THIRD QUARTERLY REPORT

Project Title: Development of Bioassay Procedures for Defining Pollution of Harbor Sediments

Project Identification No.: R804918-01

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I. WORK STATUS

A. Sampling

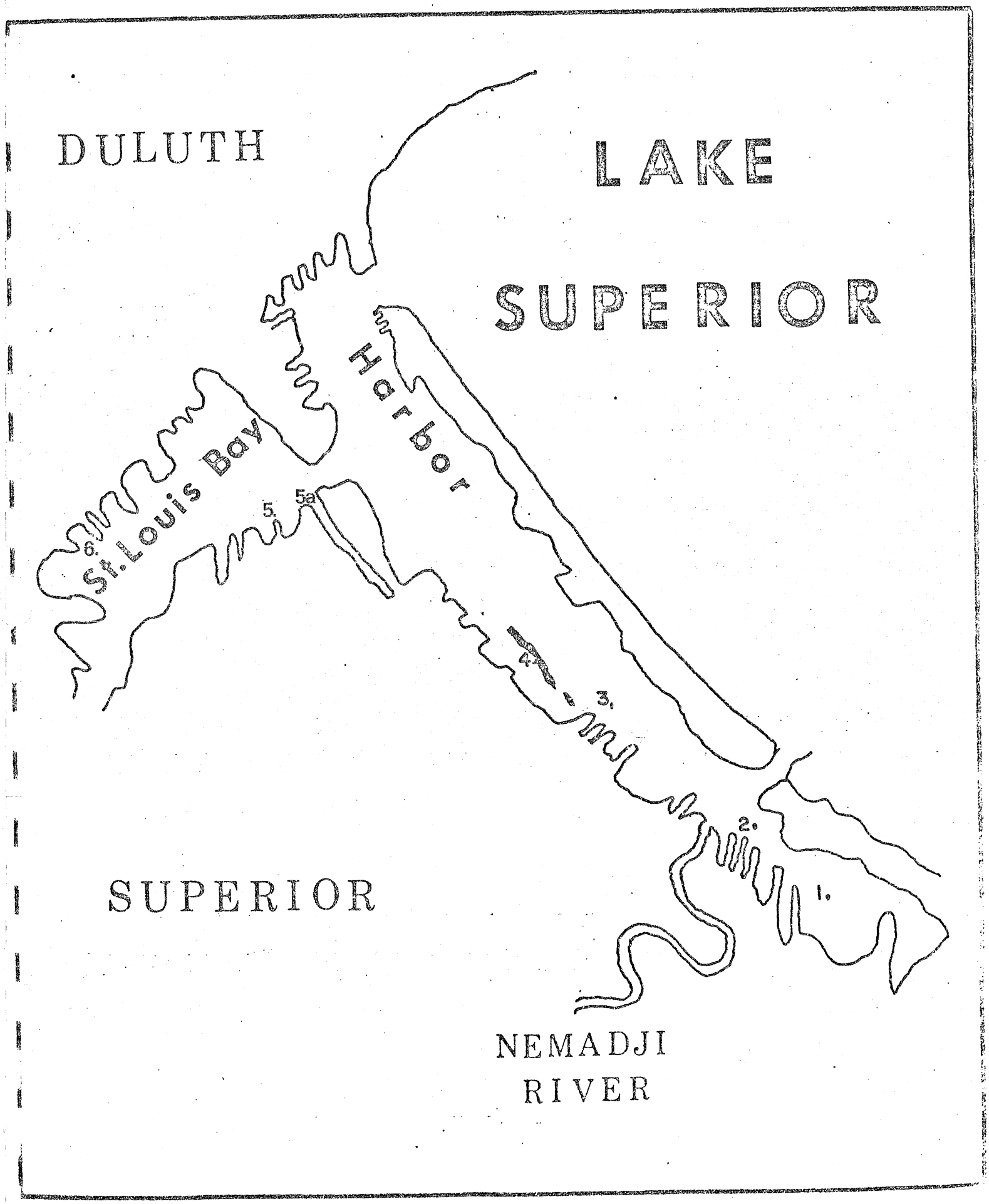
Six sites in the Duluth-Superior Harbor have been sampled. The sites 1 (Allouez Bay area), 2 (Superior Ore Docks area), 3 (Lakehead Transshipment Terminal area), 4 (Superior Sewage Treatment Plant area) and 6 (Minnesota Power and Light area) correspond to the same locations as indicated in the project proposal. The sixth site designated as 5a is located near the Farmers Union Elevator No. 1 (Figure 1).

