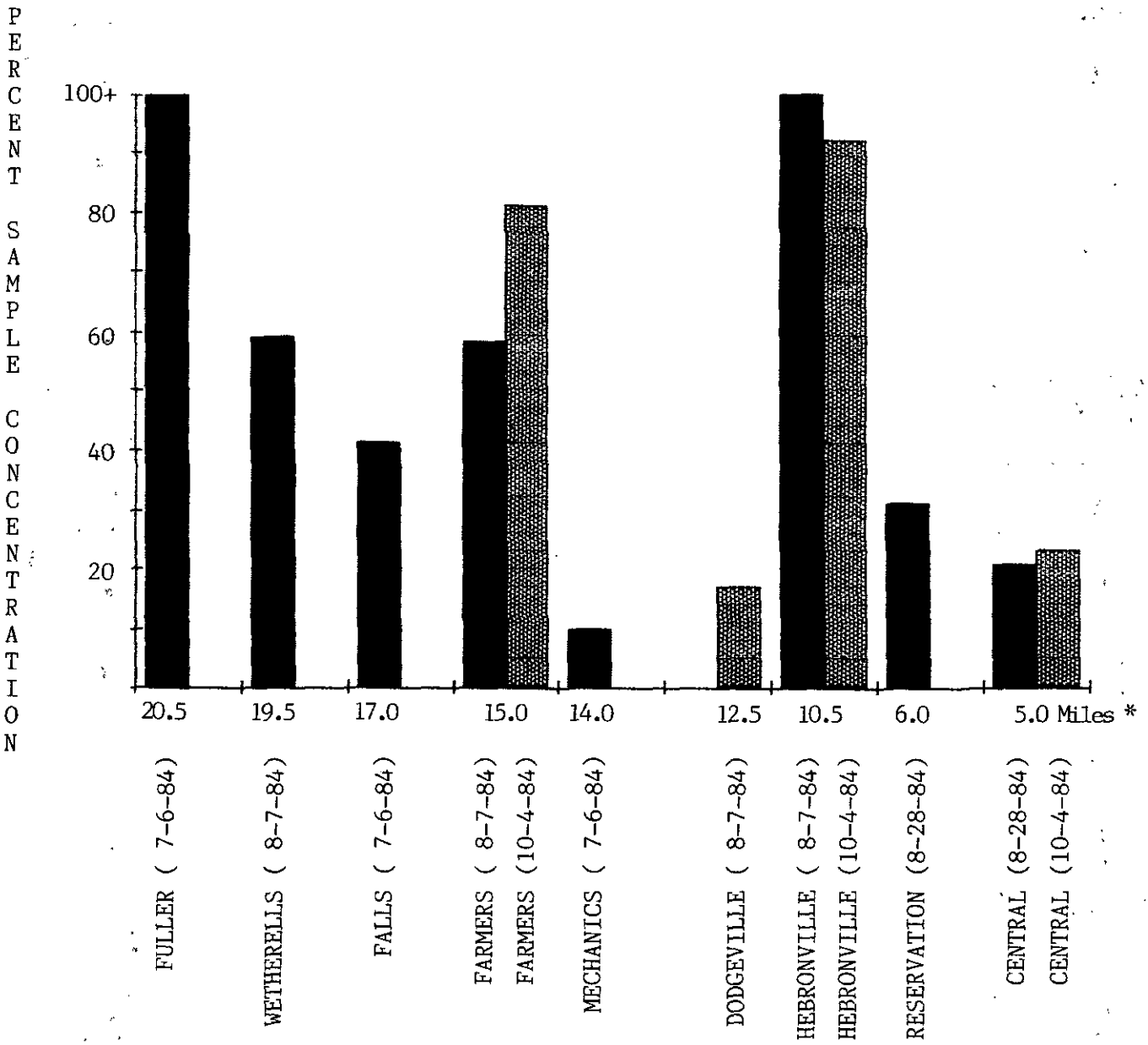

FIGURE 7 - 0.1 M HCl-KCl (Anoxic) 30EC50's (Percent Sample Concentration)

* mile from mouth

TABLE 3 - SEDIMENT DRY WEIGHT AND SPECIFIC GRAVITY

IMPOUNDMENT	DRY WT./ WET WT (%)	S.G.
FULLER (7-6-84)	16	1.05
WETHERELLS (8-7-84)	11	1.07
FALLS (7-6-84)	71	1.65
FARMERS (8-7-84)	19	1.13
FARMERS (10-4-84)	21	1.11
MECHANICS (7-6-84)	15	1.04
DODGEVILLE (6-19-84)	32	----
DODGEVILLE (8-7-84)	9	1.07
HEBRONVILLE (8-7-84)	28	1.19
HEBRONVILLE (10-4-84)	22	1.15
RESERVATION (8-28-84)	22	1.13
CENTRAL (8-28-84)	12	1.05
CENTRAL (10-4-84)	14	1.07

IX. Discussion

The Microtox analyzer appears to be a promising new tool for the assesment of sediment toxicity. The pattern of Microtox toxicity found in this study is consistant with expectations based on what is known about the distribution of effluent discharges into the river system, with greater toxicity in the downstream reaches. Sediment toxicity was demonstrated for at least some sites for each of the elutriation methods used. The most promising method studied was the 0.1 M HCl-KCl elutriation method. This method produced the most clearly defined relationship between sampling site and toxicity. For sites sampled twice, this method also demonstrated the greatest intersample correlation of toxicity results. A fairly broad range of toxicity values for the 0.1 M HCl-KCl elutriates was observed. This indicates that the test is likely to be able to distinguish between contaminated and non-contaminated samples, which is a necessary characteristic of any good toxicity testing method.

The results from the anoxic studies are interesting, but not necessarily easy to interpret. Anoxic conditions clearly had an effect on sediment toxicity as measured by Microtox. Theoretical reasons for why toxicity may be lower under anoxic conditions are beyond the scope of this report. However, this does point to the fact that the oxic or anoxic conditions of a sediment or of a proposed sediment disposal site should not be disregarded. The oxic state of a sediment sample may significantly alter either the availability or the toxicity of some toxicants. The techniques used in this investigation, however, cannot determine which of these two may be responsible for the observed decrease in toxicity. Because sediments contain a wide variety of microorganisms, aerobic and anaerobic, it may be too simplistic to assume that changes in toxicity can be attributed solely to changes in the chemical oxidation-reduction potential. Biological variables should also be considered as a possible factors contributing to the observed decrease in toxicity under anoxic conditions.

The nitrogen purge method used in this investigation was intended

to drive off ammonia in an effort to estimate the contribution of ammonia to the total toxicity. Theoretically an increase in pH should shift ammonium, NH_4^+, to ammonia, NH_3, which can then be purged by bubbling nitrogen gas through the sample. However, one of the side effects of this method was the formation of a chemical precipitate (presumably metal oxides) upon addition of the NaOH. This was especially noticable in the 0.1 M HCl-KCl elutriate samples. The solid phase removed from solution by precipitation cannot be included in the portion tested by the Microtox analyser. Instead it was allowed to settle out and the overlying supernatant used for testing. Because ammonia is clearly not the only major chemical affected, this procedure is not considered suitable for the intended purpose. Thus the observed changes in toxicity due to this procedure should not be attributed solely to ammonia.

A similar percipitation problem was observed when the pH of the 0.1 M HCl-KCl elutriate samples (non-purged) was adjusted from 2.0 to 7.0 prior to Microtox testing. The more highly toxic samples in this group appeared to form more precipitate than did the less toxic samples (quantitative measurements were not performed). It is not known whether the highest detectable toxicity is limited by maximum solubilities of constituents at the test pH. Highly toxic samples may conceivably demonstrate even greater toxicity if pH adjustments are made after sample dilutions have been performed, rather than before. This is an area which warrants further investigation.

Several other areas also require further investigation. Confirming studies should be performed comparing Microtox results to traditional sediment bioassays and to sediment chemical analysis. Statistical studies are needed to evaluate the effect of sampling error and to determine test reproducibility. If possible, tests comparing sediments contaminated with different types of toxicants should be performed. Of special interest would be tests comparing sediments contaminated with synthetic organics to sediments contaminated with heavy metals. The procedures developed in this investigation can not be assumed to work equally well for both. A final area for investigation and debate, to be pursued only after considerable further research, would be the establishment of a Microtox sediment toxicity standard, or a numerical value of a 30 minute EC50 defining allowable toxicity. Only

after all of these areas have been addressed can the Microtox toxicity analyser be fully evaluated as a method for sediment toxicity testing.

X. Summary and Conclusions

The pattern of sediment toxicity observed for sites along the Ten Mile River suggests that sediment contamination is progressively more severe in the downstream reaches. The results of this study indicate that the Microtox toxicity analyser may be useful as a new tool for evaluating sediment toxicity. The 0.1 M pH 2.0 HCl-KCl elutriate method seemed to provide the best results of the methods used. However, confirming studies are needed before firm conclusions can be drawn.

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APPENDIX I : MICROTOX DATA

FULLER POND, PLAINVILLE, MA. (7-6-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.1	6.3	2.0	6.2	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%
Highest L.L.*	23.9	0.7	16.2	9.7	16.7
Slope	.2853	-.0385	.6345	.1868	.3908
r **	.9554	-.1722	.9818	.9435	.9478
5EC50	167.0	-1313.1	97.66	268.9	128.4
5EC25	79.4	-664.0	58.3	135.0	64.4
50% Sample	16.6	-2.5	19.8	9.1	19.4
Slope	.4262	.0094	.5828	.1719	.3965
r	.9799	.0500	.9498	.8919	.9544
15EC50	118.3	5518.9	110.0	294.0	130.8
15EC25	59.63	2869.1	67.1	148.5	67.7
50% Sample	20.9	-1.6	15.0	8.1	18.0
Slope	.4585	.0241	.4396	.1869	.3407
r	.9881	.1082	.9468	.9052	.9263
30EC50	108.0	2210.9	133.0	264.0	147.9
30EC25	53.5	1173.2	76.2	130.3	74.5
50% Sample	23.4	-2.1	13.5	10.0	16.7

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

FULLER POND, PLAINVILLE, MA. (7-6-84)

N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.1				
Conc. Tested	11.2,22.5,30,45%				
Highest L.L.*	12.7				
Slope	.2267				
r **	.8772				
5EC50	230.1				
5EC25	119.8				
50% Sample	9.2				
Slope	.2280				
r	.8412				
15EC50	224.8				
15EC25	115.1				
50% Sample	10.2				
Slope	.3332				
r	.9090				
30EC50	156.9				
30EC25	81.9				
50% Sample	14.4				