At each site, 24 three-inch diameter cores with overlying water were obtained. Sediment was also dredged to use in the bioassay chambers for the Hexagenia limbata and Daphnia magna tests. Chironomids were obtained from each site except 5a and these samples have been weighed and frozen for future analysis of heavy metal and organic compound accumulation. Samples of sediment, overlying water and interstitial water have been sent to Virginia Polytechnic Institute for the enzyme study phase of the project.

Sediment has been obtained from Pokegema Bay, Wisconsin each time a harbor site was sampled. The Pokegema Bay sediment has been used primarily as a reference material in the bioassay tests.

B. Treatment of Samples

The majority of the sediment cores for each site were extruded under N_2 in a glove bag and subjected to high speed centrifugation to obtain interstitial water. Approximately 4 liters of interstitial water is obtained per site and


DULUTH
LAKE
SUPERIOR
Harbor
St. Louis Bay
6.
5.
5a
4.
3.
2.
1.
SUPERIOR
NEMADJI
RIVER

this water is used for the bluegill cough response tests, Daphnia magna bioassay tests, enzyme tests and chemical tests as indicated in Table 1. The total processing of the cores at each site was accomplished in one 16 hour day on the day immediately following the sampling.

Subsamples of several of the cores from a particular site were blended and subjected to the chemical analysis outlined in Table 2. In addition, extensive analysis on metals found in exchangeable, easily reducible, moderately reducible, organic and residual phases are being conducted. Two of the cores were used in conducting "standard elutriate tests" which are outlined in Table 3. These standard elutriate tests were carried out by procedures given in the recent "Implementation Manual for Section 103 of Public Law 92-532" entitled "Ecological Evaluation of Proposed Discharge of Dredged Material Into Ocean Waters" prepared jointly by the EPA and Corps of Engineers. The preparation of the elutriate was carried out using both air-exposed sediment and sediment isolated under N_2 using the method indicated for preparing the liquid phase bioassay medium.

Water overlying the sediment cores was immediately siphoned and preserved and stored for the chemical analysis as indicated in Table 4.

Bioassays were begun the day following the sampling using sediment for Hexagenia and Daphnia toxicity tests and interstitial water for bluegill cough response and Daphnia toxicity tests. Overlying water from the Hexagenia bioassay test chambers and 25% interstitial water--75% Lake Superior water cough response test media were analyzed at the conclusion of the bioassay tests for essentially the same chemical parameters as in Table 4. Hexagenia from the bioassay tests have been frozen for future analysis for metal and organic compound content.

TABLE 1: INTERSTITIAL WATER TESTS

INTERSTITIAL (PORE) WATER

3.7 liter (minimum)