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

WETHERELLS POND, PLAINVILLE, MA. (8-7-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	7.2	5.8	2.0	6.4	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%	11.2,22.5,30,45%	11.2,22.5,30,45%
Highest L.L.*	29.2	27.0	29.7	11.8	41.6
Slope	.5322	.1713	1.2457	.1910	1.1512
r **	.9817	.6904	.9470	.7645	.9987
5EC50	91.1	315.3	47.0	271.8	54.4
5EC25	44.1	169.4	26.9	140.9	32.7
50% Sample	28.1	4.6	53.7	7.6	44.9
Slope	.6552	.4325	1.5422	.2381	1.2378
r	.9918	.9385	.9621	.9111	.9804
15EC50	76.9	129.5	39.5	224.5	51.6
15EC25	38.8	71.7	23.3	119.5	31.4
50% Sample	32.4	15.6	66.1	8.5	48.0
Slope	.5343	.7506	1.8483	.2983	.9910
r	.9923	.9866	.9665	.9299	.9747
30EC50	87.1	76.0	32.9	173.4	59.4
30EC25	40.3	42.7	19.4	89.6	34.1
50% Sample	30.2	30.5	81.5	13.2	40.7

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

WETHERELLS POND, PLAINVILLE, MA. (8-7-84) N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	7.2	5.8	2.0		2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%		11.2,22.5,30,45%
Highest L.L.*	18.6	10.5	56.6		11.0
Slope	.3698	.1983	.8596		.1962
r **	.9546	.9005	.9946		.8820
5EC50	129.2	285.6	66.2		282.6
5EC25	61.6	159.5	37.1		155.1
50% Sample	20.7	3.3	36.1		4.4
Slope	.3242	.3092	1.1326		.2456
r	.9641	.8953	.9973		.8733
15EC50	143.2	195.6	51.2		218.4
15EC25	66.1	114.7	29.2		116.7
50% Sample	19.8	5.0	48.6		8.6
Slope	.3014	.4791	1.3053		.1624
r	.9657	.9317	.9958		.7456
30EC50	160.8	131.6	39.7		281.2
30EC25	77.9	79.4	20.5		34.8
50% Sample	16.6	10.9	63.5		12.5

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

FALLS POND, NORTH ATTLEBOROUGH, MA. (7-6-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	5.9	6.4	2.0	6.4	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%
Highest L.L.*	20.5	3.7	73.1	8.0	74.9
Slope	.1785	-.0246	.7854	.1872	.7577
r **	.7892	-.1017	.9926	.9551	.9888
5EC50	282.5	-1996.8	78.1	271.4	70.9
5EC25	142.4	-979.0	46.2	137.8	37.9
50% Sample	8.5	-0.3	27.9	8.6	34.2
Slope	.1825	-.0447	1.4423	.2082	.9464
r	.7145	-.2158	.9846	.9895	.9929
15EC50	265.9	-1087.2	52.0	249.9	59.2
15EC25	128.9	-527.6	34.7	129.9	32.8
50% Sample	10.6	-0.8	47.1	8.4	41.3
Slope	.2287	-.0567	1.9827	.2286	1.4650
r	.8246	-.2347	.9809	.9672	.9708
30EC50	204.1	-878.9	40.4	231.9	40.7
30EC25	94.8	-437.9	27.8	122.5	23.7
50% Sample	14.8	-2.7	69.0	8.4	63.6

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

FALLS POND, NORTH ATTLEBOROUGH, MA. (7-6-84) N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH			2.0		2.0
Conc. Tested			11.2,22.5,30,45%		11.2,22.5,30,45%
Highest L.L.*			70.9		95.8
Slope			.9857		.9484
r **			.9661		.9976
5EC50			51.7		56.1
5EC25			26.3		29.7
50% Sample			48.4		44.2
Slope			1.2749		1.9854
r			.9699		.9903
15EC50			39.3		28.4
15EC25			16.7		15.8
50% Sample			63.7		92.9
Slope			1.4338		1.9354
r			.9455		.9246
30EC50			27.8		17.5
30EC25			10.4		4.6
50% Sample			81.9		112.8

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

FARMERS POND, ATTLEBORO, MA. (8-7-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	7.0	5.0	2.0	6.5	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	1.4,2.8,5.6,11.2%	11.2,22.5,30,45%	5.6,11.2,22.5,30%
Highest L.L.*	75.4	60.0	98.9	71.9	22.8
Slope	1.0569	.2770	3.9258	1.3099	1.0140
r **	.9469	.8816	.9878	.9884	.9668
5EC50	17.8	175.8	15.0	29.0	59.5
5EC25	-5.9	85.5	8.6	9.9	34.8
50% Sample	84.1	15.2	187.5	77.5	40.4
Slope	1.1229	.7259	8.5486	1.4493	.9298
r	.9606	.9329	.9957	.9943	.9308
15EC50	19.9	64.3	7.6	29.0	62.1
15EC25	-2.4	29.8	4.8	11.8	35.2
50% Sample	83.9	39.6	412.3	80.4	38.8
Slope	1.0196	1.1770	9.9122	1.0897	.9274
r	.9872	.9353	.9788	.9971	.9671
30EC50	34.8	35.8	5.9	43.1	58.1
30EC25	10.3	14.6	3.4	20.2	31.1
50% Sample	65.5	66.7	487.2	57.5	42.5

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

FARMERS POND, ATTLEBORO, MA. (8-7-84) N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	7.0	5.0	2.0	6.5	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%	11.2,22.5,30,45%	11.2,22.5,30,45%
Highest L.L.*	55.1	45.8	5.2	24.2	11.1
Slope	.9382	.2901	.3447	.3973	.3690
r **	.9614	.9755	.7573	.9819	.9743
5EC50	45.3	183.4	153.9	126.8	151.6
5EC25	18.7	97.2	81.4	63.9	83.6
50% Sample	54.4	11.3	14.2	19.5	12.5
Slope	1.0577	.7173	.3594	.5376	.3677
r	.9793	.9955	.7968	.9929	.9667
15EC50	39.7	78.3	147.7	93.1	153.1
15EC25	16.0	43.4	78.1	46.6	85.1
50% Sample	60.9	29.7	14.9	26.8	12.1
Slope	.9068	1.2046	.4459	.5294	.3428
r	.9868	.9853	.7800	.9920	.9660
30EC50	46.8	47.4	121.7	95.1	160.2
30EC25	19.3	26.7	65.7	47.9	87.3
50% Sample	52.9	53.1	18.0	26.1	12.2

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

FARMERS POND, ATTLEBORO, MA. (10-4-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.0	5.9	2.0	6.4	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	1.4,2.8,5.6,11.2%	11.2,22.5,30,45%	5.6,11.2,22.5,30%
Highest L.L.*	38.1	66.6	29.4	5.2	16.2
Slope	.6665	.3585	1.6951	-.0165	.4369
r **	.9480	.9432	.9549	-.1022	.9532
5EC50	68.9	148.0	34.1	-3067.2	130.9
5EC25	31.4	78.3	19.4	-1554.8	73.7
50% Sample	37.4	14.8	76.9	-1.5	14.6
Slope	.7325	1.0448	3.0549	.0190	.5927
r	.9602	.9754	.9716	.0938	.9486
15EC50	61.4	52.5	21.4	2592.2	95.9
15EC25	27.3	28.6	13.2	1278.2	53.7
50% Sample	41.6	47.4	137.3	1.6	22.8
Slope	.6714	1.6634	4.2327	.0682	.5829
r	.9506	.9604	.9804	.2322	.6828
30EC50	70.6	32.0	16.5	731.3	80.8
30EC25	33.4	16.9	10.6	365.8	37.9
50% Sample	36.2	80.0	191.9	3.5	32.0

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

MECHANICS POND, ATTLEBORO, MA. (7-6-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.1	6.0	2.0	5.8	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	5.6,11.2,22.5,30%	11.2,22.5,30,45%	5.6,11.2,22.5,30%
Highest L.L.*	77.1	28.7	73.2	31.2	97.8
Slope	.8644	.5736	.9826	.1526	2.7700
r **	.8555	.9775	.9764	.9060	.9742
5EC50	11.1	92.1	55.7	340.5	25.6
5EC25	-17.8	48.5	30.2	176.7	16.5
50% Sample	83.6	25.9	44.4	5.7	117.7
Slope	.9312	.7336	1.6521	.4947	5.2971
r	.8690	.9898	.9721	.9621	.9966
15EC50	10.8	74.8	34.7	116.1	14.4
15EC25	-16.0	40.7	19.6	65.6	9.6
50% Sample	86.5	31.8	75.3	17.3	238.8
Slope	.9173	.7011	2.1876	.8286	7.8844
r	.9414	.9936	.9738	.9799	.9956
30EC50	23.5	81.1	25.2	75.2	10.1
30EC25	-3.7	45.4	13.8	45.2	6.9
50% Sample	74.3	28.2	104.3	29.1	364.5

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

MECHANICS POND, ATTLEBORO, MA. (7-6-84)

N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.1	6.0	2.0	5.8	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	5.6,11.2,22.5,30%	11.2,22.5,30,45%	5.6,11.2,22.5,30
Highest L.L.*	15.2	11.6	15.6	52.0	74.4
Slope	.3567	.4633	.4003	.6410	.9880
r **	.9114	.9842	.9455	.9846	.9982
5EC50	150.8	126.8	131.3	82.0	60.6
5EC25	80.7	72.7	68.8	43.0	35.2
50% Sample	14.0	14.4	17.5	29.5	39.6
Slope	.4444	.5407	.4009	.9587	2.0317
r	.9717	.9832	.9146	.9922	.9945
15EC50	125.5	120.0	130.3	58.6	31.1
15EC25	69.2	73.8	67.9	16.9	18.8
50% Sample	16.4	12.2	17.8	41.7	88.5
Slope	.4570	.6393	.2702	1.3825	3.1890
r	.9611	.9797	.9123	.9873	.9908
30EC50	125.4	108.7	191.2	42.5	21.6
30EC25	70.6	69.6	98.7	24.5	13.8
50% Sample	15.6	12.5	11.8	60.3	140.8

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

DODGEVILLE POND, ATTLEBORO,MA. (6-19-84)

Microtox Sample	Interstitial Water Core 1 (top) (first run)	Interstitial Water Core 1 (top) (second run)	Interstitial Water Core 2 (top)	Interstitial Water Core 1 (bottom)	Interstitial Water Core 1 (top) N_2 - CO_2 Purged
Initial pH	6.0	6.0	6.0	6.0	6.0
Conc. Tested	2.5,7.5,22.5,45%	2.5,7.5,22.5,45%	2.5,7.5,22.5,45%	2.5,7.5,22.5,45%	2.5,7.5,22.5,45%
Highest L.L.*	44.4	45.6	42.9	36.6	22.6
Slope	.9373	.8484	.7909	.5330	.3641
r **	.9976	.9873	.9903	.9960	.9821
5EC50	50.4	56.4	59.7	94.7	136.8
5EC25	23.8	26.9	28.1	47.8	68.2
50% Sample	7.8	9.2	9.1	19.7	27.0
Slope	.9325 +	.9566	.9009	.7238	.4274
r	.9935	.9858	.9911	.9938	.9521
15EC50	49.8	47.5	51.4	69.9	111.0
15EC25	23.0	21.4	23.6	35.4	52.5
50% Sample	6.9	5.7	7.0	14.6	17.4
Slope	.7705 ++	.8856	.8216	.7806	.4331
r	.9950	.9820	.9928	.9935	.9585
30EC50	61.6	50.4	57.6	62.0	108.6
30EC25	29.1	22.1	27.1	30.0	50.9
50% Sample	9.7	5.2	8.9	10.8	16.2