stainless steel

Cough Response
1800 ml

Sample Water Required	
10% interstitial	800 ml
25% interstitial	1000 ml

Chemical Tests
1900 ml

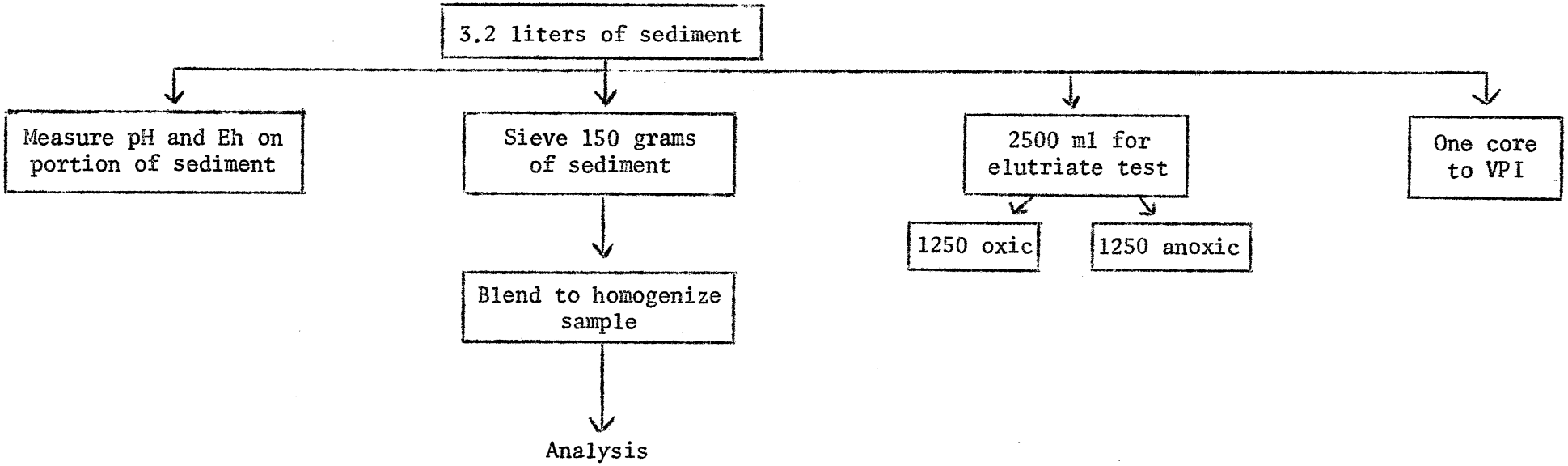
Test	Volume	Preservative	Max Holding Time
pH	30 ml		immediate
metals	15 ml	0.2% HNO_3	6 months
Hg (total)	50 ml	0.5% HNO_3-0.05% $K_2Cr_2O_7$	38 days in glass
Hg (inorg)	100 ml	same	same
COD	50 ml	H_2SO_4 (pH=2)	7 days
trace organics		extract and	4 weeks
1. chlorinated	500 ml	refrigerate	
2. PAH	500 ml		
H_2S	100 ml	zinc acetate (2 ml)	24 hrs
chloride	50 ml	none	7 days
suspended solids	obtained from filtering metals, Hg and Cl		
NH_3 & TKN	500 ml	0.8 ml H_2SO_4/ liter cool to 4 C	24 hours
duplicates	excess of 3.7 liters		

20 ml in glass
20 ml in plastic
transported on ice to VPI

NOTE: All water except that for trace organics may be obtained in plastic centrifuge bottles (900 ml).

TABLE 2: SEDIMENT ANALYSIS

SEDIMENT SAMPLES



Parameters	Amount of Sediment
COD	0.5 to 1.0 gram
total solids	10 grams
total volatile solids	
moisture	obtain by difference
ammonia	0.5 to 2 grams
TKN	
organic N_2	obtain by difference
total sulfide	1.0 to 5 grams
total phosphorus	0.5 to 0.8 gram
oil and grease	5.0 to 10 grams
phenols	10 to 50 grams

TABLE 3: ELUTRIATE WATER ANALYSIS

ELUTRIATE TEST

1.25 liter of oxygenated sediment

↓

Shake with 5 liters Lake Superior water

↓

centrifuge

↓

Analyze Lake Superior water and elutriate water

Parameter	Water Required
metals	15 ml
mercury-total	150 ml
mercury-inorg	150 ml
trace organics (PCBs, pesticides, phenols, PAH)	2 liters
H_2S	100 ml
NH_3 & TKN	500 ml
COD	50 ml
pH	50 ml

Periodic duplicates of above parameters

1.25 liter of anoxic sediment

↓

Shake with 5 liters of Lake Superior water

↓

centrifuge

↓

ANALYZE

FOR

SAME

PARAMETERS

AS FOR

OXIC

ELUTRIATE

↓

May be re-extracted for same parameters

TABLE 4: OVERLYING WATER ANALYSIS

OVERLYING WATER

32 liters

18 liters

Siphon on site into glass container. Store in dark at 4 C. Collect dissolved oxygen.