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

\+ 20 min. data ++ 35 min data

DODGEVILLE POND, ATTLEBORO, MA. (6-19-84)

Microtox Sample	RO:DI H_2O Elutriate	RO:DI H_2O Elutriate N_2 - CO_2 Purged	0.1 M HCl-KCl pH 2.0 Elutriate (first run)	0.1 M HCl-KCl pH 2.0 Elutriate (second run)	0.1 M HCl-KCl pH 2.0 Elutriate N_2 - CO_2 Purged
Initial pH	5.5	5.5	2.0	2.0	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%	1.4,5.6,11.2,22.5%	2.5,7.5,22.5,45%
Highest L.L.*	55.4	55.8	100.0	100.0	79.1
Slope	.7811	.2773	1.8330	4.0087	.6571
r **	.9840	.9384	.8743	.9781	.9105
5EC50	65.3	192.9	18.8	11.6	85.0
5EC25	33.3	102.7	5.2	5.3	47.0
50% Sample	38.1	10.4	107.1	204.1	27.0
Slope	1.0490	.6484	3.2089	9.7587	1.0160
r	.9789	.9777	.8614	.9240	.9539
15EC50	50.5	85.4	7.1	4.7	57.2
15EC25	26.5	46.8	-.7	2.1	32.6
50% Sample	49.4	27.0	187.6	492.2	42.7
Slope	1.2224	1.0840		19.9763	1.4922
r	.9829	.9936		.9990	.9665
30EC50	44.1	52.8		3.2	37.5
30EC25	23.6	29.8		1.9	20.8
50% Sample	57.1	47.0		890.4	68.6

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

DODGEVILLE POND, ATTLEBORO, MA. (8-7-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.8	4.7	2.0	6.5	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	5.6,11.2,22.5,30%	5.6,11.2,22.5,30%	1.4,2.8,5.6,11.2%
Highest L.L.*	66.1	67.8	98.6	70.4	31.6
Slope	.9966	.3629	2.4440	1.6574	3.7222
r **	.9124	.9614	.9821	.9515	.9935
5EC50	28.7	136.5	22.0	15.6	16.3
5EC25	3.6	67.6	11.8	0.6	9.6
50% Sample	71.3	18.6	118.3	107.0	175.4
Slope	1.0000	.9080	2.9989	1.6684	2.9313
r	.9522	.9495	.9651	.9573	.9893
15EC50	27.0	44.7	14.8	16.6	18.8
15EC25	2.0	17.1	6.5	1.6	10.3
50% Sample	73.0	54.8	155.5	105.8	141.4
Slope	.7117	.9991	3.6419	1.4961	3.0987
r	.9720	.9313	.9716	.9774	.9928
30EC50	35.2	23.2	9.1	26.4	17.2
30EC25	0.1	-1.8	2.2	9.7	9.2
50% Sample	60.6	76.8	199.0	85.3	151.5

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

DODGEVILLE POND, ATTLEBORO, MA. (8-7-84) N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.8	4.7	2.0	6.5	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	5.6,11.2,22.5,30%	5.6,11.2,22.5,30,%	5.6,11.2,22.5,30%
Highest L.L.*	33.9	75.5	10.8	31.2	56.4
Slope	.6191	.4482	.5582	.9912	.2350
r **	.9624	.9807	.9589	.9790	.9731
5EC50	85.8	109.8	100.4	50.8	26.2
5EC25	45.4	54.1	55.7	25.6	15.6
50% Sample	27.8	23.2	21.8	49.2	105.9
Slope	.7643	.9952	.5073	1.0674	1.8706
r	.9707	.9510	.9554	.9868	.9829
15EC50	70.2	43.2	106.8	46.8	31.7
15EC25	37.5	18.1	57.5	23.4	18.4
50% Sample	34.6	56.7	21.2	53.4	84.2
Slope	.7773	1.2688	.3352	.9035	.9139
r	.9441	.9289	.9373	.9829	.9847
30EC50	62.8	21.6	146.5	57.2	60.6
30EC25	30.7	1.9	71.9	29.5	33.3
50% Sample	40.0	86.0	17.7	43.5	40.3

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

HEBRONVILLE POND, ATTLEBORO, MA. (8-7-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.3	4.9	2.0	6.5	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%	11.2,22.5,30,45%	11.2,22.5,30,45%
Highest L.L.*	58.6	83.6	99.6	12.1	21.2
Slope	1.2002	.3888	3.4999	.1869	.7187
r **	.9288	.9641	.9775	.9401	.9836
5EC50	34.4	139.0	15.8	257.1	83.3
5EC25	13.6	74.7	8.7	123.3	48.5
50% Sample	68.7	15.4	169.6	11.3	26.1
Slope	1.2701	1.1563	4.1807	.1973	.5985
r	.9468	.9716	.8966	.9191	.9831
15EC50	36.1	38.1	10.0	239.5	96.8
15EC25	16.4	16.4	4.0	112.7	55.0
50% Sample	67.6	63.8	217.4	12.6	22.0
Slope	.8986	1.0806	8.6837	.2412	.5160
r	.9655	.9063	.9747	.9637	.9637
30EC50	56.4	10.6	6.5	201.8	110.1
30EC25	28.5	-12.5	3.6	98.2	61.7
50% Sample	44.3	92.6	427.8	13.4	19.0

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

HEBRONVILLE POND, ATTLEBORO, MA. (8-7-84) N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.3	4.9	2.0		2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	5.6,11.2,22.5,30%		11.2,22.5,30,45%
Highest L.L.*	25.8	77.2	12.2		0.4
Slope	.5857	.5660	.3609		.1662
r **	.9887	.9870	.9483		.6910
5EC50	87.0	101.3	157.2		340.9
5EC25	44.4	57.1	87.9		190.5
50% Sample	28.3	21.0	11.3		1.6
Slope	.5623	1.2193	.4686		.0631
r	.9861	.9550	.9692		.4672
15EC50	92.4	42.9	122.4		918.6
15EC25	48.0	22.4	69.0		522.7
50% Sample	26.1	58.7	16.1		-4.8
Slope	.3054	1.4912	.6170		-.0515
r	.9573	.9117	.9773		-.3266
30EC50	170.7	23.0	91.6		-1036.8
30EC25	88.6	6.3	51.1		-551.5
50% Sample	13.2	90.2	24.3		-6.0

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

HEBRONVILLE POND, ATTLEBORO, MA. (10-4-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	5.9	5.9	2.0	6.0	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	1.4,2.8,5.6,11.2%	11.2,22.5,30,45%	5.6,11.2,22.5,30%
Highest L.L.*	2.5	25.2	26.3	8.4	17.9
Slope	.0585	.1325	.8217	.0763	.3912
r **	.3886	.7987	.9638	.7252	.8298
5EC50	900.6	371.7	65.0	683.2	139.2
5EC25	473.1	183.0	34.6	355.4	75.3
50% Sample	0.3	7.4	37.7	1.7	15.1
Slope	.0659	.4195	1.8041	.1336	.5548
r	.4311	.9749	.9809	.6955	.8968
15EC50	799.2	133.2	32.2	394.8	100.0
15EC25	419.7	73.6	18.3	207.7	55.0
50% Sample	0.6	15.1	82.2	3.9	22.2
Slope	.0381	.7906	3.1321	.2338	.5777
r	.1852	.9825	.9898	.8763	.8269
30EC50	1291.3	77.9	19.0	220.6	91.6
30EC25	634.3	46.3	11.0	113.7	48.3
50% Sample	2.8	27.9	147.1	10.1	26.0

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

RESERVATION POND, EAST PROVIDENCE, RI. (8-28-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	7.2	5.7	2.0	6.4	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%
Highest L.L.*	70.5	59.2	89.3	5.4	33.8
Slope	.8323	.3532	2.8310	.0985	1.0117
r **	.8818	.9797	.9923	.8198	.9879
5EC50	18.1	158.2	24.6	503.3	57.6
5EC25	-11.9	87.4	15.7	249.4	32.9
50% Sample	76.6	11.8	122.0	5.4	42.3
Slope	.9028	.8647	4.5492	.0649	1.4419
r	.8601	.9880	.9870	.6293	.9871
15EC50	17.2	65.5	17.0	729.2	42.3
15EC25	-10.4	36.6	11.5	343.4	25.0
50% Sample	79.6	36.6	200.1	6.0	61.1
Slope	.9254	1.5247	5.3241	.1170	1.9112
r	.9281	.9788	.9771	.8490	.9821
30EC50	33.0	36.8	14.4	437.8	31.3
30EC25	6.0	20.4	9.7	210.1	18.2
50% Sample	65.8	70.2	239.8	6.3	85.8

**Highest Percent Light Loss ** Pearson product-moment correlation coefficient

RESERVATION POND, EAST PROVIDENCE, RI. (8-28-84)

N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	7.2	5.7	2.0		2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	11.2,22.5,30,45%		11.2,22.5,30,45%
Highest L.L.*	51.0	55.0	11.7		19.0
Slope	.9156	.4087	.4616		.3853
r **	.9940	.9734	.9684		.9611
5EC50	45.6	127.6	137.0		148.4
5EC25	18.3	66.4	82.9		83.6
50% Sample	54.1	18.3	9.8		12.1
Slope	.9866	.7794	.5339		.4238
r	.9875	.9781	.9690		.8906
15EC50	43.9	63.0	122.2		131.7
15EC25	18.6	31.0	75.4		72.7
50% Sample	56.0	39.8	11.4		15.4
Slope	.7380	1.2122	.5402		.5687
r	.9980	.9727	.9342		.9590
30EC50	60.2	39.5	114.2		97.6
30EC25	26.3	18.9	67.9		53.6
50% Sample	42.5	62.7	15.3		23.0

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

CENTRAL POND, PAWTUCKET, RI. (8-28-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.8	5.4	2.0	6.4	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%	11.2,22.5,30,45%	2.8,5.6,11.5,22.5%
Highest L.L.*	74.2	74.4	100.0	9.6	55.0
Slope	.9375	.3214	3.7541	.1517	.7383
r **	.8898	.9893	.9879	.8117	.9658
5EC50	15.1	156.7	14.4	328.9	76.8
5EC25	-11.6	78.9	7.8	164.1	43.0
50% Sample	82.8	15.7	183.5	7.7	30.2
Slope	1.0233	.8953	8.4551	.2746	2.0579
r	.8887	.9839	.9837	.9008	.9869
15EC50	16.9	43.8	6.6	192.6	30.2
15EC25	-7.5	15.8	3.6	101.6	18.1
50% Sample	83.8	55.6	417.2	10.8	90.7
Slope	1.0986	1.0037	7.0047	.1411	2.7321
r	.9784	.9500	.8744	.8633	.9825
30EC50	32.9	18.0	3.9	350.1	20.7
30EC25	10.1	-6.9	0.5	173.0	11.5
50% Sample	68.8	82.1	391.5	7.6	130.1