Analysis

Parameter	Water Required
trace organics (PCBs, pesticides, phenols, PAH)	8 liters
dissolved O_2	300 ml
total phenols	500 ml
duplicates	9 liters

one liter in glass
one liter in plastic
transported on ice
to VPI

3 liters

Siphon on site into plastic bottles. Store in dark at 4 C.

Analysis

Parameter	Water Required
pH	50 ml
metals	25 ml
H_2S	100 ml
NH_3 & TKN	500 ml
mercury-total	150 ml
mercury-inorg	150 ml
COD	100 ml
orthophosphate	50 ml
suspended solids	200 ml
chloride	50 ml
duplicates	1500 ml

Tests to determine the availability of chemical species to water penetrating the sediments has begun using sediment from two of the sites. After the interstitial water is removed from the sediment in stainless steel centrifuge cups, a volume of fresh Lake Superior water equal to the volume of the interstitial water removed is added to the sediment and homogenized. After a certain storage period, the interstitial water is again removed from the sediment. This process is repeated obtaining generated interstitial water at 10 hours, 1-1/2 days, 3-1/2 days, 7-1/2 days, 10-1/2 days, 15-1/2 days and 21-1/2 days following the removal of the initial interstitial water. This generated interstitial water is being analyzed for D.O., pH, metals, COD and certain individual organic species (PCB's, phenols, pesticides, PAH).

C. In situ Sediment Sampling - Bioassay Chamber

Development of a box sampler bioassay chamber is continuing. The first prototype chamber was completed by the contractor, Chester Zimm Inc., and tested in the field July 24. The first model proved effective in obtaining an undisturbed sample and could be sealed and used as a bioassay chamber. The prototype sampler was used as a bioassay chamber during tests on sediments from site 5a. A modified chamber has been designed and ordered from the contractor. The modified chamber will allow for easier deployment by divers and lower construction cost.

II. PRELIMINARY RESULTS

A. Chemical Analysis

At this time, the chemical analyses are at various stages. Analysis of the various types of water and sediment samples have been largely completed in terms of parameters such as pH, Eh, COD, total solids, volatile solids, ammonia, TKN, sulfide, phosphorus, oil and grease, total phenols, mercury, chloride and suspended solids. This data is presently being tabulated. Analyses for some samples is complete for metals but many samples have yet to be analyzed. Since these data are fragmentary at this time, no attempt will be made to submit partial tabulations here.

For organic analysis of specific compounds, some data has been obtained for PCB's, pesticides and pentachlorophenol but mainly in the water samples. All of the samples obtained thus far have been extracted but many remain to be cleaned-up by gel permeation chromatography. Work has progressed on PAH analyses as gas chromographic conditions have been worked out for PAH compounds of interest. Extractions have been tested with spiked samples. Some tentative analysis has been performed on interstitial elutriate water from sites 1 and 2 but no positive identifications of PAH compounds have been made.

B. Sediment Bioassays

Bioassay tests have been completed for six of eight proposed sites in the Superior-Duluth Harbor. To date all within and over-sediment bioassays have been conducted in 40 x 20 x 20 cm glass chambers partitioned into two 20 x 20 x 20 cm sections by a removable divider. Sediments removed from the test and