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

CENTRAL POND, PAWTUCKET, RI. (8-28-84)

N_2 - PURGED SAMPLES

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	6.8	5.4	2.0		2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%		11.2,22.5,30,45%
Highest L.L.*	36.9	52.2	13.0		13.1
Slope	.8067	.3249	.5060		.2658
r **	.9933	.9650	.9381		.9898
5EC50	62.9	178.0	147.5		218.2
5EC25	31.9	101.0	65.1		124.1
50% Sample	39.6	8.4	17.4		5.3
Slope	.7954	.7662	.6914		.4159
r	.9935	.9805	.9620		.9910
15EC50	62.0	71.9	82.8		144.1
15EC25	30.5	59.2	46.7		84.0
50% Sample	40.5	33.2	27.3		10.9
Slope	.6813	1.3439	.8387		.4752
r	.9925	.9558	.9236		.9945
30EC50	76.5	41.2	65.4		122.2
30EC25	39.8	22.6	35.6		69.6
50% Sample	32.0	61.8	37.1		15.7

* Highest Percent Light Loss

** Pearson product-moment correlation coefficient

CENTRAL POND, PAWTUCKET, RI. (10-4-84)

Microtox Sample	Interstitial Water	RO:DI H_2O Elutriate	0.1 M HCl-KCl pH 2.0 Elutriate	RO:DI H_2O Anoxic Elutriate	0.1 M HCl-KCl pH 2.0 Anoxic Elutriate
Initial pH	5.9	5.9	2.0	6.5	2.0
Conc. Tested	11.2,22.5,30,45%	11.2,22.5,30,45%	1.4,2.8,5.6,11.2%	11.2,22.5,30,45%	2.8,5.6,11.2,22.5%
Highest L.L.*	7.4	52.8	79.4	3.2	51.4
Slope	.2461	.3636	3.2514	-.0098	.6338
r **	.9719	.9048	.9910	-.0895	.9516
5EC50	227.5	148.9	17.0	-4947.6	90.2
5EC25	125.9	80.1	9.3	-2402.9	50.8
50% Sample	6.3	14.1	157.4	0.9	24.5
Slope	.2297	.7622	5.7875	.0242	1.7097
r	.9548	.9715	.9925	.2180	.9728
15EC50	242.8	70.9	10.4	1995.4	35.8
15EC25	134.0	38.1	6.1	961.1	21.2
50% Sample	5.7	34.1	279.2	3.0	74.3
Slope	.2759	1.2744	8.1518	-.0295	2.6550
r	.9520	.9810	.9929	-.3161	.9692
30EC50	202.4	40.8	7.4	-1595.4	23.1
30EC25	111.7	21.2	4.3	-747.2	13.7
50% Sample	8.0	61.7	397.2	1.5	121.4

* Highest Percent Light Loss ** Pearson product-moment correlation coefficient

APPENDIX II : CHEMICAL ANALYSIS

INTERSTITIAL		EC50	Cd	Cr	Cu	Fe	Pb	Ni	Ag	Zn	Total
Dodgeville	6/19/84	56	0.00	0.25	0.32	7.30	0.04	0.18	0.00	0.05	8.14
Dodgeville	8/7/84	35	0.00	0.07	0.23	4.50	0.06	0.24	0.00	0.12	5.22
Hebronville	8/7/84	56	0.00	0.03	0.20	4.50	0.04	0.15	0.00	0.59	5.51
Reservation	8/28/84	33	0.02	0.00	0.10	2.60	0.04	0.70	0.00	9.00	12.46
Central	8/28/84	33	0.00	0.00	0.60	3.00	0.04	0.18	0.00	10.00	13.82
Fuller	7/6/84	108	0.00	0.02	0.03	1.70	0.00	0.05	0.00	0.01	1.81
Wetherells	8/7/84	87	0.00	0.51	0.62	1.10	0.16	0.10	0.00	0.20	2.69
Falls	7/6/84	204	0.00	0.04	0.19	7.10	0.05	0.15	0.06	0.21	7.80
Farmers	8/7/84	35	0.00	0.04	0.32	2.40	0.06	0.08	0.00	0.27	3.17
Mechanics	7/6/84	24	0.02	0.26	0.37	2.40	0.10	0.02	0.00	0.12	3.29
	Linear	R^2	---	3E-04	0.061	0.153	0.007	0.045	---	0.108	0.017
	Log	R^2	---	0.084	0.082	0.053	0.001	0.002	---	0.107	0.01
RO:DI H2O		EC50	Cd	Cr	Cu	Fe	Pb	Ni	Ag	Zn	Total
Dodgeville	6/19/84	44	0.13	0.08	0.45	1.10	0.04	3.60	0.00	0.05	5.45
Dodgeville	8/7/84	23	0.30	0.03	1.10	0.50	0.07	3.50	0.00	2.60	8.10
Hebronville	8/7/84	11	0.23	0.00	0.82	0.35	0.00	2.20	0.00	0.59	4.19
Reservation	8/28/84	37	0.20	0.00	0.17	0.62	0.00	2.20	0.00	5.00	8.19
Central	8/28/84	18	0.28	0.00	0.31	0.44	0.00	3.00	0.00	5.60	9.63
Fuller	7/6/84	2210	0.00	0.00	0.03	0.50	0.00	0.00	0.00	0.00	0.53
Wetherells	8/7/84	76	0.00	0.11	0.21	0.30	0.05	0.33	0.00	0.73	1.73
Falls	7/6/84	880	0.00	0.02	0.08	0.80	0.00	0.05	0.00	0.04	0.99
Farmers	8/7/84	36	0.05	0.00	0.33	0.40	0.00	0.96	0.00	3.80	5.54
Mechanics	7/6/84	81	0.02	0.08	0.11	0.48	0.00	0.30	0.00	1.20	2.19
	Linear	R^2	---	---	0.203	0.005	---	0.291	---	0.194	0.356
	Log	R^2	---	---	0.606	0.018	---	0.647	---	0.400	0.656