and control sites by a Ponar dredge are placed in the chambers. Just prior to testing, approximately 10 cm of oxygenated water is added and the divider is removed. Hexagenia limbata are added one at a time, five over sediment from the test site and five over sediment from the control site. In all tests it was shown that the insects burrow within a few seconds of reaching the substrate. There was no evidence that the insect inspected the substrate prior to burrowing or selected control substrate over any substrate taken from the six test sites. Hexagenia did not return to the surface after burrowing to look for a superior sediment and exhibited low mortality in both the test and control site sediments (Table 5). Survival was low only during testing of sediments from site 1 in which the number of replicates was two rather than the normal four. Tests of sediments from other sites included four replicates in which usually ten organisms were submitted to test and control sediments. During these tests four replicates were run with oxygen in the overlying water held at approximately 4 ppm and four were run near saturation (7 ppm) at 18-19°C. Preliminary statistical analysis by two-way analysis of variance demonstrated that survival was similar under low and high oxygen (blocks) and with test and control site sediments.

Five Daphnia in each of two 250 ml beakers were held over the control site and sediments from six harbor sites. Two 75 mm holes were drilled in each 250 ml beaker and fitted with 50 μ screen. The screened opening allowed circulation of water through the beakers which were set inside the 20 x 20 x 20 cm bioassay chambers to maintain 200 ml volumes. Water in the bioassay chambers was circulated by the activity of Hexagenia limbata. Their respiratory activity created turbidities ranging from 9 to 944 FTU. Because turbid water circulated

TABLE 5: SURVIVAL OF HEXAGENIA LIMBATA IN HARBOR SEDIMENTS

Date	Site	Number of Reps.	Animals per Rep.	SURVIVAL %				Value of F	Level of Sign.
				Low O_2		High O_2			
				Control	Test	Control	Test		
June 6 - June 10	2	4	9-12	85.5	92.5	92.2	78.0	2.92	NS
June 13 - June 17	1	2	9	61.0	33.0	62.0	72.0	1.83	NS
June 27 - July 1	6	4	9-11	80.5	85.0	82.5	87.5	0.15	NS
July 11 - July 15	3	4	5-11	85.0	93.0	85.0	92.0	0.83	NS
July 18 - July 22	4	3 or 4	5-10	90.0	77.0	90.0	87.0	0.60	NS
July 25 - July 29	5a	4	10-12	84.0	92.5	85.2	87.5	0.33	NS

freely through the beakers during the test, it was assured that Daphnia magna were in direct contact with any pollutants from the sediments.

Survival of Daphnia magna held over sediments was generally higher over the control sediments than test site sediments (Table 6). However, statistical analysis by two-way analysis of variance (with sampling) failed to show that survival for individual harbor test sites varied from the control (Table 6; $P > 0.05$).

C. Interstitial Water Bioassays

Daphnia magna and bluegill sunfish Lepomis macrochirus were used in tests with water extracted from harbor sediments from six test sites. Static 96 hr Daphnia magna tests were conducted in 250 ml beakers containing 5 and 50% interstitial water from the test site, 5 and 50% interstitial water extracted from control site sediments and 100% Lake Superior water. Lake Superior water was used to dilute the interstitial water to the selected concentrations. All tests were conducted with five animals per chamber and five chambers per treatment.

Survival was highly variable within and among tests. During tests on sediments from sites 2, 3 and 4 most Daphnia magna came to the surface and died. Low survival during these tests was apparently caused by use of supersaturated Lake Superior water as a test medium. This problem was identified and eliminated by the start of the sixth test conducted July 25-29, 1977 on sediments from site 5a (Figure 1). Preliminary statistical testing employing a Latin Squares model showed survival varied between test sites 6, site 5a and the control site (Table 7).