EC50 - Percent Sample Metals - mg/l

HCl-KCl		EC50	Cd	Cr	Cu	Fe	Pb	Ni	Ag	Zn	Total
Dodgeville	6/19/84	3	2.20	1.20	20.00	110.0	3.90	7.50	0.02	16.00	160.8
Dodgeville	8/7/84	9	0.96	1.20	10.00	43.00	3.10	4.00	0.05	7.80	70.11
Hebronville	8/7/84	6	1.10	1.10	11.00	62.00	2.40	3.2	0.02	5.90	83.52
Reservation	8/28/84	14	1.70	0.98	12.00	120.0	1.70	4.20	0.02	15.00	155.6
Central	8/28/84	4	1.20	1.20	12.00	85.00	2.80	3.50	0.03	11.00	116.7
Fuller	7/6/84	133	0.00	0.03	0.07	30.00	0.26	0.12	0.00	0.41	30.89
Wetherells	8/7/84	33	0.07	4.00	10.00	21.00	3.70	1.10	0.06	4.80	44.73
Falls	7/6/84	40	---	---	---	---	---	---	---	---	---
Farmers	8/7/84	6	0.25	0.46	5.90	54.00	3.20	1.70	0.00	7.20	72.71
Mechanics	7/6/84	25	0.56	4.50	8.70	78.00	3.00	1.80	0.00	10.00	106.6
	Linear	R^2	0.316	0.030	0.542	0.239	0.583	0.378	---	0.459	0.345
	Log	R^2	0.496	0.564	0.929	0.291	0.842	0.879	---	0.882	0.550
RO:DI Anoxic		EC50	Cd	Cr	Cu	Fe	Pb	Ni	Ag	Zn	Total
Dodgeville	6/19/84	---	---	---	---	---	---	---	---	---	---
Dodgeville	8/7/84	26	0.04	0.33	0.58	2.80	0.12	0.16	0.05	2.10	6.18
Hebronville	8/7/84	202	0.00	0.07	0.23	3.70	0.08	0.07	0.00	0.36	4.51
Reservation	8/28/84	438	0.00	0.02	0.09	3.50	0.09	0.06	0.03	0.17	3.96
Central	8/28/84	350	0.00	0.00	0.05	2.60	0.05	0.00	0.00	0.49	3.19
Fuller	7/6/84	264	0.00	0.00	9.20	0.87	0.45	1.20	0.00	7.40	19.12
Wetherells	8/7/84	173	0.00	0.15	0.21	0.58	0.07	0.00	0.03	1.40	2.44
Falls	7/6/84	232	---	---	---	---	---	---	---	---	---
Farmers	8/7/84	43	0.00	0.03	0.20	4.20	0.12	0.04	0.00	2.50	7.09
Mechanics	7/6/84	75	0.02	0.27	26.00	1.80	1.40	1.90	0.00	18.00	49.39
	Linear	R^2	---	---	0.076	0.000	0.097	---	---	0.136	0.106
	Log	R^2	---	---	0.159	0.000	0.120	---	---	0.404	0.136

HCl-KCl Anoxic		EC50	Cd	Cr	Cu	Fe	Pb	Ni	Ag	Zn	Total
Dodgeville	6/19/84	---	---	---	---	---	---	---	---	---	---
Dodgeville	8/7/84	17.00	0.03	5.00	0.09	77.00	0.11	4.10	0.02	2.60	88.95
Hebronville	8/7/84	110.0	0.36	1.90	0.13	85.00	0.49	2.80	0.02	3.80	94.50
Reservation	8/28/84	31.00	0.24	1.30	0.14	110.0	0.24	2.40	0.00	10.00	124.3
Central	8/28/84	21.00	0.81	1.50	0.35	52.00	1.20	2.40	0.00	9.20	67.46
Fuller	7/6/84	148.0	0.03	0.02	11.00	20.00	0.54	1.80	0.00	8.40	41.79
Wetherells	8/7/84	59.00	0.03	3.00	0.09	20.00	0.95	0.60	0.02	4.40	29.09
Falls	7/6/84	41.00	0.09	0.18	3.60	20.00	2.40	0.54	0.03	4.60	31.44
Farmers	8/7/84	58.00	0.02	0.51	0.02	61.00	0.16	1.20	0.00	3.60	66.51
Mechanics	7/6/84	10.00	0.62	5.00	3.90	83.00	2.60	1.60	0.00	12.00	108.7
	Linear	R^2	0.142	0.289	0.335	0.138	0.118	0.013	---	0.036	0.132
	Log	R^2	0.109	0.473	0.054	0.168	0.012	0.001	---	0.009	0.109