Bluegill cough response was monitored for 16 fish during each test with interstitial water. Coughing was monitored for four bluefills held in 10% test

TABLE 6: SURVIVAL OF DAPHNIA MAGNA OVER HARBOR SEDIMENTS

Date	Site	Number of Reps.	Animals per Rep.	SURVIVAL %				Value of F	Level of Sign.
				Low O_2		High O_2			
				Control	Test	Control	Test		
June 6 - June 10	2	8	5	62.5	52.5	55.0	50.0	0.3	NS
June 13 - June 17	1	8	5	67.5	85.0	85.0	62.5	3.4	NS
June 27 - July 1	6	8	5	100.0	92.5	85.0	67.5	4.4	NS
July 11 - July 15	3	8	5	90.0	90.0	72.0	65.0	2.6	NS
July 18 - July 22	4	8	5	98.0	65.0	90.0	82.0	4.4	NS
July 25 - July 29	5a	8	5	80.0	82.5	95.0	77.1	0.7	NS

TABLE 7: SURVIVAL OF DAPHNIA MAGNA IN SEDIMENT INTERSTITIAL WATER

Date	Site	Number of Reps.	Animals per Rep.	SURVIVAL % Lake Superior	Control Interstitial 5%	Control Interstitial 50%	Test Interstitial 5%	Test Interstitial 50%	Value of F	Level of Sign.
June 7 - June 11	2	5	5	16	16	28	0	32	1.52	NS
June 14 - June 18	1	3	3	93	93	93	80	80	0.90	NS
June 28 - July 2	6	5	5	68	68	92	16	28	14.95	0.01
July 12 - July 16	3	5	5	12	24	52	4	16	1.13	NS
July 19 - July 23	4	5	5	28	12	44	0	15	2.82	NS
July 26 - July 30	5a	5	5	93	100	97	52	84	7.97	0.01

sediment interstitial water, 25% test sediment interstitial water, 25% control sediment interstitial water and 100% Lake Superior water. During the 96 hr tests, cough was monitored for 30-36 hrs by a Gilson IMP 5 physiograph. Coughing was monitored for 12 hr prior to initiation of the test to measure background levels. Monitoring was also carried on after test chambers had been flushed with dechlorinated water for 3 hrs.

Physiograph records from the bluegill tests have not been analyzed. However, gross observations during the tests suggest background levels of coughing are quite high. Coughing appears to remain high through the second day of the test and then is suppressed during the remainder of the test period.

Data is available for five of six sites sampled. However, infection of fish with Ick. prior to conducting tests on sediments from site 6 made data collection impractical for that location.

III. ACTIVITIES ANTICIPATED DURING THE NEXT QUARTER

Two more samplings and the accompanying biological and chemical tests will be completed during the next quarter. The sampling locations will be site 6 and a site located on Lake Superior. The sampling of site 6 will serve as a comparison to the data previously obtained on this site. The study of a Lake Superior location will give information on a site containing sediments of higher quality than those found in the harbor.

In addition to the bioassay tests conducted on samples from the previously studied sites, elutriate water will be used to study toxic effects toward

Daphnia. The elutriate water toxicity results will be compared to those run concurrently using interstitial water.

The cough response data pertaining to Lepomis macrochirus exposure to interstitial water will be analyzed for all sites.

Additional testing (and modifications if necessary) of the box sampler bioassay chamber will be carried out. Further bioassays using this chamber will be conducted.

Chemical analysis will continue on the sediment and water samples with extensive work conducted on metal and specific organic compound determinations. Analyses of Chironomids from the harbor sediments and Hexagenia samples pertaining to the bioassays will begin.

Fractionation of the sediment organic extracts (and possibly some organic extracts of water samples) will be initiated using high pressure liquid chromotography (reverse phase). Investigation of the magnitudes of chemicals in the sediments with various relative bioaccumulation potentials will be attempted.

Particle-size distributions of sediments from all of the sites will be determined using the pipet-sedimentation method.

Tabulation and study of the chemical data from the various sample types will continue. Investigation of possible sediment and water chemical relationships to the bioassay results will be initiated.

IV. EXPENDITURES

(THROUGH 31 JULY 1977)

	Federal	Non-Federal
Personnel	$21,760	$ 6,600
Fringe Benefits	4,439	1,346
Student Assistants	7,780	---
Travel	538	---
Equipment	---	4,910
Supplies	4,600	---
TOTAL	$39,117	$12,856